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The $\delta^{13}C$ trophic position isotope spectrum as a tool to define and quantify carbon pathways in marine food webs

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ABSTRACT: The trophic position isotope spectrum is a stable-isotope-based method with which to analyse food web interactions and quantify the relative magnitude of dietary pathways. The approach was developed and tested for anchovy in the Southern Benguela using $\delta^{13}C$ determinations of the protein fraction. The results confirm recent findings that anchovy in the Southern Benguela are largely zooplanktivorous and refute the traditional Ryther hypothesis that large pelagic fish populations in upwelling areas are supported mainly by direct feeding on phytoplankton.

INTRODUCTION

Modelling energy flow through food webs is a way to understand trophic relationships in an ecosystem (Winberg 1956, Ivlev 1961, Durbin & Durbin 1983). These relationships vary in time and space and their definition has led to considerable debate in the literature (reviewed e.g. in James 1988 for planktivorous clupeoids). Unless flows of energy, carbon or nitrogen are monitored on fine temporal and spatial scales, the present uncertainties concerning the importance of various pathways will persist. This is especially so in pelagic food webs, where planktivorous fish such as anchovy have a wide choice of prey and are capable of flexible and opportunistic feeding behaviour (Koslow 1981, Angelescu 1982, James 1987, James & Findlay 1989, James & Chiappa-Carrara 1990).

The concept of food web structure in upwelling areas has been dominated by the hypothesis that the large populations of pelagic fish in these regions resulted from their ability to shorten the food chain by using the primary producers directly, thereby increasing trophic efficiency (Ryther 1969). This hypothesis appeared to be supported by field studies (reviewed by James 1988a), but recent data raise questions about the validity of this argument and indicate that this pelagic food web is longer and more complex than previously thought (Cushing 1978, Koslow 1981, Angelescu 1982, James 1987, 1988, Cherry et al. 1989, James et al. 1989).

In this study we develop a method, the trophic position isotope spectrum (TPIS), with which to analyse food web interactions and estimate the relative magnitude of the different pathways. The approach was developed using $\delta^{13}C$ data collected in the southern Benguela Current (Sholto-Douglas et al. 1991 [companion article]). We then use the TPIS for anchovy protein $\delta^{13}C$ to show that its trophic link to phytoplankton is longer and more complex than that suggested by Ryther (1969).

The use of stable isotope ratios to estimate diet composition was pioneered in the laboratory by DeNiro (1977) and for marine food chains by Parker (1963), McConnaughey & McRoy (1979) and Rau et al. (1983). It was found that at each trophic position the organism was enriched in the heavier isotope relative to its diet (DeNiro & Epstein 1978). It is this enrichment, which both is quite variable (Peterson & Fry 1987) and occurs as a result of differential catabolic and anabolic rates within the organism (DeNiro 1977, Owens 1987), which allows dietary links to be deduced from food-chain samples. The study of marine food chains using stable

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isotope ratios is relatively recent (McConnaughey & McRoy 1979, Macko et al. 1982, Rau et al. 1983, Fry et al. 1984, Kiyashko 1988, Rau et al. 1989), and most reports can be classified as surveys of stable isotope ratios rather than investigations of elemental pathways (Miyake & Wada 1987, Peterson & Howarth 1987, Macko 1981).

Present stable-isotope-based food-chain studies have 2 main problems: data tend to be sparse (n = 1 to 5 samples) (Rau et al. 1983, Dunton et al. 1989) and relationships between diet and consumers are established on the basis of the means of these small data sets. The use of mean values ignores sample variability, which may be the key to the complexity of trophic interactions. Stable isotope ratios characteristic of specific trophic interactions are spatially and temporally highly variable (Owens 1987, Dunton et al. 1989, Saupe et al. 1989). Organisms may switch diets because of size, availability, ease of capture and biogeography of prey. With the TPIS approach, set out below, many of these problems are overcome and with careful sampling specific pathways may be identified and quantified.

