New 356

(1995) 87: 329-342

LIPID CHARACTERISTICS OF HYDROTHERMAL VENT ORGANISMS FROM 9°N, EAST PACIFIC RISE

Gareth Rieley[1*], Cindy L. Van Dover[2], David B. Hedrick[3], David C. White[3], and Geoffrey Eglinton[1,4]

[1]Organic Geochemistry Unit, School of Chemistry, University of Bristol, Bristol BS8 1TS, UK

[2]Department of Chemistry, Woods Hole Oceanographic Institute, Woods Hole, MA 02543, USA

[3]Center for Applied Biotechnology, Knoxville, TN., USA

[4]Biogeochemistry Research Centre, University of Bristol, Bristol BS8 1TS

Manuscript; Journal of the Geological Society Special Publication

MS. Dated; **July 26, 1994**

Running Head; Lipids of hydrothermal vent organisms

*Corresponding Author

Lipid structures and distributions, especially those of fatty acids and sterols, can be used to gain information about the ecological conditions and diets of various types of marine organisms (e.g. Sargent and Whittle; 1981; Bradshaw et al., 1990). The vast majority of the studies on marine invertebrate lipids as related to diet have been upon coastal and estuarine species which depend upon carbon derived from photosynthetic organisms, as such, the lipids of these invertebrates are generally dominated by structures which can be related directly or indirectly to corresponding structures in the dietary lipids (Bradshaw, 1988). Recent investigations on organisms which derive part of their diet from bacterial sources, such as those which harbour endosymbiotic bacteria, demonstrate that corresponding structural similarities can be observed between host and symbionts (Conway and McDowell Capuzzo, 1991; Ben Mligh et al., 1992; Fang et al., 1993).

Primary production by chemosynthetic bacteria forms the base of food webs within deep-sea hydrothermal vents and therefore a bacterial signature is expected in the lipids of vent organisms, though, carbon derived ultimately from the photic zone may play an important role (Dixon et al., 1994). Ecologically and biochemically interesting cases of eukaryotic dependence on bacterial primary production are found in associations between endosymbiotic chemoautotrophic bacteria and invertebrate hosts, with vestimentiferan tubeworms (e.g. Riftia pachyptila, the giant tubeworm from hydrothermal vents on the East Pacific Rise (EPR)) providing the most extreme example of this kind of association. R. pachyptila and other species within the Vestimentifera have lost all vestiges of a digestive system in the adult and rely almost exclusively on their endosymbionts for nutrition, although some uptake of dissolved organic material from the surrounding seawater may make a contribution to the diet of these worms. In Bathymodiolid mussels (e.g. Bathymodiolus thermophilus from eastern Pacific hydrothermal vents), a functional gut is present and nutrition from endosymbiotic bacteria in the gills is apparently supplemented by suspension feeding (Le Pennec and Prieur, 1984). Of equal interest to host/symbiont associations are the myriad species of grazing invertebrates at hydrothermal vents (small crustaceans, polychaetes, molluscs, etc.) that are ultimately dependent on chemoautotrophic production. From the same region where Riftia pachyptila and Bathymodiolus thermophilus specimens were abundant, we sampled several

Abstract

Lipid compositions are reported for three distinctive deep sea hydrothermal vent invertebrate species collected around 9°N East Pacific Rise; Riftia pachyptila Jones, a vestimentiferum tubeworm, Bathymodiolus thermophilus Kenk and Wilson, a mussel, and Halice hesmonectes Martin et al., an amphipod crustacean. The lipid compositions of all these organisms were dominated by components characteristic of diets based upon bacteria, with only very minor contributions from carbon derived from the oceanic photic zone. In all the organisms studied, large abundances of n-7 fatty acids, polyunsaturated fatty acids with unsaturations separated by more than one methylene bond, and sterol distributions dominated by cholesterol were observed. Branched fatty acids were generally of low abundance, whilst polyunsaturated fatty acids separated by single methylene groups were either absent, as in the case of R. pachyptila or in very low abundance as in the case of B. thermophilus and H. hesmonectes. Monounsaturated fatty acids were the most abundant component of R. pachyptila lipids, whilst non-methylene interrupted fatty acids were particularly abundant in the lipids of B. thermophilus (up to 45 % of total fatty acids). The lipids of H. hesmonectes were dominated by storage lipids (e.g. wax esters).

Stable carbon isotope analyses of individual sterols from the organisms examined allows specific sources to be proposed for these biochemicals. The $\delta^{13}C$ values of sterols from R. pachyptila were consistent with de novo biosynthesis, whilst that of cholesterol from B. thermophilus corresponded to that from marine phytoplankton. The $\delta^{13}C$ values of sterols from H. hesmonectes fell into two different groups and suggest at least two distinct sources of sterols are available to these crustacea in the vent ecosystem one of which derives from phytoplankton. Overall, the combination of the interpretation of lipid structure and distribution with compound specific isotope analyses can lead to valuable insights into trophic relationships within the deep sea hydrothermal ecosystem.

-end of abstract-

Samples

Sites; Samples were collected from four geographically distinct locations along the East Pacific Rise, and designated sites A to D as indicated in Table 1, a guide to the organisms sampled is as follows;

Tubeworms; The tubeworm samples were of Riftia pachyptila Jones (Phylum Vestimentifera; Class Axonobranchia). Tissues analysed were vestimentum (which harbours no symbionts) and trophosome (where sulphur oxidising bacteria live). All tissues were from single organisms.

Mussels; The mussel samples were all of Bathymodiolus thermophilus Kenk and Wilson (Phylum Mollusca; Class Bivalvia). Tissues examined were foot and adductor muscles, both of which are symbiont free, and gill which harbours sulphur oxidising bacteria. All tissues were from single organisms.

Amphipods; The amphipods were all of Halice hesmonectes Martin et al. (1993) (Phylum Arthropoda; Class Crustacea). For analyses, approximately 10 - 30 organisms were pooled (individual organisms being 1-3mm length) and so amphipod analyses represent 'snapshots' of the feeding behaviour of a larger population.

Lipid Extraction

Two main isolation procedures were used for the preparation of lipids, the first being the preparation of total fatty acids and total sterols. A second procedure was used so that fatty acids could be separated into biochemically distinct classes, ie. those bound as neutral (storage) and polar (structural/membrane) lipids.

Total lipid (TL) samples were extracted by sonication of dried samples in 2:1 dichloromethane/methanol, TL extracts were subsequently saponified in saturated KOH/methanol following the method of Bradshaw (1990). Total lipid fatty acids (TLFA) and total lipid neutrals were separated using aminpropyl phase cartridges (Bond Elut; Analytichem), eluting total lipid extracts with 2:1 dichloromethane/isopropanol for neutral lipid fractions (including sterols) and 2 % acetic acid in ethyl ether for fatty acid fractions. Prior to GC analyses total lipid fractions were derivatized using BSTFA.

populations of an amphipod crustacean, Halice hesmonectes (Martin et al., 1993), which swarms in the outflow area of warm water vents, most often above mussels or tubeworms but also above areas of bare rock (Van Dover et al. 1992; Kaartvedt et al. 1994).

Halice hesmonectes has been presumed to ingest suspended microorganisms flowing out from subsurface reservoirs (Van Dover et al. 1992), though ingestion of detritus, as is common for pelagic amphipods is also likely to be important. Therefore, additional to looking for bacterial signatures in the lipids of the amphipods, we have been interested in identifying lipids that might be derived from vent organisms or from carbon derived from the oceanic photic zone. Amphipods, and crustaceans in general, are held to be unable to synthesize sterols de novo (Goad, 1978; Kerr and Baker, 1991), and since H. hesmonectes seem to lack symbiotic bacteria (Martin et al., 1993), they must assimilate sterols either directly from their diet or from modification of dietary sterols. In order to help assign sources of individual sterols, compound specific isotope analyses (CSIA) have been employed (e.g. Hayes et al., 1990; Rieley, 1991). Stable carbon isotope compositions ($\delta^{13}C$ values) are useful because of the distinctive fractionations imparted due to carbon source and metabolic effects (Hayes, 1993).

