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Chemical Geology 205 (2004) 427–442

CHEMICAL GEOLOGY
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Archaeal lipids and anaerobic oxidation of methane in euxinic water columns: a comparative study of the Black Sea and Cariaco Basin

Stuart G. Wakeham[a,*], Ellen C. Hopmans[b], Stefan Schouten[b], Jaap S. Sinninghe Damsté[b]

[a] *Skidaway Institute of Oceanography, 10 Ocean Science Circle, Savannah, GA 31411, USA*
[b] *The Royal Netherlands Institute for Sea Research, Department of Biogeochemistry and Toxicology, P.O. Box 59, 1790 AB, Den Burg, The Netherlands*

Received 20 August 2002; received in revised form 22 June 2003; accepted 23 December 2003

Abstract

The Black Sea and the Cariaco Basin are both large, euxinic marine basins in which methane concentrations are high and where anaerobic oxidation of methane (AOM) is an important part of the carbon cycle. AOM can be recognized by lipid biomarkers that are specific to methanotrophic archaea involved and by strongly ^{13}C-depleted isotope compositions consistent with uptake of ^{13}C-depleted methane-derived carbon. The working hypothesis for our investigation was that AOM in both the Black Sea and Cariaco Basin would generate measurable diagnostic biomarkers and isotope depletions. To test this hypothesis, we analyzed particulate matter and surface sediments for intact glycerol dialkyl glycerol tetreaethers (GDGTs), components of archaeal membrane lipids, and measured stable carbon isotope compositions of their constituent biphytanes. Several GDGTs and strongly ^{13}C-depleted biphytanes indicative of AOM were present in the deep anoxic region of the Black Sea (>700 m). Unexpectedly, this biomarker signal was not detected in the upper anoxic zone of the Black Sea or in the entire water column of the Cariaco Basin, even though previous studies had shown high rates of AOM to occur in both basins. It is possible that the AOM-derived biomarker signal is masked by archaeal lipids derived from non-methanotrophic archaea which utilize ^{13}C-enriched carbon substrates. Alternately we speculate that the methanotrophic community may be highly diverse in euxinic basins, possibly producing another suite of biomarkers that we did not measure. This conclusion will require further testing by coordinated organic geochemical–microbial ecology studies.

Keywords: Black Sea; Cariaco Basin; Euxinic basins; Anaerobic oxidation of methane; Stable carbon isotopes; Biomarkers

1. Introduction

Methane plays an important role in the global biogeochemical cycle of carbon and, as a radiatively active trace gas, is important in global climate modification. In the ocean, CH_4 production in anoxic marine sediments is largely balanced by oxidation in

* Corresponding author. Tel.: +1-912-598-2347; fax: +1-912-598-2310.
E-mail address: stuart@skio.peachnet.edu (S.G. Wakeham).

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doi:10.1016/j.chemgeo.2003.12.024

near-surface sediments and the water column, with the result that on a global scale, the ocean is a relatively minor source of methane to the atmosphere (about 2% of the global methane budget; Reeburgh, 1976). However, if CH_4 release from marine gas hydrates and seeps on continental margins of the ocean were to overwhelm oxidation, major alteration of global climate could occur. Indeed, massive release of CH_4 from hydrate dissociation caused by oceanic circulation change and warming has been linked to perturbations in the ocean–atmosphere carbon cycle (Dickens et al., 1995; Katz et al., 1999; Hinrichs et al., 2003). Given the present-day concern with global warming and the potential for methane release from marine hydrates and seeps, a better understanding of the biogeochemistry of methane is warranted.

Methanotrophy that occurs in diverse aquatic environments renders a tight control on the biogeochemical cycle of methane. A growing body of work now confirms that anaerobic oxidation of methane (AOM) occurs in methane-rich sediments, especially those associated with methane hydrate areas and methane seeps (see reviews by Hinrichs and Boetius, 2002; Valentine, 2002) as well as in euxinic water columns (Ward et al., 1987, 1989; Reeburgh et al., 1991). Molecular biomarkers apparently specific to AOM-mediating archaea are both abundant and strongly depleted in ^{13}C. Since Hoehler et al. (1994) (see also Hoehler and Alperin, 1996) first proposed that AOM might be carried out by a consortium of methane-oxidizing archaea and sulfate-reducing bacteria, there is now mounting biomarker, isotopic and microbiological evidence (e.g., Boetius et al., 2000; Hinrichs and Boetius, 2002; Orphan et al., 2002; Nauhaus et al., 2002; Michaelis et al., 2002) for such a syntrophy. There is, however, also an indication that some archaea may oxidize CH_4 without a tightly coupled syntrophic partner (Valentine, 2002).

