

$^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ isotope ratios in the Southern Benguela Ecosystem: indicators of food web relationships among different size-classes of plankton and pelagic fish; differences between fish muscle and bone collagen tissues

A. D. Sholto-Douglas^{1,2}, J. G. Field¹, A. G. James³, N. J. van der Merwe²

¹ Marine Biology Research Institute, Department of Zoology, University of Cape Town, Rondebosch 7700, South Africa

² Archaeometry Research Group, Department of Archaeology, University of Cape Town, Rondebosch 7700, South Africa

³ Sea Fisheries Research Institute, P Bag X2 Rogge Bay, Cape Town 8012, South Africa

ABSTRACT: Measurements of the stable isotope ratios of carbon and nitrogen were obtained for 3 size-fractions of plankton and 2 species of pelagic fish, *Engraulis capensis* Gilchrist and *Etrumeus whiteheadi* Wongratana, from the Benguela current system on the southwest coast of Africa. There was a trend towards enrichment in ^{13}C and ^{15}N with increasing plankton size. The data are consistent with the hypothesis that larger plankton feed further up the food web than smaller plankton. Fish muscle and bone collagen tissues tended to be more enriched than the plankton. There was depletion of the heavier isotopes with increasing fish length, in contrast to the opposite tendency with increasing plankton size and towards the trophic position of fish. The fractionation of $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ ratios during the formation of muscle tissue was different from that for bone collagen. Bone collagen was richer in ^{13}C than muscle tissue, whereas muscle was richer than bone collagen in ^{15}N . For both ^{13}C and ^{15}N , the less enriched tissue displayed the stronger negative correlation with fish length.

INTRODUCTION

$^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ isotope ratios reflect a consumer's diet (Miyake & Wada 1967, De Niro & Epstein 1976, 1978, 1981). However, a consumer is slightly more enriched in ^{13}C and ^{15}N than its diet, by ca 1 to 2 ‰ for $\delta^{13}\text{C}$ and 3 to 5 ‰ for $\delta^{15}\text{N}$, leading to increasing enrichment with increasing trophic level (De Niro & Epstein 1978, 1981, McConnaughey & McRoy 1979, Rau et al. 1983, Fry & Sherr 1984, Minagawa & Wada 1984).

It is important to distinguish the general trophic trend in isotope fractionation for whole animals with differing isotope abundance in different tissues. The isotopic content of an animal's diet is fractionated to different degrees in different tissues (De Niro & Epstein 1978, 1981, Lee-Thorp et al. 1989). Lee-Thorp et al. (1989) report bone collagen to be more enriched in ^{13}C than muscle tissue by 2 to 2.5 ‰ in terrestrial animals when lipids are removed before analysis. Muscle and bone tissues were more enriched than diet by ca 2.5

and 4.5 ‰ respectively. These diet-tissue differences may be smaller if lipids are not removed from the tissues, since lipids are depleted in $\delta^{13}\text{C}$ relative to other biochemical fractions (De Niro & Epstein 1978, Tieszen et al. 1983). In the case of ^{15}N , however, there is evidence that muscle is more enriched than bone collagen (De Niro & Epstein 1981). Muscle tissue from *Mus musculus* was more enriched in ^{15}N than the diet by 2.9 ‰, whereas bone collagen from these mice fed the same artificial diet was enriched by only 2.6 ‰.

In the present study, measurements of isotope ratios are used to examine how they reflect the diets of 2 small pelagic school-fish species and their plankton prey. The diets of these fish depend upon their size and the size, concentration and availability of their prey (Koslow 1981, Angelescu 1982, James 1987, James & Findlay 1989). Gut content analyses indicate that Cape anchovy *Engraulis capensis* and roundherring *Etrumeus whiteheadi* are largely zoophagous (James 1987, Wallace-Fincham 1987). Their zooplankton prey in turn tend to have increasing proportions of zoo-

plankton and decreasing proportions of phytoplankton in their diet with increasing size (Lazarus 1975).

A major disadvantage of gut content analyses is that they only provide 'snapshots' of the diet of pelagic schooling fish, which are often highly opportunistic foragers. The stable isotopes of carbon and nitrogen in an organism's tissues, on the other hand, integrate the relative contributions of isotopically distinct dietary components over a period of time. Thus we are provided with an average estimate of an organism's preferred diet, less subject to temporal bias than gut content analyses. The period of integration is related to tissue turnover time (Fry & Arnold 1982, Tieszen et al. 1983). Tissue turnover time, in turn, is related to tissue type (metabolically active tissues display fast turnover) and the age and physiological state of an animal (younger animals have faster turnover rates than older ones). The faster the tissue turnover, the shorter the period of dietary integration and the greater the likelihood of temporal bias in isotopic dietary estimates.

Pelagic food webs may be modelled in terms of organism size relationships as well as trophic interactions (Cousins 1980, 1985, Azam et al. 1983, Platt 1985, Moloney & Field 1989). Larger organisms are generally more carnivorous than smaller ones (Lazarus 1975, Knoechel & Holtby 1986). The correlation between enrichment of ^{13}C and ^{15}N and position in a food web enables us to test the hypothesis that enrichment in these isotopes will occur with increasing organism size in the pelagic food web sampled. At the same time, we can investigate the differential isotopic enrichment in different fish tissues.

MATERIALS AND METHODS

Sampling. Samples of anchovy *Engraulis capensis* and roundherring *Etrumeus whiteheadi* were collected off the west and south coasts of South Africa during a cruise aboard RS 'Africana' in July 1987 using an Engels 308 midwater trawl. Fish from each trawl were sorted into five 10 mm size-classes. In addition, 5 *E. capensis* in the same size range, from a cruise in May 1986, were included in the data. In each trawl, the vertebrae and muscle tissue from 1 fish per size-class were removed for analysis. James (1987) found no significant differences in the composition of the gut contents of *E. capensis* in the same size-class from the same trawl. Therefore gut contents of 1 to 3 fish in the same size class and trawl were pooled to gain adequate sample sizes for each analysis.

