

$^{13}\text{C}/^{12}\text{C}$ ratios in marine invertebrates from reducing sediments: confirmation of nutritional importance of chemoautotrophic endosymbiotic bacteria

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ABSTRACT: The carbon isotope composition ($\delta^{13}\text{C}\text{‰}$) of the tissues of benthic invertebrates usually ranges from -16 to -20 . In contrast we report that several common bivalve molluscs belonging to the superfamily Lucinacea and several small species of Pogonophora show much greater depletions, ranging from -23 to -31 in the bivalves and from -35 to -46 in the pogonophores. These bivalves and pogonophores live in reducing sediments where the concentration of dissolved sulphide is low, usually $< 1\ \mu\text{M}$. The gills of the bivalves and the trophosome tissue of the pogonophores contain intracellular or sub-cuticular bacteria. The bacteria are autotrophs, as shown by ribulosebisphosphate carboxylase activity of extracts of the bacteria-containing tissues. Comparisons of the $\delta^{13}\text{C}$ values of the bacteria-containing regions and the rest of the tissues indicate substantial transfer of organic carbon, half or more of the nutritional needs of the hosts. The organic carbon is produced by fixation of CO_2 by the autotrophic bacteria, which oxidize reduced inorganic compounds, notably sulphide, to obtain energy for the process. Similar depletions of ^{13}C were previously reported for other autotrophic symbiont-containing bivalves that live in habitats where dissolved sulphide concentrations are 2 or 3 orders of magnitude higher than in the sediments analysed here. This form of nutrition, involving symbiosis with autotrophic bacteria, is evidently not dependent on high levels of dissolved sulphide and appears to be widespread in calcareous reducing sediments of the shelf and continental slope.

INTRODUCTION

Benthic communities that exist in regions where there are high concentrations of dissolved sulphide e.g. around hydrothermal vents, in sea-grass beds and near sewer outfalls, include a number of bivalve molluscs and tube-worms with tissues containing autotrophic bacteria that obtain energy by oxidizing sulphur compounds (Cavanaugh et al. 1981, Felbeck 1981, 1983, Felbeck et al. 1981, Cavanaugh 1983a,b, Berg & Alatolo 1984, Fisher & Hand 1984). Species that have been analysed for their stable carbon isotope composition all show unusual ratios of ^{13}C to ^{12}C , from which it has been inferred that much of the carbon in the host tissues is supplied by the bacteria and thus derived from autotrophically-fixed CO_2 (Rau & Hedges 1979,

Rau 1981, Williams et al. 1981, Felbeck 1983). Other invertebrates, more widely distributed in shelf and slope sediments, are now known to contain autotrophic endosymbiotic bacteria (Southward et al. 1981, Felbeck et al. 1983, Dando et al. 1985, Schweimanns & Felbeck 1985) and several of them can exist in habitats where the dissolved sulphide concentration is $\leq 1\ \mu\text{M}$ (Dando et al. 1985). The first report of stable carbon isotope ratios in symbiont-containing animals from habitats not rich in dissolved sulphide (Southward et al. 1981) concerned 3 small species of gutless Pogonophora, whose tissues were extremely depleted in ^{13}C when compared with heterotrophic benthic invertebrates and with particulate organic carbon from the habitat. In this report we extend the use of carbon isotope analyses to additional symbiont-containing animals to cover a range of habitats from the low intertidal to 1800 m depth. The results suggest that a

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wide range of invertebrates that live in reducing sediments may obtain a significant proportion of their food from chemoautotrophic endosymbiotic bacteria.

MATERIAL AND METHODS

Bivalve molluscs were collected by digging at low tide (Mill Bay, Salcombe, Devon, England) and by Van Veen Grab (Norwegian Fjords). Pogonophora were collected by Van Veen Grab and Agassiz Trawl (Norwegian Fjords) and by Plymouth Anchor Dredge (Bay of Biscay). Depths of collection are shown in the tables where appropriate. Details of habitats and sediment chemistry will be found in Southward et al. (1979), Dando et al. (1985, 1986) or will appear elsewhere. All the animals studied, whether intertidal, shelf or slope species, come from sediments containing high proportions of calcium carbonate derived from shells of molluscs or foraminiferans, constituting, in the Bay of Biscay for example, 33 to 40 % of dry weight.

