Fatty acid composition in deep hydrothermal vent symbiotic bivalves

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Abstract Fatty acids in deep hydrothermal vent bivalves have been analyzed. Their composition is completely different from that of a littoral mussel collected in the Mediterranean sea. The distribution of fatty acids in the littoral mussel is characterized by a predominance of polyunsaturated fatty acids (20:5n-3, 22:6n-3) reflecting the planktonic origin of the food. Vent bivalve fatty acid distribution is dominated by an abundance of the monounsaturated acids (double bond in the n-7 position) 16:1n-7, 18:1n-7, and 20:1n-7 which are clearly of bacterial origin and give an indication of the symbiotic bacterial activity in the bivalves. Differences between the fatty acid composition of the bivalves from two hydrothermal sites (13°N and Galapagos) and differences between the mantle and the gill were observed and are discussed with respect to vent activities at the two sites and species metabolic capacities as a function of ecological conditions.—**Ben-Mlih, F., J-C. Marty, and A. Fiala-Médioni.** Fatty acid composition in deep hydrothermal vent symbiotic bivalves. *J. Lipid Res.* 1992. ***33:*** *1797–1806.*

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Lipids are involved in important functions in metazoans. They are fundamental components of membranes and are involved in membrane transport processes; they are, in addition, used as energy storage compounds and can function as metabolic regulators and hormones (1). Fatty acids which are aliphatic components of lipids, particularly triacylglycerols and phospholipids (2), have a characteristic pattern in marine invertebrates reflecting ecological conditions and the source of trophic material (2–7). The structural diversity of fatty acids synthesized in marine algae and the relative stability of these molecules allow their use as biomarkers. Specific fatty acids (or combinations) can be associated with particular phytoplankton classes (8, 9). In this way, fatty acids have been used in the study of transfer through marine food chains and especially in bivalve molluscs (see reviews in 5, 10).

The relative proportions of particular fatty acids may differ among lipid classes, between specific tissues or organs within an animal, or due to differences in environmental variables such as temperature and salinity (11). General fatty acid composition in marine invertebrates is characterized by a predominance of two polyunsaturated acids: 20:5n-3 and 22:6n-3 (2, 3, 5, 6). This pattern reflects the fact that these organisms utilize organic material elaborated in the photic zone during photosynthesis where phytoplanktonic primary production constitutes the first step of the marine food chain.

In contrast, recently discovered luxuriant populations of invertebrates associated with deep hydrothermal vents (see reviews in references 12–16) bear witness to the existence of an alternative food chain based primarily on chemosynthetic bacterial primary production (17–20).

Bivalve molluscs constitute one of the main groups of vent populations found in the East Pacific Rise. Two principal families were encountered: Mytilidae represented by the species *Bathymodiolus thermophilus* (21) and Vesicomyidae represented by *Calyptogena magnifica* (22). These species have been found to behave as two different functional anatomical models (23) but with similar biochemical characteristics related to the fact that they obtain most of their energy from symbiotic relationships with endocellular chemosynthetic bacteria integrated in their gills (24–26).

The usefulness of biomarkers of endosymbiont activity in bivalves was first noticed by Gillan et al. (27) and recently applied to the study of littoral bivalves by Conway and McDowell Capuzzo (28). Fatty acids are particularly adapted to the study of hydrothermal sites as some of them are specifically synthesized by bacteria. Branched acids from C14 to C19 in *iso* and *ante-iso* position were

Abbreviations: PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids; GC-MS, gas chromatography-mass spectrometry.

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reported in most bacteria accounting for as much as 70% of the total fatty acids present (29, 30). While polyunsaturated fatty acids (PUFA) are absent from bacteria, monounsaturated fatty acids (MUFA) are major bacterial components: 18:1n-7 has been used as a bacterial indicator in sediment (29, 31, 32) and in estuarine and oceanic waters (33-35). Cyclopropanoid acids such as 9,10-methylene-C16 (delta 17) and 9,10-methylene-C18 (delta 19) are also good markers of bacterial biomass (36, 37). Other fatty acids have also been described as bacterial indicators: branched monounsaturated acids (32), phytanic acid (38), and some hydroxylated fatty acids (39).

Fatty acid composition of the major bivalve vent species was performed in order to compare the major features of their biochemical composition to that of the littoral mussel, to detect a possible impact of these deep and specific environments, and finally to find characteristic markers of the bacterial symbionts.

MATERIAL AND METHODS

Biological samples

The mytilid *B. thermophillus* was collected from two different sites: the 13°N site on the East Pacific Rise (2600 m) during the "Hydronaut" cruise using the French submersible "Nautile" (IFREMER, 1987), and the Galapagos Rise (2610 m) during the "Galapagos" cruise (University of California-Santa Barbara; Scripps, University of San Diego and Woods Hole) from the U.S. submersible "Alvin." The vesicomid *C. magnifica* was present only at the Galapagos site. For fatty acid analyses only three specimens of *B. thermophilus* were available from the 13°N site and two specimens from the Galapagos site. For *C. magnifica,* three specimens were available for fatty acid analyses from the Galapagos site. This limitation in the number of specimens obtained is related to technical difficulties in obtaining a number of specimens at these depths, the necessity to dispatch the material for quite different analyses as well as, for example, the small number of mussels in the 13°N site.

