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ON THE BIOLOGY OF SUBMARINE CAVES WITH SULPHUR SPRINGS: APPRAISAL OF ^{13}C/^{12}C RATIOS AS A GUIDE TO TROPHIC RELATIONS

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Submarine caves with sulphurous springs at Cape Palinuro, Campania, Italy, have a richer fauna than expected from the known oligotrophic nature of the cave habitat. Warm water containing sulphide issues from springs and rises above the cooler ambient sea-water with a sharp thermocline/chemocline between. The warm water then escapes from the caves mixed with cooler sea-water, probably inducing an inflow of ambient sea-water. Bacterial mats, often dominated by large species of attached bacteria resembling *Beggiatoa*, line the upper parts of the inner caves and act as primary producers, fixing CO_2 by means of the autotrophic enzyme ribulosebisphosphate carboxylase. Many of the animals in the innermost parts of the caves live close to the chemocline or just below, where they would experience fall-out of bacterial organic matter, and some carry filamentous bacteria on their tubes and hard parts. Dominant members of the community include sponges, cnidarians, and tubicolous polychaetes.

The inner caves form a two compartment system, with production in the upper layer of sulphurous water and consumption below. The carbon isotope ratio in the bacterial mats (range of δ^{13}C −30·1 to −31·8‰) is a good 'marker' for tracing carbon flow, contrasting with the usual enhancement of carbon-13 in benthic photoautotrophs. Animal tissue isotope ratios confirm that bacterial carbon is entering the food chain and that this is a source of food for some cave biota. The contribution from bacteria ranges from zero to virtually 100%, depending on species and variation in local habitat. Animals living close to the bacterial mats benefit most, notably a polychaete *Phyllochaetopterus*, an oligochaete *Thalassodrilides*, a podocopid ostracod *Paracypris* and certain echinoderms and bivalves. The large sponges (*Geodia, Petrosia*) may not benefit from bacterial production.

INTRODUCTION

Mediterranean littoral limestones harbour many karstic caves, some of which now extend well below present sea level. A characteristic marine fauna and flora occurs in the submarine caves. General descriptions have been provided by several authors (Riedl, 1966; Cinelli et al., 1977; Balduzzi et al., 1989; Harmelin et al., 1985). In the majority of submarine caves the richness and diversity of the organisms is reduced the further one penetrates from the sea outside so that in general cave ecosystems are classed as oligotrophic (Ott & Svoboda, 1976; Harmelin et al., 1985; Fichez, 1990a,b). Photoautotrophic organisms are the first to be lost; then the sessile fauna becomes impoverished as the particulate organic matter from production outside the cave is progressively filtered off. In some of the oligotrophic caves the fauna includes species normally associated with oligotrophic conditions in the deep sea (Vacelet et al., 1994).

However, in certain caves where there are hot springs that carry dissolved hydrogen sulphide, the innermost parts carry a richer than usual fauna (Abbiati et al., 1992, 1994). Comparable enrichment has been reported from continental caves with hot springs (e.g. Sarbu, 1992). The present shape of many of the sulphurous submarine caves may have been influenced by biological oxidation of the hydrogen sulphide with production of excess hydrogen ions, leading to dissolution of the limestone (Forti, 1985, 1989, 1993). To explain the unexpectedly richer fauna of sulphurous caves it has been hypothesized that there is an input of organic matter produced from the bacteria that oxidize hydrogen sulphide (Abbiati et al., 1994). Organic matter from this source (chemolithoautotrophy) would be independent of photoautotrophic production. Conditions in caves with sulphurous springs might be to some extent comparable to those existing at mid-ocean ridges where utilization of geochemical energy produces a locally high biomass that contrasts with typical sparse deep-sea benthic biota (Grassle, 1986; Tunnicliffe, 1991; Childress & Fisher, 1992).

This preliminary contribution examines aspects of the hypothesis that the biota in caves with sulphurous springs benefit from chemolithoautotrophic production and gives a general description of the habitat.

METHODS

Observations and sampling were carried out at three of the many submarine caves at Cape Palinuro, Cilento, Campania (Figure 1). Cape Palinuro is part of the Monte Bulgheria formation, composed of limestone and dolomitized limestone (Pescatore et al., 1985; Alvisi et al., 1994b); normally the rock is permeable only by fracture and karst formation. The tip of the Cape is separated from the main massif by recent deposits. The caves studied were: (1) Grotta Azzurra; (2) a smaller cave opening onto Cala Fetente, on the opposite side of the Cape, now named Grotta Sulfurea; and (3) Grotta Trombetta, which has no perceptible sulphur input, was therefore used as a control. Samples of the fauna and flora from outside the caves were also examined. The Grotta Azzurra, or 'Blue Grotto', is exhibited to tourists and has been well-surveyed (Alvisi, 1991, 1994a); the other sulphurous cave is smaller and more difficult to enter, and is less well-known (Muscio, 1985; Muscio & Sello, 1989; Forti, 1985, 1989, 1993). All sampling and measurement was carried out by SCUBA diving. Water temperatures were measured with a Hydronaut Srl digital thermometer. Water samples were taken with large syringes mounted on a Perspex rod, and salinity estimated with a portable sodium electrode (Horiba Compact Salt Meter, C-121). Subsamples of water and sediment were preserved with 2% zinc acetate for subsequent estimation of sulphide. Samples of the fauna, flora, and sediment from the caves were taken in May 1991, May 1992 and May and November, 1993 and February, May and October 1994. Four cylindrical sediment traps (51 mm diameter, aspect ratio 3·9) were suspended in Grotta Azzurra for eight days, 4–12 May 1993; the coarse particulate matter retained by 200-µm mesh was analysed under a stereomicroscope. Results of later sediment trapping will be reported elsewhere.

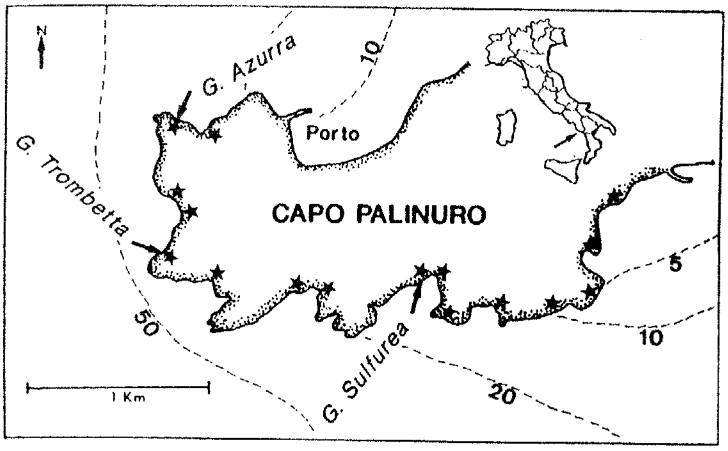

Figure 1. Sketch map of the Capo Palinuro peninsula. Most of the known submarine caves are marked with ★; the three studied are named. Depth contours are shown in metres. Inset, the location in Campania, Italy.