The autotrophic capabilities of the tissue were assessed by measuring the activity of ribulosebisphosphate carboxylase (EC 4.1.1.39) according to the modified procedure of Dando et al. (1985): fresh tissues were homogenized in distilled water and the suspension reacted with ribulose-1,5-bisphosphate and ^{14}C labelled sodium bicarbonate in Tris/HCl buffer (pH 8) containing magnesium chloride and reduced glutathione.

Tissues for carbon isotope analysis were dissected free of tube or shell and gut and then dried at 55 °C in glass or aluminium containers. The dried tissues were subsequently prepared according to Sofer (1980); briefly, 2 mg subsamples were mixed with 500 mg pretreated CuO and loaded into a quartz tube previously heated to 900 °C. The tubes were evacuated, sealed and heated in a muffle furnace for 1 h at 850 °C. The tube was broken in a Cajon flexible stainless steel bellows, water was separated in a trap cooled to -90 °C and the carbon dioxide collected for mass spectrometric analysis.

Sediment pore water was extracted from gravity core segments by filtration through 0.2 µm cellulose acetate filters under pressure of oxygen-free nitrogen. Dissolved sulphide was estimated by the method of Cline (1969). Dissolved amino acids were concentrated by means of a copper chelating resin and eluted with ammonium hydroxide (Siegel & Degens 1966) before drying. The dried samples were treated in the same way as the tissue samples above.

Dissolved bicarbonate and carbonate in filtered pore water were precipitated on addition of ammonium hydroxide/strontium chloride solution (Gleason et al. 1969): subsequently the precipitate was collected on a GF/C filter and dried, both operations being carried

out in an atmosphere of nitrogen. Core segments and sediment from the grab and dredge were dried at 55 °C. Sediment carbonate and precipitated carbonate were reacted with 100 % orthophosphoric acid by a method similar to that described by McCrea (1950).

The prepared CO_2 from all sources was analysed in a triple collector VG 903 micromass mass-spectrometer. The results were corrected according to Craig (1957) and Deines (1970), and are expressed in the usual δ notation relative to the PDB standard:

$$\delta^{13}\text{C} = \left(\frac{R_{\text{c sample}}}{R_{\text{c standard}}} - 1 \right) \times 1000 \text{‰} \quad \text{where } R_{\text{c}} = {}^{13}\text{C}/{}^{12}\text{C}$$

$$\delta^{18}\text{O} = \left(\frac{R_{\text{o sample}}}{R_{\text{o standard}}} - 1 \right) \times 1000 \text{‰} \quad \text{where } R_{\text{o}} = {}^{18}\text{O}/{}^{16}\text{O}$$

The reproducibility of the $\delta^{13}\text{C}$ determinations is 0.1 % for the tissue and carbonate analyses and 0.2 % for the bicarbonate and amino acid samples.

RESULTS

Table 1 shows the ribulosebisphosphate carboxylase activity found in the tissues of the symbiont-containing bivalves and pogonophores that have been studied for their stable carbon isotopes; some values for the leaves of photosynthetic angiosperms are given for comparison. No carboxylase activity was found in tissues without symbionts or in the tissues of 2 control bivalves lacking symbionts (*Dosinia lupinus* and *Arctica islandica*).

Table 2 gives details of the stable isotope analyses for the 6 species of bivalve mollusc examined in the present study. All 4 species containing symbionts were considerably depleted in ^{13}C compared with the 2 controls, *Dosinia lupinus* and *Arctica islandica*. Previously reported $\delta^{13}\text{C}$ values for invertebrates with endosymbiotic autotrophic bacteria in their tissues are listed in Table 3. These are all from habitats reported to have concentrations of dissolved sulphide 2 or 3 orders of magnitude higher than the sediments investigated here. It will be seen that the $\delta^{13}\text{C}$ values from the bacteria-containing bivalves we have examined, -23.4 to -31.0, fall within the range of -23.0 to -33.9 reported from environments with high concentrations of dissolved sulphide (Table 3), despite the low levels of dissolved sulphide in the habitats sampled in this study (Dando et al. 1985, 1986, unpubl.).