Plankton was collected in July 1987, but samples were too small for isotope analyses. We therefore re-sampled in late May and June 1988. Plankton samples

were collected with a multiple opening/closing rectangular midwater trawl (RMT 1 × 6). The samples were sorted into 20–200, 200–500 and > 500 μm escape diameter size-classes, by washing fresh samples through screens aboard ship with seawater. The 3 size-classes comprised mainly phytoplankton and microzooplankton, copepods, and euphausiids respectively.

The Benguela Ecosystem is characterised by a seasonal upwelling regime, favoured by a predominance of southerly winds during summer (September to March) (Andrews & Hutchings 1980). The autumn and winter seasons in the southern Benguela system extend from about the end of March to the end of September. During this time there is a predominance of westerly winds, not favourable for upwelling. Surface water is of rather uniform temperature (Andrews & Hutchings 1980), and mean monthly temperatures from the 0 to 50 m water column have shown $\leq 1^\circ\text{C}$ variations between consecutive winter months (Buys 1959, Shannon 1985). Data from Shannon & Agenbag (1990) show that mean sea-surface temperature anomalies during July 1987 and July 1988 were similar ($\leq -1^\circ\text{C}$). Using sea-surface temperatures as an index of the environment, we can assume that conditions for plankton caught in 1988 and fish caught in 1987 were similar. May 1986 showed slightly warmer anomalies ($+1$ to $+2^\circ\text{C}$), which may affect the data for anchovy collected during this month. However, the isotopic content of these organisms at the time of capture is not necessarily related to the environmental conditions at the same time. The isotopic content of plankton is likely to reflect more recent environmental conditions than that of fish, which are longer-lived and have slower tissue turnover rates. Therefore, conditions during the preceding months are also important. The first halves of 1986, 1987 and 1988 showed decreasing average temperature anomalies, from $+1^\circ\text{C}$ to -1°C (Shannon & Agenbag 1990). The interannual variation is small and thus is unlikely to cause significant temporal bias in the data.

Sample preparation. Samples were defatted in a solution of chloroform, methanol and water (2:1:0.8) by the method of Bligh & Dyer (1959). This procedure was carried out to minimise discrepancies resulting from differences in proportions of fatty tissue between different organisms. Bone samples were decalcified in 1.5 % hydrochloric acid (Sealy et al. 1987) leaving the collagen for analysis. Samples were then freeze-dried for 24 h.

Stable isotope analysis. A sample of 10 to 15 mg was weighed into a quartz breakseal tube with excess copper, copper oxide and silver foil. The tube was evacuated to 10^{-2} Torr, sealed, and combusted in a furnace at 800°C for 8 h. The isotope ratios of the resultant N_2 and CO_2 gases were measured using a VG Micromass 602E

90° sector double-collector mass spectrometer. Isotope ratios were calculated as:

$$\delta X (\text{‰}) = [(R_{\text{sample}} - R_{\text{std}})/R_{\text{std}}] \times 1000$$

where $X = {}^{13}\text{C}$ or ${}^{15}\text{N}$, $R = {}^{13}\text{C}/{}^{12}\text{C}$ or ${}^{15}\text{N}/{}^{14}\text{N}$, and std = Pee Dee Belemnite carbonate for $\delta^{13}\text{C}$ (PDB), and atmospheric (AIR) nitrogen for $\delta^{15}\text{N}$ (De Niro & Schoeninger 1983, Rau et al. 1989). The measurement error (standard deviation) for homogeneous sample materials was less than 0.08 ‰ (present data). Mann-Whitney U -tests were performed and their significance levels were calculated to compare the differences between the means of different size-classes of plankton, between fish gut contents (anchovy and roundherring separately) and the different plankton size-classes, and between the muscle and bone collagen tissues of each fish species. Spearman's rank-correlation coefficients were used to measure relationships with increasing anchovy length. Kruskal-Wallis 1-way ANOVA and nonparametric multiple comparison tests were carried out to determine differences between pooled values for gut contents, muscle and bone collagen tissues of both anchovy and roundherring together. Statistics were calculated according to Zar (1984). Nonparametric tests were chosen because of the small sample sizes and non-normal distribution of much of the data.

RESULTS AND DISCUSSION

Food web structure

The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data obtained from plankton are consistent with those reported in the literature (Deuser

1970, Haines & Montague 1970, Fry et al. 1982, 1983, Thayer et al. 1983, Fry 1984, Gearing et al. 1984; all cited in Fry & Sherr 1984). The mean $\delta^{13}\text{C}$ value for plankton in the 20–200 μm size-class (largely phytoplankton) was -17.3‰ and the range was from -14.2 to -20.6‰ (Fig. 1). These represent slightly more positive values than reported by Deuser (1970), Haines & Montague (1970) and Gearing et al. (1984) (all cited in Fry & Sherr 1984), whose data for phytoplankton (as POC) ranged from ca -18 to -24‰ with a mean of ca -21‰ . Values for plankton in the 200–500 and $> 500\text{ }\mu\text{m}$ size-classes ranged from -15.0 to -19.0‰ , with means of -17.0 and -15.9‰ respectively.

There was a trend towards ${}^{13}\text{C}$ enrichment (i.e. less negative $\delta^{13}\text{C}$ values) with increasing plankton size (Fig. 1). This suggests that larger plankton feed further up the food web than do smaller plankton. The large scatter of $\delta^{13}\text{C}$ values in the plankton data probably reflects the large number of trophic interactions, or expressed differently, the likelihood that primary producers and primary and secondary consumers are all represented in the 20–200 μm size-fraction (see Monteiro et al. 1991).

