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Spatial variation in the ^{15}N and ^{13}C stable isotope composition of plants, invertebrates and fishes on Mediterranean reefs: implications for the study of trophic pathways

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ABSTRACT: $\delta^{15}N$ and $\delta^{13}C$ were determined for plants, invertebrates and fishes collected from 3 sites on the southern coast of the Mediterranean island of Mallorca, Spain. The sites were separated by distances of 1250 to 3750 m. The mean $\delta^{15}N$ of plants was 1.1 to 4.1‰, benthic invertebrates 5.9 to 6.9‰, planktonic invertebrates 5.5 to 5.8‰ and fishes 8.4 to 13.8‰. $\delta^{15}N$ became enriched with increasing trophic level. The mean $\delta^{13}C$ of plants was −11.4 to −16.3‰ , benthic invertebrates −14.8 to −16.8‰, planktonic invertebrates −19.3 to −19.8‰ and fishes −16.1 to −19.2‰. There were significant differences in the isotopic composition of individual species within the plant, invertebrate or fish groupings at each site and there were significant differences in the isotopic composition of the same species at different sites. Depleted ^{13}C was associated with benthic food chains and enriched ^{13}C with planktonic chains. The data suggest that benthic food chains are important to the rocky reef associated fishes studied, as might be expected in a nutrient poor system where planktonic production is relatively low. However, the variance in $\delta^{13}C$ composition between sites was such that the relative significance of the 2 pathways could not be determined. ^{15}N measurements indicated that some of the fish species studied had adopted different feeding strategies at different sites and, as a result, individuals of the same species could sometimes be assigned to different trophic groups at different sites. The data suggest that these fishes exhibit plasticity in their feeding strategies and this may provide them with greater adaptive flexibility to respond to site-specific changes in food availability. Moreover, the data provide empirical support for current theories of food web dynamics which suggest that trophic 'levels' are dynamic rather than fixed and that 'multichannel omnivory' is an important feature of food webs.

KEY WORDS: Stable isotopes · Trophic interactions · Variability · Mediterranean · Reefs · Feeding strategy

INTRODUCTION

Studies of stable isotope composition can provide indications of the origins and transformations of organic matter (Fry & Sherr 1984, Owens 1987, Preston 1992) and thus stable isotope analysis provides a useful tool for investigating trophic relationships within food webs (Peterson et al. 1985, Peterson & Fry 1987, Fry 1988, Yoshioka et al. 1994). The abundance of ^{15}N in the tissues of consumers is typically enriched by 3‰ relative to their prey and thus ^{15}N studies have been used to define the trophic positions of organisms in systems where feeding relationships are not known (Hobson & Welch 1992, Fry & Quinones 1994). Conversely, ^{13}C is not enriched, or only weakly enriched, with increasing trophic level, and may act as a good indica-

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tor of sources of production (Fry & Sherr 1984, Peterson et al. 1985, Vandover et al. 1992, Bunn & Boon 1993). Combined measurements of N and C can thus provide information on source materials and trophic relationships (Peterson et al. 1985, Peterson & Fry 1987).

The seminal studies of stable isotope variations in aquatic ecosystems were based on relatively few samples and, since isotopic analysis was expensive and time consuming, few experimental designs were replicated in space or time (Fry & Sherr 1984, Owens 1987). However, with the advent of continuous flow isotope ratio mass spectrophotometry, the number of samples analysed in many studies has been increased and isotopic ratios for N and C are often determined simultaneously (Preston & Owens 1983, Preston 1992). Fry & Sherr (1984), Owens (1987) and Hemminga & Mateo (1996) reported that within-species variation in isotopic composition was apparent at almost all scales of investigation and that low levels of replication could mask ecologically significant variation. Thomas & Cahoon (1993) demonstrated significant spatial differences in the ^{15}N and ^{13}C composition of reef fishes on spatial scales of 10s of km, but there has been no attempt to compare variablility between individuals on smaller scales (100s or 1000s of metres) even though many reef-associated fishes are relatively site-attached and typically forage within areas of 1 km^2 or less (Harmelin 1987, Campbell 1995, Garcia-Rubies & Macpherson 1995, Harmelin-Vivien et al. 1995). Spatial differences in ^{15}N and ^{13}C may indicate that fishes are adopting site-specific feeding strategies. They may also reflect spatial variability in the isotopic composition of the same food material. If marked local variation in isotopic composition exists, this may reduce the probability of making useful generalisations about trophic pathways and the role of species within food webs on the basis of localised studies (Polis & Strong 1996).