While methane-rich sediments often have been the focus of studies related to AOM, its biomarkers, and the microbial ecology of AOM-mediating microorganisms, elevated concentrations of CH_4 concentrations in euxinic basins compared to oxygenated seawater (e.g., 12 μM for the Black Sea and Cariaco Basin vs. nM for normal seawater) support high rates of AOM. As for the Black Sea, inputs of CH_4 from sediments, including methane seeps on the continental margin (Luth et al., 1999; Kruglyakova et al., 2002) and methane-rich mud volcanoes on the abyssal plain of the Black Sea (Ivanov et al., 1996; Kruglyakova et al., 2002) make it the world's largest surface water reservoir of dissolved methane and drive high rates of AOM in the anoxic zone (averaging 2 nM day^{-1}; Reeburgh et al., 1991) that consume >99% of that CH_4. Methane cycling in the Cariaco Basin is less well studied but probably similar. Thus relatively little CH_4 escapes from euxinic water columns into the atmosphere, minimizing this source of atmospheric methane.

One route for investigating AOM is to use AOM-specific biomarkers and their ^{13}C-depleted isotope signatures to trace the pathways followed by methane-derived carbon (summarized by Hinrichs and Boetius, 2002, and references therein). Compounds of archaeal origin that have been found associated with methane seep sediments include acyclic C_{20} isoprenoids 2,6,10,14-tetramethylhexadecane (phytane) and 2,6,11,15-tetramethylhexadecane (crocetane), C_{25} isoprenoids 2,6,10,15,19-pentamethylicosane (PMI) and unsaturated PMI analogs, phytanol (3,7,11,15-tetramethylhexadecanol), archaeol, hydroxyarchaeol, and cyclic C_{40}-biphytanes. In a number of cases, these have $\delta^{13}C$-values as low as −120 ‰. Glycerol dialkyl glycerol tetreaethers (GDGTs), the core membrane lipids of diverse taxa of archaea, have also been detected (Pancost et al., 2000, 2001; Schouten et al., 2000, 2001). In some instances, ^{13}C-depleted fatty acids and alkyl glycerol ethers that are derived from sulfate-reducing bacteria (SRB) provide support for the archaea-SRB consortium being responsible for AOM. Given that AOM is also important in euxinic water columns, we asked the question whether there is biomarker and isotopic evidence for this process in the euxinic Black Sea and Cariaco Basin. Preliminary evidence suggested that there should be: Freeman et al. (1994) reported an unidentified hydrocarbon with a $\delta^{13}C$-value of −66 ‰ in the Cariaco Basin and Schouten et al. (2001) found ^{13}C-depleted (down to −58 ‰) archaeal biphytanes in the deep anoxic Black Sea. In this paper, we present distributions of archaeal GDGTs in both the Black Sea and Cariaco Basin, along with the limited $\delta^{13}C$ data we have for biomarkers. We find biomarker and isotope signatures for AOM in the deep anoxic zone of the Black Sea, but surprisingly in neither the shallow anoxic zone of the Black Sea nor entire water column of the Cariaco Basin. These observations raise interesting issues related to the

process of AOM in euxinic water columns and the utility of biomarkers as indicators of water column AOM, and point to avenues for future work.