Samples of the littoral mussel *Mytilus galloprovincialis,* used as reference, were collected in the Bay of Banyuls on the Mediterranean coast of France. Because the fatty acid composition of littoral mussels is well documented in the literature (see review in 5), only one sample was analyzed in order to confirm the general trends in the described composition.

Specimens were dissected (using tools rinsed with chloroform) immediately after their arrival on board and the different organs were frozen in liquid nitrogen until chemical analysis in the laboratory. Dissection was undertaken in order to separate the mantle from the gill where bacteria accumulate. Bivalve tissues were freeze-dried and ground to a powder. Preweighed aliquots were subjected to fatty acid analysis.

Fatty acid analysis

Extraction of lipids in the laboratory was performed within 2 months of sampling. Lipids were extracted according to the method of Bligh and Dyer (40) using sonication (5 min). Solvent was removed from the extract using a Speed-Vac system.

Lipids were separated into neutral and polar lipids on Sep-Pak silica cartridges (41). Fatty acids from neutral and polar lipids and from total extracts were transmethylated with 7% BF_3 in methanol in sealed tubes under argon at 70°C for 30 min. Separation of fatty acid methyl esters was carried out with Sep-Pak silica cartridges using solvents of increasing polarity. Fraction 1, eluted with 6 ml hexane contained hydrocarbons; fraction 2, eluted with 8 ml hexane plus 75 μl ethyl acetate, contained fatty acid methyl esters; fraction 3, eluted with 10 ml methanol, contained more polar compounds.

Fatty acid methyl esters were then analyzed by capillary gas chromatography on both polar and nonpolar columns using two chromatographic systems. The first one was a Perkin-Elmer Sigma 2 with a split/splitless injector equipped with a fused silica column, 25 m long, 0.32 mm ID coated with CP sil 5 (CHROMPACK), using He as carrier at an inlet pressure of 1.5 bar, and flame ionization detection; temperature was increased from 100 to 280°C at 2°C min^{-1}. The second system was a Girdel 3000, with solid (Grob) injector, equipped with a fused silica column, 25 m long, 0.32 mm ID coated with CW 20 CB (CHROMPACK), He at 0.5 bars, operated between 100 to 200°C at 1.5°C min^{-1}.

Resolved compounds were identified by comparison of their retention time with those of standards run under the same conditions on the two columns and by GC MS analysis of selected samples. Analytical conditions for GC MS analysis have been described previously (8). Quantification was achieved by integration of peaks with the Nelson Analytical system and calculation of concentrations with respect to the internal standard (5 μg of C19 methy ester) added to the sample (100 mg dry weight) prior to extraction.

RESULTS

Ecological characteristics

Comparison of bivalve species from food chains with different primary productivity bases, i.e., photosynthetic versus chemosynthetic. The littoral mussel total fatty acid concentration was 29.5 $mg \cdot g^{-1}$ dry weight for the mantle and 26.5 $mg \cdot g^{-1}$ for the gill. The composition (**Table 1**) is characterized by the predominance of the polyunsaturated acids 20:5n-3 (14.8%) and 22:6n-3 (12.1%) and the presence of other PUFA 16:4 (2.8%), 18:4n-3 (3.1%), 20:4n-6 (1.8%).

Additional compounds identified include the usual saturated 16:0 (17.3%) and monounsaturated fatty acids 20:1n-9 (3.3%), 22:1n-9 (3.0%), 16:1n-7 (2.4%), and 18:1n-7 (2.1%). The major trend of the distribution of fatty acids was similar to that observed in mussels and bivalves in general, as reported by Joseph (5); the main feature was the predominance of polyunsaturated acids. Small variations in the distribution of fatty acids were observed when comparing the gill with the mantle, e.g., the presence of 24:0 (characteristic of higher plants) and a higher abundance of some unsaturated acids (e.g., C20:4n-6) in the gill.

TABLE 1. Fatty acid distribution, expressed as a percentage of total fatty acid, for the littoral mussel (Banyuls), for deep hydrothermal vent mussels (13°N and Galapagos), and *Calyptogena*