The $\delta^{13}\text{C}$ measurements for anchovy gut contents (values representing all fish size classes) were similar to those for the 2 smaller plankton size-classes (Fig. 1). The values for $> 500\text{ }\mu\text{m}$ plankton were significantly different from those for anchovy gut contents ($U = 56$, $p < 0.05$), suggesting that most of the plankton consumed by the fish at the time of capture was $< 500\text{ }\mu\text{m}$.

There was ${}^{13}\text{C}$ enrichment from all size-classes of plankton towards the fish tissues. Thus the fish may have consumed plankton of any size despite what was present in their gut contents at the time of capture.

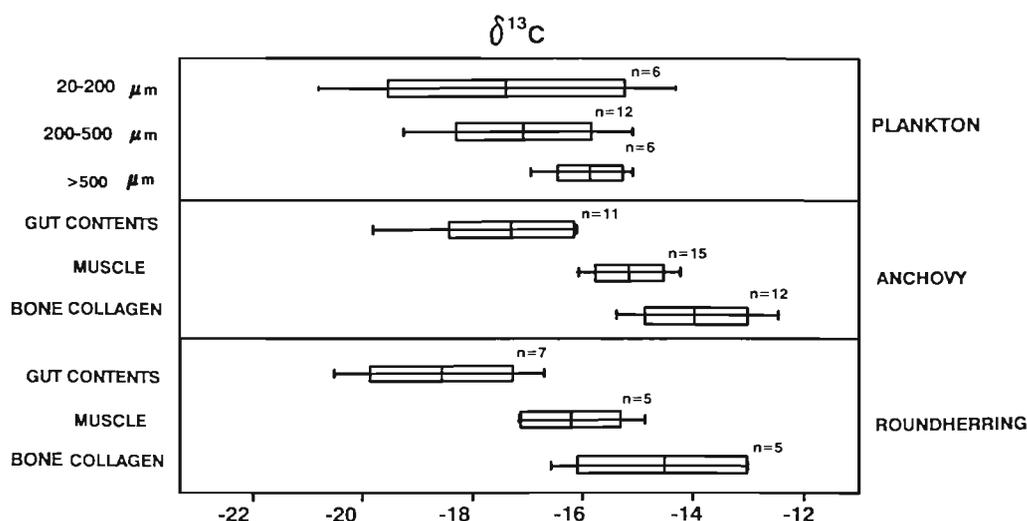


Fig. 1. $\delta^{13}\text{C}$ measurements for different size-classes of plankton and for gut contents, muscle and bone collagen tissues from Cape anchovy *Engraulis capensis* and roundherring *Etrumeus whiteheadi*. All size-classes of fish are represented in the gut content and tissue samples. Horizontal lines represent data ranges, rectangles represent standard deviations, and the means are given by vertical lines inside rectangles. Numbers of measurements (n) are also given

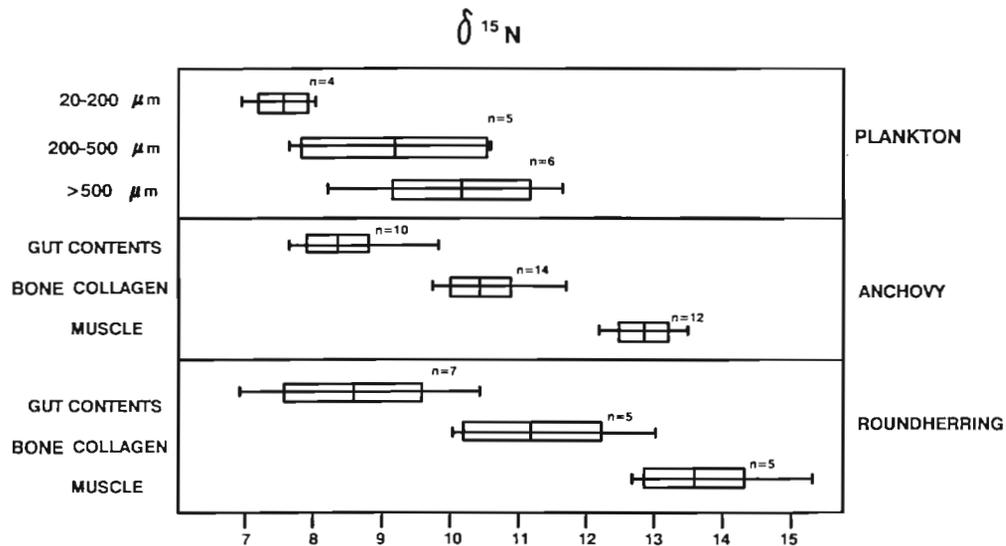


Fig. 2. $\delta^{15}\text{N}$ measurements for different size-classes of plankton and for gut contents, muscle and bone collagen tissues from all size-classes of Cape anchovy *Engraulis capensis* and roundherring *Trumeus whiteheadi*. Symbols as in Fig. 1

However, it is not possible to estimate the proportions of different plankton size-fractions in their diet from these data alone. Roundherring gut contents, muscle and bone collagen appeared to show less enrichment relative to the plankton than did the corresponding anchovy tissues.

$\delta^{15}\text{N}$ of plankton in the 20–200 μm size-class ranged from 6.8 to 7.9‰ (Fig. 2). For plankton > 500 μm , the range was from 8.2 to 11.7‰, which does not overlap with that of the 20–200 μm size-class (largely phytoplankton). These are within the ranges for data reported by Minagawa & Wada (1984) for zooplankton and primary producers in marine and freshwater ecosystems.

There was a trend towards enrichment in ^{15}N (i.e. more positive $\delta^{15}\text{N}$ values) with increasing plankton size (Fig. 2). The $\delta^{15}\text{N}$ values for 200–500 and > 500 μm plankton were significantly greater than those for 20–200 μm ($U = 20$, $p < 0.01$ and $U = 24$, $p < 0.005$ respectively) (see Table 2). The $\delta^{15}\text{N}$ data for anchovy gut contents and > 500 μm plankton were significantly different ($U = 52.5$, $p < 0.02$), as was the case for the $\delta^{13}\text{C}$ data. The values for anchovy gut contents were also significantly different from those for 20–200 μm plankton ($U = 37.5$, $p < 0.02$), so it is possible that small (< 200 μm) and large (> 500 μm) plankton formed very little of the diet of anchovy when caught, supporting James (1987), who found *Engraulis capensis* to be a selective forager of 'mesozooplankton' (0.7 to 20 mm). There was again enrichment in the heavy isotope from all size-classes of plankton towards the fish tissues.