2. Experimental

Suspended particulate matter (SPM) and surface sediments were collected in the western basin of the Cariaco Basin (10°40′N, 65°36′W; water depth 1400 m) on a cruise in 1986 of *R/V Iselin* and in the Black Sea (43°05″N, 34°00″E; water depth 2200 m) on a cruise in 1988 of *R/V Knorr*. SPM samples were collected using large volume in-situ filtration systems (see Table 1 for depths; Wakeham and Ertel, 1988 ; Wakeham and Beier, 1991 for sampling details) that filtered 500–5000 l of seawater sequentially through 53 μm Nitex™ screens and 0.7 μm glass fiber filters to give >53 and <53 μm size fractions. Only the <53 μm samples were analyzed in this study. Sediments were collected by box-coring.

Analytical details are given in Wakeham et al. (2003). Briefly, lipids were extracted in a Soxhlet extractor using chloroform–methanol (2:1, v/v). Solvent extractable lipids were fractionated on columns of silica gel using solvents of varying polarity. Polar factions were analyzed for intact GDGTs by high-performance liquid chromatography-atmospheric pressure positive ion chemical ionization mass chromatography (HPLC/APCI-MS) (Hopmans et al., 2000). Positive ion spectra were generated using an Hewlett Packard 1100 series LC/MS by scanning the mass range *m/z* 1225–1325 in 1.9 s. Individual GDGTs were quantified by integrating peaks in summed mass chromatograms of $[M+H]^+$ and $[M+H]+1^+$ ions and comparing with a standard curve obtained by serial dilution of GDGT-0[1] reference standard. Ethers were cleaved from GDGTs

Table 1
POC (μg l^{-1}) and GDGT concentrations (ng l^{-1}) in the Black Sea and Cariaco Basin normalized to volume of seawater filtered

Depth (m)	POC (μg l^{-1})	GDGT-0	GDGT-1	GDGT-2	GDGT-3 (ng l^{-1})	Cren	Cren isomer	Total
Black Sea								
10	133	3.22	0.23	0.07	0.02	3.38	0.06	6.98
30	94	8.78	0.41	0.11	0.04	7.01	–[a]	16.35
70	51	1.54	0.15	0.07	0.02	1.37	0.03	3.17
100	14	16.74	2.52	0.95	0.16	16.47	0.28	37.12
130	36	53.07	6.79	2.30	0.61	49.59	–[a]	112.36
200	26	3.42	0.23	0.12	0.04	3.42	–[a]	7.24
300	20	5.33	0.55	0.29	0.11	5.35	0.13	11.75
400	19	2.42	0.34	0.20	0.05	3.04	–[a]	6.05
700	17	1.73	1.07	1.33	0.04	1.21	–[a]	5.39
1000	15	2.94	3.36	3.59	0.05	0.65	0.02	10.62
1500	11	1.78	2.21	2.66	0.03	0.26	0.01	6.96
2000	10	4.59	7.13	5.99	0.09	0.48	0.02	18.31
Cariaco Basin								
10	53	0.04	0.01	0.03	0.01	0.14	0.01	0.25
50	40	0.59	0.29	0.24	0.14	0.65	0.08	1.98
150	10	0.35	0.17	0.26	0.04	0.56	0.13	1.51
250	16	0.77	0.28	0.25	0.04	0.89	0.10	2.33
350	20	0.82	0.31	0.21	0.06	0.72	0.07	2.20
450	18	0.10	0.03	0.03	0.01	0.18	0.01	0.37
600	18	0.22	0.05	0.06	0.01	0.31	0.03	0.68
750	16	nd[b]	0.04	0.03	0.01	0.17	0.02	0.26
900	18	0.04	0.01	0.01	0.00	0.04	0.00	0.10
1150	17	0.24	0.06	0.06	0.02	0.23	0.03	0.63

[a] Concentrations were too low.
[b] nd: could not be determined due to contamination.

by treatment of saponified (0.5 N methanolic KOH) polar fractions with HI and $LiAlH_4$ (King et al., 1998) and the released biphytanes were analyzed by gas chromatography (GC) using a Carlo Erba 4160 GC and by gas chromatography-mass spectrometry (GC/MS) using a Hewlett Packard 5890II-Finnigan Incos 50 GC/MS system. Compound-specific stable carbon isotope ratios of biomarkers were measured by isotope-ratio-monitoring gas chromatography-mass spectrometry (irm-GC/MS) using a Finnigan DELTA-plus XL irm-GC/MS. Isotope compositions are reported in standard delta notation relative to the VPDB standard.