Table 4 lists $\delta^{13}\text{C}$ values for the small Pogonophora, including new and previously published data obtained by analytical methods briefly described in Southward et al. (1981). The species examined, from a wide range of habitats, are all more depleted in ^{13}C than any of the symbiont-containing bivalves, including the gutless

protobranch *Solemya reidi* (Table 3). Values of $\delta^{13}\text{C}$ for pore-water bicarbonate and carbonate, pore-water amino acids, sediment carbonate and sediment organic

carbon for the Bay of Biscay habitat are also given in Table 4. It can be seen that the gutless pogonophores are twice as depleted as likely organic reserves in the

Table 1. Ribulosebiphosphate carboxylase activity in tissues of 7 species of symbiont-containing invertebrates from sediments with low concentrations of dissolved sulphide; compared with activity of spinach and perpetual spinach leaf prepared the same way

Species and locality	Depth m	Dissolved sulphide in pore water, μM	Tissue	Incubn. temp. $^{\circ}\text{C}$	Carboxylase activity nmol CO_2 $(\text{g wet wt.})^{-1} \text{min}^{-1}$
<i>Spinachia oleracea</i>	–	–	Juv. leaf	12.0	840.7–2028.00
<i>Beta vulgaris</i> var.	–	–	Old leaf	8.0	5.93–6.72
<i>Beta vulgaris</i> var.	–	–	Old leaf	18.0	36.53
<i>Lucinoma borealis</i> Salcombe, Devon	MLWS	0.2	Gill	12.0	115.37–224.19
<i>Lucinoma borealis</i> Ypsesund, Norway	33	<1	Gill	12.0	456.18
<i>Myrtea spinifera</i> Ypsesund, Norway	33	<1	Gill	12.0	45.60–81.26
<i>Thyasira flexuosa</i> Ypsesund, Norway	33	<1	Gill	12.0	8.52–80.02
<i>Thyasira flexuosa</i> Dolvik, Norway	55	0.9–1.5	Gill	12.2	147.30
<i>Thyasira sarsi</i> Knappen, Norway	60	0–8*	Gill	8.0	141.16–146.77
<i>Siboglinum fiordicum</i> Ypsesund, Norway	33	<1	Post-annular	12.0	22.98–112.48
<i>Siboglinum ekmani</i> Korsefjorden, Norway	700	<2	Post-annular	11.0	9.05
<i>Siboglinum atlanticum</i> Bay of Biscay	1300–1800	0.1–1.0	Post-annular	8.0	1.05–21.70

* In zone of highest density of bivalve; sediment samples were found with up to 50 μM dissolved sulphide but these had a much lower density of *Thyasira* and contained recently dead valves

Table 2. $^{13}\text{C}/^{12}\text{C}$ ratios in the tissues of 4 species of bivalve molluscs with bacterial symbionts in the gills, compared with 2 bivalves lacking gill symbionts

Species	Locality	Depth m	$\delta^{13}\text{C}$ Gill	$\delta^{13}\text{C}$ Rest of body ¹
South Devon:				
<i>Lucinoma borealis</i>	Salcombe	0	–28.1	–25.9
<i>Lucinoma borealis</i>	Salcombe	0	–29.0	–25.3
<i>Dosinia lupinus</i> ²	Salcombe	0	–17.6	–16.8
<i>Dosinia lupinus</i> ²	Salcombe	0	–17.2	–16.9
Norwegian Fjords:				
<i>Lucinoma borealis</i> ³	Ypsesund	33	–28.8	–24.1
<i>Myrtea spinifera</i> ³	Ypsesund	33	–24.2	–23.4
<i>Arctica islandica</i> ^{2,3}	Ypsesund	33	–18.8	–18.9
<i>Thyasira flexuosa</i> ³	Dolvik	55	–29.3	nd
<i>Thyasira sarsi</i> ³	Knappen	90	–31.0	–28.2