Samples of the biota were taken for measurement of their stable carbon isotopes in the expectation that food chains based on sulphur-oxidizing bacteria would show a depletion of the heavy isotope of carbon, ^{13}C. This is an effect of discrimination by the bacterial CO_2 fixing enzyme, ribulosebisphosphate carboxylase, (RubisCo) against ^{13}C (Ruby et al., 1987), and usually provides a distinct 'marker' for tracing food sources and calculation of the bacterial contribution to different species (Conway et al., 1989). Material for stable carbon isotope analysis was cleaned of epizoic organisms and dried in the field, then later acid-washed, redried and powdered. The powdered samples were combusted in sealed quartz tubes at 750–800°C. The resulting CO_2 was cryogenically purified at −78°C and analysed in a Finnigan MAT 251 isotope ratio mass spectrometer. The results are reported in the usual notation relative to the PDB standard as parts per thousand. Replicate assays and replicate samples show a precision of 0·2‰.

Organisms were identified to species as far as possible. The percentage composition of the coarser fraction of particulate matter collected by the sediment traps was estimated under a stereomicroscope. The bacterial mats were examined and photographed immediately after collection by means of a stereomicroscope and a McArthur high-power portable microscope. Assays were made of RubisCo activity in the bacterial mats, using the method of Dando et al. (1985). Uptake of CO_2 by the mats was measured on weighed aliquots added to ^{14}C labelled bicarbonate dissolved in water from above the chemocline, and incubated in closed 50-ml plastic jars with a head space containing ambient air from outside the cave. Incubations were stopped by addition of acetic acid, and the mats homogenized. The fixed component was analysed by established technique (Dando et al., 1985, 1986). The approximate biomass of the mats was assessed by scraping circular patches into plastic jars of 37 mm diameter and weighing the sample after removal of excess water.

THE ENVIRONMENT OF THE CAVES

In both sulphurous caves the hot springs issue through fissures in the floor, but not all of them have yet been located. In the inner part of the caves the warm sulphurous waters rise above the cooler ambient sea-water to form a layer of warm, slightly less saline water containing dissolved hydrogen sulphide. This warm water eventually mixes with ambient sea-water and flows out of the caves through intermediate chambers. It is probable that this outgoing flow is balanced by an inflow of ambient sea-water, a situation corresponding to estuarine-driven circulation in fjords. However, much remains to be discovered about the flow rates of the springs, the chemical composition of the fluid, and the degree of circulation in the caves. The boundary between the upper sulphurous layer and normal sea-water is sharper in calm weather and before disturbance by divers, but appears to undergo some fluctuation in position, possibly influenced by daily or longer-term changes in sea level as well as tides.

Grotta Azzurra

The underwater layout of Grotta Azzurra is shown in Figures 2 & 3, based on the survey by Alvisi et al. (1994a). The cave is normally entered by divers from the north-eastern side of the Cape, the main entrance, which is closer to the port of Palinuro (Figure 1), and where there is also access by boat at sea level. The top of the Inner Lake (Lago) can be reached on foot from the boats. The principal hot springs arise in the inner part of the cave, beyond the Snow Hall (Sala della Neve); the warm sulphurous water rises above the cooler ambient sea-water and fills the cavities eroded in the limestone. At the seaward end of the Snow Hall the warm sulphurous water flows close to the roof through the 'canal' and escapes towards the surface of the 'inner sea' (Figure 3) and eventually flows outside the cave. The lowest level in the Central Hall is just over 30 m below sea level, in the Snow Hall 18 m and in the transition zone between the two 26 m below sea level. A section of the Snow Hall is shown in Figure 3. The chemocline/thermocline between the ambient sea-water and the warm sulphurous water is ~10 m below sea level. There are accumulations of soft sediment in depressions of the floor of the Snow Hall, and sandy sediment occurs on the floor of the outermost part of the Central Hall (Figure 2). Near the secondary (western) entrance to the cave there is an accumulation of sea-grass litter (*Posidonia*).

In May the temperature difference between the sulphurous layer and the sea-water is usually 5 or 6°C. Figure 4 shows a section in the inner Snow Hall in May 1992. The temperature difference was then 5°C and the salt content corresponded to a difference in salinity of 4‰. In high summer the difference may be less.

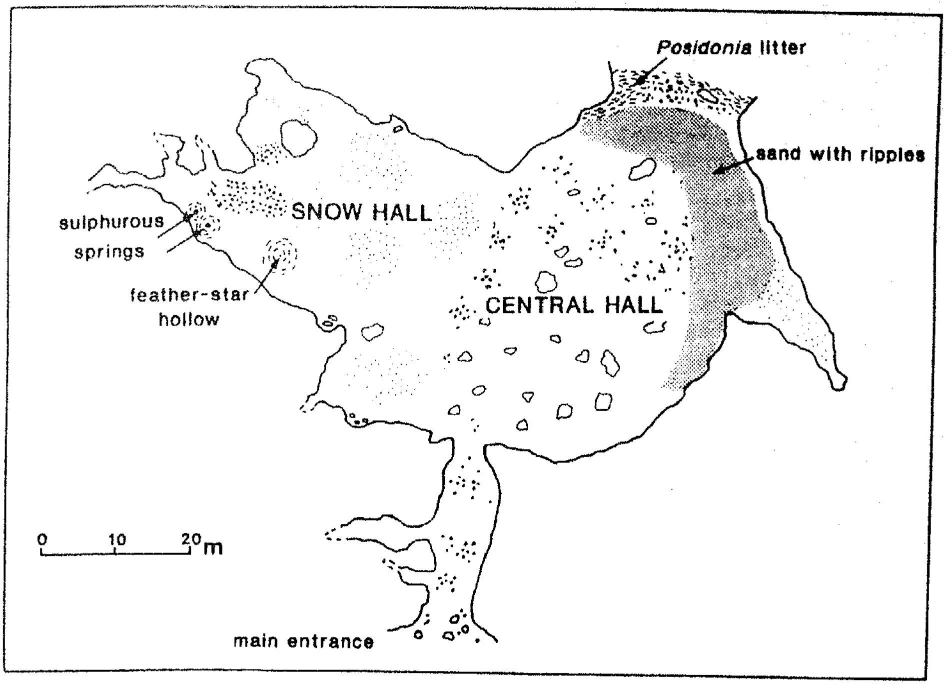

Figure 2. Plan of the underwater part of the Grotta Azzurra, based on the survey by Alvisi et al., 1994a.