Fatty Acid	Banyuls Mussel		13°N Mussel						Galapagos Mussel				Galapagos *Calyptogena*					
	M	G	M8	G8	M18	G18	M19	G19	M2	G2	M7	G7	CM5	CG5	CM7	CG7	CM11	CG11
C12:0	nd	nd	0.1	0.1	0.1	0.2	0.1	tr	0.1	nd	tr	0.1	0.2	0.2	0.2	nd	0.2	0.1
C14:0	1.3	0.4	0.1	0.1	0.3	0.2	0.1	0.1	0.5	0.2	0.2	0.2	0.9	5.1	1.0	3.4	0.4	3.0
C15:0	0.8	0.6	nd	0.1	nd	0.2	0.1	0.1	0.0	0.1	0.1	tr	0.2	0.4	0.3	0.3	nd	0.3
C16:0	17.3	8.6	6.9	6.2	7.9	8.1	6.2	8.1	10.9	6.5	8.4	6.3	11.9	9.2	11.8	9.5	11.4	8.0
C17:0	1.8	1.5	0.2	0.4	0.1	0.5	0.3	0.5	0.2	0.3	0.3	0.3	0.6	0.3	0.6	0.3	0.9	0.2
C18:0	5.5	2.8	5.4	5.6	3.9	6.0	5.2	6.0	2.4	1.6	1.5	2.7	2.1	0.9	2.3	2.0	2.3	1.7
C20:0	0.4	0.7	3.1	1.6	2.1	1.0	2.5	2.5	6.3	0.1	0.1	0.1	4.6	0.5	4.6	0.3	6.4	1.1
C22:0	0.1	0.7	nd	nd	0.1	0.2	0.2	0.7	nd	nd	nd	nd	nd	nd	0.1	tr	nd	tr
C24:0	0.5	2.6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
iC15	0.1	0.4	nd	nd	nd	nd	nd	nd	nd	nd	tr	tr	nd	0.1	nd	0.1	nd	0.1
iC17	0.4	0.8	nd	nd	nd	nd	nd	nd	nd	tr	15.6	0.1	nd	nd	nd	nd	nd	nd
aC17	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.1	8.0	0.1	nd	nd	nd	nd	nd	nd
DC17	nd	nd	1.6	1.0	0.9	1.4	3.6	1.6	0.8	0.4	0.4	0.5	nd	nd	nd	nd	nd	nd
iC19	nd	nd	1.2	14.9	8.0	11.8	4.5	6.2	2.1	1.6	1.1	1.6	4.1	1.2	4.9	1.7	5.7	3.1
DC19	nd	nd	0.6	0.2	1.7	1.6	0.9	0.9	2.5	1.8	1.7	3.4	0.7	0.6	0.7	1.0	0.6	0.8
CI4:1	nd	nd	nd	nd	nd	nd	nd	nd	0.1	0.3	0.4	0.1	0.3	0.6	nd	0.5	nd	0.3
C16:1n-5	nd	nd	0.1	0.2	nd	0.1	tr	tr	0.1	0.3	24.0	nd	0.1	0.4	0.8	0.7	0.6	0.8
C16:1n-7	2.4	1.0	18.4	10.1	22.9	13.6	5.7	7.9	51.1	56.5	19.9	39.8	24.9	56.6	24.8	47.1	23.2	43.2
C18:1n-5	nd	nd	0.9	0.2	0.9	0.5	0.6	0.1	nd	nd	nd	nd	nd	nd	nd	0.2	4.1	0.2
C18:1n-7	2.1	0.8	8.7	3.9	6.1	4.5	6.2	2.3	6.0	3.9	3.8	6.1	6.6	7.9	5.1	11.5	3.4	7.4
C18:1n-9	1.2	0.5	1.0	0.5	0.6	0.4	0.4	0.4	1.6	1.0	1.0	1.5	2.8	1.0	3.1	1.5	0.3	1.2
C20:1n-5	0.2	0.3	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
C20:1n-7	0.7	0.4	13.2	14.7	9.7	16.1	7.8	8.6	2.3	6.6	3.7	8.5	6.2	4.4	4.6	8.1	3.1	7.4
C20:1n-9	3.3	3.5	3.3	2.0	2.4	0.5	0.2	5.8	3.2	2.2	1.4	3.4	9.3	2.1	9.3	4.2	9.9	5.5
C20:1n-11	1.7	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
C22:1n-7	nd	nd	nd	nd	3.1	3.9	4.8	3.0	0.1	0.9	0.4	nd	nd	nd	nd	0.1	nd	0.1
C22:1n-9	3.0	6.7	nd	nd	nd	nd	nd	nd	nd	0.1	nd	nd	nd	nd	nd	nd	nd	nd
C16:4	2.8	6.3	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
C18:2	nd	nd	4.9	6.0	5.3	1.2	6.7	7.6	0.4	0.4	0.3	0.4	0.5	0.3	0.1	0.3	nd	0.3
C18:2n-6	1.5	1.0	0.9	0.9	0.4	0.4	1.2	2.2	0.7	0.8	0.2	0.9	0.2	0.2	0.1	nd	nd	0.2
C18:3n-3	0.9	3.6	nd	nd	nd	nd	nd	nd	nd	0.1	0.1	0.1	nd	nd	nd	nd	nd	nd
C18:4n-3	3.1	7.6	5.5	4.1	4.5	6.8	11.9	6.9	1.8	2.6	1.5	4.9	nd	nd	nd	nd	nd	nd
C18:5n-3	0.4	0.2	2.2	2.9	1.9	0.1	2.9	3.0	2.7	4.0	0.6	3.3	0.1	0.1	0.4	nd	0.2	nd
C20:2	1.1	1.4	2.2	3.0	4.3	5.9	4.9	6.3	nd	4.7	3.0	3.3	14.5	5.0	14.1	5.5	16.2	7.4
C20:3	0.3	0.4	2.7	3.1	2.3	2.5	5.0	4.0	0.6	0.3	0.1	5.0	6.2	0.2	0.6	0.2	0.3	3.2
C20:4	nd	nd	nd	0.4	0.3	0.5	0.2	0.5	1.4	2.9	nd	0.2	0.2	nd	6.2	0.7	6.8	1.7
C20:4n-6	1.8	6.2	nd	nd	nd	nd	nd	nd	nd	0.2	0.5	0.4	nd	1.1	nd	nd	nd	nd
C20:5n-3	14.8	5.8	5.8	6.9	5.4	5.8	8.9	7.5	tr	0.1	tr	0.2	nd	nd	nd	nd	nd	nd
C22:2	nd	nd	0.4	nd	0.5	0.8	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
C22:3	nd	nd	0.2	nd	0.1	0.2	nd	nd	nd	nd	nd	nd	0.2	0.1	0.1	tr	0.2	tr
C22:4	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.2	nd	nd	0.5	0.2	0.3	0.1	0.3	0.4
C22:5	1.0	0.8	2.0	2.3	1.3	1.5	2.3	1.8	nd	tr	nd	0.3	nd	nd	nd	nd	nd	nd
C22:6n-3	12.1	15.3	2.4	2.6	1.6	1.7	2.2	1.8	0.1	0.1	0.1	0.1	nd	nd	nd	nd	nd	nd
n.i.	17.5	19.3	6.1	6.1	1.5	2.0	4.4	3.6	2.1	2.6	1.3	6.3	2.3	1.8	4.0	0.8	3.6	2.5
Total, mg/g	29.5	26.5	19.7	23.5	18.6	17.3	18.9	20.6	74.2	65.6	45.4	27.8	60.4	69	57	87.1	58.1	64.7
Sum n-7	5.3	2.2	40.3	28.7	41.8	38.0	24.6	21.8	59.5	67.8	27.9	54.4	37.6	68.9	34.5	66.9	29.7	58.0
Sum sat	23.2	12.8	15.4	13.5	14.0	15.4	14.0	17.3	19.6	8.2	10.0	9.0	18.6	10.5	18.8	11.7	20.2	10.8
n-7/sat	0.23	0.17	2.62	2.12	2.99	2.47	1.75	1.26	3.04	8.32	2.78	6.05	2.02	6.55	1.84	5.69	1.47	5.36