Fractionation into fish tissues

The $\delta^{13}\text{C}$ difference between gut contents and tissues was similar for both anchovy and roundherring (Table 1). The ^{13}C enrichment from gut contents to tissues (estimated from mean values) was of the order of 2.1 to 2.3‰ for muscle and 3.2 to 3.9‰ for bone collagen. Thus, bone collagen was more enriched in ^{13}C than muscle tissue. The difference between the mean values for anchovy muscle and bone collagen was 1.1‰ (significantly different, $U = 139$, $p < 0.0025$) and for roundherring tissues 1.6‰ (not statistically significant). The degree of ^{13}C enrichment from diet (as gut contents at capture) to muscle tissue was comparable to the data from Lee-Thorp et al. (1989), but bone collagen was less enriched relative to muscle tissue values. Average ^{13}C enrichment for whole animals is likely to be weighted towards values for muscle tissue, which makes up a larger part of fish mass. Furthermore, the inclusion of body organs and fat tissue would yield more negative $\delta^{13}\text{C}$ results (De Niro & Epstein 1978). Thus a trophic enrichment value of usually < 2‰ for whole bodies of animals, as is reported in the literature, seems likely (De Niro & Epstein 1978, McConnaughey & McRoy 1979, Rau et al. 1983).

The confidence intervals were narrower for $\delta^{15}\text{N}$ than for $\delta^{13}\text{C}$ (Tables 1 & 2). The ^{15}N enrichment from gut contents to bone collagen was of the order of 2.0 and 2.5‰ for anchovy and roundherring respectively, and 4.3 to 4.9‰ from gut contents to muscle (Table 2). Thus in the case of ^{15}N , muscle was more enriched than bone

Table 1. Carbon isotope ratio statistics for plankton, anchovy *Engraulis capensis* and roundherring *Etrumeus whiteheadi*, showing number of measurements, mean $\delta^{13}\text{C}$ values (‰), their standard deviations, differences between successive categories listed ('enrichment', ‰), standard error of the difference (SE), and the significance of the differences from Mann-Whitney U -tests (U = significant for 1-tailed test, p = probability value for 1-tailed test; ns = not significant)

Category	n	Mean	SD	Enrichment (SE)	Significance
Plankton					
20–200 μm	6	–17.3	2.5	–	
200–500 μm	12	–17.0	1.3	0.3 (1.1)	ns
> 500 μm	6	–15.9	0.6	1.1 (0.5)	ns
Total enrichment <200 to >500 μm				1.4 (1.0)	ns
Anchovy					
Gut contents	11	–17.2	1.1	–	
Muscle	14	–15.1	0.6	2.1 (0.4)	$U = 153, p < 0.0005$
Bone	12	–14.0	0.9	1.1 (0.3)	$U = 139, p < 0.0025$
Total enrichment (gut to bone)				3.2 (0.4)	
Roundherring					
Gut contents	7	–18.5	1.4	–	
Muscle	5	–16.2	0.9	2.3 (0.6)	$U = 32, p < 0.01$
Bone	5	–14.6	1.6	1.6 (0.8)	$U = 21, p < 0.05$
Total enrichment (gut to bone)				3.9 (0.9)	

collagen. The difference between the means for muscle and bone collagen tissues was 2.3 and 2.4 ‰ for anchovy and roundherring respectively (statistically significant, $U = 168, p < 0.0005$ and $U = 23, p < 0.025$). The gut-contents/tissue disparity was greater for roundherring than anchovy, but the bone-collagen/muscle disparity for both species was the same. Furthermore, roundherring gut contents and tissues are not less enriched in ^{15}N than are the corresponding anchovy tissues, as was the case for ^{13}C . It appears that the $\delta^{13}\text{C}:\delta^{15}\text{N}$ ratios of the diet

and tissues of these 2 fish species are different, or that their carbon and nitrogen assimilation efficiencies differ.

The average whole-fish trophic enrichment, weighted towards the abundant muscle tissue, would result in a $\delta^{15}\text{N}$ difference of ca 4 to 5 ‰. Again, this is consistent with values reported in the literature. De Niro & Epstein (1981) and Minagawa & Wada (1984) report $\delta^{15}\text{N}$ diet/whole body relationships ranging from –0.5 to +9.2 ‰ (3.4 ± 1.1 ‰) and +1.3 to +5.3 ‰ (3.0 ± 2.6 ‰) respectively.

Table 2. Nitrogen isotope ratio statistics for plankton, anchovy *Engraulis capensis* and roundherring *Etrumeus whiteheadi*, showing number of measurements, mean $\delta^{15}\text{N}$ values (‰), their standard deviations, differences between successive categories listed ('enrichment', ‰), the standard error of the difference (SE), and the significance of the differences from Mann-Whitney U -tests (U = significant for 1-tailed test, p = probability value for 1-tailed test; ns = not significant)

Category	n	Mean	SD	Enrichment (SE)	Significance
Plankton					
20–200 μm	4	7.5	0.4	–	
200–500 μm	5	9.0	0.8	1.5 (0.4)	$U = 20, p < 0.01$
> 500 μm	6	10.2	1.1	1.2 (0.6)	ns
Total enrichment <200 to >500 μm				2.7 (0.5)	$U = 24, p < 0.005$
Anchovy					
Gut contents	10	8.6	0.7	–	
Bone	12	10.6	0.5	2.0 (0.3)	$U = 119, p < 0.0005$
Muscle	14	12.9	0.4	2.3 (0.2)	$U = 168, p < 0.0005$
Total enrichment (gut to muscle)				4.3 (0.2)	
Roundherring					
Gut contents	7	8.8	1.2	–	
Bone	5	11.3	1.1	2.5 (0.7)	$U = 32, p < 0.01$
Muscle	5	13.7	0.8	2.4 (0.6)	$U = 23, p < 0.025$
Total enrichment (gut to muscle)				4.9 (0.6)	