¹ Gut removed
² No gill symbionts
³ Pooled specimens
nd No data

Table 3. Published $^{13}\text{C}/^{12}\text{C}$ ratios (as $\delta^{13}\text{C}\text{‰}$) of tissues of symbiont-containing animals living in high-sulphide environments

Species and tissue	Locality	$\delta^{13}\text{C}$	Source
Group A: bivalve molluscs depleted in ^{13}C compared with full heterotrophs			
<i>Lucina nassula</i> , whole animal	Crystal Beach, Grand Bahama	–23.0	(1)
<i>Codakia orbicularis</i> , gill	Crystal Beach, Grand Bahama	–23.9	(1)
<i>Codakia orbicularis</i> , body	Crystal Beach, Grand Bahama	–23.2 to –23.8	(1)
<i>Codakia orbicularis</i> , gill	Gold Rock Creek, Grand Bahama	–28.3	(1)
<i>Codakia orbicularis</i> , body	Gold Rock Creek, Grand Bahama	–28.1	(1)
<i>Solemya reidi</i>	Santa Monica, California, 100 m	–30	(2)
<i>Calyptogena magnifica</i> , mantle	East Pacific Rise, ca 2500 m	–32.6, –32.7	(3)
<i>Calyptogena magnifica</i> , mantle	Galapagos, ca 2500 m	–32.0, –32.1	(3)
Unnamed mytilid, foot & mantle	Galapagos, ca 2500 m	–32.7 to –33.9	(4)
Group B: giant Pogonophora, less depleted in ^{13}C			
<i>Riftia pachyptila</i> , trophosome	Galapagos, ca 2500 m	–10.9, –11.1	(3)
<i>Riftia pachyptila</i> , vestimentum	Galapagos, ca 2500 m	–10.8 to –11.0	(3)
Sources: (1) Berg & Alatalo 1984; (2) Felbeck 1983; (3) Rau 1981; (4) Rau & Hedges 1979			

Table 4. $^{13}\text{C}/^{12}\text{C}$ ratios in the tissues of small Pogonophora compared with $^{13}\text{C}/^{12}\text{C}$ ratios of sediment

Source of carbon	Locality (depth, m)	$\delta^{13}\text{C}$, ‰
Norwegian Fjords:		
<i>Siboglinum fiordicum</i> whole animal	Ypsesund (33)	–35.5 ¹
<i>Siboglinum ekmani</i> whole animal	Korsfjorden (700)	–45.3 ¹
Continental slope in Bay of Biscay:		
<i>Siboglinum atlanticum</i> whole animal	N. Biscay (1730)	–44.6 ¹
<i>Siboglinum atlanticum</i> whole animal	N. Biscay (1850)	–45.8 ¹
<i>Siboglinum atlanticum</i> anterior end	S. Biscay (1700)	–44.7
<i>Siboglinum atlanticum</i> postannular region	S. Biscay (1700)	–43.8
<i>Siboglinum atlanticum</i> anterior part of tube	S. Biscay (1700)	–39.8
<i>Siboglinum atlanticum</i> posterior part of tube	S. Biscay (1700)	–38.2
Sediment from Bay of Biscay pogonophore habitat:		
Sediment: organic carbon	N. Biscay (1750)	–20.5 ¹
Sediment: inorganic carbonate, mean for 0 to 60 cm depth	S. Biscay (1700)	0.9 ± 0.1
Pore water: bicarbonate, mean for 0 to 60 cm depth	S. Biscay (1700)	–17.8 ± 3.3
Pore water: free amino acids, mean for 0 to 60 cm depth	S. Biscay (1700)	–18.1 ± 2.3
¹ From Southward et al. (1981) and pers. comm. G. H. Rau		

sediment. These small pogonophores are also greatly depleted in ^{13}C compared with their giant relative *Riftia pachyptila* from the hydrothermal vents in the Pacific (Table 3).