M, mantle; G, gill; number is the specimen reference. Total, total fatty acid concentration expressed in milligrams per gram dry weight of tissue; sum n-7, 16:1n-7+18:1n-7+20:1n-7+22:1n-7; sum sat, 16:0+18:0+20:0; n-7/sat, (16:1n-7+18:1n-7+20:1n-7+22:1n-7)/(16:0+18:0+20:0+22:0); i, *iso*; a, *ante-iso;* D, delta; nd, not detected; n.i., not identified; tr, trace.

The ratio of monounsaturated acids (belonging to the n-7 series) to the corresponding saturated acids (16:1+ 18:1+ 20:1)/(16:0+ 18:0+ 20:0) was low in the littoral mussel (0.17 for the gill, 0.23 for the mantle). These results are in both **Fig. 1** and Table 1.

Deep hydrothermal vent samples had total fatty acid concentrations in the range of 17.3 to 23.5 mg • g^{-1} (13°N) and 45.4 to 87.1 mg • g^{-1} (Galapagos). The 13°N and Galapagos samples had a very particular fatty acid composition when compared with littoral bivalve sample (Table 1). The following features are of note.

There was an absence or very low values of phytoplankton-derived fatty acids and especially the most common found in marine invertebrate: 20:5n-3 (not detected in *Calyptogena* samples; traces to 0.2% in Galapagos mussels, 5.4 to 8.9% in 13°N mussels) and 22:6n-3 (not detected in *Calyptogena*, 0.1% in Galapagos mussels, and 1.6 to 2.6% in 13°N mussels). Generally speaking, concentrations of long-chain PUFA were much lower than in the littoral mussel.

There was a predominance of the n-7 series monounsaturated fatty acids (the sum of n-7 MUFA varied from 21.8 to 68.9%) with very high values for 16:1n-7 especially in Galapagos samples (19.9 to 56.6%). As a consequence, the ratio of total n-7 MUFA (16:1n-7+18:1n-7+20:1n-7+ 22:1n-7) to the corresponding saturated acids (16:0+18:0+20:0+22:0) was unusually high (Fig. 1), e.g., 1.26 to 8.32 for the hydrothermal bivalves compared with 0.23-0.17 for littoral samples.

There was an occasional presence of odd branched *iso* and *ante-iso* fatty acids: *iso* 17 (not detected to 15.6%), *ante-iso* 17 (not detected to 8.0%) and *iso* 19 (1.1 to 14.9%).

There were also traces of 9,10-methylene-C16 (not detected to 3.6%) and 9,10-methylene-C18 (0.2 to 3.4%).