Fractionation with fish length

Fig. 3 shows the relationship between fish size and the $\delta^{13}\text{C}$ ratios of fish tissues for anchovy and roundherring. Contrary to the plankton data and plankton/fish data on a gross size-scale, within the fish size range of 70 to 160 mm there appeared to be ^{13}C depletion in muscle tissue with increasing size of anchovy ($r_s = -0.69$, $df = 14$, $p < 0.01$). There was a suggestion that this might be the case for anchovy bone collagen too (Fig. 3b), but this was not a clear trend, nor was it statistically significant ($r_s = -0.47$, $df = 12$, $p > 0.05$). Sample sizes for roundherring were too small for correlations, but the same trend of ^{13}C depletion with increasing fish length appeared to occur in both muscle and bone collagen tissues.

Fig. 4 depicts the relationship between $\delta^{15}\text{N}$ ratios and fish length. In contrast to the $\delta^{13}\text{C}$ data, anchovy bone collagen showed a stronger negative correlation than muscle with increasing fish length ($r_s = -0.71$, $df = 12$, $p < 0.02$), but there was little sign of a size relationship in the more ^{15}N -enriched anchovy muscle tissues ($r_s = -0.08$, $df = 14$, $p > 0.05$). There appeared to be a trend of ^{15}N depletion with increasing fish size in both the muscle and bone collagen tissues of roundherring. This size-related depletion may tend to increase the scatter in the pooled tissue data for different-sized fish, thus hampering statistical tests for tissue-diet differences.

The differences between the fractionation of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in the different tissues, and how these differences are associated with increasing fish size, are difficult to explain. It is interesting to note that the tissue less enriched in ^{13}C or ^{15}N showed a stronger relationship with fish length. This suggests that the reason for the negative relationship between isotope fractionation and fish size has a physiological rather than trophic basis. We have found no reference to differential incorporation of ^{13}C and ^{15}N by muscle and bone tissues. Nevertheless, there is reference to $\delta^{15}\text{N}$ relationships with body size. Rau et al. (1981) found a strong positive correlation between the $\delta^{15}\text{N}$ values of muscle tissue from Dover Sole and increasing body weight. However, Minagawa & Wada (1984) found no $\delta^{15}\text{N}$ correlation with age in mussels. Differences in growth efficiencies or assimilation efficiencies, as described by Owens (1987), may be responsible for ^{13}C and ^{15}N variability with age/body size, but we cannot explain the different directions of the relationships found by these authors and ourselves. This clearly requires further study both in the field and laboratory.

If the depletion with fish length has a trophic basis, and larger fish are consuming greater proportions of an isotopically depleted diet such as phytoplankton, then the results, to some extent, support the findings of King

& Macleod (1976), who found that anchovy switch their diet at about the 80 mm size-class, from zoophagy to phytophagy. However, our data do not support their suggestion that anchovy become predominantly phytophagous (Figs. 1 & 2). The isotopic variability of anchovy in the 70 to 80 mm size-class may be

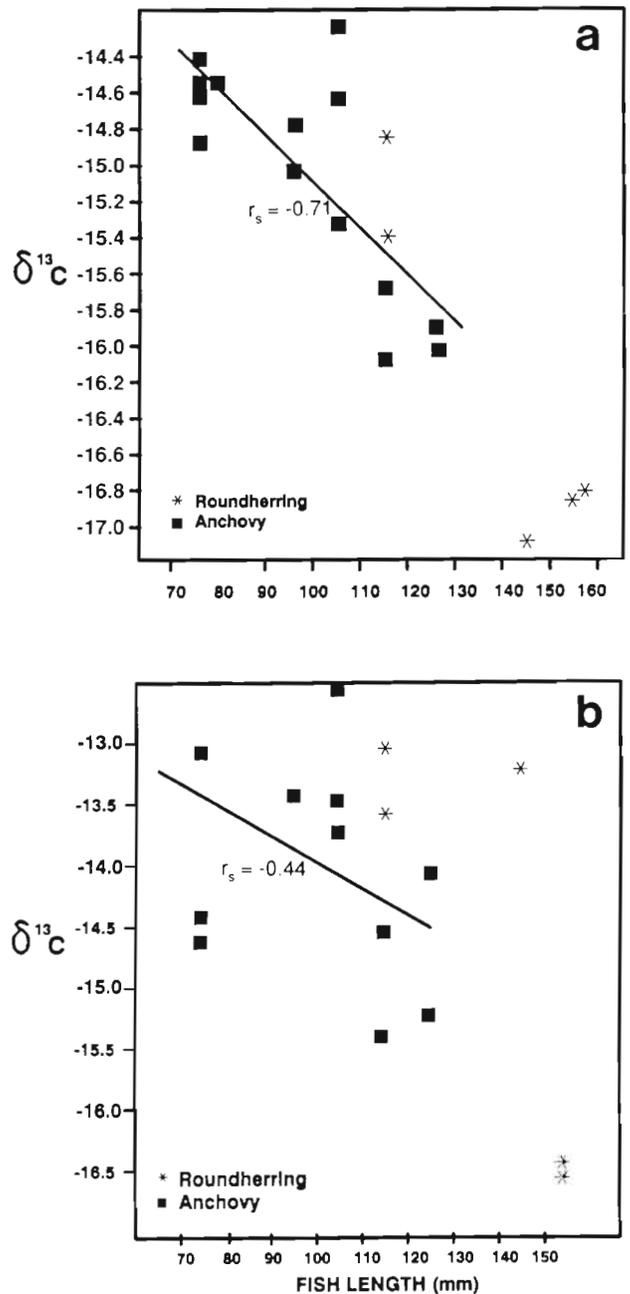


Fig. 3. *Engraulis capensis* and *Etrumeus whiteheadi*. $\delta^{13}\text{C}$ ratios for (a) muscle tissue and (b) bone collagen of different size-classes of anchovy and roundherring. Regression lines are given for anchovy, as are Spearman's rank-correlation coefficients (r_s)

explained by the possibility that some of these fish still reflect the integrated isotopic signature of a different juvenile diet. The elimination of these values would result in good negative correlations with increasing fish length in both muscle and bone tissue, for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$.