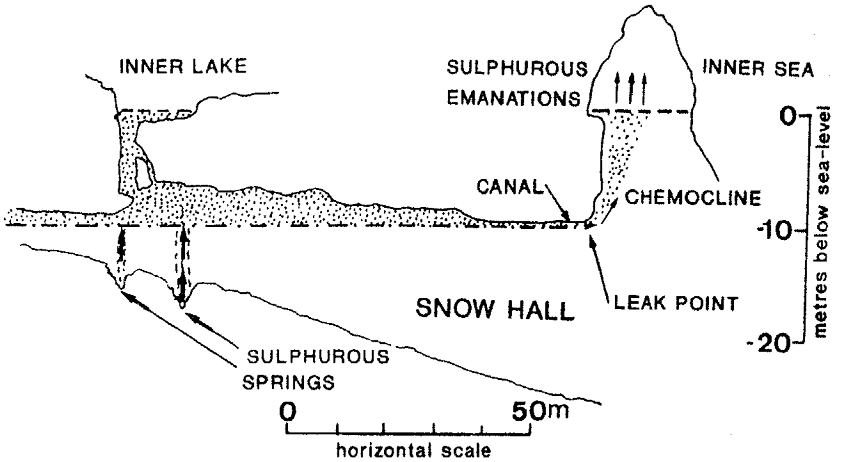

Figure 3. Section down the Snow Hall from the sulphurous springs towards the leak point.

Grotta Sulfurea

The Grotta Sulfurea is smaller than the Grotta Azzurra; a plan of the cave is shown in Figure 5A. As in Grotta Azzurra, the springs arise in the extreme inner part of the cave, and the upper layer of warm sulphurous water escapes to the exterior through a similar

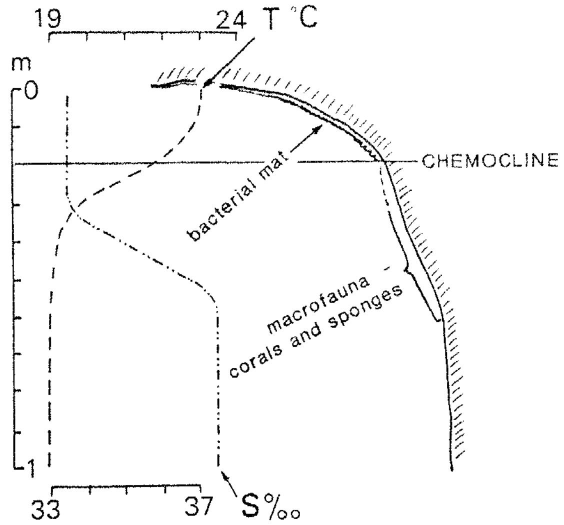

Figure 4. Diagram of the location of the bacterial mats and films in the Grotto Azzurra in relation to temperature and salinity profiles measured in May 1992.

'leak point' or 'canal', as shown in Figure 5B. The difference in temperature of the two layers in May 1992 was also 5°C, but the difference in salt content corresponded to a salinity difference of 7‰ (Figure 6). As for Grotta Azzurra there is boat access to the aerial part of the cave and the lakes can be reached on foot.

Sulphurous content

The Grotta Sulfurea is relatively more sulphurous than the Grotta Azzurra, and the little bay on which it opens is aptly named Cala Fetente or 'Stinking Cove'. In the parts of this cave above sea-level the atmosphere smells strongly of hydrogen sulphide, well above the olfactory perception limit of 0·1 μM reported by Dando et al. (1985). In calm weather the rocks and intertidal organisms outside the cave are coated with elemental sulphur for a few metres either side of the opening of the cave, from which the diluted sulphurous water flows. Such sulphur can be formed by reaction between different stages of oxidation of the hydrogen sulphide. The fauna and flora around the cave are not noticeably affected by the sulphurous effluent, and the splash-zone barnacle *Chthamalus (Euraphia) depressus* (Poli) is common in the upper opening to the cave system, above mean sea level, where there is a strong smell of hydrogen sulphide. Millimolar levels of sulphide were present in samples of the overlying warm layer from Grotta Sulfurea.

In Grotta Azzurra levels of hydrogen sulphide are lower and by olfactory perception seem to be more variable than in the Grotta Sulfurea. In May 1992 there were only micromolar levels of dissolved sulphide in the upper warm layer in the Snow Hall above the hot springs. More detailed chemical measurements are in progress and will be reported elsewhere. Sulphide has not been detected in Grotta Trombetta.

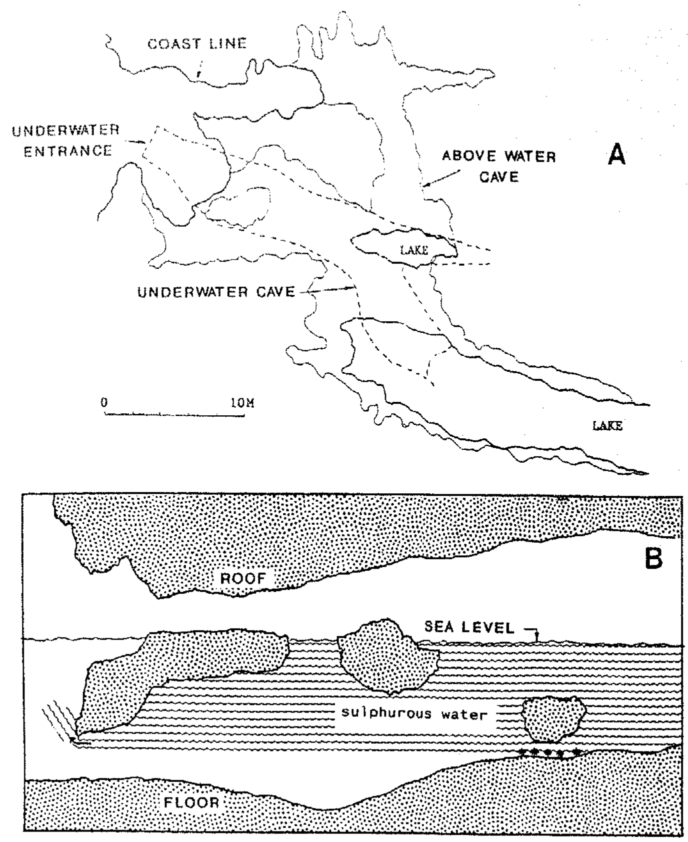

Figure 5. La Grotta Sulfurea. (A) A plan based on Abbiati et al. (1994); (B) a section down the underwater part of the cave, showing the location of the colonies of *Phyllochaetopterus* at the oxic/anoxic boundary, and the leak point (arrowed) where the warm sulphurous water escapes above the colder sea-water.

BACTERIAL MATS

The main bacterial mats occur chiefly above the chemocline, in the main body of sulphurous water, but smaller local patches may be found elsewhere where there is mixing of the water layers or there are minor sulphurous springs, as in the entrance to the Grotta Azzurra. Usually, in Grotta Azzurra (Figure 4) the roof or vault of the cave carries a thin film of bacteria which becomes bubbly in places; on the side walls the bacterial mat becomes thicker and may be essentially a community of *Beggiatoa*-like attached filaments. Similar *Beggiatoa*-like mats occur at the leak point, in the chimney, around the upper margin of the lake and near the chemocline. These are places where both sulphide and oxygen are present as a result of mixing or contact with the atmosphere. The walls below the chemocline carry a brown film. There is considerable variation in mat development and the filamentous sulphur bacteria may overlap the fauna. Where there is an aerial chamber above the sulphurous water (e.g. Inner Lake), the uppermost part of the marine system, above the bacterial mat proper, carries a coating of brown film, apparently partly oxidized iron in addition to bacteria; below this, close to the average water line, there are thick mats of attached filamentous bacteria which can be partly exposed to the air at low tide, and form an extension of the mats in the chimney. There is a slightly more complex arrangement of the bacterial development in Grotta Sulfurea (Figure 6). The roof of the cave carries a thin bacterial film, below which there is a thicker hairy mat. Below this again is a less hairy bacterial coating. This latter gives way to a bubbly coating near the chemocline, while below the chemocline the bare parts of the wall have a brown film like that in Grotta Azzurra. In places where the cave water level is accessible from the aerial chambers the rocks carry filamentous growths of bacteria similar to those reported in a similar setting in Grotta Azzurra.