Comparison of the same species from different sites. As pointed out earlier, the importance of the bacterial signature remains the major feature in all vent samples. However, *B. thermophilus* from the Galapagos site showed clear differences in fatty acid concentration and composition when compared to samples collected at the 13°N site. The following features are worthy of comment. The specific total fatty acid concentrations were generally two or three times higher in the Galapagos samples than at the 13°N site (Table 1). While the dominant fatty acids, the monounsaturated n-7 series, were present in all samples, they constituted only 21.8-41.8% of the total fatty acids in the 13°N samples but 27.9-67.8% in the Galapagos mussels. For the 13°N samples, this produced a lower ratio of total monounsaturated n-7 to saturated fatty acids (Fig. 1), i.e., from 1.26 to 2.99 with only slight differences between gill and mantle. By contrast, Galapagos mussels had (Fig. 1) a higher ratio in the gill (8.32-6.05) than in the mantle (2.78-3.04). 13°N samples were also characterized by the absence of *iso* and *ante-iso* 17 (present in considerable quantities in one of the Galapagos mussels) (Table 1) and an unusual presence of high concentrations of *iso* 19 (up to 14.9% in the gill and 8.0% in the mantle). At both sites, samples did not show important differences

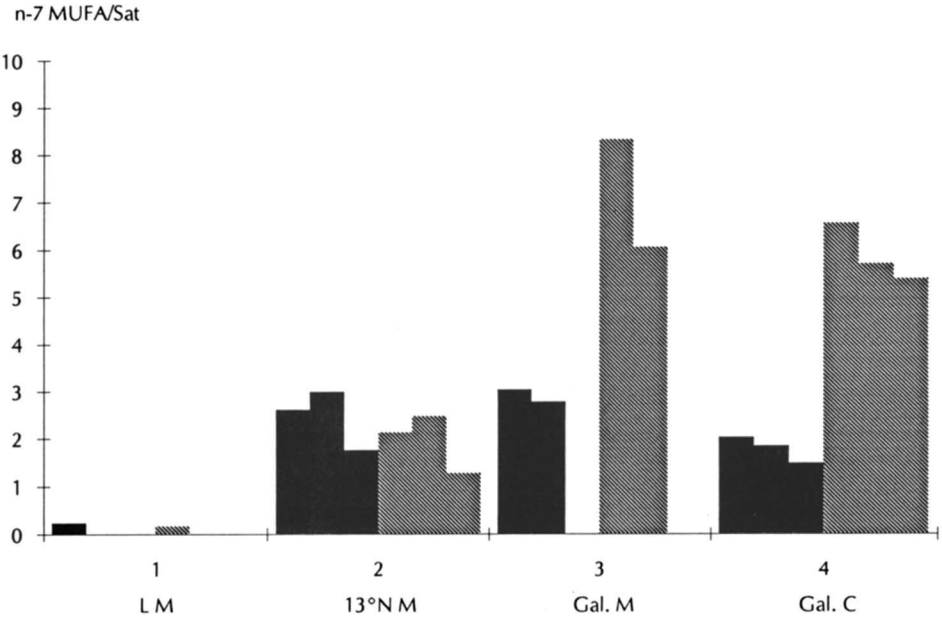

Fig. 1. Ratios (16:1n-7+18:1n-7+20:1n-7+22:1n-7)/(16:0+18:0+20:0+22:0) for the littoral mussel (LM) and for deep hydrothermal vent mussels (13°N: 13°NM, Galapagos: Gal M) and *Calyptogena* (Galapagos: Gal C). Black columns, mantle; hatched columns, gill.

in concentrations of monounsaturated 20:1n-9 and polyunsaturated 18:5n-3, 20:2, and 20:3 fatty acids. In contrast, the percentages of 18:2, 20:5n-3, 22:6n-3, and 22:5 were higher in the 13°N mussels than in the Galapagos ones (for example levels of 20:5n-3 were between 5.8 and 7.5% in the gill and 5.4 and 8.9% in the mantle of 13°N bivalve samples, but less than 1% in Galapagos mussels).

Species-specific characteristics

The fatty acid compositions of the two species *B. thermophilus* and *C. magnifica* reflect primarily the ecological constraints of the hydrothermal Galapagos site (Table 1), i.e., low level or absence of the more characteristic phytoplankton-derived fatty acids 20:5n-3 and 22:6n-3 and predominance of a bacterial signature, i.e., very high percentages of the monounsaturated n-7 fatty acids, due to the presence of endosymbionts in the gills of both species. As in *Bathymodiolus, Calyptogena* has a high (16:1n-7 + 18:1n-7 + 20:1n-7 + 22:1n-7)/(16:0 + 18:0 + 20:0 + 22:0) ratio; 1.47 to 2.02 in the mantle and 5.36 to 6.55 in the gill. Of note is the presence of 9,10-methylene-C18 at low levels.

The following differences observed between the two species confirm the hypothesis of two different metabolic pathways. There was a lower diversity of fatty acids in *Calyptogena* samples, in particular the absence of a number of polyunsaturated acids (20:4, 20:5n-3, 22:5, 22:6n-3) and branched C17 acids. In *Calyptogena* polyunsaturated 18:4n-3 and 18:5n-3, which are well represented in the mussel, were absent or at low levels. In *Calyptogena* tissues, there were very low levels of the PUFA 22:4 and 22:3, which are absent in the mussel.

DISCUSSION

The fatty acid composition of hydrothermal vent bivalves confirms the existence of an alternative food chain, i.e., one based on bacterial chemosynthetic rather than planktonic photosynthetic primary production (17, 26, 42, 43).