Nutrient limitation in the Mediterranean Sea leads to relatively low primary productivity (Thingstad & Rassoulzadegan 1995), and it is not clear whether much of the inshore fish production is ultimately supported by pelagic (plankton) or benthic food chains based on the seagrass and benthic algae which flourish in nutrient enriched inshore areas (Ros et al. 1985, Duarte 1989, Margalef 1989, Alcoverro et al. 1995). Conventional assessments of diet, based on the analysis of stomach contents, have not resolved such debate because it is often difficult to distinguish planktonic and benthic invertebrates in partially digested stomach contents and, even when stomach contents can be identified, it is not clear whether they will be assimilated and therefore contribute to production (Bell & Harmelin-Vivien 1983, Verlaque 1985, Khuory 1987). Furthermore, temporal sampling constraints generally prevent the accurate description of diet over ecologically meaningful periods of months or years (Hobson & Welch 1992, 1994). The ^{15}N and ^{13}C composition of fish muscle tissue will reflect the composition of assimilated food and provide a long-term indication of feeding strategy by integrating any differences in assimilated food over time (Hobson & Welch 1992, 1994, Thomas & Cahoon 1993) Analysis of the isotopic composition of muscle tissue has already been used to identify fishes supported by different trophic pathways (Sholto-Douglas et al. 1991, Rau et al. 1992, Thomas & Cahoon 1993, Bootsma et al. 1996) and may offer a means by which to determine the relative importance of planktonic and benthic pathways in supporting Mediterranean fishes.

The aims of the present study were the following: (1) to determine the ^{15}N and ^{13}C composition of Mediterranean shallow littoral plants, invertebrates and fishes; (2) to determine whether spatial variations in ^{15}N and ^{13}C composition affect the statistical probability of discriminating between species using stable isotopic analyses; and (3) to determine whether the trophic positions of species are site-specific and whether relationships between the isotopic composition of fishes and their prey can indicate the relative significance of planktonic and benthic pathways in supporting reef fishes in one region of the Mediterranean Sea.

METHODS

Plants, benthic and planktonic invertebrates and fishes were collected from 3 sites on the southern coast of the Mediterranean island of Mallorca, Spain, from 30 July to 4 August 1995. Sampling design was based on 3 scales: between individuals, between species and between sites. Intensive efforts were made to make the collections in a short time period in order to minimise the possibility of results being confounded by temporal changes in the isotopic composition of the target organisms (Owens 1987, Flynn & Davidson 1993, Hemminga & Mateo 1996). The sites were separated by distances of 1250 to 3750 m (Fig. 1). We collected those species of fishes and plants which appeared to be most abundant at the study sites. Adult reef-associated fishes were caught with spears and stored on ice immediately after capture. Juvenile *Chromis chromis* were caught with 2 mm mesh hand nets. The seagrass *Posidonia oceanica*, and the dominant benthic macroalgae, *Cytoseira balearica*, *Dictyopteris membranacea* and *Halopteris scoparia*, were collected by divers. Individual plants were treated as replicates. Twenty or more whole plants of each of these species were also collected in plastic sacks for the susbsequent removal of invertebrate fauna. Plankton were collected at night

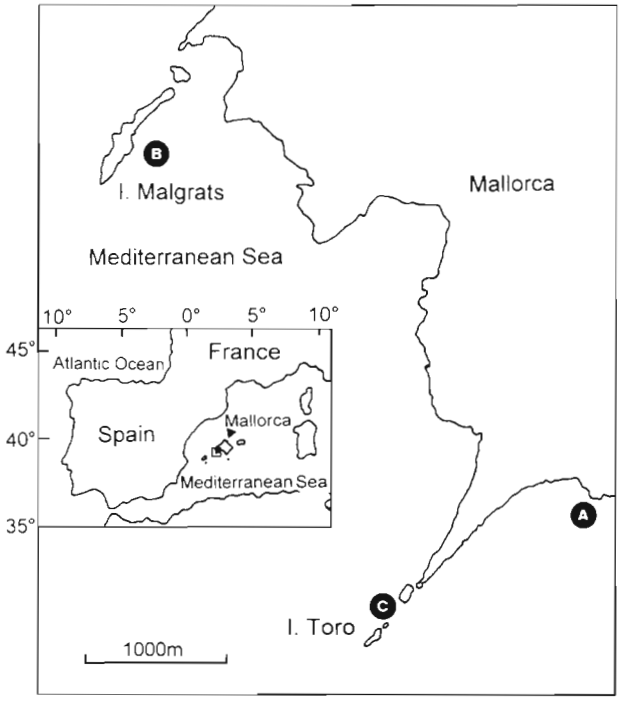

Fig. 1. General location of Mallorca and the 3 study sites. A = Cala d'Ast, B = Malgrats, C = Es Pans

by towing a 0.6 m diameter, 250 µm mesh net at the surface for 5 to 10 min.

