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Origins of long-chain polyunsaturated fatty acids in the hydrothermal vent worms *Ridgea piscesae* and *Protis hydrothermica*

David W. Pond[1,*], Cathy E. Allen[1], Michael V. Bell[2], Cindy L. Van Dover[3], Anthony E. Fallick[4] , David R. Dixon[1], John R. Sargent[2]

[1]Southampton Oceanography Centre, Empress Dock, Southampton SO14 3ZH, United Kingdom
[2]Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, United Kingdom
[3]Biology Department, The College of William and Mary, PO Box 8795, 328 Millington Hall, Williamsburg, Virginia 23187, USA
[4]Scottish Universities Research and Environmental Centre, East Kilbride, Glasgow G75 OQF, United Kingdom

ABSTRACT: Long-chain polyunsaturated fatty acids (PUFAs) are essential nutrients for all marine animals that have previously been studied. Most marine animals obtain long-chain PUFAs from their diets (i.e. as products of photosynthetic processes) and few are known to be able to produce these compounds *de novo*. Deep-sea vent organisms live in an environment that is relatively isolated from photosynthetic sources of PUFAs, yet some species are known to contain substantial amounts of these compounds. To further understand the origins of PUFAs in deep-sea vent animals, we studied 2 annelid species; a vestimentiferan tubeworm (class Pogonophora) with endosymbiotic bacteria, and a tubicolous serpulid polychaete that feeds heterotrophically. The vestimentiferan tubeworms *Ridgeia piscesae* from the Juan de Fuca Ridge are rich in the monounsaturated fatty acids 16:1n-7 and 18:1n-7, the polyunsaturated fatty acids (PUFAs) 20:5n-3 and 20:4n-6, and the non-methylene-interrupted PUFAs 20:2Δ5, 13. They also contain small but significant amounts of the PUFAs 18:2n-6, 18:3n-3 and 22:6n-3. $\delta^{13}C$ values of ca –14‰ for 16:1n-7 and 18:1n-7 suggest that these fatty acids are synthesised by chemosynthetic processes at the vent site. A $\delta^{13}C$ value of –22‰ for 20:5n-3 in *R. piscesae* makes it impossible to conclusively attribute the origin of this fatty acid to either the photic zone or hydrothermal vents. The lipids of the serpulid polychaete *Protis hydrothermica* from the East Pacific Rise are also rich in 16:1n-7 and contain the unusual PUFAs 18:3n-7. They also contain significant amounts of 20:1n-13 and the PUFAs 20:3n-9, 20:5n-3 and 22:6n-3. Other than 22:6n-3, which had a $\delta^{13}C$ of –22‰, the fatty acids in *P. hydrothermica* had $\delta^{13}C$ values ranging from –33‰ to –41‰. This is consistent with the fatty acids of *P. hydrothermica*, other than 22:6n-3 but including 20:5n-3, originating from a chemoautotrophic carbon source in the hydrothermal vent. It cannot be excluded that the 22:6n-3 in *P. hydrothermica* originates in the photic zone. Potential origins of the long-chain PUFAs in hydrothermal vent animals, whether produced by photo- or chemotrophic processes or by pro- or eukaryotic organisms, are considered.

KEY WORDS: Hydrothermal vent worms · Nutrition · PUFAs · Stable carbon isotope

INTRODUCTION

The abundant invertebrate communities endemic to hydrothermal vent sites in the eastern Pacific are largely nourished by bacterial chemosynthesis (Fisher 1990). One paradox of the trophic biochemistry of vent invertebrates that remains to be resolved is the source of their long-chain n-3 polyunsaturated fatty acids (PUFAs), 20:5n-3 and 22:6n-3. These PUFAs, especially 20:5n-3, are known to be essential nutrients for all marine animals so far studied, the majority of which have been fishes related to aquaculture (Sargent et al.

*E-mail: dwpo@soc.soton.ac.uk

1995). Photosynthetic microplankton are the predominant source of 20:5n-3 and 22:6n-3 in the marine environment (Sargent et al. 1995), but the distribution of these light-dependent organisms is limited to the upper ocean. Long-chain n-3 PUFAs are labile and do not persist in organic matter transferred to the deep ocean (Wakeham et al. 1997), so that their supplies to deep-sea hydrothermal vents are inevitably limited.