Three commercially important engraulids – *Engraulis mordax* (Koslow 1981, James & Chiappa-Carrara 1990), *E. anchoita* (Angelescu 1982, Angelescu & Anganuzzi 1981) and *E. capensis* (James 1987, James & Findlay 1989) – are all size-selective predators, the bulk of whose diets are composed of meso- (200 to 2000 µm) and macro-zooplankton (> 2000 µm), and not phytoplankton. Although recent data indicate that clupeoids are largely zooplanktivorous in upwelling areas, these data are not entirely conclusive. Field studies are restricted to analyses of stomach contents and selectivity under the observed environmental conditions, and thus are neither temporally nor spatially integrated – they essentially provide 'snapshots' of a diet and feeding behaviour. It is difficult to draw concrete conclusions from these data or extend them beyond the scope of the study, especially when the flexibility of engraulid feeding behaviour is considered. Secondly, while both field and laboratory data are used to deduce the diet and energetics of these fishes, they do not define the food pathways between the primary producers and fish, and interactions between the intermediate trophic positions remain a matter of conjecture. What is required is a method that defines energy flows over known space and time scales. These data may be used to assess the importance of the various potential carbon or nitrogen pathways from primary producers to consumers. Such data would supplement information gained from more conventional studies, or indeed challenge existing paradigms. We believe that the variability in stable isotope ratios expressed as TPIS rather than means and standard deviations can provide this information. This model was formulated using a data set which is described more fully in the companion paper of Sholto-Douglas et al. (1991).

METHODS AND MATERIALS

Sampling and analysis. Samples collected for stable isotope analysis were obtained from stations in the Southern Benguela (Sholto-Douglas et al. 1991). For $\delta^{13}C$ measurements, the lipid fractions were extracted from the sample using a chloroform:methanol:water (2:1:0.8) mix (Bligh & Dyer 1959), and the residual muscle protein was freeze-dried, then combusted under vacuum with a Cu/CuO mix at 800 °C. The gas combustion products were isolated by cryogenic distillation and carbon stable isotope ratios measured with a VG602E mass spectrometer. Although $\delta^{15}N$ data were also available, the total number of samples was not sufficient for satisfactory application of the TPIS. The details of sampling and analysis are given in the companion paper of Sholto-Douglas et al. (1991).

$\delta^{13}C$ stable isotope ratios are expressed as ‰ difference from the PDB standard (Hoefs 1980):

$$\delta^{13}C = \frac{(^{13}C/^{12}C)_{Sample} - (^{13}C/^{12}C)_{Standard}}{(^{13}C/^{12}C)_{Standard}} \times 1000$$

The trophic position isotope spectrum. The TPIS is based on the hypothesis that each stable isotope data point is representative of the dietary history of the organism, integrated over the turnover time of the chosen tissue, and that variability among organisms represents differences in their dietary histories. It addresses the 2 basic requirements of food-web studies: to define and to quantify the flow of carbon fixed by primary producers to successive trophic positions.

The terms 'trophic category' and 'trophic position' are used in accordance with the nomenclature of Field et al. (1989). The term 'category' corresponds to the classical term 'level', and 'position' refers to intermediate locations between 'trophic levels' arising from omnivory. This change of nomenclature was invoked to emphasise the dynamic nature of food web trophic relationships against the classical static relationships associated with the term 'level'. An organism is seen to occupy a trophic category relative to its food web and it will, at a particular time or place, have a unique trophic position as a function of its feeding behaviour. The TPIS is an analytical tool which helps clarify the extent and predominance of this variability.

We hypothesise that the number of pathways along which carbon or nitrogen reaches a given compartment in the food chain will be reflected by the distribution

and frequency of occurrence of isotope ratios from organisms in that particular compartment. The isotope ratios cluster around δ values which are representative of the dominant input pathways during the period over which the organism's tissue integrates dietary history. Because the technique distinguishes between individuals with different dietary histories, it is essentially applicable to individuals from different populations. This is especially so when sampling schooling fish, because individuals of the same age and length classes presumably have similar diets. Therefore it is necessary to sample several temporally and spatially separated populations to produce a TPIS. The hypothesis is described in Fig. (1).