3. Results

3.1. Biogeochemical setting

The Black Sea is characterized by a strong density-controlled chemocline in which dissolved oxygen concentrations decrease to 0 by about 110 m depth and hydrogen sulfide concentrations increase in the anoxic zone, reaching about 400 μM near the sea floor (Fig. 1a). Methane that is released from the sediments accumulates in the anoxic water column to about 11 μM by about 500 m and remains relatively constant to the seafloor (Fig. 1b). High rates of AOM were measured (Reeburgh et al., 1991) throughout the anoxic zone, yielding a range of 2–5 nM day^{-1} (Fig. 1c). Only small amounts of CH_4 escapes being oxidized and CH_4 concentrations are very low (5–10 nM) at the chemocline and in the overlying oxic zone.

The oxic–anoxic boundary in the Cariaco Basin is located at about 275 m (Fig. 1d), but unlike the Black Sea, H_2S concentrations only reach ~60 μM at the bottom of the anoxic zone. Methane concentrations in the deep anoxic Cariaco Basin were ~12 μM (Fig. 1e), similar to the Black Sea, although the shape of the CH_4 profiles in the two basins are different. Methane oxidation rates in the Cariaco Basin were about 0.2 nM day^{-1} (Fig. 1f; Reeburgh, 1976; Ward et al., 1987).

[1] GDGTs are named according to the number of cyclopentyl rings; thus GDGT-0 contains no rings (structure **I** in the Appendix), GDGT-1 contains 1 ring, etc.

3.2. GDGT distributions

GDGTs consisting of acyclic, bicyclic, and tricyclic biphytanes (Appendix A) were present in suspended particulate matter and sediment samples from both the Black Sea and the Cariaco Basin. Total GDGT concentrations (volume normalized, Table 1; organic carbon-normalized, Table 2) were uniformly higher in the Black Sea than in the Cariaco Basin (Fig. 2), although particulate organic carbon concentrations (Table 1) were similar. Both profiles were characterized by high concentrations at the respective chemoclines (70–130 m in the Black Sea and 250–350 m in the Cariaco Basin), secondary concentration peaks at the chlorophyll-*a* maxima (30 m in the Black Sea; 50 m in the Cariaco), and reduced concentrations in the anoxic zones.

In the Black Sea, GDGTs in SPM in the water column shallower than 400 m and in surface sediments were dominated by GDGT-0 (**I** in Appendix) containing two acyclic biphytanes and crenarchaeol (**V**) with its characteristic cyclohexyl-containing biphytane **d** (Sinninghe Damsté et al., 2002b). Together, these two GDGTs comprised nearly 95% of total GDGTs (Fig. 3). Volume-normalized concentrations of crenarchaeol were high in surface waters (Table 1), still higher at the top of the anoxic zone where microbial production is high (Wakeham and Beier, 1991), but low in the deep anoxic zone. Like crenarchaeol, concentrations of GDGT-0 were highest at 130 m, but unlike crenarchaeol generally increased in the deep anoxic zone of the Black Sea. Particles deeper than 700 m in the Black Sea showed markedly increased relative abundances (up to nearly 40% of total GDGTs from only a few percent in other samples) of GDGT-1 (**II**) and GDGT-2 (**III**) (Fig. 3). This shift in composition resulted in substantially reduced relative abundance of crenarchaeol (decreasing to 5% of total GDGTs) and a twofold reduction in the relative abundance of GDGT-0. Furthermore, absolute concentrations (both volume-normalized and OC-normalized; Tables 1 and 2) of GDGT-1 and GDGT-2 increased in the deep anoxic zone over concentrations in shallower samples.