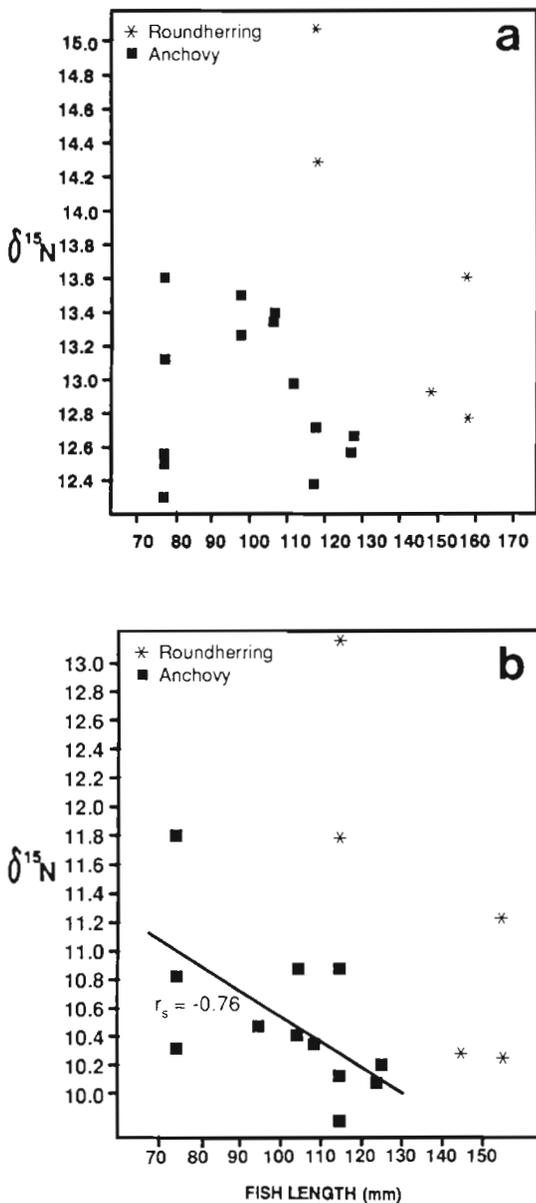


Fig. 4. *Engraulis capensis* and *Etrumeus whiteheadi*. $\delta^{15}\text{N}$ values for (a) muscle tissue and (b) bone collagen of different size-classes of anchovy and roundherring. The regression line for anchovy bone collagen is given, as is the Spearman's rank-correlation coefficient (r_s)

No relationship with fish length was exhibited by the results for gut contents. Small and larger fish were consuming diets with isotopic signatures on either, or both, sides of the means for the pooled values in Figs. 1 & 2. It appears, therefore, that the gut contents at the time the fish were caught were not representative of their average dietary history. If, however, the different size-classes of fish had been consuming similar average diets, as indicated by the gut contents, then the isotopic depletion with fish length would be entirely physiological.

CONCLUSIONS

These data are consistent with the hypothesis that trophic relationships in pelagic food webs are governed largely by organism size (Cousins 1980, 1985, Azam et al. 1983, Platt 1985). Within the plankton community, there is a trend of ^{13}C and ^{15}N enrichment with increasing organism size, and the muscle and bone collagen tissues of plankton-consumer fish species are more enriched than the plankton. The complexity of the plankton food web is reflected in the large standard

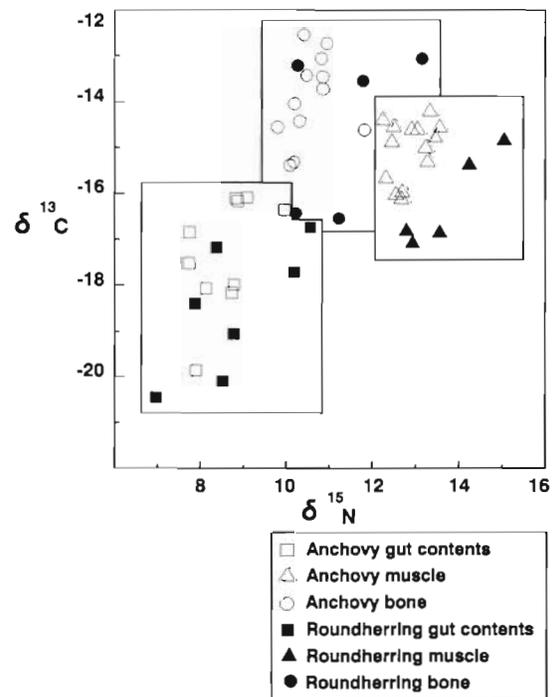


Fig. 5. *Engraulis capensis* and *Etrumeus whiteheadi*. $\delta^{13}\text{C}$ vs $\delta^{15}\text{N}$ isotope ratios for gut contents, bone collagen and muscle tissues of anchovy and roundherring, in the southern Benguela region. The gut contents, bone collagen and muscle data points have been enclosed in boxes to clarify the grouping

deviations and large standard errors of the differences (i.e. of the enrichment from one size-class to the next), particularly for $\delta^{13}\text{C}$ data.