The food chain in Fig. 1 has 3 trophic categories above the primary producer, the highest being a planktivorous fish (P) utilizing phytoplankton carbon via 3 pathways (Fig. 1). In this hypothetical example, the vertical axis represents relative frequency of occurrence and the horizontal axis represents increasing prey trophic category. Each pathway has a different number of trophic categories and will therefore produce a unique isotopic 'signature' in the fish. If all 3 pathways coexist simultaneously the isotope signatures in the fish will be a combination of all 3 inputs, the actual δ value being dependent upon the importance of each pathway. If the pathways are separated in time and space by factors such as seasonality, biogeography or size-selective feeding behaviour so that different individuals get their diet from different pathways, then a suitable sampling strategy could isotopically resolve individual pathways. Each pathway has a characteristic isotope ratio (P1, P2 and P3) ranging from the isotopically 'light' single-step P1 route to the 'heaviest' 3-step P3 pathway. The frequency of occurrence of the ratios quantifies the proportion of individuals receiving their main carbon supply along each pathway. It is possible that the δ of P2 could reflect a 50:50 diet mix of P1 and P3. This would be unlikely without the presence of δ values at P1 and P3, which would then be a clue as to the pathways contributing to P2. To clarify this problem direct inspection of gut contents would be essential, emphasising the complementary nature of the 2 techniques. To define a food web, all trophic positions between the primary producer and relevant consumers must be sampled and a TPIS constructed for each. Pathways are defined by the positions of the peaks of each TPIS and the isotopic trophic enrichment for a given step.

The choice of biological tissue at each trophic position is important. Tissues from different parts of the organism will have different turnover rates. These rates determine the period over which the 'dietary memory' persists. For instance, muscle protein reflects a shorter-term dietary history than bone collagen (protein), which has a slower turnover rate (Tieszen et al. 1983), whereas isotopically lighter lipids are subject to seasonal fluctuations as storage products (Attwood & Peterson 1989). For this reason, lipids have been extracted from the present samples (Sholto-Douglas et al. 1991).

RESULTS

The results from the carbon TPIS for anchovy (muscle tissue and gut contents) and 3 planktonic size classes are shown in Figs. 2 & 3. For clarity, Fig. 2 is limited to anchovy muscle and gut content $\delta^{13}C$, but Fig. 3 includes the plankton part of the food chain. For the purposes of this paper, the plankton samples have been divided into 3 size classes: > 500, 200–500 and 20–200 μm escape diameter, which correspond to the MZ, Z and μZ fractions respectively The isotope ratios were rounded to within ± 0.125 ‰ of each 0.25 ‰ distribution interval. This was the maximum estimated

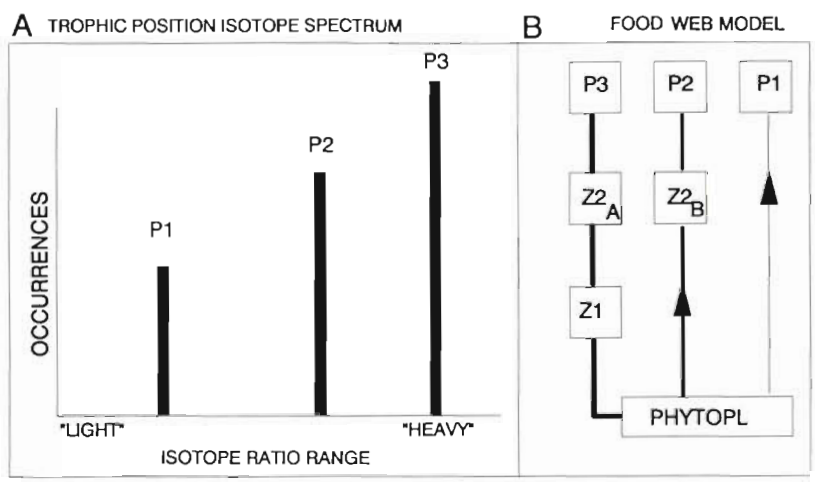

Fig. 1. Conceptual diagram of the relationship between (A) the trophic position isotope spectrum of a predator (P), and (B) its dietary relationship with the food chain. It shows that for each pathway there is a characteristic isotope ratio (P1, P2, P3) which is a function of the number of intermediary trophic steps and whose height indicates the number of individuals that have fed via that pathway. It is possible that an intermediate peak (P2) could also represent a mix of 2 extremes (P1 and P3); gut content studies would be required in order to clarify this posibility. PHYTOPL: phytoplankton; Z1 and Z2 are zooplankton trophic categories in this hypothetical food web. Z1 and $Z2_B$ are grazer categories and $Z2_A$ is a predator category