GDGT distributions in the Cariaco Basin (Fig. 3) were remarkably uniform throughout the entire water column and in surface sediments. As in the Black Sea, crenarchaeol and GDGT-0 were the predominant

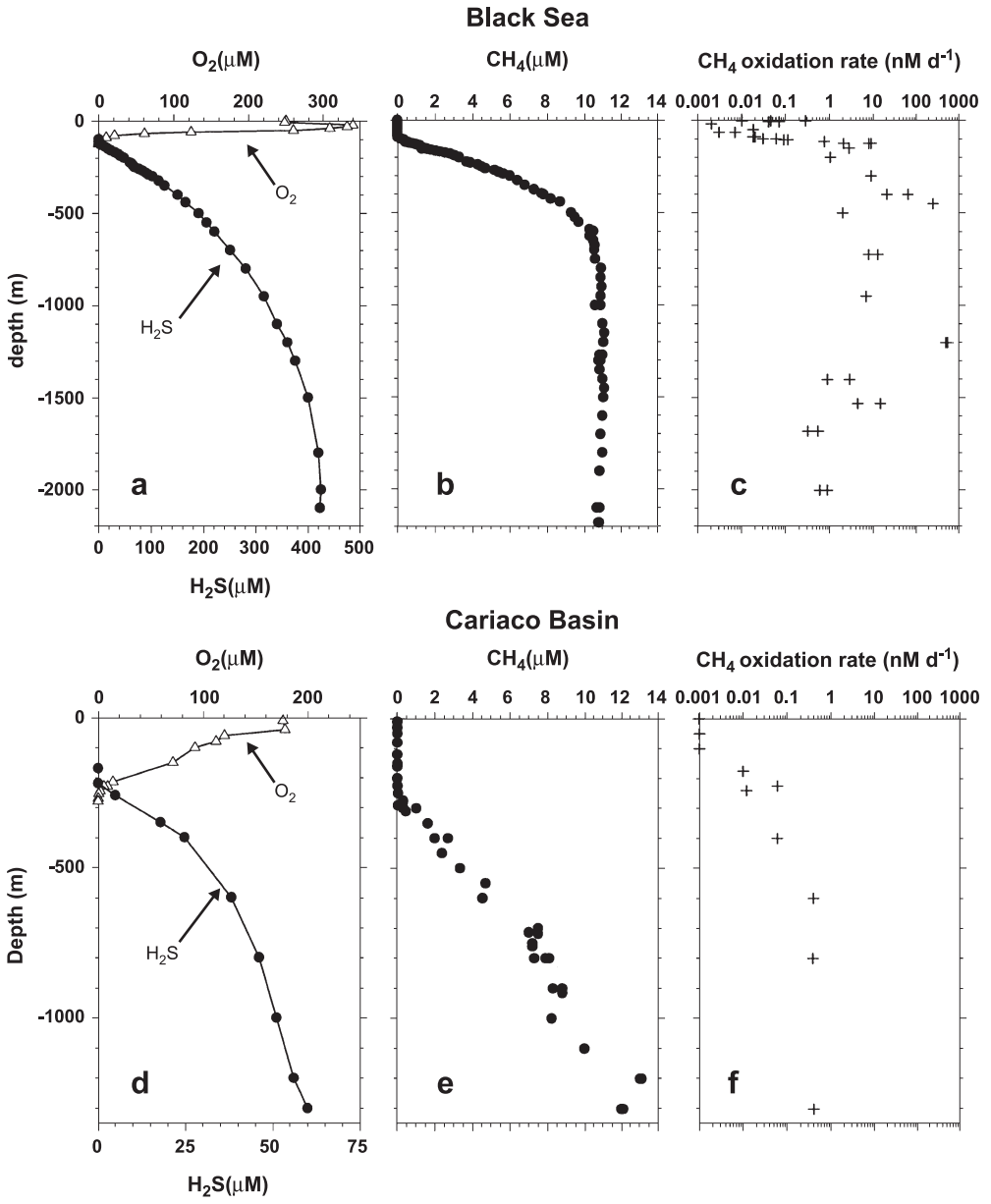


Fig. 1. Bulk parameters in the Black Sea and Cariaco Basin water columns. (**a**, **d**) Dissolved oxygen (μM, △) and sulfide (μM, ●), (**b**, **e**) methane concentrations (μM) and (**c**, **f**) methane oxidation rates obtained from $^{14}CH_4$ incubations. Black Sea O_2 and H_2S data are replotted from data collected by White et al. (1989) and Friederich et al. (1990) and Cariaco Basin O_2 and H_2S data are from Zafiriou et al. (unpublished cruise report). Black Sea CH_4 concentration and CH_4 oxidation rate data replotted from Reeburgh et al. (1991): Cariaco Basin Sea CH_4 concentration and CH_4 oxidation rate data are from Reeburgh (1996); Scranton (1988) and Ward et al. (1987).