DISCUSSION

The $^{13}\text{C}/^{12}\text{C}$ ratio in the tissues of an animal is usually close to that of its food source, and comparisons of $\delta^{13}\text{C}$ have been used to trace trophic pathways in several groups of marine invertebrates (Rau 1982, Rau et al. 1982, 1983, Fry & Sherr 1984, Ziemann et al. 1984, Siemenstad & Wissmar 1985). The tissues of an animal

are usually very slightly enriched in ^{13}C compared to its food, and this has been attributed to preferential loss of ^{12}C in respired CO_2 , to selection of ^{13}C during assimilation of food or to a property inherent in all enzyme-mediated biochemical reactions (McConnaughey & McRoy 1979, Rau 1982, Fry & Sherr 1984, Galimov 1985). The 4 species of symbiont-containing bivalves listed in Table 2 were considerably depleted in ^{13}C compared with the presumed heterotrophic controls, hence much of their food must be obtained in other ways than by digestion of organic particles from the water column or the sediment or by uptake of dissolved organic matter. The gills of all 4 species

showed substantial activity of the CO_2 -fixing enzyme ribulosebiphosphate carboxylase (Table 1). This enzyme has not been found in animal tissues lacking symbiotic bacteria and we may assume that the endosymbionts are autotrophic. Indeed, recent studies (Dando et al. 1985, 1986, unpubl.) suggest that they are probably chemolithotrophic sulphur-oxidisers, despite the low level of dissolved sulphide in the habitats. Sulphide stimulates carbon dioxide fixation by the gill of *Myrtea spinifera* (Dando et al. 1985), to a lesser degree by the gill of *Lucinoma borealis* (Dando et al. 1986) and to a greater degree by the gill of the proto-branch *Solemya velum* (Cavanaugh 1983a).

It is established that ribulosebiphosphate carboxylase discriminates against ^{13}C during fixation of carbon dioxide (e.g. Benedict 1978, Estep et al. 1978, Galimov 1985). Photoautotrophic plankton from middle latitudes usually shows $\delta^{13}\text{C}$ ‰ values of -18 to -23 compared to -2.7 to -6.7 for dissolved bicarbonate, though individual algae in culture show a slightly wider range; marine angiosperms such as *Zostera* are usually less depleted, $\delta^{13}\text{C}$ ‰ from -7 to -15 , owing to their active uptake of bicarbonate (Sackett et al. 1965, Degens et al. 1968, Benedict 1978, Rau et al. 1982, Fry & Sherr 1984, Descolas-Gros & Fontugne 1985, Galimov 1985, Zieman et al. 1984). If we assume that the endosymbiotic bacteria have access to dissolved bicarbonate with a $\delta^{13}\text{C}$ ‰ of around -18 (Table 4), and if there is free exchange with the fixation site, then the organic carbon produced by the bacteria should have a $\delta^{13}\text{C}$ ‰ ≥ -40 . The simplest explanation for the ^{13}C depletion observed in the bivalve gills is therefore that much of the organic carbon originates from carboxylase fixation of carbon dioxide derived from pore-water bicarbonate or respired carbon dioxide. The other tissues of the bivalves are slightly enriched in ^{13}C compared to the gill. This could happen if more of the carbon in these tissues originated from digested particulate food. The symbiont-containing bivalves listed in Table 2 do possess a gut, albeit simplified (Allen 1958), and their stomachs have been observed to contain particles such as diatoms.

Tissue $\delta^{13}\text{C}$ values are not easy to evaluate in terms of the proportion of carbon derived from alternative sources (Teeri & Schoeller 1979, Rau & Anderson 1981). Spies & DesMarais (1983) have related quite small decreases in the heavy isotope abundance of benthic animals in oil-seep regions to their use of organic matter derived via sulphide-linked chemoautotrophic pathways. Similar arguments might be applied to data from polluted environments in general (cf. Rau et al. 1981). Galimov (1985) suggests that all enzyme-mediated reactions are selective, and hence the ratios observed in animal tissues will

depend on the length of the reaction pathway. However, since the gutless symbiont-containing bivalve *Solemya reidi* has a $\delta^{13}\text{C}$ ‰ of -30 , and our non-symbiont-containing bivalves are depleted between -16.8 and -18.9 , we may postulate that in the symbiont-containing bivalves examined here, where the body $\delta^{13}\text{C}$ ‰ ranges from -23.4 to -28.2 , half or possibly more of the body carbon is derived from bacterial autotrophy.