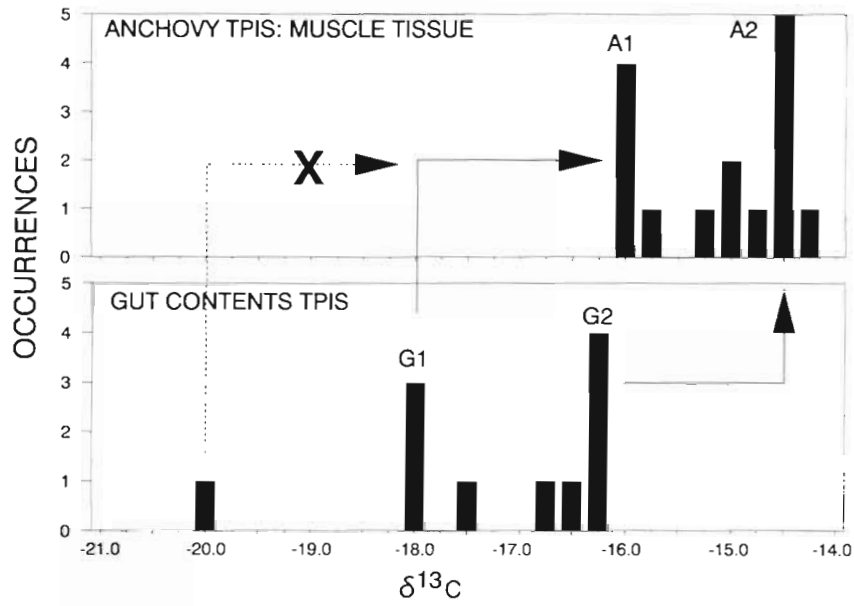

Fig. 2. *Engraulis capensis*. TPIS for anchovy muscle tissue samples and for anchovy gut content samples. Both TPIS's display strong bimodality, which is indicative of the fact that the anchovy carbon input occurs predominantly along 2 main pathways (arrowed). It indicates that a $\delta^{13}C$ enrichment of 2‰ separates the 2 TPIS's and that A1 individuals have fed from G1 whereas A2 individuals preyed mainly on G2. It also shows that phytoplankton is not a significant direct carbon source for anchovy. A1 and A2, and G1 and G2, designate the main peaks in the anchovy and gut content TPIS's respectively; dashed line marked with 'X' indicates unlikely pathway

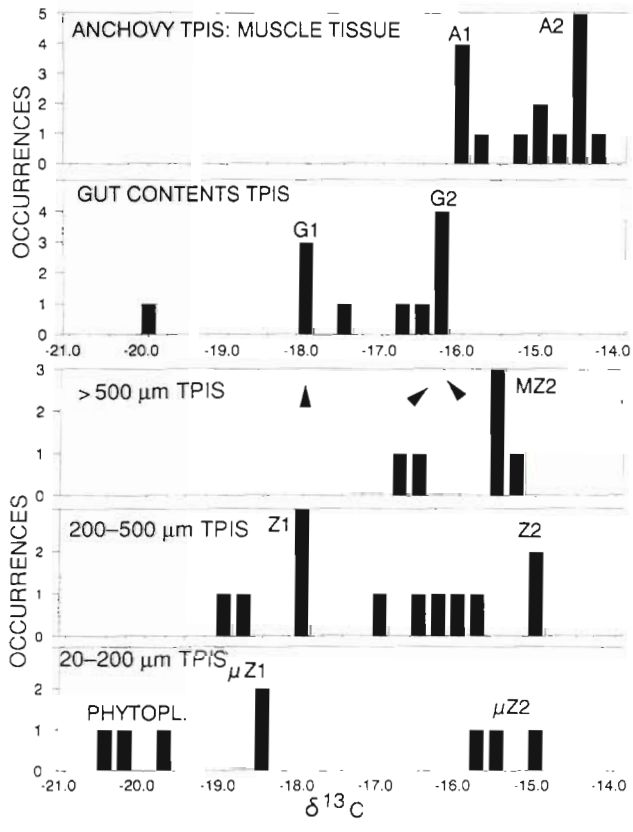

Fig 3. Composite TPIS diagram, which includes anchovy *Engraulis capensis* and 3 size classes of plankton samples. It shows that besides phytoplankton ($\delta^{13}C$ from −19.75 to −20.5 ‰) there are 2 trophic positions in all 3 planktonic size classes (ca −18 ‰ and ca −15 ‰). The $\delta^{13}C$ trophic enrichment per level is 2 to 3 ‰. PHYTOPL: phytoplankton; arrows indicate trophic pathways; dashed rectangle in top panel encloses intermediary $\delta^{13}C$ values, which are indicative of omnivory

experimental error, and for this relatively small sample size it improved the signal-to-noise ratio without reducing the significance of natural differences.