The fatty acid composition of the littoral mussel that was examined is similar to compositions reported for other species of *Mytilidae* from different geographic zones (5, 10, 44-47), as well as for other marine invertebrates (2, 3, 5, 48). This composition is characterized by a fatty acid pattern reflecting the nature of the ingested food, i.e., high levels of polyunsaturated fatty acids 20:5 and 22:6 (the main acids found in phyto- and zooplankton) and noticeable amounts of other PUFA, 18:3, 18:4, etc. The presence of these acids can be related to various planktonic classes as described by a number of authors (8, 9, 41, 49, 50). The phytoplanktonic (16:4n-3, 18:4n-3, 18:3n-3) and zooplanktonic (20:1n-9, 22:1n-9) acids, as well as the ubiquitous 22:6n-3, are more abundant in the gill than in the mantle of the littoral mussel. This is probably due to the presence in the gill of fresh planktonic cells.

The presence in the littoral mussel, and particularly in the gill, of 24:0 which is characteristic of terrestrial higher plants (51, 52) but also of *Posidonia* (41) can be explained by its shallow water habitat; as expected, this marker is absent in hydrothermal vent species.

The very high concentrations of monounsaturated fatty acids of the n-7 series in vent mussels clearly indicate a bacterial contribution to host nutrition. The dominant monounsaturated fatty acid, 16:1n-7, was not thought to be strictly bacterial as it constitutes a major monoenic fatty acid in most algal classes (9, 41, 53, 54). However, the combination of an absence or low levels of the usual phytoplankton markers, e.g., 20:5n-3 or 22:6n-3; the high levels of fatty acids considered typically as bacterial biomarkers, e.g., 18:1n-7, the most common fatty acid in many sulphur-oxidizing bacteria (35, 55, 56); as well as the presence of 20:1n-7 and 22:1n-7, led to the conclusion that all the n-7 series is of bacterial origin.

In *Solemya velum,* a littoral symbiont-bearing bivalve, Conway and McDowell Capuzzo (28, 48) found that the bacterial fatty acid *cis*-vaccenic acid (18:1n-7) accounted for 20% of the total fatty acids and almost 1.5% of the animal dry weight. They concluded that this fatty acid was derived from sulphur-oxidizing bacterial symbionts. Similar results were obtained for *Codakia orbicularis,* a littoral symbiont-bearing *Lucinidae* (57).

The hypothesis of a bacterial origin for the monounsaturated fatty acid n-7 series also agrees with the results of Oliver and Colwell (58) who found 16:1 and 18:1 to be the most common fatty acids in a marine gram-negative bacteria, with proportions close to those found in hydrothermal vent species, 41.2 and 25.4%, respectively. The increase in total monounsaturated when compared to saturated fatty acids, seems to be a general feature in barophilic bacteria and is induced by high pressure acting in a way similar to the temperature-induced changes in lipid composition of bacteria (59). The ratio of n-7 monounsaturated to total saturated fatty acids has been shown by these authors to increase from 1.9 at 1 bar, to 3 at 690 bars. The very high ratio observed in our samples, in vent species, is consistent with the low temperatures (2-10°C) and high pressure (260-280 bars) experienced by the hydrothermal vent animals.

The evidence of a major bacterial contribution to the nutrition of host tissues is enhanced by the presence of other bacterial biomarkers: branched *iso* and *ante-iso* 17 acids.

The evidence of association of bacterial markers with vent animals is in complete agreement with *a)* ecological data from vents demonstrating high concentrations of chemosynthetic bacteria (17) constituting the major part of the organic material present in this environment (this particulate organic material is characterized by high levels

of monounsaturated fatty acids 16:1 and 18:1 (60, 61); *b)* isotopic ratios of the main vent species indicating the predominance of chemosynthetic processes in their nutrition (62); and *c)* ultrastructural observations showing the importance of gill colonization by sulphur-oxidizing bacterial symbionts (26, 43) as well as evidence of carboxylase activities associated with these symbionts (20).

Long chain PUFA are considered to be absent from prokaryotes (63-65) and therefore to be characteristic of eukaryotes. In vent species, the lower percentage of PUFA found when compared to littoral mussels is probably a consequence of the organic carbon contribution originating predominantly from symbiotic bacteria. The contribution of bacterial chemosynthetic carbon to the total bivalve host requirements can clearly be seen by the presence of the same signature in symbiont-lacking tissues such as the mantle and in the gill.

Polyunsaturated fatty acids in vent mussels (especially 20:5n-3 and 22:6n-3 and more generally the n-3 series) are considered to be essential for growth and normal metabolism in invertebrates that are unable to synthesize them (6, 66-69). Their absence or low values in vent bivalves raises the question about the alternative to these compounds for optimal growth. These vent mussels are larger than littoral ones. Other organisms from the same environment (annelids) have been shown to contain low amounts of polyunsaturated fatty acids (61, 70).