The tissues of the small gutless Pogonophora show much greater depletion of ^{13}C , from -36 to -46 ‰. In these animals the bacteria are largely confined to the postannular (posterior) part of the body, where ribulosebiphosphate carboxylase activity is located (Southward et al. 1981; Table 1), yet both ends of the body are highly depleted, as is the organic material of the tube (Table 4). The $\delta^{13}\text{C}$ data for sediment from the deep water pogonophore habitat show that the dissolved bicarbonate and amino acids are only slightly less depleted in ^{13}C than sediment organic carbon, and differ remarkably from the pogonophore tissues and from the carbonate in the sediment. The carbon isotope ratio of the pore-water bicarbonate (-17.8 ± 3.3) is similar to that measured in the sulphate-reducing zone of near-shore sediments off California (Preseley & Kaplan 1968), but more depleted in ^{13}C than the total dissolved carbon in pore water from sediments of the Equatorial Pacific (McCorkle et al. 1985). The free amino acids in the pore water have an isotopic composition ($\delta^{13}\text{C}$ -18.1 ± 2.3) similar to the average for marine amino acids (Degens 1969). The carbonate fraction of the sediment from the deep water pogonophore habitat consists largely of the tests of planktonic Foraminifera; its stable isotope composition ($\delta^{13}\text{C}$ ‰ 0.88 ± 0.14 ; $\delta^{18}\text{O}$ ‰ 1.22 ± 0.44) falls into the range of marine carbonates, reflecting the planktonic input without noticeable diagenetic effects.

As the gutless pogonophores are so highly depleted in ^{13}C compared with the organic reserves in the sediment, they cannot be living entirely on dissolved organic matter as previously suggested (Southward & Southward 1980, 1981). Instead it seems likely that most of the pogonophore tissue carbon is derived from carbon fixed by their autotrophic symbionts. The much greater depletion of ^{13}C in the pogonophores compared with any of the bivalves, including the gutless *Solemya reidi*, can be explained by 2 major factors. One is their small size (diameter < 0.5 mm) and the highly vascularized nature of the bacteria-containing tissues (Southward 1982), which will allow rapid exchange of CO_2 and thus enable the carboxylating enzyme to exercise greater isotope selectivity. Another possibility, underlined by the slightly lesser depletion of the shallow-water pogonophore *Siboglinum fiordicum*, is that the low temperatures experienced by the

pogonophores, which live below the seasonal thermocline, will result in greater isotope selectivity by the carboxylating enzyme in comparison with warm water bivalves such as *Solemya reidi* (Sackett et al. 1965, Galimov 1985).

The symbiont-containing animals from sulphide-rich environments (Table 3) fall into 2 groups according to whether the ^{13}C depletion is greater or lesser than that of typical benthic animals ($\delta^{13}\text{C}\text{‰}$ –16 to –19). Tissues of the gutless protobranch bivalve *Solemya reidi* and the 2 bivalve species from hydrothermal vents show slightly greater depletion of body ^{13}C than the 4 bivalves studied here. This may indicate a greater reliance on bacterial autotrophy. However, there are habitat differences that suggest there may be differences in the $\delta^{13}\text{C}$ values of the CO_2 sources utilized by the bacteria. It is also possible that the hydrothermal vent mytilid is not exposed to as high a level of dissolved sulphide as once supposed, though its growth rate is evidently higher closer to the vents (Smith 1985). The giant Pogonophora from the hydrothermal vents show considerably less depletion of tissue ^{13}C than typical benthic animals. This has been attributed either to use of crustal inorganic sources of CO_2 rather than biogenic, or to lack of selection at the ribulosebiphosphate carboxylase stage, due to the long diffusion pathway and the mechanisms of the internal CO_2 supply (Rau 1981, Felbeck 1981, 1983). This does not entirely explain the large difference in their ^{13}C depletion compared with the bivalves from the hydrothermal vents and it seems possible that the bacteria-containing tissues (posterior end of *Riftia*, gills of the bivalves) experience different temperatures: high temperatures are known to reduce selectivity of the carboxylase enzyme (Sackett et al. 1965, Galimov 1985).