Strong bimodality was evident in both the anchovy gut content and muscle tissue $\delta^{13}C$ data. The gut content samples had main peaks at −18.0 and −16.25 ‰, and the muscle at −16.0 and −14.5 ‰ (Fig. 2).

The muscle tissue, which probably integrates dietary changes over a period of weeks (Tiezen et al. 1983), has intermediate peaks, suggesting diet mixing or switching. The $\delta^{13}C$ values at −20.0 ‰ in the gut content TPIS demonstrate that phytoplankton can be a dietary input. However, allowing for a 2 ‰ enrichment of $\delta^{13}C$ in this trophic step, this is not reflected in the anchovy TPIS. It suggests that, over the tissue turnover time, phytoplankton are an insignificant carbon source. Stomach content data have confirmed that phytoplankton are not an important component of the diet of *Engraulis capensis* (James 1987).

The distribution and number of $\delta^{13}C$ peaks for each size class are an indication of the average number of trophic steps along which carbon reaches that particular size class. The $\delta^{13}C$ peaks are indicative of the number of trophic positions within the size class. The 20–200 µm size fraction includes phytoplankton (mainly diatoms) and microzooplankton (mainly ciliates and crustacean nauplii). Its TPIS (Fig. 3) appears to show 3 trophic positions in this size class: phytoplankton at −19.75 to −20.5 ‰, and 2 microzooplankton trophic positions (µZ1 at −18.5 ‰ and µZ2 at −15.5 to −15.75 ‰, Fig. 3). The assumed phytoplankton $\delta^{13}C$ ranged from −19.75 to −20.5 ‰, which is within the range measured in the Southern Benguela Current (Monteiro unpubl.). The ^{13}C enrichment per

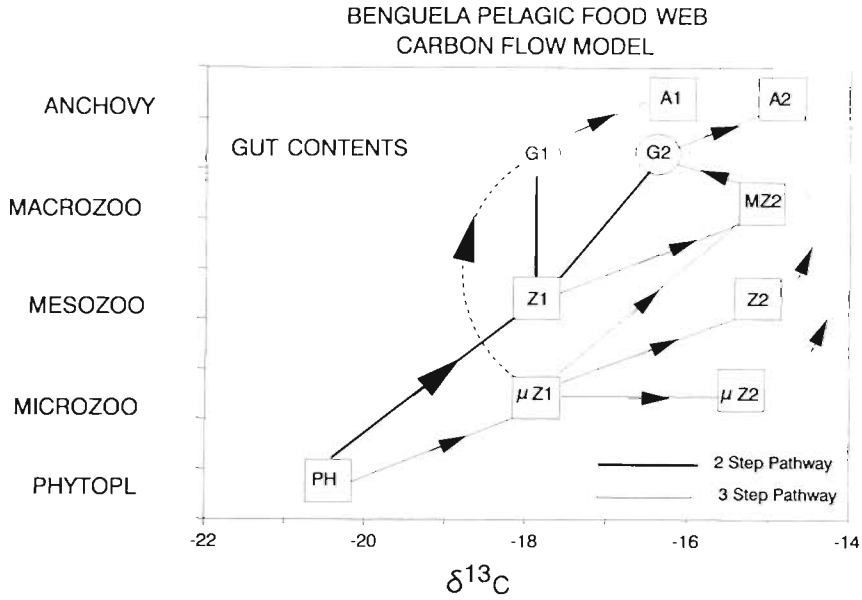

Fig. 4. Hypothesized isotope-based pelagic foodweb, constructed on the basis of the relationships of the compartments in the composite TPIS's. Bold lines: 2-step pathways; thin lines: 3-step pathways; hatched lines: possible but unlikely pathways. Abbreviations in the pathways as in Fig. 3

trophic category is thus ca 3‰ for the 20–200 μm plankton part of the pelagic food web.