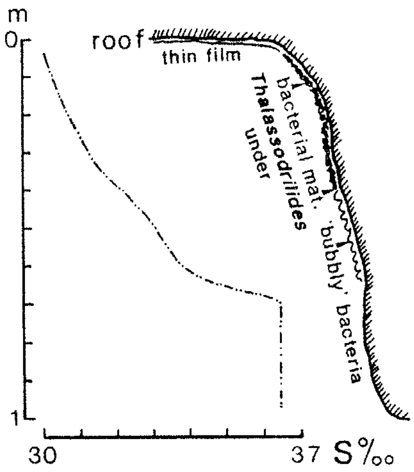

Figure 6. The location of the bacterial mats in the Grotta Sulfurea, in relation to the salinity profile in May 1992.

Under the microscope the thick films or hairy mats resolve into *Beggiatoa*-like colonies containing filaments of various diameters, possibly distinct species, with a few inclusions of other bacteria (Figure 7). The larger examples of *Beggiatoa*-like bacteria, almost 100 µm in diameter, are close to a size referred to as 'giant' when encountered in other sulphide-rich habitats (Jannasch et al., 1989). The thin films and bubbly mats appear to be mostly clusters of certain other rounded sulphur bacteria comparable in form to *Thiovulum*.

In Grotta Sulfurea (Figure 6) the thick filamentous bacterial mats are colonized by large numbers of a marine oligochaete worm, *Thalassodrilides gurwitschii*. In both caves, the mats are inhabited by rotifers.

Parts of the bacterial mats in both caves, predominantly the thinner mats and the bubbly types, shed flakes of material when disturbed by wave-surge or divers, and the falling flakes can resemble snow, giving rise to the name used for the inner chamber of Grotta Azzurra, Sala della Neve or Snow Hall. Sometimes the flakes flow from the cave with the outgoing water and can be seen in the sea immediately outside the entrance; they are then as intangible as real snow and difficult to sample.

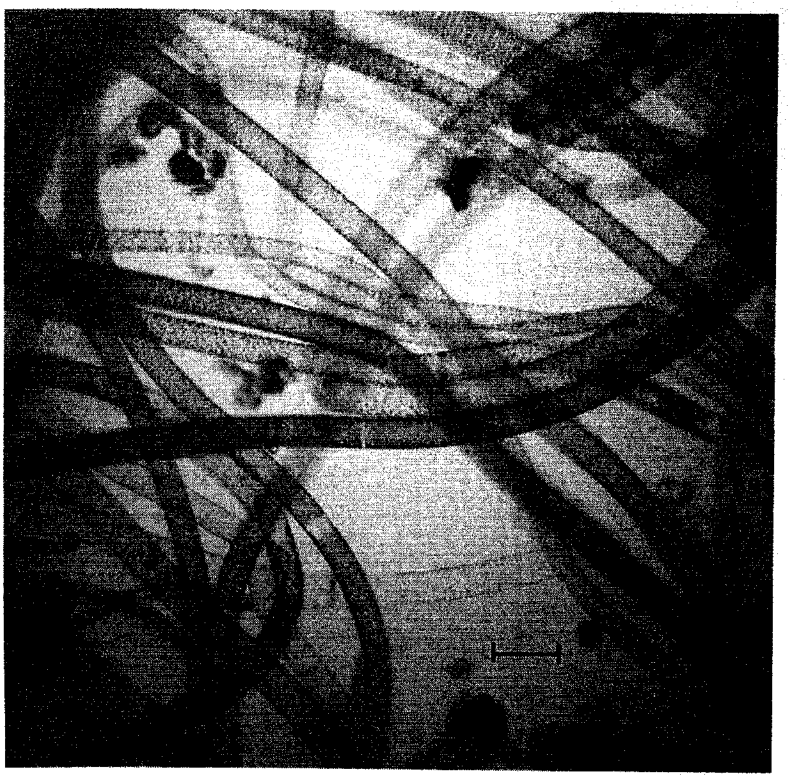

Figure 7. Photomicrograph of the large forms of *Beggiatoa*-like filamentous bacteria seen in samples of the thick 'hairy' mats from the Grotta Sulfurea. Scale bar: 100 μm.

Table 1. *Carbon dioxide fixation and ribulosebisphosphate carboxylase (RubisCo) activity in bacterial mats from the Cape Palinuro caves. One hour incubations. As μmoles CO_2 g^{-1} min^{-1}.*

experiment	average temperature °C	biomass g. wet wt. per dm^2	fixation rate	RubisCo activity
A	23.2	24.72	0.0058	trace
B	23.8	19.76	0.0054	0.0096
D	24.5	59.47	0.0671	0.0511

A and B, white mats from Grotta Azzurra, containing filaments of *Beggiatoa*-like bacteria and clumps of rounded bacteria, with much inert amorphous particles. D, long ('hairy') filaments of *Beggiatoa*-like bacteria from the Grotta Sulfurea, with a smaller amount of inert material. The biomass estimations are very approximate.

Carbon dioxide fixation and RubisCo activity were measured on three samples of bacterial mats (Table 1), at a temperature close to that of the upper layer of sulphurous water, in May 1993. The fixation rate and RubisCo activity were highest in the long ('hairy') filamentous mat sample from the Grotta Sulfurea. This mat also had the

Table 2. *^{13}C/^{12}C of biota of submarine caves at Capo Palinuro. The Grotta Trombetta lacks sulphurous springs. Collection dates: 1, May 1991; 2, May 1992; 3, May 1993; 3a, November 1993; 4a, February 1994; 4, May 1994; 4b, October 1994. Samples associated with clumps of the sponge* Geodia *are marked *. The numbers in the first column refer to entries in Figure 10.*