Conway and McDowell Capuzzo (28) working on *Solemya velum,* a littoral bivalve containing endosymbiotic chemoautotrophic bacteria, raise the same question, as 20:5 and 22:6 are also absent from their fatty acids. But in this case the authors found, together with high contents of monounsaturated fatty acids of the n-7 series indicating a bacterial origin, high levels of 20:4n-6 which is supposed to have the same function as 20:5 and 22:6. In the samples of the present study, 20:4n-6 was absent; we must suppose that other fatty acids play the essential role in bivalve metabolism devoted to n-3 series in the littoral mussel. The high concentrations of the monounsaturated acids of the n-7 series suggest that these acids could fulfill this role. This hypothesis is not confirmed by the partition of these acids between neutral and polar lipids (**Table 2**). Monounsaturated fatty acids of the n-7 series generally occur in lower percentages in the structural lipids than in the neutral lipids, while higher percentages of polyunsaturated (20:2, 20:3, 20:4) fatty acids are observed in structural rather than in neutral lipids (Table 2). In fact, there is no real tendency of special distribution of fatty acids between polar and neutral acids. Perhaps this is associated with the low number of samples obtained and analyzed. More experiments are needed to answer this question of essential fatty acids in vent bivalves.

It has become increasingly clear that the fatty acid composition of molluscs, as well as of other marine invertebrates, is influenced by ecological factors such as temperature and the quality of available food (5, 11). The main ecological conditions encountered at both the Galapagos and 13°N sites (high pressure and low temperature associated with a predominance of bacterial primary production) are so unique that they are reflected in the main features of the fatty acid composition of bivalves colonizing such sites. Mussels at both sites show the same major features with a predominance of n-7 MUFA series.

Despite the similarities, inter-site differences do occur between species from 13°N and Galapagos. In the 13°N samples, the ratio of the sum of n-7 MUFA to corresponding saturated fatty acids (Fig. 1) is about the same in both the mantle and the gill (about 2); however, in Galapagos samples, while this ratio is also 2 for the mantle, it is as high as 6 to 8 for the gill. This very high ratio in the mussel and *Calyptogena* gills, which are the site for bacterial symbiont activity, at the Galapagos site is probably related to a very high bacterial activity in the animals.

In the 13°N mussels, the lower concentrations of fatty acids than in Galapagos samples (Table 1), as well as the lower ratio of n-7 MUFA to corresponding saturated fatty acid in the gill and mantle, are in agreement with ultrastructural observations in the gill showing a regression in the number of bacterial symbionts (71) when compared with observations made during earlier cruises at the site (72, 73). This observation suggests a deterioration in the physiological condition for this species (71) which is probably related to a drastic reduction in vent activity at this site (74). A complementary explanation for these differences could also be the result of a colonization by different strains of bacteria than at the Galapagos site; this is supported by the unusual concentration of *iso* 19 which is a very uncommon bacterial tracer and whose concentration is higher in 13°N samples than in Galapagos samples (Table 1), particularly in the gill.

The other important inter-site difference is the presence 20:5n-3 and 22:6n-3 in the 13°N samples. These acids, components of phytoplanktonic origin in the 13°N waters, were not detected in Galapagos samples or were present only in trace amounts. The presence of these fatty acids is probably related to the capacity of the vent mussel to filter-feed on external particulate material (73, 75, 76). This particulate material must contain components of phytoplanktonic origin at 13°N to explain the presence of these PUFA. Sediment trap experiments at the 13°N site (70) have shown the presence of low levels of PUFA in the material collected. This ability may partly explain the capacity of this species to maintain itself on a site with decreasing vent activity a long time after the disappearance of other vent species relying more strictly on symbiotic relationships (74). Thus, the higher percentages of polyunsaturated fatty acids present in the 13°N samples probably indicate differences in species metabolic capacities in response to ecological conditions.

TABLE 2. Fatty acid distribution, expressed as a percentage of total fatty acid, for neutral lipids (NL) and polar lipids (PL) from deep hydrothermal vent mussels (13° and Galapagos) and *Calyptogena*