The TPIS of the 200–500 μm fraction has 2 main peaks, at −18.0‰ (Z1) and −15.0‰ (Z2), which correspond to dietary inputs from phytoplankton (−20.5‰) and μZ1 (−18.5‰) respectively, assuming a trophic enrichment of 2.5 to 3.5‰. The intermediary peaks are thought to indicate omnivory.

The > 500 μm size class (MZ2) has a TPIS clustered around −15 to −15.5‰, indicating that the principal input pathways originate from organisms in the second trophic position (either μZ1 or Z1; $\delta^{13}C$ values of ca −18‰). The major components of this size category are euphausiids and amphipods. The more depleted values (< −16.5‰) in the macrozooplankton TPIS suggest that some macrozooplankton have mixed diets in the range −18 to −20‰, although the frequency of occurrence was low (2 out of 6; Fig. 3). The planktonic (< 2000 μm) TPIS's also show that the highest degree of ^{13}C enrichment in this part of the food web is −15‰ (Fig. 3). This indicates that, assuming a 2 to 3‰ trophic enrichment, the components of the planktonic food web are linked by no more than 2-step pathways.

All the above TPIS's can now be compiled into a carbon flow network, as shown in Fig. 4. The hypothesized pelagic food web was constructed using 2 basic rules: (1) each compartment has a $\delta^{13}C$ value obtained from the TPIS of its size class (Fig. 3), and (2) the compartments are linked according to the $\delta^{13}C$ difference between diet and consumer compartments. This difference is 2 to 3‰ unless, as is the case for the G2 (gut contents) compartment, it is a combination of 2 inputs. Applying these rules, a food web with 10 compartments was constructed (Fig. 4), including anchovy gut contents G1 and G2, and 15 possible pathways.

DISCUSSION

The proposed Southern Benguela pelagic food web is a highly complex one, although not all pathways are of equal importance (Fig. 4). The main shortcoming of this data set is that, using carbon alone, it is not possible to separate the contributions of μZ1 and Z1 within the first trophic position and μZ2, Z2 and MZ2 in the second. In Fig. 4 it can be seen that $\delta^{13}C$ values indicate the trophic position of food web components, and that there may be more than 1 size class in each trophic position.

Although $\delta^{15}N$ values were available for some of the samples, there were not enough for a similar TPIS treatment to be applied for $\delta^{15}N$. The application of TPIS to $\delta^{15}N$ may face other difficulties related to differences in the nature of the metabolism of protein carbon and nitrogen after assimilation (Hare 1988). Transamination, which appears to characterize amino acid metabolism, may scramble the dietary signal so as to make a TPIS analysis less clear. This requires further investigation with a larger data set.

The trophic enrichments proposed in this study are at the upper end of the published ranges (Peterson & Fry 1987). This issue is still not resolved but an important factor which probably accounts for the high values observed here could be that many previous $\delta^{13}C$ measurements were made on whole tissue samples, which included the $\delta^{13}C$-depleted lipid fraction (Saupe et al. 1989). It is well known that the protein:lipid ratio of organisms can vary as a function of environmental conditions. This is especially serious for smaller organisms, and neglecting this problem might conceivably bias findings because of the large $\delta^{13}C$ differences between lipids (ca −26‰) and protein (ca −16‰). We

believe that having made our $\delta^{13}C$ measurements specifically on defatted protein carbon gives our findings a good degree of internal consistency.

Stomach content data have helped assess the importance of some of the pathways. Pathways which have been confirmed by stomach content observations are shown as continuous lines and those that have not as dashed lines in Fig. 4.

The food web model shows that, in the Southern Benguela region, phytoplankton carbon only reaches anchovy along a 2 or 3-step food chain. In the case of a 2-step food chain, the anchovy forage on the first trophic position (μZ1, Z1), and for the 3-step one, on the second trophic position (μZ2, Z2, MZ2). The relative magnitudes of the 2- and 3-step pathways may be quantified using the anchovy TPIS. Based on the $\delta^{13}C$ values of each anchovy main peak (A1 and A2) and a 2 ‰ enrichment of ^{13}C relative to the diet, the 2- and 3-step pathways are calculated to contribute 57 and 43 % of the anchovy carbon respectively. The mass balance equations are:

$$\delta^{13}C_{A1} = \delta^{13}C_{G1} + 2\text{ ‰; and}$$
$$\delta^{13}C_{A2} = 0.14\ (\delta^{13}C_{G1} + 2\text{ ‰}) + 0.86\ (\delta^{13}C_{G2} + 2\text{ ‰})$$

The mass balance equation for $\delta^{13}C_{A2}$ reflects the proportions of G1 (0.14) and G2 (0.86) required to give the value −14.5 ‰ for A2. Then, total G1 contributing to A1 and A2 = 1 + 0.14 = 1.14; and total G2 contributing to A2 = 0.86.