Bresiliid shrimp, which dominate the fauna of many hydrothermal sites along the Mid-Atlantic Ridge (MAR), obtain substantial amounts of photosynthetically derived 20:5n-3 and, especially, 22:6n-3 during their dispersive, planktotrophic larval phase (Pond et al. 2000). Adult shrimp either do not require or are not able to exploit a source of long-chain n-3 PUFAs at the vent sites (Pond et al. 2000). Analysis of free-living filamentous bacteria attached to bivalve shells and detrital material has provided no evidence of a bacterial source of 20:5n-3 and 22:6n-3 at MAR vent sites (Pond et al. 1998, 2000).

At eastern Pacific vents, vestimentiferan tubeworms often dominate the fauna (Fisher 1990, Tunnicliffe et al. 1990). These tubeworms possess a gut only during their early life stages (Jones & Gardiner 1988), and as adults their nutrition is derived from substantial populations of endosymbiotic sulphur-oxidizing bacteria (Cavanaugh et al. 1981, Felbeck 1981, Rau 1981, deBurgh et al. 1989). *Ridgeia piscesae*, a major component of the macrobenthos at the Juan de Fuca Ridge in the northeast Pacific (Tunnicliffe & Fontaine 1987, Southward et al. 1996), contains substantial amounts of 20:5n-3 and also 20:4n-6 (Fullarton et al. 1995, Allen 1999). The lack of a gut in adult *R. piscesae*, the reliance of the tubeworm on endosymbiotic sulphur-oxidizing bacteria, and its benthic existence deeply removed from photosynthetic sources of long-chain n-3 PUFAs make it difficult to reconcile the high levels of 20:5n-3 in its tissues with a photosynthetic origin. Furthermore, vestimeniferans produce lecithotrophic larvae (Tyler & Young 1999) and their post-larvae settle at suitable vent sites when very small (Southward 1988). It seems improbable, therefore, that their larvae could accumulate substantial reserves of photosynthetically-derived material. This contrasts with the bresiliid shrimp found at Mid-Atlantic Ridge vents which, as noted above, accumulate relatively high levels of photosynthetically-derived long-chain n-3 PUFAs during larval and early juvenile development (Pond et al. 1997a,b,c, 2000, Allen et al. 1998).

Characterization of $\delta^{13}C$ values of lipid biomarkers in hydrothermal vent organisms is a valuable technique for studying nutritional relationships between the animal and bacterial communities that exploit vent environments and cold-seep environments (Abrajano et al. 1994, Jahnke et al. 1995, Pond et al. 1998). Lipid biomarker techniques are also useful in understanding the coupling of vent ecosystems with processes occurring in the surface layers of the ocean (Rieley et al. 1995, Pond et al. 1997a,c, 2000, Allen et al. 1998). To investigate the origin(s) of the high levels of 20:5n-3 in *Ridgea piscesae*, we have employed gas-liquid chromatography coupled with isotope ratio-mass spectrometry (GC-IRMS) to determine the $\delta^{13}C$ values of individual fatty acids in adult tubeworms collected from active vent sites on the Juan de Fuca Ridge. For comparison, we also analysed the $\delta^{13}C$ values of fatty acids in a species of tubicolous polychaete (*Protis hydrothermica* f. Serpulidae) from the East Pacific Rise (EPR). *P. hydrothermica* lives in dense colonies on basalt in peripheral regions of active vent fields. It is presumed, by analogy to its shallow-water serpulid relatives, to be a suspension-feeder with a functional gut. There is no evidence to suggest that this species relies on endosymbiotic bacteria for its nutrition, although dietary roles of episymbiotic and gut bacteria have not been eliminated.

MATERIALS AND METHODS

Station locations and description. In 1995, five intact specimens of *Ridgeia piscesae* were collected from the High Rise hydrothermal vent field, on the Endeavour segment of the Juan de Fuca Ridge (48° 57.1′ N, 129° 05.2′ W, approx. 2200 m depth). The worms were cut immediately below the vestimentum to provide 2 sections of tissue for lipid analysis. The anterior section comprised the obturaculum and vestimentum that supports the branchial plume, and is not known to contain bacterial endosymbionts. The posterior section contained the opisthosome, trophosome and gonad tissues. Three specimens of *Protis hydrothermica* were collected from the Genesis site on the EPR during the 'HOPE 1999' cruise (12° 48.7 N, 103° 56.4 W, Dive 1383, 2600 m: Lallier et al. 1999). The calcareous tubes of the polychaetes were attached to basalt located within 1 m of the base of the vent discharge. Intact polychaetes were removed from their tubes and dissected into crown (including thorax) and abdominal sections. All tissues were frozen (–70°C) immediately on board ship and transported in dry ice to the UK, where they were stored at –70°C until analysis.