Experimental

Sampling

The organisms in this study were collected at active venting sites along the East Pacific Rise at 9°N during December 1991, using the DSRV Alvin (Support ship; RV Atlantis II, Chief Scientists; Mullineaux and Van Dover). Vestimentiferum tubeworm (Riftia pachyptila) and mussel (Bathymodiolus thermophilus) samples were collected using the manipulator arm of the submersible. Amphipods (Halice hesmonectes) were collected using a vacuum pump system. On board ship, these samples were individually sorted, and oven dried at 60°C for 12 hours. On reaching the surface these samples were dissected on board ship and either dried (Add and Vest) as for the amphipods or stored at -70°C until further analysis on land (Gill, Foot and Troph). A key to the samples examined is given in Table 1.

Results

Fatty Acid Compositions

Fatty Acid Nomenclature

Fatty acids are conventionally written as a:b(n-c), where a is the carbon number of the fatty acid, b the number of double bonds, and c the number of carbons to the first double bond from the terminal methyl. Unless otherwise noted, all double bonds are separated by one methylene group. The prefixes i and a indicate methyl branching at positions 2 and 3 respectively from the terminal methyl.

Tubeworm (Riftia pachyptila);

The fatty acid compositions of the tubeworm samples were characterised by very high abundances of (n-7) fatty acids with up to 84 % of the total fatty acids of the tubeworm trophosome storage lipids having unsaturations seven carbons from the terminal methyl (Tables 2 to 5). The major (n-7) fatty acids identified in all lipid extracts were 16:1(n-7) and 18:1(n-7) (Tables 3 to 5). Only minor amounts of (n-9) fatty acids were observed, with a maximum of 2 % of trophosome structural lipids being of this type (Table 2). Small amounts of non-methylene interrupted (NMI) dienoic fatty acids were observed in all the tubeworm samples, at around 3 to 4 % of total fatty acids (Tables 2 to 5). No single-methylene interrupted (SMI) polyunsaturated fatty acids were detected in the tubeworm samples. Only very small abundances of branched fatty acids (0.1 to 0.5% of total fatty acids; Table 2) were observed in the lipids of all the tubeworm tissues examined, the most abundant component generally being i19:0 (Tables 3 to 5).

Mussel (Bathymodiolus thermophilus)

The fatty acids of the mussel samples were characterised by very large abundances of non-methylene interrupted (NMI) polyunsaturated fatty acids, as can be observed upon the gas chromatogram displayed in Figure 1. This class of fatty acid represented up to 45 % of total fatty acids identified in the mussel tissues (Tables 2 to 5). The most abundant components observed were 20:2(n-7, n-15) and 22:2(n-7, n-15). Fatty acids with n-7 unsaturations were by far the most dominant type observed, being up to 45 % of total identified (Table 2), this

For the separation of structural and storage lipids, dried samples were extracted using a modified Bligh/Dyer method (Bligh and Dyer, 1959; Rieley, 1993). The resultant extract was separated into the required lipid extracts using silicic acid column chromatography, sequentially eluting with chloroform, acetone and methanol to give neutral lipid (NL) from the chloroform elution sterp and polar lipid (PL) fractions from the methanol elution step (Hedrick et al., 1991).

Neutral lipids; NL fractions were saponified as for total lipid (TL) samples, as outlined above, though instead of derivatizing free fatty acids with BSTFA, the acids were converted to neutral lipid fatty acid (NLFA) methyl esters using BF_3/Methanol (Bradshaw et al., 1990).

Polar lipids; Fatty acids were released from the polar lipids by heating PL fractions at 100°C for 1 h in 0.05 N KOH in methanol giving polar lipid fatty acid (PLFA) methyl esters (Hedrick et al., 1991).

Lipid Characterisation

GC / GC-MS; Lipids were analysed by gas chromatography (GC) using a 50m bonded methyl silicone phase capillary column (CP Sil 5CB; Chrompack), 0.12 μm thickness, 0.32 mm internal column diameter. Fatty acid samples were also analysed upon a Supelco Omegawax 320, 30 m x 0.32 mm i.d., 0.3 μm phase thickness capillary column. Hydrogen was used as carrier gas. Lipids were identified using GC- mass spectrometry (GC-MS), using a GC system as above, except using helium as carrier gas. The GC was attached to a Finnigan Mat 4500 mass spectrometer (70eV ionisation voltage, scan time 1s). Temperature program for the methyl silicone column was 40 - 150°C at $10°min^{-1}$ 150 - 300°C at $4°min^{-1}$, isothermal for 25 min. Temperature program for the Omegawax 320 column was 40 - 150°C at $10°min^{-1}$ 150 - 240°C at $4°min^{-1}$, isothermal for 10 min.

Positions of fatty acid unsaturation were determined by the formation of dimethyl disulphide (DMDS) adducts of fatty acid methyl esters, followed by GC-MS identification (Yruela et al., 1990).

GC-IRMS; Isotopic compositions of sterols were determined using a Finnigan MAT delta-S isotope ratio mass spectrometer as described by Rieley et al. (1993). Values reported are corrected for the addition of trimethyl silyl groups (cf. Rieley, 1994).

Mussel (Bathymodiolus thermophilus)

A variety of sterols were observed in the mussel samples (Table 6), the most abundant component being cholesterol (68 % of total sterols; Table 6). As well as cholesterol, a wide variety of minor sterols were observed (Table 6), such as cholesta-5,22-dienol, 5α-cholestanol and cholest-7-enol. The $\delta^{13}C$ value of cholesterol from B. thermophilus was -26.5 ‰ as compared to a bulk tissue $\delta^{13}C$ value of -33 ‰ (Table 7).

Amphipods (Halice hesmonectes)

A large variety of sterols were also observed in the amphipod samples (Table 6), their distribution being similar to those of the mussel samples (Table 6). The sterol distributions were dominated by cholesterol in accord with distributions observed in other marine crustacea (Kerr and Baker, 1991). High abundances of 5α-cholestanol were observed in one of the amphipod samples (A160; Table 7).

$\delta^{13}C$ values of the amphipod cholesterol fell into two categories one around -16 ‰ the other around -21 ‰ (Table 7), these categories corresponding to two sets of bulk tissue $\delta^{13}C$ values (-14 ‰ and -24 ‰; Table 7).

Discussion

Fatty Acid Compositions

Tubeworm (Riftia pachyptila);

Vestementiferum tubeworms provide a striking example of eukaryotic dependence on carbon fixed by chemoautotrophic bacteria. Adults have no digestive systems and rely almost exclusively upon their endosymbionts for nutrition, though uptake of carbon gained from dissolved organic carbon may also make a minor contribution (Childress and Fisher, 1992). Riftia pachyptila, the giant tubeworm associated with East Pacific vent sites, is representative of this dependence, being fixed at sites of expulsion of warm vent water rich in dissolved sulphide.

The dependence of R. pachyptila upon carbon fixed by endosymbiotic bacteria is apparent in the lipid compositions identified in this study, and especially in the fatty acid compositions. Very high abundances of (n-7) fatty acids (Table 2), and corresponding minor amounts of (n-9)

being in contrast to the low abundance of n-9 fatty acids (around 5 to 13 %; Table 2). Small amounts of the single methylene interrupted polyunsaturated fatty acid arachidonic acid 20:4(n-6) were detected (0.7 % of structural PLFA; Table 5).

Minor abundances of branched fatty acids were observed (around 1 to 3 %; Table 2), the most abundant component being i19:0 (Tables 3 to 5). Greater abundances of branched fatty acids were observed in the gill tissues than in the foot and adductor tissues (Table 2).