In the laboratory, white dorsal muscle tissue was dissected from the fishes, lyophilised and ground to a fine powder, which was thoroughly mixed before analysis. Epiphytes were removed from algae and *Posidonia oceanica* with a rubber spatula, washed in distilled water, lyophilised and ground. *P. oceanica* and algal leaves were cleaned of epiphytes with a spatula, washed in distilled water, lyophilised and ground. Invertebrates were obtained by gently agitating the *P. oceanica* plants or macroalgae in distilled water for 2 to 3 h, and straining the water through 250 µm mesh. Invertebrates were sorted from other debris using fine tweezers. Tissue was separated from calcareous invertebrates beneath a dissecting microscope and stomach contents were also removed if possible. Acid was not used to remove other inorganic carbonate (Bunn et al. 1995). Plankton samples were washed in distilled water, lyophilised and ground. Samples were only stored as dry powder when lyophilisation and grinding were complete.

The ^{15}N and ^{13}C composition of the samples was determined using a continuous flow isotope ratio mass spectrometry (CF-IRMS) (Preston & Owens 1983, Preston 1992). Weighed samples of ground material (from 0.7 mg for fish and benthic invertebrates to 1.2 mg for plants and algae) were oxidised and the N_2 and CO_2 passed to a single inlet dual collector mass spectrometer [Automated Nitrogen Carbon Analysis (ANCA) SL 20-20 system]. This was a continuous flow system, so 2 samples of reference material (an internal standard) were analysed after every 5 tissue samples in order to calibrate the system and compensate for drift with time (ANCA-SL Dual Isotope v3.4 software). Home Pride Flour was used as the reference material; this is a stable material and its isotopic composition has been determined by Europa Scientific, which manufacture the ANCA system.

Ratios of ^{15}N:^{14}N and ^{13}C:^{12}C were expressed relative to N_2 in air for nitrogen and to the PeeDee Belemnite (PDB) standard for carbon. The PDB standard was adopted to allow our data to be compared with that reviewed or reported in existing studies. It is important to note that that this standard differs from the recently proposed Vienna PeeDee Belemnite (V-PDB) standard (Anon 1995). Ratios of ^{15}N:^{14}N and ^{13}C:^{12}C were calculated as:

$$\delta X = (R_{sample}/R_{standard} - 1) \times 10^3$$

where X is ^{15}N or ^{13}C and R is ^{15}N:^{14}N or ^{13}C:^{12}C.

Kruskal-Wallis tests were used to test for species-specific differences in the length-frequency distributions of fishes speared at the 3 sites. Multivariate analysis of variance (MANOVA) was used to test the null hypotheses that there were no significant differences in the isotopic composition of individual species (for brevity, 'species' also refers to multi-species samples of epiphytes and benthic and planktonic invertebrates) within plant, invertebrate or fish groupings at each site. If the null hypothesis was rejected, ANOVA was used to test the null hypothesis that there were no significant differences in either the $\delta^{15}N$ or $\delta^{13}C$ composition of each species at the 3 sites and to quantify $\delta^{15}N$ and $\delta^{13}C$ variation both within and among sites. Parametric discriminant analysis was used to determine criteria for classifying given species of plants, invertebrates or fishes to specific sites on the basis of their $\delta^{15}N$ and $\delta^{13}C$ composition. The accuracy of each classification criterion was evaluated by assessing its performance in the classification of future observations. The entire data set was used to define and evaluate the criteria.

RESULTS

Two to eight fishes of each species were collected at each site (Table 1) and the species-specific length-frequency distributions of these fishes did not differ significantly among sites (Kruskal-Wallis $p > 0.1$), except for *Oblada melanura* and *Thalassoma pavo* ($p < 0.05$). For these 2 species neither $\delta^{15}N$ or $\delta^{13}C$ were correlated with length within the size range sampled ($p >$

Table 1. Trophic groups, size ranges (cm) and numbers of fishes (in parentheses) collected for stable isotope analysis. Trophic group codes based on underwater observations of target species: hb = herbivore, iv = benthic invertebrate feeder, pi = fish feeder, pk = pelagic plankton feeder