Lipid analyses. Total lipid was extracted from the tissues using chloroform:methanol (2:1 vol:vol) (Folch et al. 1957). Aliquots of total lipid were transesterified in methanol containing 1.5% sulphuric acid at 50°C for 16 h to generate fatty acid methyl esters (FAMEs; Christie 1982). FAME were purified by thin-layer chromatography using a hexane:diethyl ether:acetic acid (90:10:1, vol:vol:vol) solvent system. Purified FAMEs

were dissolved in hexane to a concentration of 2 mg ml^{-1} and analyzed by gas chromatography on a 6000 Vega series (Carlo Erba, Milan, Italy) fitted with a BP20 fused silica capillary column (30 m by 0.32 mm i.d.) using hydrogen as the carrier gas. FAMEs were identified using the procedures detailed in Pond et al. (1998) including, where necessary, GC-MS of their dimethyldisulphide and picolinyl esters.

GC-IRMS. Stable carbon isotope ratios (^{13}C:^{12}C) were determined for individual fatty acids by GC-IRMS with a VG Isochrom II instrument equipped with a column similar to that described above (Eakin et al. 1992). The $\delta^{13}C$ value of the methanol derivatization reagent was determined by conventional closed-tube combustion (Sofer 1980) and the contribution of derivatized carbon to specific fatty acids was calculated by rearranging the equation from Abrajano et al. (1994):

$$\delta^{13}C_{FA} = \frac{\delta^{13}C_{FAME} - (1 - x)\delta^{13}C_{CH_3OH}}{x}$$

where: $\delta^{13}C_{FA}$ is the isotopic composition of the free fatty acid, $\delta^{13}C_{FAME}$ is the isotopic composition of the fatty acid methyl ester, x is the fractional carbon contribution of the free fatty acid to the ester, and $\delta^{13}C_{CH_3OH}$ is the isotopic composition of the methanol derivatization reagent. All isotope data are reported as $\delta^{13}C$ and relative to that of Peedee Belemnite (‰ v-PDB). Replicate injections indicate that analytical precision was ± 0.4‰ or better.

RESULTS

The fatty acid compositions of the anterior and posterior tissues of *Ridgea piscesae* were similar, with both containing high proportions of 16:0, 16:1n-7, 18:1n-7, 20:5n-3 and the non-methylene interrupted dienoic fatty acid (NMID), 20:2Δ5, 13 (Table 1). Unsaturated fatty acids of the n-6 series, 18:2n-6 and 20:4n-6 were also present in substantial, although lower, amounts. The fatty acid compositions of the 2 different tissue types within *Protis hydrothermica* were also similar, although the abdomen contained a much higher proportion of 16:1n-7 than the crown (Table 1). Dienoic fatty acids and, particularly, NMIDs were only minor components, whilst the unusual trienoic fatty acids 18:3n-7 and 20:3n-7, and also the well known 'Mead acid' 20:3n-9 comprised approx. 3.0% of the total in *P. hydrothermica*. The 18:3n-7 was identified by GC-MS as 18:3Δ5,8,11 and the 20:3n-7 as 20:3Δ7,10,13. In contrast to the fatty acid composition of *R. piscesae*, *P. hydrothermica* contained similar and quite substantial amounts of both 22:6n-3 and 20:5n-3 (2.8 to 4.7%; Table 1).