Amphipods (Halice hesmonectes)

The fatty acids of H. hesmonectes were characterised by high abundances of 'storage' fatty acids (NLFA; Table 2) indicating high concentrations of wax esters in these organisms as observed in many deep sea crustacea (Sargent et al., 1976). As with the mussel samples, NMI fatty acids were observed in relatively high abundance (up to 25 %; Tables 2 to 5). NMI fatty acids were present in high abundance in the structural fatty acids (PLFA) of the amphipod samples (up to 11 % of total fatty acids; Table 2). In general, greater abundances of NMI fatty acids were identified in the amphipod storage lipids (NLFA) as compared to that in the structural fatty acids (Table 2). As for the mussel samples, minor amounts of the 20:4(n-6) SMI fatty acid were detected in the amphipod samples (up to 1% of total; Tables 3 to 5). Branched fatty acids constituted up to 5 % of total fatty acids (Table 2), i17:0, i19:0 and a19:0 were the most abundant components (Tables 3 to 5). In general, branched fatty acids were in greater abundance in the storage NLFA lipids than in the structural PLFA (Table 2).

Sterol Compositions

Tubeworm (Riftia pachyptila)

The major sterols identified in the R. pachyptila tissues were cholesterol, cholesta-5,24-dienol, and 24-methylcholesta-5,24(28)-dienol (Table 6), the major tubeworm sterols had $\delta^{13}C$ values of -15.1 ‰, -15.4 ‰ and -16.4 ‰ for cholesterol, cholesta-5,24-dienol, and 24-methylcholesta-5,24(28)-dienol respectively, compared to a total tissue value of -11 ‰ (Table 7), a difference of 4-5 ‰, which is well within that observed for lipids isolated from autotrophic organisms (Abelson and Hoering, 1961; Park and Epstein, 1961).

Indeed, the occurrence of NMI fatty acids is widely reported for bivalves which contain endosymbiotic bacteria, though the abundance of these lipids observed in this study is much larger than previously observed (eg. Klingensmith, 1982; Berg et al., 1985; Zhukova and Svetashev, 1986; Zhukova et al., 1992). The presence of the SMI polyunsaturated fatty acid 20:4(n-6) in the mussel samples (Tables 3 to 5), albeit in very low abundance, demonstrates the assimilation of phytoplankton derived carbon in the deep sea vent ecosystem.

Branched fatty acids are indicative of a wide variety of bacteria, and have been proposed by Zhukova et al. (1992) to be markers for symbiotic bacteria in bivalves. In this study minor abundances of this lipid type were observed (around 1 to 3 %; Table 2) and indicate direct incorporation of bacterial lipids by the bivalves examined. Greater abundances of branched fatty acids were observed in the gill tissues than in the foot and adductor tissues (Table 2), this observation may be due to a combination of two factors; the gills harbour the endosymbiotic bacteria associated with B. thermophilus and also filter particulate bacteria from the water column (Cavanaugh, 1985; Le Pennec and Prieur, 1984; Page et al., 1991).

Amphipods (Halice hesmonectes)

The fatty acid compositions of all the H. hesmonectes samples were remarkably consistent given that they were all taken fom geographically distinct vent sites within the Venture hydrothermal fields (9°N EPR; Tables 1 and 2; Figure 2), which suggests similar food sources for this crustacean in all sites examined. The fatty acids of H. hesmonectes were characterised by high abundances of 'storage' fatty acids (NLFA; Table 2) indication high concentrations of wax esters as observed in many deep se crustacea (Sargent et al., 1976). Wax esters have been proposed to provide both bouyancy and energy storage in such organisms (ibid). As with the mussel and tubeworm samples, NMI fatty acids were observed in relatively high abundance (up to 25%) and may indicate a trophic link between the pelagic amphipod species and the benthic mussel species, i.e. detrital feeding by the amphipod, however, de novo biosynthesis may be a factor. The generally greater abundance of NMI fatty acids in storage lipids (NLFA) than in the structural fatty acids (Table 2) reinforces the contention that these acids are predominately derived from diet (fatty acids surplus to membrane structural requirements being stored). The

fatty acids were observed (Table 2). Bacteria generally have high abundances of (n-7) monounsaturated fatty acids, and studies of marine organisms largely dependent upon bacteria for food reflect the assimilation of this class of fatty acid (Conway and McDowell Capuzzo, 1991; Ben-Mligh et al., 1992; Fang et al., 1993). Small amounts of non-methylene interrupted (NMI) dienoic fatty acids were observed in all the tubeworm samples, at around 3 to 4 % of total fatty acids. No single-methylene interrupted (SMI) polyunsaturated fatty acids were detected, which indicates the lack of filter feeding as a food source for vestimenferum tubeworms. Only very small amounts of branched fatty acids, usually considered bacterial markers, were observed in the lipids of all the tubeworm tissues examined, and indicates that branched fatty acids are only minor components of the sulphur oxidising symbiotic bacteria associated with R. pachyptila (c.f. Conway and McDowell Capuzzo, 1991 who did not identify branched fatty acids in a thiotrophic bacterium isolated from a deep sea hydrothermal vent).

Mussel (Bathymodiolus thermophilus)

The fatty acids of the mussel samples were characterised by very large abundances of non-methylene interrupted (NMI) polyunsaturated fatty acids (Table 2). In the hydrothermal vent mussels examined this large abundance of NMI fatty acids apparently replaces single-methylene interrupted (SMI) polyunsaturated fatty acids which are observed in similarly large abundances in coastal and estuarine bivalves (Joseph, 1982; c.f. Fang et al., 1993), e.g. arachidonic acid; 20:4(n-6). The significance of these observations is that SMI fatty acids are characteristic of marine algae, and the abundance of these acids in marine invertebrates are indicative of a diet based primarily upon carbon fixed by phytotrophs (eg. Bradshaw, 1988). In contrast, SMI fatty acids are very rare in bacteria and therefore invertebrates which depend upon a diet based upon bacteria appear to desaturate bacterially derived monounsaturated (n-7) fatty acids which are extended and desaturated by the invertebrates to produce non-methylene interrupted polyunsaturated fatty acids (c.f. Zhukova, 1986; Fang et al., 1993). The high abundance of NMI fatty acids identified in this study are in accord with the findings of Fang et al. (1993) who reported NMI fatty acid abundances of 14 to 22 % of total fatty acids in bivalves containing endosymbiotic bacteria living around hydrocarbon seeps of the Gulf of Mexico.

seep mussels from the Gulf of Mexico a product/precursor relationship between bacterially produced 4-methyl sterols and 4-methyl sterols observed in the mussels was proposed (Fang et al., 1992). However, only methylotrophic bacteria have been demonstrated to have the ability to biosynthesise sterols and since B. thermophilus has thiotrophic symbiotic bacteria, additional evidence other than sterol distribution and structure is needed to help assign particular sources of sterols in these organisms, such as stable carbon isotopic analyses. Solemya velum a coastal clam, containing symbiotic bacteria, was reported to contain sterols with $\delta^{13}C$ values around -38.5 ‰ compared to a tissue $\delta^{13}C$ value of -33 ‰ (Conway and McDowell Capuzzo, 1991) and as discussed earlier this is within the range expected if de novo biosynthesis is being undertaken. However, cholesterol isolated from B. thermophilus adductor tissues in this study had a $\delta^{13}C$ value of -27 ‰ compared to a tissue $\delta^{13}C$ value of -34 ‰ (Table 7). The 7 ‰ higher $\delta^{13}C$ value for the mussel cholesterol compared to total tissue rules out de novo biosynthesis as a major source and thus requires that the mussel studied derive sterols from filter feeding (Goad, 1978). Dixon et al. (1994) have demonstrated that phytoplankton are a significant proportion of the detritus associated with deep sea hydrothermal vents. Furthermore, electron microscopic studies on the gut of a mytilid from 12°N EPR showed that phytoplankton are present, and a source for the B. thermophilus sterols from phytodetritus is proposed, this proposal being reinforced by published $\delta^{13}C$ values for marine phytoplankton (Abelson and Hoering, 1961; Park and Epstein, 1961) which are in the range of -23 ‰ (ibid).