			δ^{13}C (‰)		
			Grotta Azzurra	Grotta Sulfurea	Grotta Trombetta
	BACTERIA				
1		^{1}general bacterial mat	−30.6	−30.1	
2		^{3}thick mat, round and filamentous	−30.7		
3		^{1}selected filamentous mat	−30.7		
4		^{3}hairy filamentous mat		−31.8	
5		^{4b}long filaments		−30.5	
6		^{2}white crust	−30.1		
7		^{2}softer mat, including sediment	−29.1		
8		^{2}scummy film	−29.9		
9		^{2}bubbly bacteria		−25.8	
10		^{3}brown film	−22.3		
11					
	SPONGIA				
13		^{1}*Ircinia* sp.	−17.4		
14		^{1}*Geodia cydonium*	−18.5		
15		^{2}*Geodia cydonium*	−19.4		
16		^{2}*Petrosia ficiformis*	−18.8		−18.9
	COELENTERATA				
18		^{3a}*Eudendrium racemosum**	−19.4		
19		^{1}*Eunicella cavolinii*	−19.7		
20		^{1}*Astroides calycularis*	−21.3		−21.0
21		^{2}*Leptopsammia pruvoti*	−21.2		−19.0
	POLYCHAETA				
23		^{1}*Sigalion* sp.	−21.0		
24		^{4b}polynoid		−19.8	
25		^{1}sabellid on *Pinna* shell	−20.6		
26		^{2}*Sabella pavonina*, branchiae	−19.2		
27		^{2}*Sabella pavonina*, thorax	−20.2		
28		^{2}*Sabella pavonina*, small	−21.2		
29		^{1}*Pectinaria* sp.	−21.5		
30		^{2}*Terebellides stroemi*	−20.8		
31		^{2,3}*Lumbrineris* sp.	−22.5		
32		^{2}stylocheirid	−21.6		
33		^{2}*Apomatus similis*	−21.2		
34		^{2}*Serpula* sp.	−20.6		−20.4
35		^{1}serpulid from *Astroides*	−28.9		
36		^{2}*Phyllochaetopterus socialis*	−21.8		
37		^{2,3}*Phyllochaetopterus* at chemocline	−25.0	−30.9	
38		^{3}capitellids with *Phyllochaetopterus*	−23.7		
39		^{3a}*Eunice harassi**	−19.2		
40		^{3}*Eunice* sp.	−23.5		
	OLIGOCHAETA				
42		^{2}*Thalassodrilides gurwitschii*		−27.6	
	CRUSTACEA				
44		^{3}*Paracypris complanata*		−28.3	
45		^{3a}*Lysmata seticaudata**	−18.6		
46		^{3a}*Pilumnus hirtellus**	−19.7		
47		^{3a}*Pagurus anachoretus**	−21.9		
48		^{4a}*Herbstia condyliata*			−19.0
49		^{2}*Pinnotheres pinnotheres*	−21.1		
50		^{2}*Dromia personata*	−17.1		

	MOLLUSCA			
52	^{4b}'oyster'			–20.5
53	^{1}*Pinna nobilis*, adductor muscle	–19.6		
54	^{1}*Pinna nobilis*, foot	–20.6		
55	^{1}*Pinna nobilis*, gill	–21.2		
56	^{2}*Pinna nobilis*, adductor muscle	–19.2		
57	^{2}*Pinna nobilis*, gill	–20.5		
58	^{1}*Arca noae*, with *Pinna*	–21.2		
59	^{1}*Arca* and *Striarca*, pooled	–20.4		
60	^{2}*Arca noae*, adductor muscle	–19.3		
61	^{2}*Chama gryphoides*	–20.4		
62	^{3}*Cardita calyculata*	–19.3	–23.8	
63	^{4b}*Lithophaga* sp.			–20.7
64	^{2}*Pitar rudis*	–20.9		
65	^{2}*Corbula gibba*	–20.4		
66	^{2}*Diodora* sp.	–18.0		
67	^{2}*Luria lurida*	–19.8		
68	^{2}*Hinia limata*	–18.0		
69	^{4a}*Gardinia garnoti*			–19.6
70	^{4a}*Barbatia barbata*			–18.3
71	^{3a}*Buccinulum corneum**	–18.1		
72	^{4b}vermetid		–24.7	
	BRACHIOPODA			
74	^{3}*Crania* sp.	–19.4		
75	^{3}*Argyrotheca* sp.	–20.3		
	SIPUNCULA			
77	^{3a}*Golfingia vulgaris**	–19.2		
	ECHINODERMATA			
79	^{1}*Antedon mediterranea*	–20.2		
80	^{2}*Antedon mediterranea*	–19.7		
81	^{1}small echinoid	–21.2		
82	^{1}*Echinaster sepositus*	–17.9		
83	^{2}*Arbacia*, gut	–23.4		
84	^{2}*Arbacia*, test and lantern	–21.6		
85	^{2}*Ophioderma longicaudum*	–20.8		
86	^{3a}*Ophiothrix fragilis**	–19.1		
87	^{2}*Amphiura* sp.	–23.1	–25.9	
88	^{2}*Holothuria forskalii*	–21.5		
89	^{3}*Cucumaria* sp.	–25.0		
	ASCIDIACEA			
91	^{1}*Clavelina lepadiformis*	–21.4		
92	^{2}*Polycarpa gracilis*	–20.6		
	SEDIMENT ORGANIC MATTER			
94	^{1}sediment with *Pinna*	–22.1		
95	^{2}core sample, 0–5 cm	–21.2		
96	^{2}core sample, 15–25 cm	–22.7		

greatest biomass per unit area. The rate of RubisCo activity is an order of magnitude less than that shown at corresponding temperatures by the symbiotic bacteria in the gills of the bivalve molluscs *Lucinoma* and *Calyptogena* (Southward, 1991). There is a possibility that the enzyme was not being efficiently extracted from the bacterial filaments by the homogenization procedures considered suitable for gill and tube-worm tissue (Dando et al., 1985; Southward et al., 1986).

THE CAVE BIOTA

The animal species recovered from the caves and near the caves for analysis are listed in Tables 2 & 3.

Table 3. *$\delta^{13}C$ values for carbonate from the caves at Capo Palinuro and of organic carbon in biota from outside the caves. Dates of collection: 1, May 1991; 2, May 1992; 3, May 1993; 3a, November 1993; 4a, February 1994; 4, May 1994; 4b, October 1994. Replicate analyses are shown where available. See Table 2 for further explanation.*

	$\delta^{13}C$ ‰
CARBONATE	
[1]limestone from Grotta Azzurra	1.9
mollusc shells from Grotta Azzurra	
[1]*Pinna nobilis*	1.6
[2]*Diodora*	1.2, 1.0
[2]*Chama*	1.6
[2]*Callista*	0.3
[2]*Corbula*	0.4
[2]*Arca*	2.5
ORGANIC MATTER FROM OUTSIDE THE CAVES	
PHANEROGAMIA	
[2]*Posidonia* litter, 5 km away from cave	–14.3
PHAEOPHYCEAE	
[1]*Cystoseira* sp., 5 km away from cave	–15.4, –15.4
[4]*Dictyota dichotoma* near Grotta Azzurra	–18.6, –18.9
[4]*Halopteris filicina* near Grotta Azzurra	–25.4, –25.8
RHODOPHYCEAE	
[4]*Peyssonnelia* sp. near Grotta Azzurra	–24.0, –23.9
[4]'lithothamnia' near Grotta Azzurra	–20.4, –21.0, –20.7
MOLLUSCA	
[1]*Mytilus galloprovincialis*, 5 km from cave	–18.3, –18.4

Grotta Azzurra

The distribution of the major species assemblages of the fauna and flora of the Grotta Azzurra has been summarized by Cinelli et al. (1994), on which this account is based.