Therefore the total contribution from the 2-step pathway to the anchovy pool, represented by the major peaks A1 and A2, is 1.14/(1.14 + 0.86) = 57 %, and for the 3-step pathway 0.86/(1.14 + 0.86) = 43 %. This shows that in the Southern Benguela the 2- and 3-step pathways contribute ca 50 % each to the anchovy diet.

The $\delta^{13}C$ values of the bimodal anchovy TPIS are consistent with the finding that a direct phytoplankton-anchovy pathway does not significantly contribute to anchovy energy requirements (James 1987, James et al. 1989). If this pathway were significant, then a $\delta^{13}C$ peak of ca −18 ‰ would occur in the anchovy TPIS. Although one stomach contents sample did contain phytoplankton ($\delta^{13}C$ −20 ‰, Fig. 2), it is not reflected in the anchovy TPIS. We conclude that this input is likely to be occasional and insignificant, at least in the Southern Benguela Ecosystem. The data (Fig. 4) indicate that *Engraulis capensis* feed on Z1 and MZ2. Carbon from the 200–500 μm size class, in this plankton sample, makes up 100 % of the isotopically 'lighter' diet reflected in the muscle protein, and 14 % of the contribution to the 'heavier' peak. MZ2 comprises 86 % of the 'heavier' diet (Fig. 4). This finding is consistent with field observations of *Engraulis capensis* gut contents in the Southern Benguela (James 1987).

Two important questions arise from the use of such a model to interpret complex food webs: the required sample size to make the TPIS quantitatively useful, and the temporal or spatial mismatch between samples from the various trophic categories. This study has shown that to make TPIS a more routine quantitative ecological tool there should be 20 to 30 samples in each size class. The sample sizes used here were, however, adequate to achieve the objectives of the study, which were to demonstrate the rules of TPIS construction and function and to use TPIS to confirm the absence of a direct link between phytoplankton and anchovy in the Southern Benguela. As was discussed in Sholto-Douglas et al. (1991), temporal mismatch between samples is unlikely to account for significant variability in the data. The most important environmental factor potentially affecting $\delta^{13}C$ is temperature, which can change the value in phytoplankton at the base of the food web. In the Southern Benguela, however, the seasonal temperature range is very small. Furthermore, the $\delta^{13}C$ of muscle tissue integrates the dietary signal over months. Thus, while it is important to have a sample set of prey which is representative of its isotope variability, simultaneous sampling, while desirable, is not essential. We have to emphasise that the TPIS is not an alternative to more established techniques such as gut content analysis but an additional method for the ecological 'tool box'.

A remaining problem is to identify the causes of the strong bimodality in the anchovy carbon TPIS. A number of natural factors might influence the isotopic composition of the diet, including biogeography, size of both anchovy and prey, season, prey abundance, vertical distribution of predator and prey and predator feeding behaviour. It is surprising that, with so many potentially interlinked factors, the bimodal signal is still prominent, suggesting that the factors regulating anchovy diet are simpler than initially thought. It would appear from the available data that switching occurs fairly rapidly, but each diet is sustained long enough to isotopically characterise the muscle protein.

CONCLUSION

The results of this study refute the traditional hypothesis of Ryther (1969) that large pelagic fish populations in upwelling areas are supported mainly by direct feeding on phytoplankton. The isotope ratios show that the pelagic food web is a highly complex one, with widespread omnivory in all consumer positions. This supports recent findings that anchovy are not only capable of flexible feeding behaviour but are also size-selective predators (James 1987, James & Findlay 1989). Despite this complexity the strong bimodality of the anchovy TPIS indicates that certain

pathways are consistently important. Anchovy carbon requirements are supplied primarily through 2 or 3 trophic category transfers within the pelagic food web. The major advantage of the TPIS is that it provides a method by which isotope ratios can be used to quantify trophic interactions within food webs rather than through simplistic food chain models (Sholto-Douglas et al. 1991).

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