Amphipods (Halice hesmonectes)

The large variety of sterols observed in the amphipod samples (Table 6), had a distribution similar to those of the mussel samples and to those of marine crustacea from coastal and estuarine habitats (Goad, 1978; Bradshaw et al., 1990; Kerr and Baker, 1991). The significance of the sterol distributions in a trophic context is that crustacea are held to be unable to biosynthesise sterols de novo and must assimilate these vital biochemicals from their diet (Goad, 1978; Kerr and Baker, 1991). In more conventional marine settings, crustacean sterols derive from algae which are rich in a variety of sterols (ibid). However, in deep sea hydrothermal vents phytoplankton are unable to exist, and bacteria replace them as the base of

presence of NMI fatty acids in high abundance in the structural fatty acids (PLFA) of the amphipod samples (up to 11 % of total fatty acids; Table 2) demonstrates that this class of fatty acid plays a vital structural role in the amphipod cellular membranes. As for the mussel and tubeworm samples, SMI fatty acids were not detected in the amphipod samples.

Sterol Compositions

Tubeworm (Riftia pachyptila)

The major sterols identified in the R. pachyptila tissues were the sterols cholesterol, cholesta-5,24-dienol, and 24-methylcholesta-5,24(28)-dienol (Table 6). The presence of these sterols suggests de novo biosynthesis by the tubeworm symbiosis (most likely biosynthesis by the tubeworm proper rather than thiotrophic endosymbiotic bacteria), the latter sterols being intermediates in the conversion of the sterol precursor lanosterol to cholesterol (Lehninger, 1978; Kerr and Baker, 1991). Stable carbon isotopic analyses of the R. pachyptila sterols provides strong evidence that this tubeworm species does indeed biosynthesise sterols de novo. The $\delta^{13}C$ values of the major tubeworm sterols were 4-5 ‰ lower than that of the total tissue value of -11 ‰ (Table 7), in the range for that observed for lipids isolated from autotrophic organisms (Abelson and Hoering, 1961; Park and Epstein, 1961; Hayes, 1993). If the tubeworm sterols were derived from marine phytodetritus (or from phytoplankton derived organic matter), a value of around -27 ‰ may be expected (ibid). Minor sterols were also observed, such as 24-ethylcholesterol (Table 6), which have known phytoplankton sources (Kerr and Baker, 1991) and may indicate the assimilation by the tubeworm of a small amount of dissolved organic carbon.

Mussel (Bathymodiolus thermophilus)

The most abundant sterol identified in the mussel samples was cholesterol (Table 6), in line with observations on other bivalves collected from coastal environments (Goad, 1978; Conway and McDowell Capuzzo, 1991). As well as cholesterol, a wide variety of minor sterols were observed (Table 6), including a number of sterols which are indicative of algae, such as cholesta-5,22-dienol (Goad, 1978; Kerr and Baker, 1991). Biosynthesis of sterols by bivalves is controversial (Conway and McDowell Capuzzo, 1991), and in the case of cold hydrocarbon

have at least two sources in the vent system examined, one possibly deriving ultimately from the photic zone of the ocean.

This work was supported by funds from the Natural Environment Research Council (Grant numbers GR3/2951, GR3/3748 and GR3/7731) and by funds from the National Science Foundation (USA).

the food chain. Bacteria are generally devoid of sterols and therefore the sterol distributions and the stable carbon isotopic composition of those sterols in vent crustacea are of particular interest.

$\delta^{13}C$ values of the amphipod cholesterol fall into two categories one around -16 ‰ the other around -21 ‰ (Table 7), these categories corresponding to two sets of bulk tissue $\delta^{13}C$ values (-14 ‰ and -24 ‰; Table 7). The separation of the amphipod bulk tissue and cholesterol $\delta^{13}C$ values into two classes is presumably determined by diet, and suggests that at least two sources of dietary sterols are available to the amphipods, one which is similar to that observed in the tubeworm sample (i.e. most likely to derive from vent organisms) and one similar to that observed in the mussel sample (i.e. most likely derived from phytoplankton). Different proportions of these dietary sources will lead to the $\delta^{13}C$ values observed in this study.

Overview

Overall, of all lipid types fatty acids give the greatest indication as to the main diet of hydrothermal vent organisms. In this study a diet based primarily upon bacteria is associated with high abundances of (n-7) fatty acids, low abundances of (n-9) fatty acids, high abundances of NMIP fatty acids and low abundances of MIP fatty acids. These findings are in accord with similar studies upon cold hydrocarbon seep organisms, and studies upon molluscs which contain symbiotic bacteria. The relative abundance of NMI to SMI in marine organisms gives a measure of the relative contributions of bacteria and phytoplankton in their diet. The deep sea hydrothermal vent organisms analysed in this study from 9°N East Pacific Rise are an extreme example of almost total dependence upon bacterially fixed carbon which is reflected by very high abundances of NMI fatty acids and correspondingly low abundances of SMI fatty acids.

In contrast to the fatty acids, sterols, due to their absence in the majority of bacteria and due to limitations on de novo biosynthesis in certain invertebrates, provide an indication of assimilation of carbon from organisms from the photic zone of the ocean. Stable carbon isotopic analyses of individual compounds can provide additional evidence in order that the source of individual lipids can be assigned. For example, cholesterol has been demonstrated to

Fång J., Comet P. A., Wade T. L., and Brooks J. M. (1992) Gulf of Mexico hydrocarbon seep communities- IX. Sterol biosynthesis of seep mussels and its implications for host-symbiont association. Org. Geochem. **18**, 861-867

Fang J., Comet P. A., Brooks J. M., and Wade T. L. (1993) Nonmethylene - interrupted fatty acids of hydrocarbon seep mussels: ocurrence and significance. Comp. Biochem. Physiol. **104B**, 287-291

Goad, L.J. (1978) In Marine Natural Products (ed. P.J. Scheuer). pp76-172. Academic Press, London.

Hayes J. M. (1993) Factors controlling ^{13}C contents of sedimentary organic compounds: principles and evidence. Mar. Geol. **113**, 111-125.

Hayes J.M., Freeman K.H., Popp B.N., and Hoham C.H. (1990) Compound-specific isotopic analyses: A novel tool for reconstruction of ancient biogeochemical processes. Org. Geochem. **16**, 1115-1128

Hedrick D. B., Guckert J. B., and White D. C. (1991) Archaebacterial ether lipid diversity analyzed by supercritical fluid chromatography: integration with a bacterial lipid protocol. J. Lipid Res. **32**, 659-666

Joseph J. D. (1982) Lipid composion of marine and esturarine invertebrates. Part II: Mollusca. Prog. Lipid Res. **22**, 109-153

Kaartvedt S., Van Dover C.L., Mullineaux L.S., Wiebe P.H. and Bollens S.M. (1994) Amphipods on a deep-sea hydrothermal treadmill. Deep Sea Res. I **41**, 179-195

Klingensmith J.S. (1982) Distribution of methylene and nonmethylene-interrupted dienoic fatty acids in polar lipids and triacyglycerols of selected tissues of the hardshell clam (Mercenaria mercenaria). Lipids **17**, 976-981

Kerr R. G. and Baker B. J. (1991) Marine Sterols. Natural Products Reports **8**, 465-497

References

Abelson P. H. and Hoering T. C. (1961) Carbon isotope fractionation in formation of amino acids by photosynthetic organisms. Proc. Nat. Acad. Sci. **47**, 623-627

Ben-Mligh F., Marty J.-C., and Fiala-Medioni A. (1992) Fatty acid composition in deep hydrothermal vent symbiotic bivalves. J. Lipid. Res. **33**, 1797-1806

Berg C.J., Kryzynowek J., Alatalo P. and Wiggin K. (1985) Sterol and fatty acid composition of the clam, Codakia orbicularis, with chemoautotrophic symbionts. Lipids **20**, 116-120

Bligh E. G. and Dyer W. J. (1959) A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. **37**, 911-917

Bradshaw S. A. (1988) The biogeochemistry of lipids in model marine food chains. Ph.D. Thesis, University of Bristol, Bristol, U.K.