Fig. 5 draws together the paired data of those fish for which we measured both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ on the same samples. A clear pattern emerges which overrides any species-specific differences. The pooled values for gut contents, fish muscle and bone collagen tissues of both species of fish are distinct from one another. Following Kruskal-Wallis 1-way ANOVA (statistically significant, $H_c = 48.32$, $p < 0.001$ for $\delta^{13}\text{C}$; $H_c = 42.64$, $p < 0.001$ for $\delta^{15}\text{N}$), nonparametric Tukey-type multiple comparisons with adjustments for tied ranks (Zar 1984) were carried out to ascertain which groups differed significantly (Tables 3 & 4). Significant differences existed for all comparisons between gut contents, muscle tissue and bone collagen, for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. This reveals a clear pattern of trophic enrichment in the heavier isotopes from the gut contents towards the tissues of both fish species.

Ratios of $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ are differentially fractionated into the muscle and bone collagen tissues of both species of pelagic fish measured in this study. Bone collagen acquires more ^{13}C than muscle, and

Table 3. *Engraulis capensis* and *Etrumeus whiteheadi*. Results from a nonparametric multiple comparison test (Tukey type) to detect the differences between the $\delta^{13}\text{C}$ results for gut contents (G), muscle tissue (M) and bone collagen tissue (B), for anchovy and roundherring pooled together (see Fig. 5). Diff. = the difference between the mean ranks (rank sum/sample size), SE = the standard error (corrected for tied ranks), Q = the test statistic

Comparison	Diff.	SE	Q	$Q_{\alpha,0.5,3}$	Conclusion
G vs M	19.2	4.3	4.4	2.394	Reject H_0
G vs B	30.6	4.5	6.9	2.394	Reject H_0
M vs B	11.4	4.4	2.6	2.394	Reject H_0

Table 4. *Engraulis capensis* and *Etrumeus whiteheadi*. Results from a nonparametric multiple comparison test (Tukey type) to detect the differences between the $\delta^{15}\text{N}$ results for gut contents (G), muscle tissue (M) and bone collagen tissue (B), for anchovy and roundherring pooled together (see Fig. 5). Diff. = the difference between the mean ranks (rank sum/sample size), SE = the standard error (corrected for tied ranks), Q = the test statistic

Comparison	Diff.	SE	Q	$Q_{\alpha,0.5,3}$	Conclusion
G vs M	33.6	5.2	6.5	2.394	Reject H_0
G vs B	16.0	5.3	3.0	2.394	Reject H_0
M vs B	17.6	5.2	3.4	2.394	Reject H_0

muscle tissue gains more ^{15}N than bone collagen. It is interesting to note that the magnitude of ^{13}C enrichment to muscle and that of ^{15}N enrichment to bone collagen are similar. The ^{13}C enrichment to bone collagen is larger, and approaches that of the ^{15}N enrichment to muscle. In the Cape anchovy and possibly roundherring, the degree of fractionation is related to fish size. The tissue less enriched in ^{13}C or ^{15}N (i.e. muscle or bone respectively) shows the stronger negative correlation with increasing fish length.

The data for plankton and pelagic schooling fish reported here re-affirm the usefulness of stable isotope ratios as indicators of trophic relationships in marine food webs. The potential of stable isotope ratios for elucidating pelagic food webs is explored further in a companion study (Monteiro et al. 1991); the present study reveals differences between the uptake of ^{13}C and ^{15}N by different fish tissues. The physiological basis for these differences and their relationships with fish size require further exploration.

Acknowledgements. We thank Amos Winter, John Lanham, Julia Lee-Thorp, Judy Sealy and Pedro Monteiro for helpful discussions and assistance. Thanks are also due to Ian Hampton, Mike Armstrong and Bill Peterson for assistance in collecting samples, and to Tim Dunne for helping with statistical analyses. Financial support was provided by the Foundation for Research Development through its Benguela Ecology Programme and Core Programme funds.

LITERATURE CITED

- Andrews, W. R. H., Hutchings, L. (1980). Upwelling in the southern Benguela current. *Prog. Oceanog.* 9: 1–81
- Angelescu, V. (1982). Ecologia trophica de la anchoita del mar Argentino (*Engraulidae*, *Engraulis anchoita*). Parte II. Alimentacion, compartamento y relaciones trophicas en el ecosistema. *Contr. Inst. nac. Invest. Desar. Pesq.* 409: 1–83
- Azam, F., Fenchel, T., Field, J. G., Gray, J. S., Meyer-Reil, L.A., Thingstad, F. (1983). The ecological role of water-column microbes in the sea. *Mar. Ecol. Prog. Ser.* 10: 257–263
- Bligh, E. G., Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* 37: 911–917
- Buyts, M. E. L. (1959). The South African pilchard (*Sardinops ocellata*) and maasbanker (*Trachurus trachurus*): hydrographical environment and the commercial catches 1950–1957. *Invest. Rep. Div. Sea Fish. S. Afr.* 37 1–176
- Cousins, S. (1980). A trophic continuum derived from plant structure, animal size and a detritus cascade. *J. theor. Biol.* 82: 607–618
- Cousins, S. (1985). The trophic continuum in marine ecosystems: structure and equations for a predictive model. In: Ulanowicz, R. E., Platt, T. (eds.) *Ecosystem theory for biological oceanography*. *Can. Bull. Fish. Aquat. Sci.* 213: 76–93
- De Niro, M. J., Epstein, S. (1976). You are what you eat (plus a few‰): the carbon isotope cycle in food chains. *Geol. Soc. Am. Conference Abstract* 8: 834–835