The symbiont-containing bivalves and the small species of Pogonophora studied here are not restricted to environments rich in dissolved sulphide but are widely distributed in reducing sediments. The subsurface layers of nearly all sediments other than certain coarse sands in high-energy shore environments show corrected redox values ranging from +50 to –250 mV, and offer a potential habitat to symbiont-containing species. In contrast to previous suggestions of high levels of dissolved sulphide (Fenchel & Riedl 1970), these subsurface reducing layers need not be rich in dissolved sulphide. The level of dissolved sulphide in a sediment depends on the amount of ferrous iron present and on the rate of sulphate reduction. Most of the sediments that we have sampled show some free ferrous iron, and hence the dissolved sulphide levels are below 1 μM . The chemistry of such sediments will be described fully elsewhere, but for the present purposes it can be said that the most likely reduced inor-

ganic energy sources are elemental sulphur, acid-labile sulphide (mostly ferrous sulphide) and ammonia. In some way the symbiosis between bacteria and invertebrates in these sediments is able to exploit such sources of energy. From the widespread distribution of reducing sediments and of the autotrophic symbiont-containing animals able to exploit them it appears that this form of nutrition may make a significant contribution to benthic production processes of shelf and continental slope environments. Such production, of course, is not truly primary, unlike that at the hydrothermal vents, but it is of wider occurrence. It is a secondary process (Fenchel & Riedl 1970) since the reduced sulphur compounds that fuel the system are produced by anaerobic heterotrophic bacteria that use organic matter originally formed in the euphotic zone or on land. Hence organic production based on oxidation of sulphur compounds in shelf and slope sediments is ultimately dependent on photosynthetic sources.

In conclusion, even though no new source of energy is being exploited, we should note that, as a result of the association between metazoans and autotrophic bacteria, energy that would otherwise be lost from the system is being tapped by biological activity within the sediment. It is agreed that much of the reduced sulphur produced in sediments is reoxidized chemically or by free-living chemolithotrophic bacteria (Jørgensen 1977, 1980, 1982, Fenchel & Blackburn 1979, Trüper 1982). However, these processes are restricted to the interface between the oxidized and reduced layers, or the channels cut in the sediment by the ordinary biota. The symbiont-containing invertebrates extend the habitat available for oxidation of reduced sulphur species below the oxic/anoxic interface to considerable depths in the sediment, depths where it would not otherwise be expected that sulphur oxidation would occur. Thus, oxidation of reduced sulphur compounds by the symbioses between bacteria and invertebrates deviates from the normal diagenetic pathway whereby reducing shelf and slope sediments act as sinks for reduced mineralized sulphur (e.g. Berner 1984, Volkov & Rozanov 1983). It remains to be seen whether such sulphur-oxidizing associations are restricted to calcareous sediments or if they also occur in iron-rich clay sediments and thus form a significant component in the ocean sulphur cycle.

ADDENDUM

As this contribution was being revised for press, news arrived of carbon isotope data for the vent-like animal communities recently found in the Gulf of Mexico at hydrocarbon seeps and abyssal brine seeps

where temperatures are low (Kennicutt et al. 1985, Paull et al. 1985). Samples from these sites show differences between the giant pogonophores ($\delta^{13}\text{C}$ -27 to -47‰) and the bivalves ($\delta^{13}\text{C}$ -31 to -74‰). As at the hydrothermal vents, the bivalves are more depleted than the pogonophores, a reversal of the situation in the Norwegian fjords. This difference points again to circulation restrictions limiting isotope selectivity in the giant pogonophores. However, both sites in the Gulf of Mexico show much greater depletions of tissue ^{13}C than the hydrothermal vent faunas and it is not yet clear how much of this is due to assimilation of already depleted carbon dioxide and how much to the lower temperatures experienced.

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