Bradshaw S. A., O'Hara S. C. M., Corner E. D. S., and Eglinton G. (1990) Changes in lipids during simulated herbivourous feeding by the marine crustacean Neomysis integer. J. Mar. Biol. Ass. **70**, 225-243

Cavanaugh C.C. (1985) Symbioses of chemoautotrophic bacteria and marine invertebrates from hydrothermal vents and reducing sediments. Biol. Soc. Wash. Bull. **6**, 373-388

Childress J. J. and Fisher C. R. (1992) The biology of hydrothermal vent animals: physiology, biochemistry, and autotrophic symbioses. Oceanogr. Mar Biol. Annu. Rev. **30**, 337-441

Conway N. and McDowell Capuzzo J. (1991) Incorporation and utilization of bacterial lipids in the Solemya velum symbiosis. Mar. Biol. **103**, 277-291

Dixon D.R., Dixon L.R.J., Jollivet D., and Holland P.W.H. (1994) Molecular identification of early reproductive stages of hydrothermal vent organisms. J. Geol. Soc. this volume

Sargent J. R., Lee R. F., and Nevenzel J. C. (1976) In Chemistry and biochemistry of natural waxes. pp50-91. Elsevier, Amsterdam.

Sargent J. R. and Whittle K. J. (1981) In Analysis of marine ecosystems. (ed. A. R. Longhurst), pp491-533. Academic Press, London.

Van Dover C.L., Kaartvedt S., Bollens S.M., Wiebe P.H., Martin J.W. and France S.C. (1992) Deep-sea amphipod swarms. Nature 358, 25-26

Yruela I., Barbe A., and Grimalt J. O. (1990) Determination of double bond position and geometry in linear and highly branched hydrocarbons and fatty acids from gas chromatography - mass spectrometry of epoxides and diols generated by stereospecific resin hydration. J. Chomatogr. Sci. **28**, 421-427

Zhukova N. M. (1986) Biosynthesis of non-methylene-interrupted dienoic fatty acids from [^{14}C] acetate in molluscs. Biochimica Biophysica Acta, **878**, 131-133

Zhukova N. M. and Svetashev V. I. (1986) Non - methylene - interrupted dienoic fatty acids in molluscs from the Sea of Japan. Comp. Biochem. Physiol. **83B**, 643-646

Zhukova N. M., Kharlamenko V. I., Svetashev V. I., and Rodionov I. A. (1992) Fatty acids as markers of bacterial symbionts of marine bivalve molluscs. J. Exp. Mar. Biol. Ecol. **162**, 253-263

Lehninger A. L. (1978) Biochemistry, 2nd edition. pp1104. Worth, New York.

Martin J.W., France S.C., Van Dover C.L. (1993) Halice hesmonectes, a new species of pardaliscid amphipod (Crustacea. Peracarida) from hydrothemal vents in the eastern Pacific. Can. J. Zool. **71**, 1724-1732

Page H.M, Fiala-Médioni A., Fisher C. and Childress J.J. (1991) Experimental evidence for filter-feeding by the hydrothermal vent mussel, Bathymodiolus thermophilus. Deep Sea Res., **38**, 1455-1461

Park R. and Epstein S. (1961) Metabolic fractionation of ^{13}C and ^{12}C in plants. Plant Physiol. **36**, 133-138

Le Pennec M. and Prieur D. (1984) Biologie marine - observations sur la nutrition d'un mytilidae d'un site hydrothermal actif de la dorsale du Pacifique orental. C. R. Acad. Sc. Paris Série III **17**, 493-498

Rieley G. (1993) Molecular and isotopic studies of natural environments. Ph.D. Thesis, University of Bristol, UK.

Rieley G. (1994) Derivatization of organic compounds prior to gas chromatographic - combustion -isotope ratio mass spectrometric analysis: identification of isotope fractionation processes. Analyst **119**, 915-919

Rieley G., Collier R. J., Jones D. M., Eglinton G., Eakin P. A., and Fallick A. E. (1991) Sources of sedimentary lipids deduced from stable carbon-isotope analyses of individual compounds. Nature **352**, 425-427

Rieley G., Collister J.W., Stern B. and Eglinton G. (1993) Gas chromatography - isotope ratio mass spectrometry of leaf wax n-alkanes from plants of differing carbon dioxide metabolisms. Rapid Commun. Mass Spectrom. **7**, 488-491

Sample	A158	A162	A160	A202	A214	A187	Add	Vest
Site	B	C	C	D	D	A	A	C
TLFA								
Total	41761	40253	28455	35534	31022	25972	9728	21897
sats	21.4	17.7	16.9	17.4	21.3	16.9	30.3	17.0
unsats	78.6	82.3	83.1	82.6	78.7	83.1	69.7	80.1
polyuns	9.2	12.6	8.9	17.2	6.5	9.1	40.0	3.8
monouns	69.3	69.7	74.2	65.4	72.2	74.0	29.7	76.3
n-7	60.6	60.3	66.5	60.6	58.9	66.6	38.1	78.7
n-9	3.4	3.9	9.2	12.0	8.9	8.9	4.8	0.7
NMI	8.9	12.2	8.2	17.0	6.5	9.1	40.0	3.8
branched	1.1	2.6	2.0	1.2	1.7	2.0	1.0	0.4

Sample	A158	A162	A160	A202	A214	Gill	Foot	Troph
Site	B	C	C	D	D	D	D	C
NLFA								
Total	9208	22259	13487	21498	21718	1453	1861	11280
sats	31.3	3.7	20.4	18.4	12.4	21.9	36.2	10.7
unsats	68.7	96.3	79.7	81.6	87.6	78.1	63.8	86.2
polyuns	14.1	4.4	24.8	22.7	18.9	42.4	31.5	3.0
monouns	54.7	91.9	54.9	58.9	68.7	35.7	32.3	83.2
n-7	43.5	89.0	36.8	34.5	46.8	37.0	25.8	84.4
n-9	9.71	3.4	19.1	32.0	22.5	7.6	6.2	0.5
NMI	14.0	4.0	22.5	22.5	17.0	42.4	31.5	3.0
branched	0.8	1.1	5.1	1.7	1.6	1.9	1.1	0.5

Sample	A158	A162	A160	A202	A214	Gill	Foot	Troph
Site	B	C	C	D	D	D	D	C
PLFA								
Total	8497	6413	8147	9562	5040	1091	2074	2957
sats	34.3	21.2	16.0	20.6	19.1	21.0	20.7	17.9
unsats	65.7	78.9	84.0	79.4	80.9	79.0	79.3	82.1
polyuns	5.7	10.5	12.5	13.9	11.7	44.5	45.2	7.6
monouns	60.0	68.4	71.5	65.5	69.2	34.5	34.1	74.5
n-7	52.7	62.1	57.5	54.7	56.4	42.1	44.8	73.2
n-9	2.2	3.0	7.7	8.5	4.9	13.0	11.2	2.4
NMI	5.3	9.6	10.7	11.9	10.2	43.8	44.5	4.4
branched	0.7	0.3	0.7	0.5	0.4	3.8	1.3	0.1

Table 2 Total fatty acid concentrations, in $\mu g\ g^{-1}$ total extract, and relative concentrations for different classes of fatty acid, as % of total fatty acids identified, for total lipid (TLFA), neutral lipid (NLFA) and polar lipid fatty acid (PLFA) fractions of the organisms studied. Sites refer to geographical localities given in Table 1a.