Table 1. *Ridgeia piscesae* and *Protis hydrothermica*. Percent (SD) mean weight of fatty acids in the 2 vent worms. –: absent

Fatty acid	*Ridgeia piscesae* (n = 5)		*Protis hydrothermica* (n = 3)	
	Trunk	Obturaculum	Abdomen	Crown
14:0			1.7 (0.3)	1.1 (0.5)
14:1			1.5 (0.6)	1.0 (0.9)
15:0			0.3 (0.1)	0.3 (0.1)
16:0	9.0 (0.4)	9.0 (0.5)	6.6 (2.2)	6.2 (1.4)
16:1(n-7)	9.8 (1.8)	7.2 (0.8)	23.6 (4.3)	12.2 (1.8)
16:2(n-4)	0.3 (0.1)	0.4 (0.1)	0.3 (0.1)	0.3 (0.1)
18:0 DMA	5.0 (0.5)	5.6 (0.3)		
18:0	0.9 (0.1)	0.9 (0.1)	4.6 (0.9)	5.5 (1.1)
18:1(n-9)	1.2 (0.1)	1.3 (0.2)	2.5 (0.2)	3.2 (1.4)
18:1(n-7)	19.0 (1.0)	18.1 (1.2)	6.4 (0.6)	6.5 (0.7)
18:2(n-6)	3.6 (0.6)	3.7 (0.5)	0.1 (0.1)	0.1 (0.1)
18:3(n-7)		15.3 (1.1)	16.6 (1.7)	
18:3(n-3)	1.5 (0.1)	1.7 (0.1)		
20:1 DMA	7.1 (0.7)	7.4 (0.3)	11.1 (1.9)	15.1 (0.9)
20:1(n-13)	3.7 (0.2)	4.6 (0.4)	7.8 (1.2)	11.6 (1.6)
20:1(n-7)	3.7 (0.4)	3.2 (0.2)	3.1 (0.7)	2.8 (0.4)
20:2(5, 13	10.7 (0.4)	10.5 (0.5)	0.7 (0.1)	0.7 (0.1)
20:3(n-9)		3.1 (0.2)	3.2 (0.3)	
20:3(n-7)		1.7 (0.2)	2.3 (0.1)	
20:4(n-6)	3.2 (0.6)	3.0 (0.6)	0.5 (0.1)	0.8 (0.2)
20:5(n-3)	16.8 (1.6)	18.1 (0.7)	3.6 (0.5)	4.7 (0.2)
22:2Δ7, 15	2.9 (0.2)	3.9 (0.3)	0.8 (0.1)	1.2 (0.1)
22:5(n-3)	0.9 (0.1)	1.1 (0.1)	1.0 (0.1)	1.2 (0.2)
22:6(n-3)	0.5 (0.2)	0.3 (0.1)	3.3 (0.5)	2.8 (0.4)
Saturated	14.9	15.5	13.2	13.1
Monounsaturated	44.5	41.8	56.0	52.4
Polyunsaturated	40.4	42.7	30.1	33.9

The $\delta^{13}C$ values of fatty acids in the 2 species were different: $\delta^{13}C$ values in *Ridgea piscesae* ranged from –14.3‰ for 18:1n-7 in the obturaculum to –22.2‰ for 20:5n-3 in the posterior tissue (Fig. 1). Fatty acids in *Protis hydrothermica* were comparatively depleted in ^{13}C (from –28.6‰ for 16:0 in the crown to –43.4‰ for 18:3n-7 in the abdomen (Fig. 2), with the exception of 22:6n-3 ($\delta^{13}C$ = –21.3 to –22.0‰).

Carbon isotopic compositions of individual fatty acids within tissues differed by up to 8‰ in *Ridgea piscesae* and 22.0‰ in *Protis hydrothermica*. Between tissue types, carbon isotopic compositions of any given fatty acid were relatively consistent, differing at most by ~2‰ in *R. piscesae* and by ~4‰ in *P. hydrothermica* (Figs 1 & 2).

In *Ridgea piscesae*, the monounsaturated fatty acids 16:1n-7 and 18:1n-7 were the most isotopically enriched in ^{13}C, with $\delta^{13}C$ values of ca –14.5‰ for both posterior and anterior tissues (Fig. 1). The PUFAs 20:4n-6 and 20:5n-3 in the anterior tissue were the most depleted in ^{13}C, with $\delta^{13}C$ values of ca