The immediate vicinity of the entrance to the cave carries fully photophilic communities dominated by the turf-forming algae *Dictyota dichotoma*, *Jania rubens*, *Padina pavonica* and *Amphiroa rigida*. *Halopteris filicina* is also present and its abundance increases inside the entrance to the cave. There are some minor sulphurous springs in the entrance, but the fluids appear to be immediately mixed into the ambient sea-water. Along the entrance channel light becomes reduced (Figure 8) and the normal turf-forming algae listed above are replaced by encrusting forms, notably *Peyssonnellia* sp. and non-articulated corallines, but after a few metres inside the entrance the algae disappear. In the main part of the cave system, the Central Hall (Salone Centrale), as in other caves, no photoautotrophic benthic species are found (cf. Cinelli et al., 1977; Balduzzi et al., 1989).

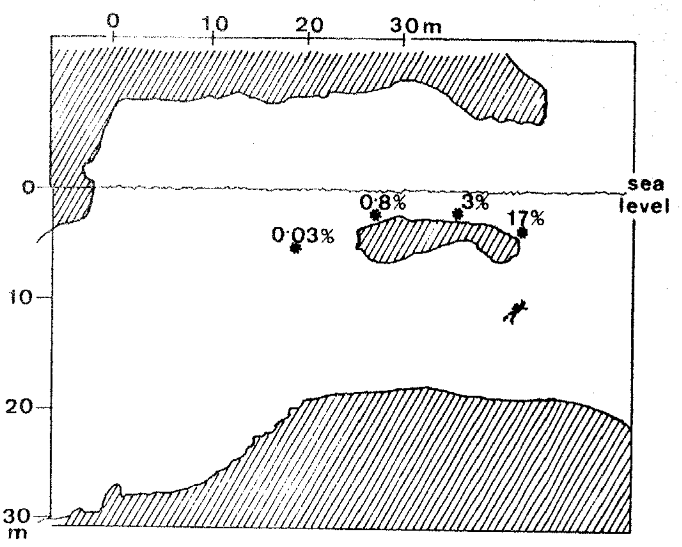

Figure 8. The Grotta Azzurra. Section through the main entrance showing illumination levels in May 1992 as a percentage of that in the sea outside the cave. The algal communities change from brown turf-forming species at the 17% point to encrusting reds at 3%; beyond the 0·8% point only animals are found.

The benthic fauna of the Central Hall is comparatively rich, with 100% cover, dominated by passive filter feeders, such as *Eunicella cavolinii* and *Eudendrium,* and active filter feeders such as sponges. There is appreciable water flow, possibly a result of the tunnel shape, and this may help maintain the fauna, as shown by Harmelin (1969) for other tunnel-shaped caves.

Between the Central Hall and the innermost part of the cave (Snow Hall) there is a transition zone where a typical reduction in organismal cover occurs (first of passive filter feeders, then more active filter feeders) as described for non-sulphurous caves (Harmelin et al., 1985). The innermost part of the cave, the Snow Hall, is completely dark and has the stratified waters already described. The most noticeable biological features of the Snow Hall are the sponge, coral and tubicolous polychaete communities extending up the walls close to the chemocline (Figure 9). The large masses of the sponge *Geodia* are inhabited by other animals, including ophiuroids. The orange-coloured coral, *Astroides calycularis,* is the predominating coral and occurs most abundantly in the outer part of the Snow Hall, where it may be accompanied by the bivalve *Cardita calyculata,* thin films of sponges and spionid polychaetes. A sample taken from the canal region, where the sulphurous water escapes, showed, per dm^2 198 coral polyps and 236 *Cardita.* This bivalve ranged in size from 1 mm to 11 mm shell length, but two-thirds of the population was 5 mm or less so that its contribution to community biomass was less than that of the coral.

The yellow coral, *Leptopsammia pruvoti,* appears to be most abundant at 0·5 m below the chemocline in the inner part of the Snow Hall, and occurs together with two species of brachiopods; a sample contained, per dm^2, 17 coral polyps, 23 brachiopods and one *Cardita,* with some small sabellid and serpulid polychaetes. The polychaete *Phyllochaetopterus* occurs in dense clumps near the chemocline; it has stiff tubes that provide anchorage for other polychaetes and for ophiuroids.

The distribution of these species is very heterogenous at spatial scales of metres, and it is not always possible to tie in their distributions with distance from the sulphurous water boundary. Both corals can occur close to the bacterial mats and filaments.

The floor of the Snow Hall near some of the sulphur springs is covered with a very soft sediment containing much organic matter, inhabited by the fan-mussel *Pinna* and various polychaetes including *Sabella pavonina*. In depressions on the floor there are colonies of feather-stars, *Antedon mediterranea* (Figure 9). At various places on the rock walls and on shells of *Pinna* there are examples of the bivalves *Chama* and *Arca*. Less abundantly there are cowries *Luria lurida*, crabs *Dromia personata* and a gobiid fish, *Thorogobius ephippiatus*. Occasionally one finds predators that appear to have entered the cave fortuitously, including the large starfish, *Echinaster sepositus*.

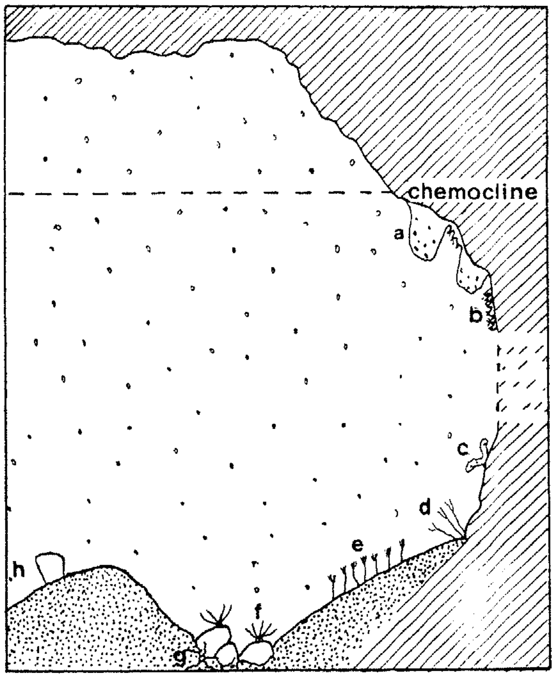

Figure 9. The Grotta Azzurra. Section through part of the Snow Hall, with typical dominants of the animal communities indicated. A, *Geodia*; B, *Astroides*; C, *Petrosia*; D, *Phyllochaetopterus*; E, *Sabella*; F, *Antedon*; G, *Ophioderma*; H, *Pinna*.

Specimens of *Geodia* and of *Astroides* in the Snow Hall are significantly larger than outside, indicating favourable trophic or environmental conditions (Morri et al., 1994).