Sites	Samples	Latitude	Longitude
Site A	A123 A187 Add	9°48.40'	104°17.16
Site B	A158	9°49.86'	104°17.41
Site C	A160 A162 Vest Troph	9°50.56'	104°17.49
Site D	A200 A202 A214 A216 Gill Foot	9°30.88'	104°14.67

Table 1. Sample key to hydrothermal vent organisms studied, by site. Axxx indicate amphipod (Halice hesmonectes) samples; Foot = mussel (Bathymodiolus thermophilus) foot; Add = mussel adductor; Gill = mussel gill; Vest = tubeworm (Riftia pachyptila) vestimentum; Troph = tubeworm trophosome

GC #[1]	Fatty Acid	A158 B	A162 C	A160 C	A202 D	A214 D	Gill D	Foot D	Troph C
-	14:0						0.61	0.66	
1	15:0						0.11	0.39	
3	16:0	19.40	1.95	5.43	9.49	8.29	6.99	16.17	9.47
6	17:0								
12	18:0	8.58	0.51	4.23	4.73	2.29	8.98	16.69	0.80
-	19:0						0.14	0.10	
22	20:0	2.44	0.17	5.62	2.47	0.19	2.17	0.34	
29	22:0						0.95	0.74	
-	i14:0								
4	i17:0	0.28	0.26	0.31	0.09	0.25	0.47		
-	a17:0						0.44	0.42	
14	i19:0	0.23	0.16	1.17		0.32	0.97	0.37	0.45
14	a19:0	0.06	0.58	1.80	0.68	0.94			
-	i21:0						0.31	0.36	
-	14:1(n-7)								
2	16:1(n-7)	9.43	76.84	13.02	8.01	11.83	4.15	4.49	61.69
5	17:1(n-7)	0.25	0.10	1.79	0.94	0.10			
-	18:1(n-5)								
11	18:1(n-7)	26.94	10.49	15.39	20.95	28.85	2.32	2.01	19.71
10	18:1(n-9)	3.64	2.29	9.12	8.71	8.94	0.76	0.76	0.50
10	18:1(n-13)	0.91	0.13	0.62	0.45	0.49	1.56	1.71	0.30
15	19:1(n-7)						5.14	2.75	
21	20:1(n-7)	9.76	0.97	6.34	5.21	5.97	9.20	8.53	0.45
20	20:1(n-9)	1.22	0.55	5.76	10.35	7.72	2.63	2.20	
20	20:1(n-13)	2.76	0.57	4.61	5.21	4.94	6.41	7.77	0.57
-	21:1(n-7)						0.71	0.48	
28	22:1(n-7)						0.19	0.09	
-	22:1(n-9)						2.18	1.13	
9	18:2(n-7,13)	0.22	1.01	0.71	0.12	1.12	0.14	0.32	0.80
9	18:2(n-7,15)	0.49	0.13	0.93		0.46			
8	18:2(n-9,15)	2.90	0.26	1.13	0.90	1.43	0.51	0.34	
19	20:2(n-7,13)	2.90	0.06	0.35	0.36	1.20			
19	20:2(n-7,15)		0.03	0.55	0.28	1.50	11.79	7.40	1.39
18	20:2(n-9,15)	1.52	0.18	1.29	10.35	2.23	3.69	2.84	
24	21:2(n-7,14)	2.28	0.30	3.68	3.81	0.25	1.59	1.40	
24	21:2(n-7,16)						2.12	1.02	
-	21:2(n-?)						0.45	0.78	
27	22:2(n-7,15)	1.25	0.23	2.15	0.93	1.61	8.5	5.53	0.83
27	22:2(n-9,15)	0.43	0.16	1.84	1.70	2.14			
-	24:2(n-?)	1.06	0.48	1.53	2.85	0.55			
7	18:3		0.05	0.77	0.12	0.45	7.26	7.53	
-	18:3	0.96	1.15	6.87	1.10	2.64			
17	20:3		0.03	0.69		1.36	5.32	3.31	
25	22:3						0.80	0.44	
26	22:3						0.16	0.53	
16	20:4(n-6)	0.08	0.36	2.30	0.17	1.93			

Table 4. Relative concentrations of neutral lipid fatty acids, as % total neutral lipid fatty acids identified as their methyl esters (NLFAME). Unidentified isomers are given in order of elution (CP Sil 5CB, 50m). [1]Numbers refer to GC peaks in Figure 1.

GC #[1]	Fatty Acid	A158 B	A162 C	A160 C	A202 D	A214 D	A187 A	Add A	Vest C
-	14:0	0.86	6.39	0.17	1.01	0.97	0.78	0.65	3.23
1	15:0			0.23	0.07		0.23		
3	16:0	14.11	7.49	12.1	10.74	15.49	12.07	15.10	12.35
6	17:0		0.67		0.15	0.13			
12	18:0	2.49	0.33	1.75	1.86	2.31	1.77	12.35	1.03
-	19:0					0.70			
22	20:0	2.85	0.20		2.36	0.03		0.31	
29	22:0							0.97	
-	i14:0		1.67						
4	i17:0	0.27		0.46	0.22	0.36	0.47	0.46	
-	a17:0					0.25			
14	i19:0	0.87	0.94	1.12	1.02	0.43	1.14	0.96	0.38
14	a19:0			0.42		0.62	0.43		
-	i21:0								
-	14:1(n-7)	0.51		0.32	0.38	0.29	0.36		
2	16:1(n-7)	15.74	33.50	23.3	19.27	25.99	23.35	5.31	54.27
5	17:1(n-7)	0.78	0.13		0.49			0.19	
-	18:1(n-5)	1.10	3.93	1.15		0.70	1.15		
11	18:1(n-7)	34.24	21.81	32.16	23.91	25.95	32.06	2.91	20.19
10	18:1(n-9)		2.67	6.79	6.37	6.00	6.42	0.67	0.74
10	18:1(n-13)	4.71	2.35	0.92	0.95		0.40	1.04	1.03
15	19:1(n-7)	0.14						3.53	
21	20:1(n-7)	5.34	3.07	5.36	5.00	5.61	5.43	4.05	0.45
20	20:1(n-9)	1.84	0.34	1.57	4.06	2.39	2.70	2.02	
20	20:1(n-13)	4.41	1.92	2.65	4.98	5.32		7.61	0.62
-	21:1(n-7)							0.49	
28	22:1(n-7)	0.50						0.63	
-	22:1(n-9)							1.21	
9	18:2(n-7,13)	0.15				0.41			0.81
9	18:2(n-7,15)			0.85	1.98		0.87	0.32	
8	18:2(n-9,15)	0.87		0.71	0.21	0.45	0.72	2.10	
19	20:2(n-7,13)				0.23			1.02	
19	20:2(n-7,15)	0.75	1.13	3.86	1.80	0.56	3.94	10.16	2.04
18	20:2(n-9,15)	0.85		0.77	2.99	2.23	0.79	2.98	
24	21:2(n-7,14)	1.81	0.24		3.56	0.03		1.48	
24	21:2(n-7,16)							1.32	
-	21:2(n-?)							0.69	
27	22:2(n-7,15)	1.11	0.38	0.61	1.21	0.10	0.62	7.28	0.95
27	22:2(n-9,15)	0.72	0.93	018	1.39	0.10	0.19	0.03	
-	24:2(n-?)	0.69	0.29		2.74	0.02			
7	18:3	1.04	9.19	0.94	0.67	0.25	0.96	8.38	
-	18:3								
17	20:3	0.94		1.01	0.20	0.51	1.03	3.43	
25	22:3					1.78		0.35	
26	22:3							0.17	
16	20:4(n-6)	0.29	0.39		0.20	0.04			

Table 3. Relative concentrations of fatty acids, as % of total lipid fatty acid extract (TLFA). (Identified as fatty acid silyl esters). Unidentified isomers are given in order of elution (CP Sil 5CB, 50m). [1]Numbers refer to GC peaks in Figure 1.

sterol	A158 B	A162 C	A160 C	A202 D	A214 D	A187 A	Add A	Vest C
C27 Δ5,22[1]	0.52				0.59	0.45	11.55	
C27 Δ22[2]	0.61					0.53		
C27 Δ5[3]	80.22	40.04	94.16	92.43	90.91	90.14	67.53	39.73
C27 Δ0[4]	0.14	32.37	1.06		2.22	1.91	8.66	
C27 Δ5,24[5]	1.04	8.49	0.81	0.60	0.62	1.55	1.18	37.06
C27 Δ24[6]		6.41	0.08	0.03	0.09		0.49	
C27 Δ7[7]	4.68	1.13	0.81	1.20	1.13	0.68	6.22	1.02
C28 Δ5,24[8]		4.42			1.12	0.23		15.48
C28 Δ24[9]		5.24						
C28 Δ5[10]	4.21	1.93	1.01	1.20	0.35	1.86	1.58	2.21
C28 Δ0[11]			0.89	0.10			0.91	
C28 Δ5,22[12]				0.33		0.37		
C29 Δ5[13]	6.66		1.00	2.34	1.86	1.31	1.48	3.50
C29 Δ5,24[14]	1.92		0.19	1.76	1.11	0.35	0.41	1.01
Total	2944	1291	7833	6914	5272	4096	225	5605

Table 6. Relative abundances of sterols, as % of total identified. Total concentrations given are μg total sterols / g sample.

