

## STABLE ISOTOPE ANALYSIS TO DETERMINE TROPHIC RELATIONSHIPS IN THE SPENCER GULF, SOUTH AUSTRALIA

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### ABSTRACT

A study of the food web in the Spencer Gulf, South Australia has been undertaken with the primary aim of determining the effect of discarding 1000 tons per year of by-catch from a prawn fishery. Previously, food web studies involved extensive analysis of gut contents but recently the use of stable isotope ratios has shown to be effective in helping to characterise food web structure and assign species groups to trophic levels. By using the stable isotopes  $^{13}\text{C}$  and  $^{15}\text{N}$  we determined the trophic position of the dominant filter feeding bivalves *Trichomya hirsuta* and *Equichlamys bifrons*, the blue crab *Portunus pelagicus* and the prawn *Penaeus latisulcatus*. In addition, gut samples from a deposit feeding holothurian and gut samples from the two bivalves are compared. The paper describes the preliminary results of this work and discusses some problems associated with using stable isotope ratios in food web analysis.

### INTRODUCTION

A study of a marine food web usually involves gut content analysis of the dominant species in combination with direct observations of feeding. More refined methods of quantifying trophic pathways, using radio-tracer techniques, are also sometimes used. Unfortunately, all of these methods are time consuming and may not adequately allow researchers to separate or identify the com-

ponents of the different trophic levels. The reason for this is usually that rates of digestion vary between organisms, food items are often difficult to identify from stomach contents, and dietary analyses only give a snapshot of consumption of an individual's immediate past. In contrast, the analysis of stable isotope ratios provides an alternative whereby an organism's trophic position (level) may be identified by a simple tissue sample, which provides an integrated signal of diet over a longer period (Peterson & Fry, 1987; Lajtha & Michener, 1994).

The basis for using stable isotope ratios is founded on the observation of a regular and consistent pattern of isotopic enrichment with increasing trophic level. However, the isotopic composition of tissues between organisms and within an organism may vary, reflecting a change in diet or selective feeding (Peterson & Fry, 1987; Boon & Bunn, 1994). The general purpose of the study is to describe the food web structure in areas where a prawn fishery operates, and which are likely to be influenced by discarded by-catch. The discarded by-catch, which consist mostly of 10 fin fish species, is quickly but selectively consumed on the surface by dolphins (*Delphinus delphis*) and on the bottom, primarily by schools of Degens leather jackets (*Thamnaconus degeni*), blue crabs (*Portunus pelagicus*) and the sea lice *Natatolana woodjonesi* but also by a variety of lesser common species. Through in-

creased faeces production these consumers may contribute locally to the load of particulate organic matter, in conjunction with increased resuspension by trawling of the seabed, thereby affecting the energy flow. The organisms that may be affected by an increased load of resuspended matter are the filter feeding bivalves *Equichlamys bifrons* and *Trichomya hirsuta*.

In this preliminary study we selected the isotopes  $^{13}\text{C}$  and  $^{15}\text{N}$  to describe patterns of pelagic-benthic coupling in a trawled ecosystem and to investigate the patterns of variation in isotopic composition. We compare isotope ratios of macro- and mesoplankton,