- De Niro, M. J., Epstein, S. (1978). Influence of diet on the distribution of carbon isotopes in animals. *Geochim. cosmochim. Acta* 42: 495–506
- De Niro, M. J., Epstein, S. (1981). Influence of diet on the distribution of nitrogen isotopes in animals. *Geochim. cosmochim. Acta* 45: 341–351
- De Niro, M. J., Schoeninger, M. J. (1983). Stable carbon and nitrogen isotope ratios of bone collagen. Variations within individuals, between sexes, and within populations raised on monotonous diets. *J. archaeol. Sci.* 10: 199–203
- Fry, B., Arnold, C. (1982). Rapid $^{13}\text{C}/^{12}\text{C}$ turnover during growth of brown shrimp (*Penaeus aztecus*). *Oecologia* 54: 200–204
- Fry, B., Sherr, E. B. (1984). $\delta^{13}\text{C}$ measurements as indicators of carbon flow in marine and freshwater ecosystems. *Contr. mar. Sci.* 27: 13–47
- James, A. G. (1987). Feeding ecology, diet and field based studies on feeding selectivity of the cape anchovy, *Engraulis capensis* Gilchrist. In: Payne, A. I. L., Gulland, J. A., Brink, K. H. (eds.) *The Benguela and comparable ecosystems*. S. Afr. J. mar. Sci. 5: 673–692
- James, A. G., Findlay, K. P. (1989). Effect of particle size and concentration on feeding behaviour, selectivity and rates of food ingestion by the Cape anchovy *Engraulis capensis*. *Mar. Ecol. Prog. Ser.* 50: 275–294
- King, D. P. F., Macleod, P. R. (1976). Comparison of the food and filtering mechanism of the pilchard, *Sardinops ocellata*, and the anchovy *Engraulis capensis*, off South West Africa, 1971–1972. *Invest. Rep. Div. Sea Fish. S. Afr.* 111 1–29
- Koslow, J. A. (1981). Feeding selectivity of schools of anchovy, *Engraulis mordax*, in the southern California bight. *Fish. Bull. U.S.* 79: 131–142
- Knoechel, R., Holtby, L. B. (1986). Cladoceran filtering rate: body length relationships for bacterial and large algal particles. *Limnol. Oceanogr.* 31: 195–200
- Lazarus, B. I. (1975). The inshore plankton of the western Cape. Ph.D. thesis. University of Stellenbosch, South Africa
- Lee-Thorp, J. A., Sealy, J. C., van der Merwe, N. J. (1989). Stable carbon isotope ratio differences between bone collagen and bone apatite, and their relationship to diet. *J. archaeol. Sci.* 16: 585–599
- McConnaughey, T., McRoy, C. P. (1979). ^{13}C label identifies eelgrass (*Zostera marina*) carbon in an Alaskan estuarine food web. *Mar. Biol.* 53: 263–269
- Minagawa, M., Wada, E. (1984). Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between $\delta^{15}\text{N}$ and animal age. *Geochim. cosmochim. Acta* 48: 1135–1140
- Miyake, Y., Wada, E. (1967). The abundance ratio of $^{15}\text{N}/^{14}\text{N}$ in marine environments. *Rec. oceanogr. Wks Japan* 9: 37–53
- Moloney, C. L., Field, J. G. (1989). General allometric equations for rates of nutrient uptake, ingestion and respiration in planktonic organisms. *Limnol. Oceanogr.* 34: 1290–1299
- Monteiro, P. M. S., James, A. G., Sholto-Douglas, A. D., Field, J. G. (1991). The $\delta^{13}\text{C}$ trophic position isotope spectrum as a tool to define and quantify carbon pathways in marine food webs. *Mar. Ecol. Prog. Ser.* 78: 33–40
- Platt, T. (1985). Structure of the marine ecosystem: its allometric basis. In: Ulanowicz, R. E., Platt, T. (eds.) *Ecosystem theory for biological oceanography*. *Can. Bull. Fish. Aquat. Sci.* 213: 55–64
- Owens, N. J. P. (1987). Natural variations in ^{15}N in the marine environment. *Adv. mar. Biol.* 24: 389–451
- Rau, G. H., Mearns, A. J., Young, D. R., Olson, R. J., Schafer, H. A., Kaplan, I. R. (1983). Animal $^{13}\text{C}/^{12}\text{C}$ correlates with trophic level in pelagic food webs. *Ecology* 64: 1314–1318
- Rau, G. H., Sweeney, R. E., Kaplan, I. R., Mearns, A. J., Young, D. R. (1981). Differences in animal ^{13}C , ^{15}N and D abundance between a polluted and an unpolluted coastal site: likely indicators of sewage uptake by a marine food web. *Estuar. coast. Shelf Sci.* 13: 701–707
- Rau, G. H., Takahashi, T., Des Marais, D. J. (1989). Latitudinal variations in plankton $\delta^{13}\text{C}$: implications for CO_2 and productivity in past oceans. *Nature, Lond.* 341: 516–518
- Sealy, J. C., van der Merwe, N. J., Lee Thorpe, J. A., Lanham, J. L. (1987). Nitrogen isotopic ecology in southern Africa: implications for environmental and dietary tracing. *Geochim. cosmochim. Acta* 51: 1–11
- Shannon, L. V. (1985). The Benguela ecosystem. Part I. Evolution of the Benguela physical features and processes. *Oceanogr. mar. Biol. A. Rev.* 23: 105–182
- Shannon, L. V., Agenbag, J. J. (1990). A large scale perspective on the interannual variability in the environment in the South-East Atlantic. *S. Afr. J. mar. Sci.* 9: 161–168
- Tieszen, L. L., Boutton, T. W., Tesdahl, K. G., Slade, N. A. (1983). Fractionation and turnover of stable carbon isotopes in animal tissues: implications for $\delta^{13}\text{C}$ analysis of diet. *Oecologia* 57: 32–37
- Wallace-Fincham, B. P. (1987). The food and feeding of *Etrumeus whiteheadi* Wongratana 1983, off the Cape Province of South Africa. M.Sc. thesis. University of Cape Town, Rondebosch
- Zar, J. H. (1984). *Biostatistical analysis*. Prentice Hall, Englewood Cliffs

This article was submitted to the editor

Manuscript first received: August 30, 1990

Revised version accepted: September 24, 1991