[1]cholesta-5,22-dienol; [2]cholest-22-enol; [3]cholesterol; [4]5α-cholestanol; [5]cholesta-5,24-dienol; [6]cholest-24-enol; [7]cholest-7-enol; [8]24-methylcholesta-5,24(28)-dienol; [9]24-methylcholest-24(28)-enol; [10]24-methylcholesterol; [11]24-methyl-5α-cholestanol; [12]24-methylcholesta-5,22-dienol; [13]24-ethylcholesterol; [14]24-ethylcholesta-5,24(28)-dienol

GC #[1]	Fatty Acid	A158 B	A162 C	A160 C	A202 D	A214 D	Gill D	Foot D	Troph C
-	14:0								
1	15:0	0.17	0.07	0.06	0.06	0.06	0.08	0.13	0.02
3	16:0	24.30	17.19	11.52	15.24	16.19	5.82	9.53	16.30
6	17:0								
12	18:0	5.91	2.55	2.75	3.05	1.75	9.99	9.29	1.37
-	19:0	0.02	0.05	0.03	0.02	0.02	0.15	0.07	
22	20:0	2.89	0.79	0.78	1.24	0.56	0.91	0.46	0.14
29	22:0	0.12	0.04	0.14	0.36	0.06	0.32	0.26	0.04
-	i14:0								
4	i17:0	0.42	0.19	0.22	0.24	0.22	0.15	0.21	0.02
-	a17:0								
14	i19:0	0.12	0.08	0.49	0.29	0.19	3.40	0.70	0.03
14	a19:0	0.19	0.04		0.02		0.30	0.42	0.04
-	i21:0								
-	14:1(n-7)								
2	16:1(n-7)	9.93	15.51	8.97	13.59	14.21	4.81	9.52	42.76
5	17:1(n-7)	0.73	0.39	0.22	0.32	0.23	0.30	0.24	0.01
-	18:1(n-5)	1.03	0.43	0.46	0.23	0.18	0.16		0.12
11	18:1(n-7)	29.08	34.83	34.65	25.98	29.18	1.72	1.83	27.98
10	18:1(n-9)	1.11	0.70	0.61	0.59	0.63	0.07	0.15	0.03
10	18:1(n-13)	5.28	8.22	10.91	8.75	11.91	2.69	2.10	1.60
15	19:1(n-7)	0.19	0.57	1.37	1.42	1.27	3.43	1.95	
21	20:1(n-7)	9.92	4.26	4.09	5.32	3.59	7.04	5.26	0.45
20	20:1(n-9)	0.48	0.57	5.00	3.03	2.27	2.24	1.25	0.01
20	20:1(n-13)	1.61	2.64	4.72	6.19	5.51	9.72	10.28	1.36
-	21:1(n-7)								
28	22:1(n-7)	0.71	0.18	0.12	0.12	0.06	0.10	0.11	0.03
-	22:1(n-9)								
9	18:2(n-7,13)	0.59	0.80	0.76	0.63	1.06	0.02	0.08	0.83
9	18:2(n-7,15)	0.58	1.20	0.64	1.73	0.41	0.34	0.32	0.04
8	18:2(n-9,15)	1.68	1.46	0.73	0.76	0.64	0.37	0.36	0.08
19	20:2(n-7,13)	0.02	0.20	0.83	0.55	0.60	4.92	5.46	
19	20:2(n-7,15)	0.99	3.75	5.20	3.15	4.84	15.18	13.14	2.24
18	20:2(n-9,15)	0.03	0.81	1.06	2.55	1.08	5.37	3.90	0.09
24	21:2(n-7,14)	0.07	0.03	0.09		0.11	0.50	0.78	
24	21:2(n-7,16)		0.05	0.18		0.15	1.31	0.88	
-	21:2(n-?)								
27	22:2(n-7,15)	0.22	0.72	1.13	0.90	0.99	6.54	9.96	1.02
27	22:2(n-9,15)			0.07	1.61	0.27	0.08	0.27	0.02
-	24:2(n-?)								
7	18:3	0.92	0.18	0.43	1.51	0.31	8.00	8.00	0.01
-	18:3								
17	20:3	0.03	0.19	0.25	0.06	0.36	0.14	0.10	
25	22:3	0.10	0.08	0.05	0.01	0.02	0.14	0.44	0.10
26	22:3	0.06	0.14	0.11	0.20	0.11	0.85	0.75	0.02
16	20:4(n-6)	0.40	0.89	1.00	0.25	0.71	0.72	0.73	3.16

Table 5. Relative concentrations of polar lipid fatty acids, as % total polar lipid fatty acids, identified as their methyl esters (PLFAME). Unidentified isomers are given in order of elution (CP Sil 5CB, 50m). [1]Numbers refer to GC peaks in Figure 1.

Figure Legends;

Figure 1. Gas Chromatogram of the fatty acid methyl esters of the polar lipids isolated from the hydrothermal vent mussel B. Thermophilus. 50m CPSil 5CB, 0.32mm i.d., 0.1μm film, temperature program 40 - 150°C @12°Cmin^{-1} 150-240°C @4°Cmin^{-1}. Peak numbers refer to those given in Tables 2 to 5.

Figure 2 Relative abundances of different amphipod lipid classes as a percentage of total fatty acids identified for neutral lipid fatty acids (NLFA) and polar lipids fatty acids (PLFA). A = n-7 fatty acids, B = n-9 fatty acids, C = saturated fatty acids, D = unsaturated fatty acids, E = monounsaturated fatty acids, F = polyunsaturated fatty acids, G = non-methylene interrupted polyenoic fatty acids, H = branched fatty acids. Fatty acids were identified as fatty acid methyl esters.

	A123 A	A187 A	A158 B	A160 C	A162 C	A200 D	A202 D	A216 D	Add A	Vest C
Bulk $\delta^{13}C$	-23.5	-23.1	-13.6	-23.7	-14.2	-25.1	-25.0	-25.8	-33	-11
Sterols										
C27 $\Delta5$[1]	-21.5(0.1)	-21.5(0.6)	-16.3(0)	-22.7(0.3)	-16.6(0)	-19.8(0.3)	-20.7(0.3)	-21.6(0.8)	-26.5(0.6)	-15.1(0.2)
C27 $\Delta5,24$[2]										-15.4(0.3)
C27 $\Delta7$[3]										-12.8(0.5)
C28 $\Delta5,24$[4]										-16.4(0.3)

Table 7. Corrected $\delta^{13}C$ values for sterols isolated from hydrothermal vent organisms (9°N East Pacific Rise). Values are corrected for the addition of derivative carbon(trimethyl silyl groups). Values in parentheses are standard deviations of uncorrected $\delta^{13}C$ values. [1]cholesterol; [2]cholesta-5,24-dienol; [3]cholest-7-enol; [4]24-methylcholesta-5,24(28)-dienol