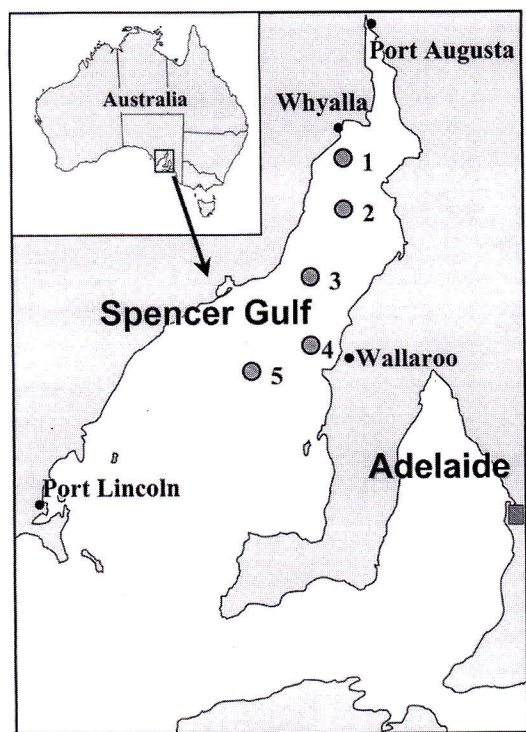


Fig. 1. Map showing the Spencer Gulf, South Australia and the sampling localities.

plankton consumed by bivalves (microplankton) and bivalve muscle tissues. These values are compared to important micro- and macropredators (sea cucumber, blue crab and prawn).

## MATERIAL & METHODS

Replicated samples were collected in the upper Spencer Gulf, South Australia (Fig. 1) during a two-week period in June 1999 using SARDI's research vessel R/V Ngerin. Blue crabs (*Portunus pelagicus*), western king prawns (*Penaeus latisulcatus*), the sea cucumber (*Stichopus ludwigi*), the scallop (*Equichlamys bifrons*) and the mytilid (*Trichomya hirsuta*) were collected using a beam trawl equipped with a 3 m wide and 1 m high iron frame opening. The depth at the sampled stations was 25-30 m. Plankton sampling to obtain particulate organic matter (POM) was done by three replicate vertical hauls using a VP2 plankton net equipped with an 85  $\mu\text{m}$  filter.

After collection, individuals sampled by beam trawl were dissected and the gut content and muscle tissue sampled and immediately thereafter frozen. On return to the laboratory, all tissue material were freeze-dried. POM samples were also frozen immediately after collection, but were defrosted in the laboratory and centrifuged to reduce the water content before re-freezing and freeze-drying the supernatant. All samples were ground and subsequently analysed for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  at Waikato Stable Isotope Unit, Department of Biological Sciences, University of Waikato, Hamilton, New Zealand.

The results were calculated as delta values, which are expressed as deviation from standard reference material (stable isotope ratio) where

$$\delta X = [(R_{\text{sample}}/R_{\text{std}}) - 1] \times 1,000;$$

$$X = {}^{13}\text{C} \text{ and } {}^{15}\text{N}$$

$$R = {}^{13}\text{C}/{}^{12}\text{C}, {}^{15}\text{N}/{}^{14}\text{N}$$

The standards used were Canyon Diablo Troilite limestone and nitrogen in air, respectively.

## RESULTS

The data on the  $^{13}\text{C}$  ratios of plankton, gut content and muscle tissue are depicted as ascending values in Fig. 2.

The results showed that the lowest ratios

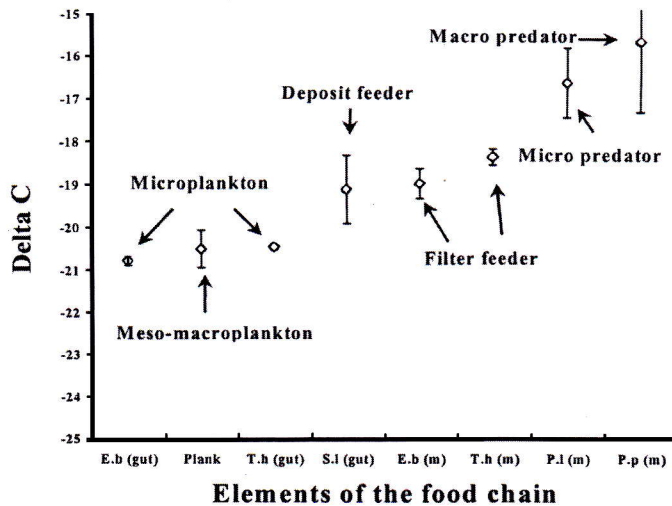


Fig. 2. Separation of trophic levels in the Spencer Gulf using stable isotope ratios of  $^{13}\text{C}$ . Vertical bars are 95% C.I. (n=3). E.b. = *Equichlamys bifrons*; T.h. = *Trichomya hirsuta*; S.l. = *Stichopus ludwigi*; P.l. = *Penaeus latisulcatus*; P.p. = *Portunus pelagicus*; Plank = plankton.