Fishes	Trophic group	Size range and number sampled by site		
		Site A	Site B	Site C
Apogon imperbis	iv/pk	6.3–9.0 (3)	8.2–9.9 (3)	8.5–9.5 (4)
Atherina presbyter	iv/pk	9.0–9.2 (3)	9.0–9.8 (2)	8.3–9.0 (3)
Chromis chromis (juvenile)	iv/pk	1.2–1.5 (3)	1.1–1.4 (3)	1.2–1.5 (3)
Chromis chromis (adult)	iv/pk	9.0–11.5 (3)	9.0–9.9 (3)	11.1–12.0 (3)
Coris julis	iv/pi	8.2–15.3 (6)	9.0–11.6 (4)	9.5–13.7 (5)
Crenilabrus tinca	iv	11.1–22.5 (6)	10.7–19.0 (6)	14.0–22.7 (6)
Diplodus annularis	iv	9.4–15.3 (6)	7.6–13.8 (5)	11.0–14.5 (5)
Diplodus vulgaris	iv	10.2–21.8 (5)	13.2–20.0 (6)	11.1–21.8 (6)
Oblada melanura	iv	11.3–16.0 (6)	11.6–11.9 (4)	13.1–18.8 (5)
Sarpa salpa	hb/iv	14.2–16.2 (4)	15.6–26.2 (5)	15.9–25.8 (5)
Scorpaena porcus	iv/pi	12.3–15.1 (3)	12.0–18.6 (2)	10.0–14.2 (4)
Serranus scriba	iv/pi	11.4–14.6 (6)	12.8–14.8 (6)	10.1–16.1 (6)
Thalassoma pavo	iv/pi	11.0–14.2 (6)	7.4–11.3 (4)	8.2–14.2 (8)

Table 2. $\delta^{15}N$ and $\delta^{13}C$ composition of plants, invertebrates and fishes at the 3 study sites (mean ± SE)

Species or type	Site A		Site B		Site C	
	$\delta^{15}N$ ‰	$\delta^{13}C$ ‰	$\delta^{15}N$ ‰	$\delta^{13}C$ ‰	$\delta^{15}N$ ‰	$\delta^{13}C$ ‰
Plants						
Cytoseira balearica	3.0 ± 0.06	−13.9 ± 0.35	1.4 ± 0.98	−13.1 ± 0.58	3.0 ± 0.22	−16.3 ± 0.20
Dictyopteris membranacea	3.3 ± 0.13	−16.0 ± 0.11	2.9 ± 0.19	−15.4 ± 0.26	2.8 ± 0.15	−16.0 ± 0.50
Halopteris scoparia	2.9 ± 0.30	−13.8 ± 0.45	2.4 ± 0.73	−15.7 ± 0.16	3.0 ± 0.22	−16.3 ± 0.20
Posidonia oceanica	3.5 ± 0.89	−12.8 ± 0.17	4.1 ± 0.05	−11.4 ± 0.30	2.8 ± 0.72	−13.2 ± 0.10
Epiphytes	2.3 ± 1.27	−13.6 ± 0.24	1.1 ± 0.42	−15.2 ± 0.58	2.8 ± 0.49	−14.4 ± 0.71
Invertebrates						
Benthic invertebrates (algae)	5.9 ± 0.47	−16.5 ± 0.30	6.9 ± 0.42	−16.0 ± 0.46	6.2 ± 0.02	−14.9 ± 0.13
Benthic invertebrates (*Posidonia*)	6.3 ± 0.15	−16.8 ± 0.19	6.2 ± 0.29	−16.3 ± 0.46	6.6 ± 0.35	−14.8 ± 0.29
Zooplankton	5.8 ± 0.16	−19.8 ± 0.17	5.7 ± 0.32	−19.3 ± 0.31	5.5 ± 0.36	−19.6 ± 0.08
Fishes						
Apogon imperbis	13.4 ± 0.18	−17.1 ± 0.23	11.0 ± 0.21	−16.2 ± 0.16	12.4 ± 0.17	−16.8 ± 0.20
Atherina presbyter	11.6 ± 0.71	−17.6 ± 0.17	10.6 ± 0.02	−17.2 ± 0.05	10.8 ± 0.43	−17.7 ± 0.11
Chromis chromis (juvenile)	8.7 ± 0.12	−18.3 ± 0.17	8.4 ± 0.49	−18.9 ± 0.20	8.9 ± 0.06	−19.1 ± 0.04
Chromis chromis (adult)	10.5 ± 0.22	−18.0 ± 0.29	9.3 ± 0.08	−17.7 ± 0.13	11.2 ± 0.03	−18.5 ± 0.06
Coris julis	12.1 ± 0.27	−16.5 ± 0.43	10.7 ± 0.11	−16.1 ± 0.11	12.2 ± 0.11	−16.9 ± 0.26
Crenilabrus tinca	11.7 ± 1.42	−16.2 ± 0.14	10.8 ± 0.86	−17.4 ± 0.74	11.5 ± 0.14	−16.9 ± 0.06
Diplodus annularis	12.7 ± 0.29	−16.6 ± 0.15	11.1 ± 0.13	−16.9 ± 0.21	12.7 ± 0.20	−18.1 ± 0.20
Diplodus vulgaris	13.6 ± 0.80	−16.3 ± 0.40	11.2 ± 1.39	−17.8 ± 1.49	11.6 ± 1.74	−18.5 ± 1.44
Oblada melanura	11.9 ± 0.09	−17.9 ± 0.09	11.3 ± 0.16	−17.7 ± 0.21	10.1 ± 1.97	−19.2 ± 1.52
Sarpa salpa	8.9 ± 0.30	−17.4 ± 0.05	10.0 ± 0.31	−17.1 ± 0.31	9.7 ± 0.10	−16.5 ± 0.37
Scorpaena porcus	13.1 ± 0.39	−17.2 ± 0.06	13.0 ± 0.39	−16.6 ± 0.04	12.1 ± 0.43	−16.9 ± 0.15
Serranus scriba	13.1 ± 0.23	−16.9 ± 0.10	13.3 ± 0.17	−16.3 ± 0.14	11.5 ± 0.29	−16.8 ± 0.10
Thalassoma pavo	13.2 ± 0.14	−17.2 ± 0.05	13.8 ± 0.11	−17.0 ± 0.22	12.1 ± 0.14	−16.7 ± 0.28