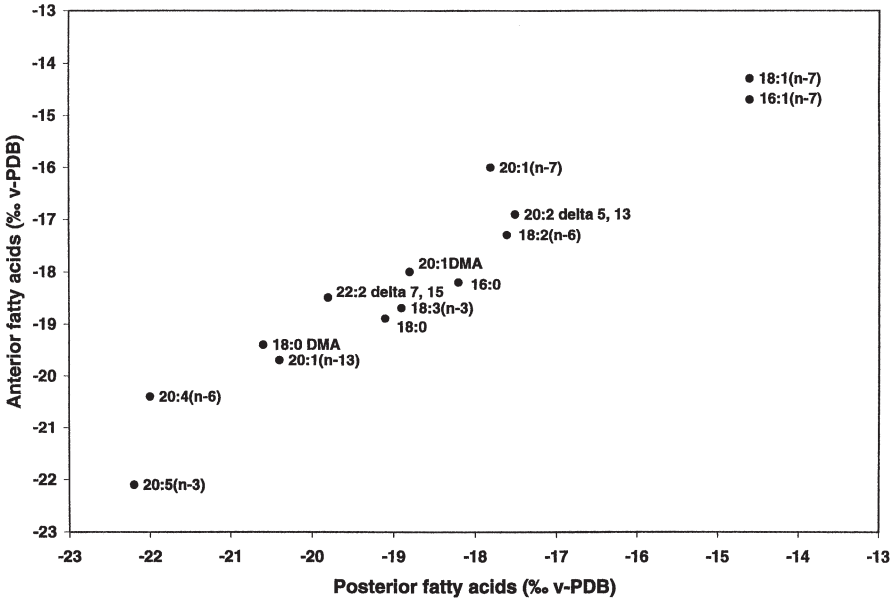

Fig. 1. *Ridgeia piscesae* (n = 5). $\delta^{13}C$ values of fatty acids of polar lipid from anterior (obturaculum and vestimentum) and posterior (opisthosome, trophosome and gonad) tissues in the vestimentiferan worm

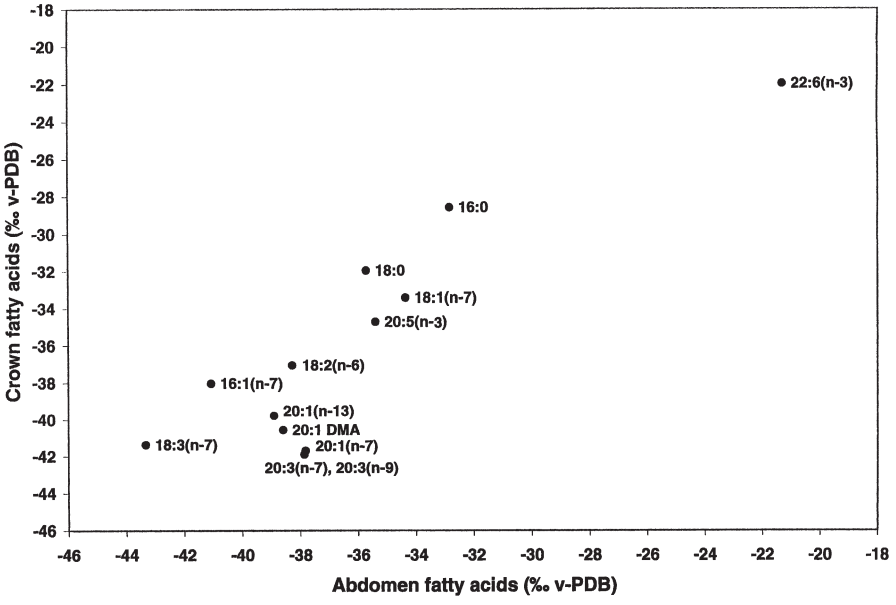

Fig. 2. *Protis hydrothermica* (n = 3). $\delta^{13}C$ values of fatty acids of total lipid from abdomen and crown (including thorax) tissues in the tubicolous polychaete

–20.4 to –22.2‰. $\delta^{13}C$ values of the saturated fatty acids 16:0, 18:0 and the NMIDs 20:2Δ5, 13 and 22:2Δ7, 15 were intermediate, with values of ca –18.0‰.