Grotta Sulfurea

The entrance to the Grotta Sulfurea shows the same changes in turf-forming algae described for Grotta Azzurra. Inside the cave there is a less diverse fauna than in Grotta Azzurra, possibly related to the inhibiting effects of the higher sulphide content of the overlying warm water. The fauna appears strongly related to the chemolithoautotrophic production. There are three basic communities that have been sampled so far. The most obvious comprises clumps of the tubicolous polychaete *Phyllochaetopterus socialis*, which live attached to the rock close to the oxic/anoxic interface, as reported by Abbiati et al. (1994); these tubes have a coating of filamentous bacteria, and bear small serpulid polychaetes. Associated with the worm tubes is another polychaete (*Capitella* sp.) and a small brittle-star (*Amphiura* sp.). A second community is found under the thick bacterial mats, and includes the oligochaete *Thalassodrilides gurwitschii* and rotifers; this oligochaete has been reported previously from organically enriched sediments, e.g. the Bay of Naples (Erséus, 1981). A third community occurs in the 'canal' where there is an escape of warm sulphurous water to the exterior of the cave (Figure 6); here are thin coatings of sponges, clumps of the small bivalve *Cardita calyculata,* and in crevices, abundant ostracods (*Paracypris complanata*). The capitellids, the oligochaetes and the ostracods in Grotta Sulfurea are strongly coloured red by what appears to be a respiratory pigment. The sea-urchin *Arbacia lixula* has been seen in this cave as well as in the Grotta Azzurra but is not quantitatively important.

STABLE CARBON ISOTOPE RATIOS

The results of analyses for stable carbon isotope ratios for the Grotta Azzurra and the Grotta Sulfurea are listed in Table 2, together with values for the Grotta Trombetta, a cave that appears to lack sulphurous springs. Additional analyses of the carbonate in the limestone rock and in the shells of the bivalve molluscs are shown in Table 3 which also gives isotope ratios for examples of fauna and flora outside the caves. The inorganic carbonate isotope ratio values are all positive, ranging from 0·3 to 2·5‰, and provide a guide to the relative depletion of the dissolved carbon dioxide in the cave waters that is available for fixation by the bacteria.

In both caves the thick bacterial mats dominated by long filaments of *Beggiatoa*-like bacteria show depletion in the heavy isotope (range, –29·1 to –31·8‰; means, Grotta Azzurra –30·2, Grotta Sulfurea –30·8). Considering the positive ratio of the inorganic carbonate, this means a fixation depletion of between –31 and –33‰. Such depletion is typical of chemolithoautotrophic bacteria (Ruby et al., 1987). The other bacterial coatings, notably the thinner and bubbly mats, showed somewhat less depletion (–25·8 to –29·9‰), and the brown films were even less depleted (–22·3‰). It should be noted that the thinner and bubbly mats are poorly attached to the cave walls and probably form the major export from the sulphurous zone to the water column i.e. the 'snow'.

Experiments confirm that the thick mats are autotrophic (Table 1). These autotrophic producers thus provide a 'marker' with which to trace utilization of bacterial food sources by higher organisms in the caves. In contrast, there is much less depletion in *Posidonia* litter (–14·3‰) and the dominant algae from outside the caves (–15·4‰). Food from this source is easily distinguished from the bacterial sources. However, as shown in Table 3, some of the smaller slow-growing algae, particularly the sciaphilous reds and *Halopteris*, are more depleted than *Posidonia* and *Cystoseira*. We have no analyses of phytoplankton penetrating the cave from outside, but these can be expected to show depletions in the range from –22 to –33‰, depending on species composition, temperature during fixation of CO_2 and pCO_2 in the ambient sea-water; a mixed population would show an average closer to the less depleted end of this range (Wong & Sackett, 1978; Fontugne & Duplessy, 1981; Descolas-Gros & Fontugne, 1985; Kerby & Raven, 1985; see also Childress et al., 1993).

From the tables it is clear that most of the animals in the sulphurous caves show more depletion of ^{13}C than would be expected if they were entirely dependent on local sea-grass debris and dominant macroalgae for food, and are more depleted than typical active filter-feeding animals (*Mytilus galloprovincialis*) from outside the caves (–18·3‰) that would be feeding on mixed local plankton. If this was the sole basis for comparison we could conclude that a substantial contribution to the nutrition of the cave fauna was being made by the sulphur bacteria.

The animals from the non-sulphurous control cave, Grotta Trombetta, show a range of depletions, several of them close to the value for *Mytilus* from outside the caves. Some showed more depletion, comparable to values found in animals from the sulphurous caves (–19 to –21‰). The cause of this depletion in the control cave has not yet been investigated. Nevertheless, it is apparent that some of the animals in the sulphurous caves are consuming organic carbon produced by the bacteria; organic matter from this source also seems to be accumulating in the sediment ($\delta^{13}C$ –22·1 to –22·7‰). There is considerable variation between the two sulphurous caves and also between taxa and this is particularly evident in the wide range of animals analysed from the Grotta Azzurra. Some of this variation may be related to local variation in the development of the bacterial mats.

Grotta Azzurra

The least depletions occurred in the sponges, two of which did not differ significantly from controls; one sample of *Geodia cydonium*, had somewhat more depletion, closer to that of the other animals living in the cave, but the biological significance of this difference is not yet clear. Heavy values were found in the crab *Dromia personata*, which is known as a component of the submarine cave fauna and lives in the outer, non-sulphurous part (Central Hall). It may somehow avoid predation on the sulphide-dependent components of the biota or may feed outside at night.

Both species of corals, *Astroides calycularis* and *Leptopsammia pruvoti* were somewhat depleted on average, –21·2, –21·3‰. The widest range of $\delta^{13}C$ values was provided by the polychaete worms, from –19·2 to –28·9‰, connected with local habitat variation and food source; the greatest depletion in *Phyllochaetopterus*, for example, was from clumps closest to the oxic/anoxic boundary, sampled in 1993 for comparison with similarly-situated clumps in the Grotta Sulfurea.

The filter-feeding bivalve molluscs from the Grotta Azzurra, including two large examples of *Pinna nobilis*, show somewhat more depletion than *Mytilus* from outside the caves, –19·2 to –21·2‰, but less than *Cardita* from the Grotta Sulfurea, as noted below.

The echinoderms, other than the starfish *Echinaster*, which can be regarded as a temporary wanderer into the caves, range from –19·1 to –25‰, with the filter-feeding feather star, *Antedon mediterranea*, from the bottom of the Snow Hall, less depleted than *Amphiura* and *Cucumaria* collected from places with a flow of sulphurous water.

The depletion in Ascidians, which are known to be capable of filtering bacteria, ranged from –20·6 to –21·4‰, not remarkably more depleted than the controls.

Grotta Sulfurea

The animals from the Grotta Sulfurea were consistently more depleted in ^{13}C than those from the Grotta Azzurra. The values ranged from –23.7 to –25‰ in the polychaetes and echinoderms associated with the clumps of *Phyllochaetopterus*. At –30·9‰ the *Phyllochaetopterus* themselves were close in value to the depletion found in the bacterial mats. The oligochaetes (–27·7‰), found under the filamentous mats, are less depleted than the bacteria on which they must be dependent for food, whether directly by predation or by absorption of organic matter. The sample of the bivalve *Cardita*, from the outflowing sulphurous water in the canal, was more depleted (–23·8‰) than the same species in the Grotta Azzurra. The podocopid ostracod found in crevices in the canal, *Paracypris complanata*, is very depleted (–28·3‰) compared with other crustaceans from Grotta Azzurra.