0.1). The mean $\delta^{15}N$ of plants was 1.1 to 4.1‰, benthic invertebrates 5.9 to 6.9‰, planktonic invertebrates 5.5 to 5.8‰ and fishes 8.4 to 13.8‰ (Table 2). There was general enrichment in $\delta^{15}N$ with increasing trophic level across groups (Table 1, Fig. 2). The mean $\delta^{13}C$ of plants was −11.4 to −16.3‰, benthic invertebrates −14.8 to −16.8‰, planktonic invertebrates −19.3 to −19.8‰ and fishes −16.1 to −19.2‰ (Table 2).

The $\delta^{15}N$ of juvenile *Chromis chromis*, which were often observed feeding on small pelagic plankton by day, was lower than that of other fishes, including *Sarpa salpa* and adult *C. chromis*. The ^{15}N of *S. salpa*, a fish known to eat plant material, was around 6‰ higher than the plants which it appeared to eat, whilst the ^{15}N of the juvenile *C. chromis* was only 3‰ higher than the plankton they appeared to eat. The range in

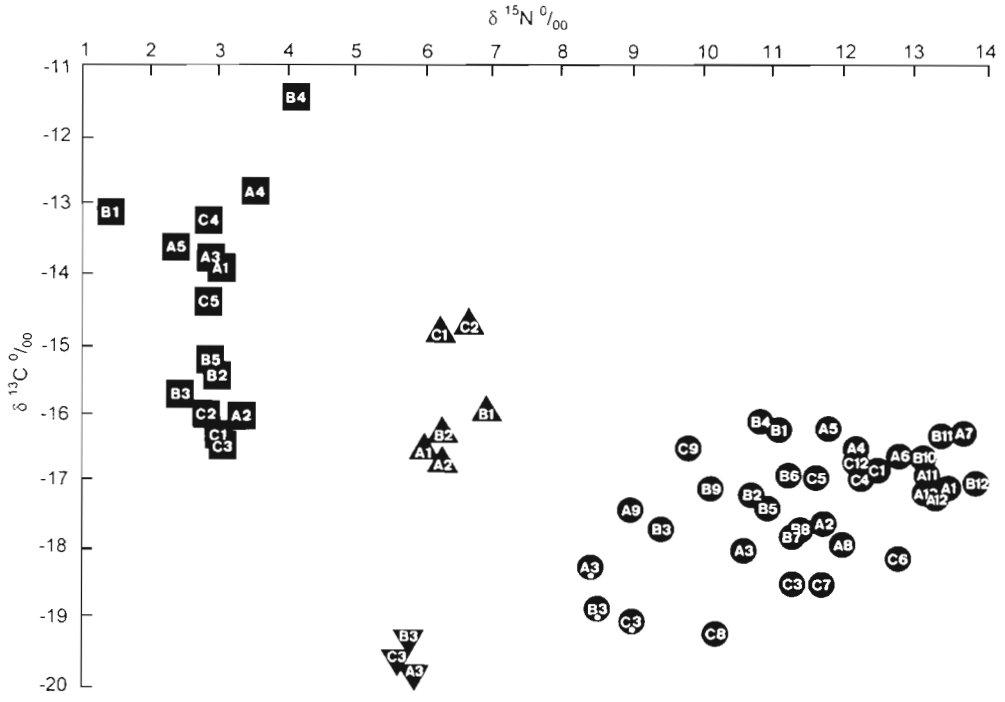

Fig. 2. Relationship between mean $\delta^{15}N$ and mean $\delta^{13}C$ for plants (■), benthic invertebrates (▲), plankton (▼) and fishes (●) at the 3 study sites. Site codes: A = Cala d'Ast; B = Malgrats; C = Es Pans. Plants: 1 = *Cytoseira balaerica*; 2 = *Dictyopteris membranacea*; 3 = *Halopteris scoparia*; 4 = *Posidonia oceanica*; 5 = Epiphytes. Invertebrates and plankton: 1= Invertebrates on algae; 2 = invertebrates on *Posidonia*; 3 = plankton; Fishes: 1 = *Apogon imperbis*; 2 = *Atherina presbyter*; 3 = *Chromis chromis* (juveniles indicated with an asterisk); 4 = *Coris julis*; 5 = *Crenilabrus tinca*; 6 = *Diplodus annularis*; 7 = *Diplodus vulgaris*; 8 = *Oblada melanura*; 9 = *Sarpa salpa*; 10 = *Scorpaena porcus*; 11 = *Serranus scriba*; 12 = *Thalassoma pavo*. Fish C2 is obscured by B2 and B5, C11 by C5 and C10 by C4

mean $\delta^{15}N$ of all fishes was 5.4‰ and thus considerably greater than the 1.0‰ and 0.3‰ ranges in their potential benthic and planktonic food sources respectively. The range in mean $\delta^{15}N$ of the same species at different sites often approached 2‰.