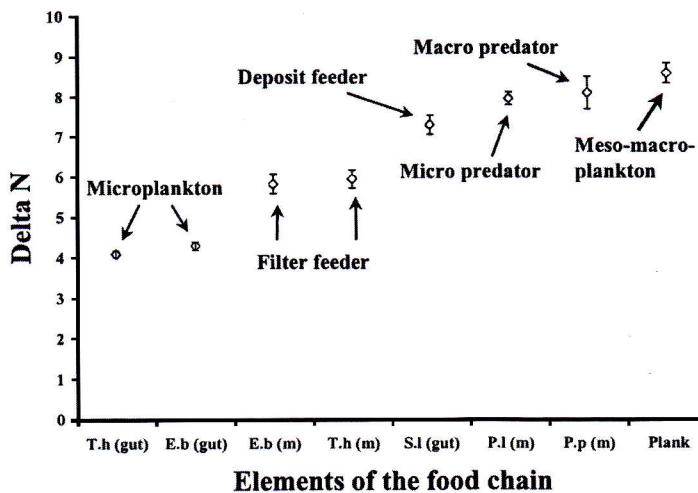


Fig. 3. Separation of trophic levels in the Spencer Gulf using stable isotope ratios of  $^{15}\text{N}$ . Vertical bars are 95% C.I. (n=3). E.b. = *Equichlamys bifrons*; T.h. = *Trichomya hirsuta*; S.l. = *Stichopus ludwigi*; P.l. = *Penaeus latisulcatus*; P.p. = *Portunus pelagicus*; Plank = plankton.

were found in the plankton samples and the samples taken from the gut of the two filter feeding bivalves, *E. bifrons* and *T. hirsuta*. The  $^{13}\text{C}$  ratios of gut content of these two species were clearly separated from muscle tissue demonstrating isotopic enrichment. As expected,  $^{13}\text{C}$  ratios from the muscle tissue of both bivalves were lower than muscle tissue from the prawn *P. latisulcatus* and the blue crab *P. pelagicus*. The values for the gut content of the deposit feeding holothurian *S. ludwigi* were found to be

slightly lower, but not significantly, than the muscle tissue from the two bivalves. Accordingly, the  $^{13}\text{C}$  ratios are distributed among trophic levels as expected. All data points are shown as the mean of three samples from individual animals or plankton samples. It is noteworthy that the largest variations were found for the predatory animals (prawns and crabs) and the gut content of the deposit feeding holothurian indicating that these animals consume a variety of organisms from higher trophic levels. A sig-

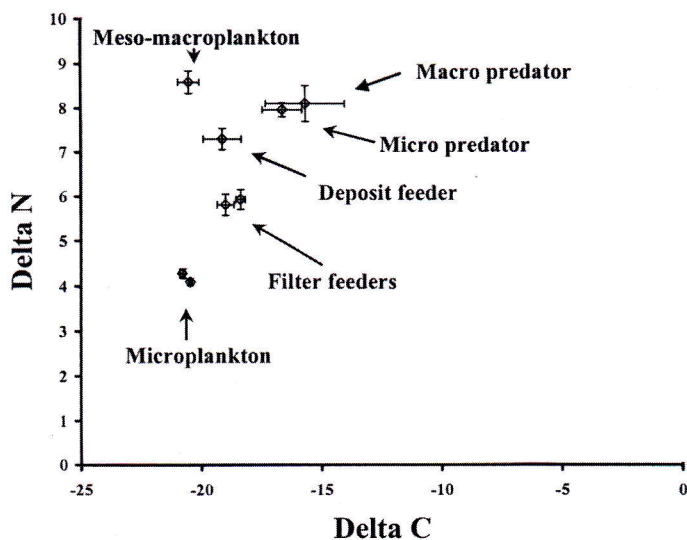


Fig. 4. Two-dimensional separation of trophic levels in the Spencer Gulf using stable isotope ratios of  $^{13}\text{C}$  and  $^{15}\text{N}$ . Vertical and horizontal bars are 95% C.I. (n = 6).