TROPHIC RELATIONS

In theory there are several sources of food for the animals in the caves: (a) suspended phytoplankton and zooplankton derived from normal marine production outside the caves; (b) suspended particulate matter derived from benthic algal and sea-grass photoautotrophic production outside the caves; (c) coarser particles such as algal fronds and sea-grass blades washed in also from photoautotrophic production outside the cave; (d) bacteria living in suspension in the sulphurous water (autochthonous plankton production); and (e) the bacterial mats (also autochthonous production, but attached to surfaces), consumed by direct 'grazing' or as detached fragments ('snow') picked up by predators and filter feeders. Material taken in sediment traps gives some idea of the relative importance of the coarser components of these categories. The study has only just begun, and investigations are still in progress. In samples taken in May 1993 the material retained by the 200 µm meshes divides up by microscopic estimation into: animal matter 5%, algal material 1·7%, *Posidonia* fragments 5% and faecal pellets 30%. The remainder (58·3%) is an amorphous aggregate that presumably includes bacteria, broken down faecal matter and remains of plankton. This suggests that of the suspended coarser particulate food available to the fauna in the Snow Hall, quite a large proportion might be derived from bacterial production. Many animals in both caves would appear to be able to feed on such suspended particles, even if filter-feeding is not the 'correct' trophic designation of the corals and some of the echinoderms and polychaetes. To this input from suspended particles we need to add that obtained by grazing. Mobile species can make temporary feeding excursions out of the lower layer of oxygenated water to graze on the bacterial mats in the upper sulphurous zone.

That many species in the caves rely on at least some bacterial food is confirmed by the stable carbon isotope ratios. The exception seems to be the large sponges, discussed later. The depletion of comparable species from the Grotta Sulfurea is usually greater than in the Grotta Azzurra. This fact, taken together with the higher concentration of sulphide in the Grotta Sulfurea points to a greater importance of bacterial food in the smaller cave. After allowing for changes in isotope ratios as a result of metabolic reactions, those species with depletions greater than –27‰ would appear to obtain all or nearly all of their nutritional needs from the bacterial sources, notably the oligochaete *Thalassodrilides*, the polychaete *Phyllochaetopterus*, and the ostracod. Species with depletions between –27 and 23‰ appear to be obtaining half of their carbon from bacterial sources.

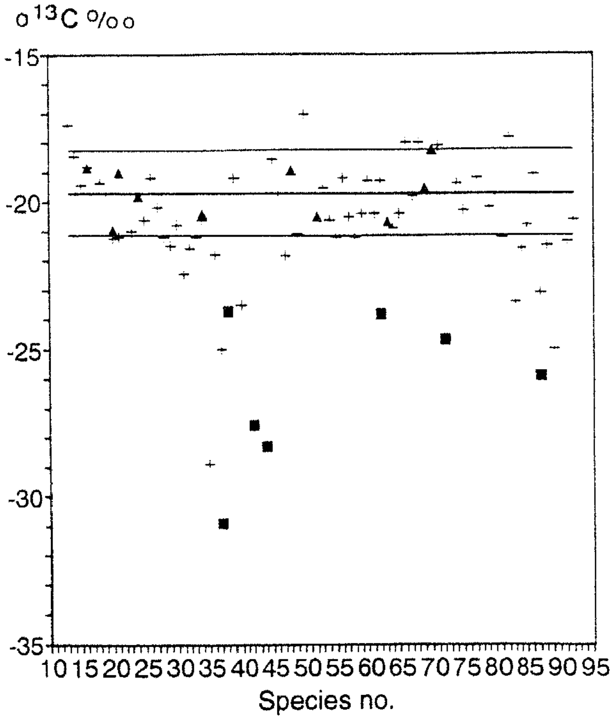

Figure 10. $^{13}C/^{12}C$ of the fauna of the three caves compared. ▲, Grotta Trombetta, control cave; crosses, Grotta Azzurra; ■, Grotta Sulfurea. The numbers are those given to the species as listed in Table 2. The thick line is the mean for Grotta Trombetta, with 99% confidence limits either side.

The values between –23 and –21‰, found in many of the animals in the Grotta Azzurra, may not in themselves look very exciting when compared with the values for the control cave, Grotta Trombetta (Figure 10). The few animals that have lesser depletions than the controls (e.g. the crab *Dromia personata*, the gastropod *Buccinulum corneum* and the starfish *Echinaster sepositus*) are either strong predators that would be expected to show some increase in the heavy isotope due to metabolic conversion (Fry & Sherr, 1984) or are temporary immigrants to the cave system. Equivalent species were not always available from the control cave. The carbon isotope values of quite a few species from the Grotta Azzurra fall within the 99% confidence limits calculated for the control samples. However, two-thirds of the Grotta Azzurra species are more depleted than the average for the Grotta Trombetta animals and nine of them are significantly more depleted ($P=0{\cdot}05$). All the Grotta Sulfurea animals are significantly more depleted than the control cave animals ($P<0{\cdot}05$). The organic matter in the Grotta Azzurra sediment ($\delta^{13}C$ −22‰) is most likely of faecal origin and could be the result of recycling of bacterial carbon sources, with a corresponding enrichment in the ^{13}C content with each step. Coincidentally or not, it represents the mean between the values for the *Posidonia* litter and the filamentous sulphur bacteria.

It can be concluded that there is input of bacterially-produced carbon into the food chains in both sulphurous caves. The greatest input is found in those species that are closely associated with the bacterial mats, especially in the Grotta Sulfurea. In contrast, the input to the corals is not statistically significant, a $^{13}C/^{12}C$ difference of −0·3‰ in *Astroides* and 1·2‰ in *Leptopsammia*; more investigation is needed on the nutrition of these species which might be capable of using several food sources (Schlichter, 1982). The sponges, which as active fine filter feeders should be expected to consume bacteria (Reiswig, 1975), have carbon isotope ratios that apparently preclude the possibility that they obtain any advantage from the sulphur bacteria, yet they grow better in the sulphurous caves (Morri et al., 1994). A number of reasons can be advanced to explain this paradox; (1) there may be autochthonous bacterioplankton production in the sulphurous layer that lacks the distinct isotope signature of the filamentous mats; (2) the major input to the sponges (and corals) might come from the 'snow' falling from the thinner bacterial mats that do not have such a marked isotopic depletion; (3) the sponges could be selectively consuming only fine phytoplankton brought in from outside the caves – the larger sizes of the colonies could then be related to hydrologic factors such as improved water flow induced by the outflowing warm water from the sulphurous springs; and (4) non-trophic growth factors may be involved, including increased silicate content of the hydrothermal fluids.

Further research is needed to quantify bacterial production and assess the proportion of cave biomass supported by chemolithoautotrophy and the role of water circulation. Such research is now in progress.

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