Posidonia oceanica was enriched with ^{13}C in comparison with the other plants, but the invertebrates living on *P. oceanica* were of similar isotopic composition to those found on algae. The $\delta^{13}C$ of *Sarpa salpa*, which were observed feeding on *P. oceanica* and algae, and had gut morphology similar to that of other herbivorous fishes, was greater than that of *Chromis chromis*, which were often observed feeding on pelagic plankton on the outer reef slopes. However, the variability in $\delta^{13}C$ within many species of fishes and between sites was often similar to the variability in their potential food sources. Thus the difference between minimum and maximum mean $\delta^{13}C$ values was 2‰ and 0.7‰ for benthic and planktonic invertebrates respectively and 3.1‰ for fishes.

There were significant differences in the isotopic composition of individual species within the plant, invertebrate and fish groupings at each site (MANOVA, $p < 0.001$), and thus ANOVA was used to test the null hypothesis that there were no significant differences in the ^{15}N or ^{13}C composition for each of the species at the 3 sites (Table 3). Differences in the ^{15}N composition of plants and invertebrates among sites were all non-significant but differences in the ^{13}C composition of 3 of the 5 plant species and 2 of the 3 invertebrate species were significant (Table 3). Within the fishes, differences in ^{15}N and ^{13}C composition were significant for 7 and 4 of the 13 species respectively, the high values of F indicating that there was considerably more variation among than within sites (Table 3).

The discriminant analysis indicated that the probability of correctly classifying plants or fishes on the basis of their isotopic composition was rarely 100%, but was better within individual sites than when data were pooled across sites (Table 4). This was consistent with the observation that variation in the ^{15}N or ^{13}C isotopic composition of many species of fishes within sites was less than that among sites (Table 3). The probability of correctly classifying invertebrates within sites was always 100%, but was less than 100% for benthic invertebrates living on algae or *Posidonia oceanica* when the sites were combined (Table 4). This was consistent with the significantly greater variation in $\delta^{13}C$ of benthic invertebrates among sites than within sites (Table 3).