nificant effect of species on  $^{13}\text{C}$  ratios was found (one-way ANOVA,  $p < 0.05$ ). The plankton samples from vertical hauls showed also a large variation compared to plankton from the gut of the mussels. This indicates that the samples obtained by vertical hauls are unspecific and contain a variety of organisms from different trophic levels in the pelagic food chain.

The data on the  $^{15}\text{N}$  ratios are depicted as ascending values in Fig. 3. These values showed the same pattern as for  $^{13}\text{C}$  with the exception of the plankton value (meso-macroplankton), which showed high  $^{15}\text{N}$  ratios. Variation about the means was generally small but highest for the blue crab. A significant effect of species on  $^{15}\text{N}$  ratios was found (one-way ANOVA,  $p < 0.05$ ). The  $^{15}\text{N}$  ratios of gut content of the two bivalve species were clearly separated their muscle tissue demonstrating isotopic enrichment as shown for carbon.

Combining the two data sets, a clear picture emerged separating the different trophic levels (Fig. 4). The effect of high  $^{15}\text{N}$  ratios in the unspecific plankton samples from vertical hauls (macro-mesoplankton) is clearly seen, displacing these values from a linearly relationship.

## DISCUSSION

The preliminary data presented here show that measurement of stable isotope ratios is a useful tool in characterising trophic levels in the Spencer Gulf ecosystem. In order to get a full picture more samples are needed, especially of those organisms that constitute the by-catch elements and their scavengers.

Variations within samples were generally small but were highest among samples from the predatory prawns and crabs. Adults of the prawn *P. latisulcatus* are deposit feeders and examination of the gut content revealed sand grains and hard fragments of a variety of meiofaunal species explaining the relatively large variation in stable isotope ratios. The blue crab, *P. pelagicus*, is known to feed on discarded by-catch during fishing periods and at other times consume a variety of food items (Wassenberg & Hill 1987) thus explaining the large variation in stable isotope ratios. The  $^{13}\text{C}$  ratio for the gut content of *S. ludwigi* were somewhat low, which may be explained by the fact that the analyses were performed on dry gut content that have a large protein component relative to carbohydrates. These variations, however, did not obscure separations of trophic levels. The plankton samples, how-

ever, were collected as vertical hauls using a 85  $\mu\text{m}$  filter in the cod end and consist of a mixture of many larger organisms like ctenophores, copepods and invertebrate larvae, which constitute major elements of a pelagic food chain. These samples contained much larger particle sizes than 85  $\mu\text{m}$  (meso-macroplankton) with an apparently much higher N content presumably originating from protein. The gut samples from the bivalves had significantly lower stable isotope ratios and contain primarily partly digested phytoplankton organisms smaller than 80  $\mu\text{m}$ , which probably accounts for the lower variability in the bivalve measurements. These results demonstrate that bivalves feed selectively within a small specific fraction size of plankton (Møhlenberg & Riisgård 1979, Nielsen *et al.* 1993). The two-dimensional data of stable isotope ratios of  $^{13}\text{C}$  and  $^{15}\text{N}$  clearly separate the studied organisms along a gradient of isotopic enrichment at higher trophic levels (Fig. 4). Variations among individual organisms were low thus making the method useful in characterising the food web. The high N-ratio of the vertical plankton samples compared to the plankton from the gut of the bivalves stresses the importance of clearly fractionate plankton samples before measurement to avoid mixing of trophic levels.

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