The pattern of $\delta^{13}C$ values for fatty acids in *Protis hydrothermica* was not as distinct as that in *Ridgea piscesae*, and no obvious trend relating either to fatty acid chain length or degree of unsaturation was apparent. Notably, the $\delta^{13}C$ value for 22:6n-3 (–21.3 to –22.0‰) was different from other fatty acids in the crown and abdominal tissue, including 20:5n-3 (ca –35.0‰) and was within the range of carbon isotope compositions of fatty acids in *R. piscesae* (cf. Figs 2 & 3).

DISCUSSION

The nutrition of hydrothermal vent fauna has been intensively studied since the discovery of deep-sea vent ecosystems in 1977 (Corliss et al. 1979). Early biological investigations centered on carbon fixation by symbiotic chemoautotrophic bacteria and their impor-

tance for the invertebrate host (Cavanaugh et al. 1981, Rau 1981, Cavanaugh 1983). More recent studies have focused on the specific nutritional requirements of vent invertebrates and the sources of the nutrients (Pranal et al. 1995, 1996, Rieley et al. 1995, Pond et al. 1997a,b, 1998, 2000, Allen 1999).

The 'essential fatty acids' which, in terrestrial animals, are 18:2n-6 and 18:3n-3, are particularly interesting nutrients (see review by Sargent et al. 1995, on which the following is based). These PUFAs originate in photosynthetic organisms, mainly plants and algae, and cannot be formed de novo by most animals, which lack the Δ12 and Δ15 fatty acid desaturases necessary for their biosynthesis from 18:1n-9. Most terrestrial animals have an absolute dietary requirement for these PUFAs to enable them to form 20:4n-6, 20:5n-3 and 22:6n-3 which have essential and fundamental roles in animal cell membrane structure and function. Most terrestrial animals can convert 18:3n-3 to 20:5n-3 and thence to 22:6n-3, and they can also convert 18:2n-6 to 20:4n-6 by a series of linked fatty acid desaturation and chain-elongation reactions which are complex, particularly so for the formation of 22:6n-3. Freshwater animals are generally similar to terrestrial animals in that they too can convert dietary 18:3n-3 to 20:5n-3 and finally to 22:6n-3. They can also convert 18:2n-6 to 20:4n-6. However, marine animals can only convert dietary C18 PUFAs to C20 and thence to C22 PUFAs to a strictly limited extent, if at all. This probably reflects the luxus in marine animal diets of 20:5n-3 and 22:6n-3, which are formed abundantly at the base of the marine food chain by photosynthetic, single-cell eukaryotes, mainly diatoms rich in 20:5n-3 and a range of flagellates rich in 22:6n-3.

20:4n-6, 20:5n-3 and 22:6n-3 are all present in hydrothermal vent animals (Fullarton et al. 1995, Allen et al. 1998, this study), in which it is presumed they have the same roles in cellular membrane structure and function as in other marine animals. However, the source of PUFAs in hydrothermal vent animals from Pacific vent sites is unknown. Do hydrothermal animals obtain biologically active PUFAs preformed from the photic zone? If so, in terms of nutrition at least, animal life at hydrothermal vents depends on life in the photic zone and has not evolved to be independent of it. Alternatively, do hydrothermal vent animals produce biologically active PUFAs themselves, or do they obtain them from sources at the vents? If the latter is the case, what organisms/processes are involved at the vents and did they evolve independently of the same or related organisms/processes in the photic zone?

We have previously shown that bresiliid shrimp *Rimicaris exoculata* which dominate the fauna of many hydrothermal sites along the Mid-Atlantic Ridge, obtain substantial amounts of photosynthetically derived 20:5n-3 and, especially, 22:6n-3 during their dispersive planktotrophic larval phase (Pond et al. 2000). However, neither of the 2 vent worms *Ridgea piscesae* and *Protis hydrothermica* studied here has a prolonged planktotrophic larval phase during which they develop to a significant size, so an input of photosynthetic PUFAs by this route is excluded. In addition, since adult vestimentiferan tubeworms lack a mouth and a gut, an input by this route is also excluded. Tubicolous serpulid polychaetes inhabiting vents on the EPR do retain a functional gut post-settlement, and apparently have the capacity to suspension-feed (ten Hove & Zibrowius 1986), so that a particulate input from the water column is possible for these animals.