Table 3. Results of ANOVA tests of the null hypothesis that there were no significant differences in the $\delta^{15}N$ and $\delta^{13}C$ composition of species at the 3 study sites. ns: not significant

Species or type	$\delta^{15}N$			$\delta^{13}C$		
	F	df	p	*F*	df	p
Plants						
Cytoseira balearica	2.60	2,6	ns	16.20	2,6	<0.01
Dictyopteris membranacea	2.83	2,6	ns	1.01	2,6	ns
Halopteris scoparia	0.37	2,6	ns	18.37	2,6	<0.01
Posidonia oceanica	1.06	2,6	ns	18.84	2,6	<0.01
Epiphytes	1.04	2,6	ns	2.19	2,6	ns
Invertebrates						
Benthic invertebrates (algae)	1.92	2,6	ns	10.57	2,6	<0.05
Benthic invertebrates (*Posidonia*)	0.69	2,6	ns	10.75	2,6	<0.05
Zooplankton	0.21	2,6	ns	1.50	2,6	ns
Fishes						
Apogon imperbis	39.26	2,7	<0.001	4.80	2,7	<0.05
Atherina presbyter	1.01	2,5	ns	2.25	2,5	ns
Chromis chromis (juvenile)	0.67	2,6	ns	8.95	2,6	<0.05
Chromis chromis (adult)	37.88	2,5	<0.001	3.27	2,5	ns
Coris julis	13.34	2,12	<0.001	1.22	2,12	ns
Crenilabrus tinca	0.64	2,15	ns	2.06	2,15	ns
Diplodus annularis	15.29	2,13	<0.001	30.71	2,13	<0.001
Diplodus vulgaris	0.74	2,14	ns	0.75	2,14	ns
Oblada melanura	0.72	2,12	ns	0.77	2,12	ns
Sarpa salpa	5.04	2,11	<0.05	2.04	2,11	ns
Scorpaena porcus	1.85	2,6	ns	4.24	2,6	ns
Serranus scriba	16.82	2,15	<0.001	6.64	2,15	<0.01
Thalassoma pavo	34.74	2,15	<0.001	1.08	2,15	ns

DISCUSSION

The enrichment in ^{15}N across plant, invertebrate and fish groups was consistent with the widespread recognition that ^{15}N provides a measure of trophic level (Owens 1987). $\delta^{13}C$ was a good indicator of benthic and planktonic source material because plants and invertebrates associated with benthic food chains were relatively enriched with ^{13}C and easily distinguished from the plankton. The ^{13}C content of epiphyte, *Posidonia oceanica* and algal source materials was, however, very variable between sites; this may reflect differences in the degree of shading and other environmental factors which are known to determine the ^{13}C of plants (Durako & Hall 1992, Hemminga & Mateo 1996). The marked differences in $\delta^{13}C$ between *P. oceanica* and the invertebrates which live on *P. oceanica* would not be consistent with the assumption that they fed on the *P. oceanica*. Rather, it appears that the invertebrates may have been feeding on epiphytes which have a similar $\delta^{13}C$ to that of the invertebrates.

Given that the range in mean $\delta^{13}C$ composition of plants and invertebrates often exceeded that of the fishes, an accurate assessment of the relative significance of benthic and planktonic pathways in supporting fish production at the different sites was not possible. Differences in the $\delta^{13}C$ of fishes may simply have resulted from variability in $\delta^{13}C$ within one type of prey. However, given that there are relatively small changes in $\delta^{13}C$ between predators and their prey (Fry & Sherr 1984, Peterson et al. 1985, Vandover et al. 1992, Bunn & Boon 1993) and that the range of mean $\delta^{13}C$ in the fish assemblage we studied was between that of the benthic and planktonic source material, we suggest that benthic pathways are important for many of the inshore fishes studied. These fishes include *Atherina presbyter* and *Chromis chromis*, which are often seen gathered in shoals around reefs and are often assumed to feed solely on pelagic plankton.

The difference between the $\delta^{15}N$ of small planktonic fishes (*Chromis chromis*) and their likely prey was 3‰, in accordance with existing evidence (Owens 1987). However, the $\delta^{15}N$ of *Sarpa salpa* is around 6‰ higher than the plants on which they were often observed to feed. Existing understanding of $\delta^{15}N$ changes between predators and their prey (Owens 1987) suggests that *S. salpa* was 2 trophic levels higher than these plants, and indicates that diatoms or invertebrates on the plants may have been an important part of the assimilated diet. Conversely, the $\delta^{15}N$ increase from algae and epiphytes to the invertebrates which live on them was around 3‰, suggesting that they fed directly on these plants. In order to improve our understanding of the relationships between benthic invertebrates or plankton and their prey it will be necessary to determine the $\delta^{13}C$ and $\delta^{15}N$ of individual species. Such detailed analyses were beyond the scope of the present study.