Fatty Acid	13°N Mussel (18)				Galapagos Mussel (7)				Galapagos *Calyptogena* (5)			
	M NL	M PL	G NL	G PL	M NL	M PL	G NL	G PL	M NL	M PL	G NL	G PL
C12:0	0.1	0.1	0.4	0.2	0.1	tr	0.1	0.1	tr	0.1	0.4	0.5
C14:0	0.1	0.1	1.5	0.4	0.3	1.0	0.6	0.5	1.8	1.0	3.8	9.2
C15:0	0.6	0.1	0.1	0.3	0.1	nd	0.1	tr	0.2	0.2	0.4	0.8
C16:0	8.8	7.5	11.3	11.0	21.8	10.8	12.7	14.4	9.7	14.5	11.4	20.6
C17:0	0.5	0.2	nd	nd	0.3	0.5	0.5	0.6	0.3	0.6	0.5	0.3
C18:0	2.4	3.1	3.5	3.6	2.0	3.8	2.7	4.8	2.2	1.9	3.6	3.0
C20:0	nd	nd	nd	nd	0.1	0.1	0.1	0.3	4.3	2.1	nd	0.2
iC15	nd	nd	nd	nd	nd	0.1	0.1	nd	nd	nd	nd	nd
iC17	nd	nd	nd	nd	6.7	0.1	0.1	nd	nd	nd	nd	nd
aC17	nd	nd	nd	nd	0.1	0.1	0.1	nd	nd	nd	nd	nd
DC17	0.1	2.2	0.4	2.2	nd	2.3	0.1	nd	nd	nd	nd	nd
iC19	nd	8.5	nd	7.2	nd	nd	nd	nd	0.2	9.7	0.2	0.1
DC19	1.1	1.2	0.9	0.9	3.1	3.2	2.8	2.9	1.3	0.8	1.5	0.5
C14:1	nd	nd	nd	nd	0.2	0.1	0.1	tr	0.3	tr	0.1	tr
C16:1n-5	0.3	0.1	0.4	0.1	nd	0.2	nd	nd	0.3	0.7	3.1	1.1
C16:1n-7	44.6	15.0	21.5	21.8	29.6	35.6	49.1	27.3	37.2	27.2	20.3	32.9
C18:1n-5	nd	0.1	nd	0.2	nd	nd	nd	nd	nd	nd	nd	nd
C18:1n-7	7.0	4.9	4.3	4.5	6.6	5.0	5.7	4.5	11.5	4.0	15.5	4.6
C18:1n-9	2.7	1.4	6.9	1.7	1.4	1.6	1.2	2.1	1.9	3.2	1.9	1.5
C20:1n-7	8.2	2.5	6.3	2.0	0.2	4.0	10.2	7.9	8.4	2.6	18.1	6.8
C20:1n-9	2.2	3.8	3.1	2.9	4.8	4.7	2.6	5.3	4.3	8.1	4.0	6.9
C22:1n-7	0.2	nd	0.5	nd	1.0	1.6	nd	nd	nd	nd	nd	nd
C18:2	1.1	0.6	0.7	0.7	0.4	0.3	0.3	0.8	0.5	0.6	0.3	0.3
C18:2n-6	0.2	0.7	1.0	0.5	0.7	0.5	0.2	0.4	0.2	0.2	0.2	0.1
C18:3n-3	nd	nd	nd	nd	nd	0.6	nd	0.1	nd	nd	nd	nd
C18:4n-3	7.0	11.0	11.2	11.2	1.0	3.4	1.3	11.7	nd	nd	nd	nd
C18:5n-3	nd	4.7	2.2	4.8	4.8	2.2	0.7	1.6	0.1	0.1	0.9	0.7
C20:2	1.4	5.2	1.4	4.0	1.6	2.3	3.9	6.5	7.8	7.7	4.1	6.4
C20:3	3.9	6.7	7.3	6.4	0.1	5.4	0.3	0.4	3.4	2.2	0.4	0.4
C20:4	0.7	2.0	3.8	1.6	nd	nd	0.4	nd	0.2	5.6	nd	nd
C20:4n-6	nd	nd	nd	nd	0.2	0.1	0.3	0.9	nd	nd	0.2	tr
C20:5n-3	3.5	9.4	6.4	5.9	0.1	0.1	0.4	nd	nd	nd	nd	nd
C22:2	0.5	0.4	0.4	0.2	nd	nd	nd	nd	nd	nd	nd	nd
C22:3	0.8	2.4	1.3	1.1	nd	nd	nd	nd	0.1	0.1	0.3	0.1
C22:4	nd	nd	nd	nd	nd	nd	nd	nd	0.1	0.1	0.1	0.2
C22:5	nd	1.6	0.3	0.6	nd	nd	0.2	nd	nd	nd	nd	nd
C22:6n-3	0.7	1.8	1.3	0.8	0.1	0.1	0.2	nd	nd	nd	nd	nd
n.i.	1.3	2.6	1.2	2.7	13.3	7.5	2.9	7.0	3.8	6.7	9.0	2.6
Sum n-7	59.9	22.5	32.7	28.4	37.4	46.2	65.0	39.7	57.1	33.8	53.9	44.2
Sum sat	12.3	11.9	15.7	15.4	26.9	17.8	18.2	22.1	13.2	17.2	16.5	24.1
n-7/sat	4.87	1.89	2.08	1.85	1.39	2.60	3.57	1.80	4.33	1.97	3.26	1.83

M, mantle; G, gill; sum n-7, sum sat, n-7/sat, as defined in Table 1; i, *iso;* a, *ante-iso;* D. delta, nd, not detected; n.i., not identified; tr, trace.

Similar fatty acid composition characteristics are found in *Bathymodiolus* and *Calyptogena* and can be related to the very special constraints of the vent environment. Despite the evident comparable signature of ecological conditions, differences in composition confirm that these two species represent different functional anatomical models as can be seen in ultrastructural observations (23, 72) and enzymatic analyses (20).

The lower diversity of fatty acids observed in *Calyptogena* with respect to mussels is consistent with the greater dependency of this species on chemosynthetic processes through symbiotic relationships. The greater diversity of fatty acid distribution found in the mussel is in agreement with the possibility that this species can incorporate external particulate material with a different signature than those of its bacterial symbionts. This is suggested by the presence in the mussel tissues of fatty acids such as 18:5n-3 and 18:4n-3 which are completely absent in *Calyptogena.* The other main difference is the possibility that *Calyptogena* can produce long-chained polyunsaturated fatty acids such as 22:4, 22:3 or 22:0 (absent in the mussel) which demonstrate different metabolic abilities in the different species.

Despite the fact that the low number of specimens

provided from these deep sites is a limitation and did not allow results confirmed by statistical analysis, it is clear that there are evident differences in the main features of the fatty acid composition between different species as well as between the same species from different sites. The bacterial markers also clearly differentiate the deep vent mussel from the littoral one.

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