In *Ridgea piscesae*, the species in which input of photosynthetically-derived PUFAs is seemingly excluded, the abundant 16:1n-7 and 18:1n-7 fatty acids typical of the sulphur-oxidising symbiotic bacteria have $\delta^{13}C$-enriched values of ca −14.5‰. This value is typical of bulk carbon isotope compositions of tubeworms and some other sulfur-oxidizing symbiotic associations (Fisher 1990, van Dover & Fry 1994). However, $\delta^{13}C$ values for 20:4n-6 and 20:5n-3 of −20 to −22‰ are ambiguous because, although these values fall within the range of photosynthetic microplankton (Goericke et al. 1994), they can also be typical of vent-associated fauna (Fisher et al. 1994, Southward et al. 1994). If the 20:5n-3 and 22:6n-3 in *R. piscesae* are formed within the hydrothermal vent ecosystem, then the results here are consistent with 2 assumptions: (1) the $\delta^{13}C$ value of the primary carbon dioxide source is the same in the photic zone and at the vent; (2) carbon isotope discrimination in the biosynthetic pathways generating these PUFAs in the photic zone and vent are similar. Our present limited knowledge precludes a more detailed interpretation of potential carbon isotope discriminations associated with the complex elongation and desaturation pathways generating long-chain PUFAs.

In *Protis hydrothermica*, the species where an input of photosynthetically-derived PUFAs is possible, the $\delta^{13}C$ value of (35.0‰ for 20:5n-3 is lighter than values expected for photosynthetically generated fatty acids, and is consistent with 20:5n-3 in this vent serpulid polychaete being synthesized from a chemosynthetically reduced carbon source at the vent. However, a $\delta^{13}C$ value of −21‰ for 22:6n-3 is within the range expected for photosynthetic microplankton (Goericke et al. 1994), so that a photosynthetic origin for this fatty acid is also possible.

In attempting to resolve these seemingly paradoxical results, we note that both species studied here are well able to further desaturate and chain-elongate C18 fatty acids. Thus, *Ridgea piscesae* is rich in the NMID

20:2Δ5,13, which is considered to be formed by animals that chain-elongate and desaturate bacterially derived 18:1n-7 (Conway & McDowell Capuzzo 1991, Rieley et al. 1995, Pond et al. 1998), especially when bacterial input to the animals is high (Ackman & Hooper 1973, Zhukova 1991, Fang et al. 1993). *R. piscesae* also has notable amounts of both 18:3n-3 and 18:2n-6. It is reasonable to propose, therefore, that this tubeworm can also desaturate and elongate these fatty acids to generate, respectively, 20:5n-3 and 20:4n-6. A similar argument can be applied to *Protis hydrothermica*, which has notable amounts of the 'Mead acid', 20:3n-9, which is produced in terrestrial animals from 18:1n-9 in response to a dietary deficiency of 18:3n-3 and 18:2n-6 (Mead 1981). The same enzymic pathways generate 20:3n-9, 20:4n-6 and 20:5n-3 from their respective 18:1n-9, 18:2n-6 and 18:3n-3 precursors. Thus, it is feasible that the relatively small amounts of 20:4n-6 and 20:5n-3 in *P. hydrothermica* are formed from 18:2n-6 and 18:3n-3. The picture then emerges of both vent worms studied here experiencing a large input of bacterially derived 18:1n-7, resulting in the production of relatively unusual, further elongated and desaturated products of this fatty acid, i.e. NMIDs in *R. piscesae* and 18:3n-7 and 20:3n-9 in *P. hydrothermica*. This suggests a relative deficiency of 18:2n-6 and 18:3n-3 in these worms caused by a relative excess of bacterially-derived 18:1n-7. However, the worms may continue to produce 20:5n-3 and 20:4n-6 from their limited amounts of 18:2n-6 and 18:3n-3. In this scenario, hydrothermal vent animals differ from marine animals in general in not having a luxus of dietary, preformed long-chain n-3 PUFAs. Rather, they have evolved, like freshwater animals, in environments where it is advantageous to retain the capacity to convert C18 PUFAs to biologically active C20 and C22 PUFAs. This does not exclude hydrothermal vent animals benefiting from externally derived, preformed C20 and C22 PUFAs, as and when available, as occurs in bresiliid shrimp (Pond et al. 2000).