The variance in $\delta^{15}N$ of plants, invertebrates and plankton among and within sites and species was considerably less than that of the fish assemblage. We suggest, therefore, that general conclusions regarding trophic level of fishes are not unduly biased by $\delta^{15}N$ variations in the source material. The $\delta^{15}N$ of fishes suggests that the assemblage crosses 2 trophic levels, which is consistent with the suggestion that juvenile fishes such as the highly abundant *Chromis chromis* are consumed by *Serranus scriba* and other partially piscivorous species (Harmelin-Vivien et al. 1989, Arculeo et al. 1993). There was significantly more variation in the $\delta^{15}N$ of *Apogon*

Table 4. Probability (%) of correctly classifying individual types of plants, types of invertebrates or species of fish, from sites 1, 2 and 3, or from all sites combined, on the basis of $\delta^{15}N$ and $\delta^{13}C$ measurements

Species or type	Site A	Site B	Site C	Mean (by site)	All sites
Plants					
Cytoseira balearica	33	100	0	44	44
Dictyopteris membranacea	100	67	67	78	78
Halopteris scoparia	0	33	0	11	22
Posidonia oceanica	100	100	100	100	89
Epiphytes	33	100	67	67	89
Invertebrates					
Benthic invertebrates (algae)	100	100	100	100	56
Benthic invertebrates (*Posidonia*)	100	100	100	100	78
Zooplankton	100	100	100	100	100
Fishes					
Apogon imperbis	33	100	50	61	10
Atherina presbyter	33	100	67	67	38
Chromis chromis (juvenile)	100	100	100	100	100
Chromis chromis (adult)	100	67	67	78	56
Coris julis	0	100	60	53	13
Crenilabrus tinca	67	0	0	22	17
Diplodus annularis	50	40	60	50	0
Diplodus vulgaris	40	17	0	19	0
Oblada melanura	100	0	20	40	7
Sarpa salpa	100	0	80	60	93
Scorpaena porcus	67	50	25	47	0
Serranus scriba	17	17	50	28	17
Thalassoma pavo	50	75	13	46	56

imperbis, adult *Chromis chromis*, *Coris julis*, *Diplodus annularis*, *Sarpa salpa*, *Serranus scriba* and *Thalassoma pavo* among sites than within sites. This, in conjunction with the magnitude of variation in mean $\delta^{15}N$, indicated that fishes of a given species at a given site may have held positions in the trophic hierarchy which differed by almost one level from those of the same species at another site. Within sites, however, the data suggested that individuals of the same species were adopting similar feeding strategies. We suggest that many of these inshore reef-associated fishes have the capacity to switch their trophic position within food webs in response to local circumstances. Whilst it is well known that many fishes undergo ontogenetic changes in diet, and may therefore occupy a number of trophic levels in the course of their life history (Winemiller 1990), the within-species variations in $\delta^{15}N$ observed in this study suggest that fishes at the same life history stage can also switch between different trophic levels. This observation is in accordance with the suggestion that the trophic positions of species in food webs are dynamic rather than fixed (Polis & Strong 1996). Plasticity in feeding strategy of fishes would reflect that in growth rates, age at maturity and other life history parameters (Roff 1984, Stearns & Crandall 1984, Jennings & Beverton 1991, Beverton 1992) and provide them with greater adaptive flexibility to respond to site-specific changes in food availability.

Variability in $\delta^{15}N$ and $\delta^{13}C$ within species was largely attributable to collection site differences. Thomas & Cahoon (1993) reported similar spatial variation in the $\delta^{15}N$ and $\delta^{13}C$ of reef fishes at sites which were separated from one another by distances an order of magnitude greater than those examined in our study. Their analysis of isotopic composition grouped fishes into feeding types as predicted from knowledge of feeding types and gut contents. As in the present study, Thomas & Cahoon (1993) demonstrated that discriminant analysis usefully distinguished 5 species of reef fishes supported by different trophic pathways, but working with the Mediterranean assemblage of 12 species such an analysis is relatively powerless when sites are not treated as a separate variable. If the total area of collection used in our study (a radius of 2 km encloses all sites) had been treated as a single site, then the variance in $\delta^{15}N$ and $\delta^{13}C$ would have masked the evidence for localised feeding strategies of these reef fishes and reduced the possibility of identifying reef fishes on the basis of their isotopic composition. Our data provide support for Fry & Sherr (1984), Owens (1987) and Hemminga & Mateo (1996), who emphasised that large within-species (>2‰) isotopic variations occur on most scales studied. They are clearly correct to state that great caution should be exercised before assigning average $\delta^{15}N$ and $\delta^{13}C$ values on the basis of a few measurements. Furthermore, the results suggest that it would be difficult to make reliable and non-trivial generalisations about the feeding strategies of these reef fishes without spatial replication on a number of scales. Increased automation of ^{15}N and ^{13}C analysis makes it possible to address such issues in contemporary experimental design.

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