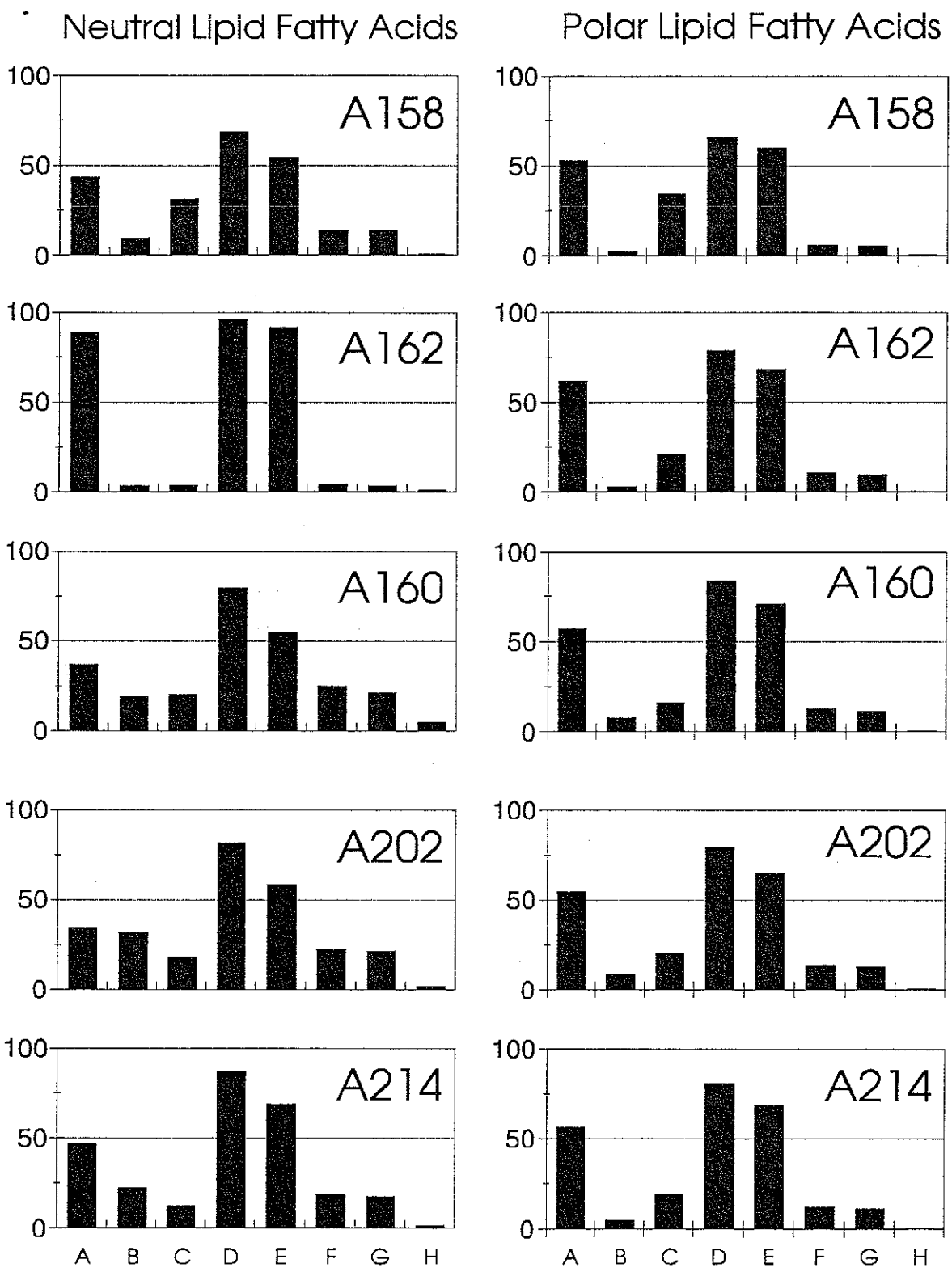
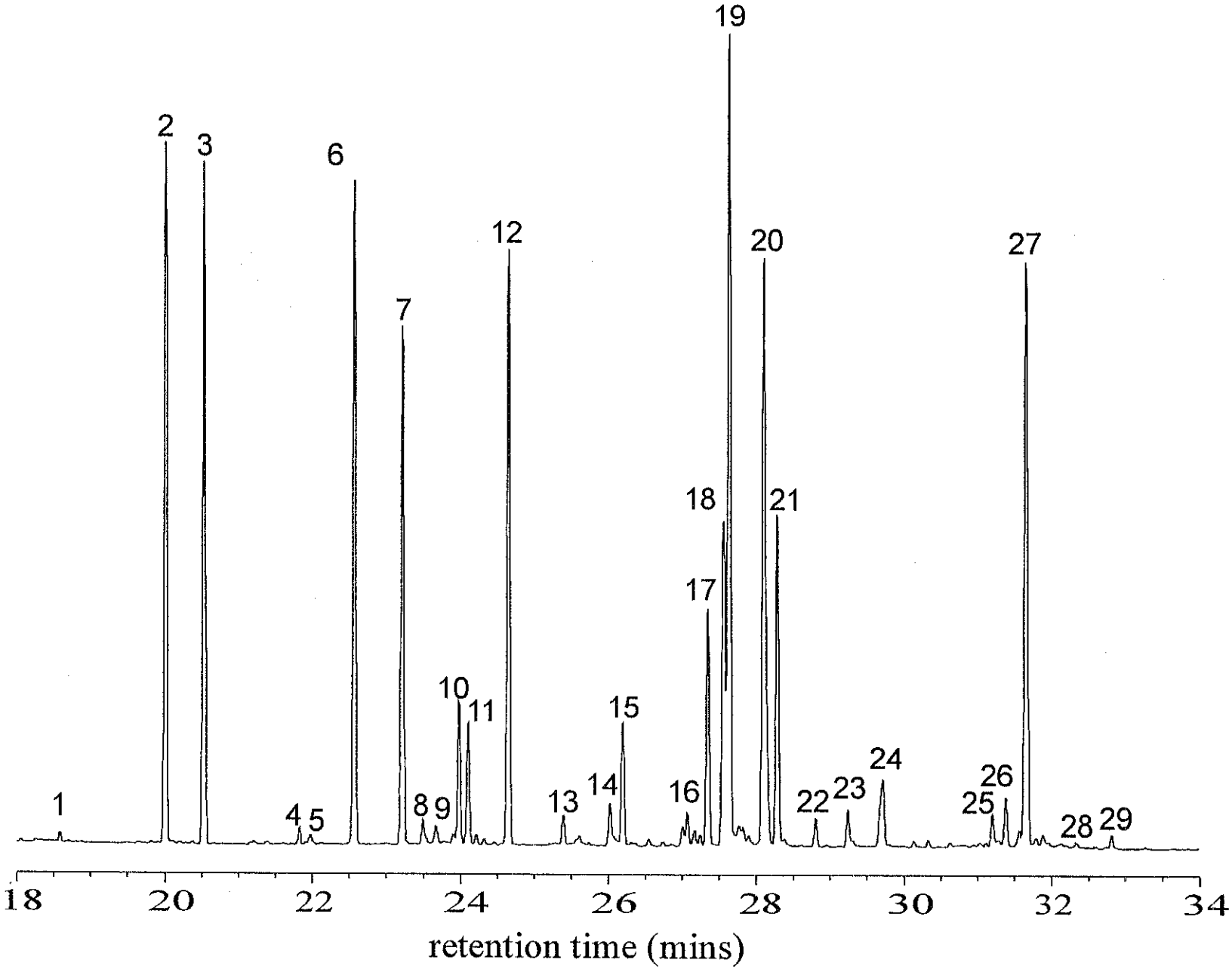


Figure 2 Relative abundances of different amphipod lipid classes as a percentage of total fatty acids identified for neutral lipid fatty acids (NLFA) and polar lipidsfatty acids (PLFA). A = n-7 fatty acids, B = n-9 fatty acids, C = saturated fatty acids, D = unsaturated fatty acids, E = monounsaturated fatty acids, F = polyunsaturated fatty acids, G = non-methylene interrupted polyenoic fatty acids, H = branched fatty acids. Fatty acids were identified as fatty acid methyl esters.


1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
18
20
22
24
26
28
30
32
34
retention time (mins)