The foregoing argument leaves the source of 18:3n-3 and 18:2n-6 in these 2 vent animals undefined. Most animals cannot biosynthesise these PUFAs *de novo* because they lack Δ12 and Δ15 desaturases. However, an increasing literature suggests that some aquatic and terrestrial invertebrates do possess Δ12 and Δ15 desaturases and are capable of synthesizing C18 PUFAs from 18:1n-9 (Rothstein & Gotz 1968, Lubzens et al. 1985, Cripps et al. 1986, Weinert et al. 1993, Ito and Simpson 1996). It is possible, therefore, that vent animals biosynthesise 18:1n-9 and thence 18:2n-6 and 18:3n-3, but this is not readily consistent with their receiving a large input of 18:1n-7 from their bacterial endosymbionts.

A more plausible alternative is that the requisite 18:2n-6 and 18:3n-3 derive from microorganisms associated with the animals. Sulphur-oxidising bacteria, are known to produce C18 PUFAs, both as free-living (Jacq et al. 1989) and intradigestive forms (Temera et al. 1991). Molecular sequencing of the bacterial symbionts of *Ridgea piscesae* has suggested that the species contains only a single type of sulfur-oxidizing bacterium (Feldman et al. 1997). It is the case, however, that 2 morphologically distinct types of bacterial endosymbionts are present in *R. piscesae* from the Explorer Ridge (deBurgh et al. 1989), and it cannot be rigorously excluded that one of these forms produces C18 PUFAs. Microorganisms contributing 18:2n-6 and 18:3n-3 to vent animals could also be single-cell eukaryotes including fungi and yeasts, marine forms of which are well known to produce these fatty acids (Cooney et al. 1993, Brown et al. 1996). Such organisms are heterotrophic, and in a hydrothermal vent ecosystem the source of organic carbon, whether from detritus, exudates or prokaryotes, is likely to be of chemolithotrophic origin in the vent rather than of phototrophic origin in the water column. However, the trophic role of single-cell eukaryotes in hydrothermal vent ecosystems, and in particular their association (if any) with vent animals is unexplored.

A final possibility is that vent animals have a close association, whether symbiotic or not, with bacteria that generate end-product 20:5n-3 and 22:6n-3 directly. Marine bacteria isolated from polar (Nichols et al. 1993, Jøstensen & Landfald 1997) and deep-sea (deLong & Yayanos 1986, Yano et al. 1994) environments are known to produce 20:5n-3 and 22:6n-3. The bacteria in question are often intimately associated with animals, as in the guts of deep-sea fishes (Yano et al. 1994) and more generally in Arctic invertebrates (Jøstensen & Landfald 1997). A characteristic of deep-sea bacteria that produce long-chain n-3 PUFAs is that they generally produce either 20:5n-3 or 22:6n-3 (deLong & Yayanos 1986), while planktonic sources generally contain both fatty acids. Equally, marine single-cell eukaryotes rich in 22:6n-3 rather than 20:5n-3 are well known (Findlay et al. 1986). It is interesting in this context that *Protis hydrothermica* contains appreciable quantities of 22:6n-3, with a $\delta^{13}C$ value for this fatty acid (–22.0‰) considerably enriched in ^{13}C compared to all other fatty acids including 20:5n-3. This strongly suggests that the 22:6n-3 and 20:5n-3 in *P. hydrothermica* originate from different sources, consistent with the species being mixotrophic (ten Hove & Zibrowius 1986). However, the possibility that vent animals have access to microbially preformed 20:5n-3 and 22:6n-3 is not readily consistent with their actively chain-elongating and desaturating C18 fatty acids (as discussed previously),

since the end-product 20:5n-3 and 22:6n-3 are potent inhibitors of such processes.

The nutrition of some invertebrates from deep-sea hydrothermal vent sites in the eastern Pacific is much more complex than previously realized. It is clear that chemoautotrophic bacteria are the primary source of nutrition for these animals. It is much less clear how they obtain their long-chain n-3 PUFAs and, in particular, whether they do so independently of phototrophic processes, or from prokaryotic or eukaryotic microorganisms. The same uncertainty exists for other key animal nutrients (e.g. retinoids and tocopherols), that are derived overwhelmingly from phototrophic eukaryotic sources in other marine and terrestrial ecosystems. Resolution of these issues is essential for fully understanding the nature of trophic processes and the evolution of animal life at deep-sea hydrothermal vents.

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