tetraethers, but unlike the Black Sea, GDGT-1 and GDGT-2 were more abundant throughout the water column and sediment of the Cariaco Basin, comprising up to ~25% of total GDGTs. The enrichment in GDGT-1 and GDGT-2 along with the depletion in crenarchaeol that was observed in the deep anoxic

Table 2
GDGT concentrations (mg gOC^{-1}) in the Black Sea and Cariaco Basin normalized to organic carbon of seawater filtered

Depth (m)	GDGT-0	GDGT-1	GDGT-2	GDGT-3 (mg gOC^{-1})	Cren	Cren isomer	Total
Black Sea							
10	24.2	1.7	0.5	0.1	25.4	0.5	52.5
30	93.4	4.3	1.2	0.4	74.6	–[a]	174
70	30.3	2.9	1.3	0.3	26.8	0.5	62.1
100	1200	180	68	12	1180	20.1	2660
130	1475	189	64	17	1380	–[a]	3130
200	130	8.9	4.6	1.6	132	–[a]	280
300	267	27.5	14.5	5.4	267	6.3	590
400	127	18.1	10.4	2.6	161	–[a]	320
700	102	63	78	2.6	71.2	–[a]	320
1000	197	224	240	3.5	43.1	1.1	710
1500	162	200	240	2.7	23.6	1.2	630
2000	460	710	599	8.5	48.3	2.5	1830
Cariaco Basin							
10	0.8	0.3	0.5	0.2	2.6	0.2	4.7
50	14.7	7.3	5.9	3.5	16.2	2.0	49.6
150	35.4	16.6	25.7	4.4	56.0	12.9	150
250	48.1	17.6	15.9	2.4	55.9	6.1	146
350	41.0	15.6	10.7	2.9	36.0	3.6	110
450	5.7	1.5	1.8	0.4	10.1	0.7	20.3
600	12.1	2.8	3.1	0.8	17.1	1.5	37.5
750	–[a]	2.2	2.0	0.6	10.3	1.0	16.0
900	2.1	0.4	0.5	0.1	2.2	0.2	5.5
1150	13.9	3.3	3.5	1.0	13.8	1.8	37.3

nd: could not be determined due to contamination.
[a] Concentrations were too low.

zone of the Black Sea was not seen in the deep anoxic zone of the Cariaco Basin. In addition, the Cariaco samples contained two additional GDGTs (GDGT-3 and an isomer of crenarchaeol in Table 1) that were only minor components in the Black Sea.

3.3. Stable carbon isotope compositions of archaeal lipids

In Black Sea particulate matter, biphytanes released by HI/$LiAlH_4$ treatment of saponified polar fractions varied significantly in both composition and isotopic composition (Fig. 4). Biphytanes **a** and **d**, derived partially from GDGT-0 and only from crenarchaeol, respectively, dominated the biphyane distribution in SPM from the oxic zone and the upper anoxic zone (130–400 m). All four biphytanes (**a**–**d**) had δ^{13}C-values generally ranging from −25‰ to −21‰ (−31‰ for biphytane **b** in the upper anoxic zone). Phytane that would derive from phytanyl glycerol ethers (including archaeol and hydroxyarchaeol) of various archaea (De Rosa and Gambacorta, 1988; Koga et al., 1993) was also present in the saponified and HI/$LiAlH_4$ treated fractions and was slightly depleted in ^{13}C (−32‰ to −28‰) relative to the biphytanes. Crenarchaeol's tricyclic biphytane **d** was isotopically uniform down the entire water column (~ −22‰), but δ^{13}C-values of the other biphytanes and phytane were strongly depleted, although to varying degrees, in the deep anoxic zone. Biphytane **b** derived from GDGT-1 and GDGT-2 was the most strongly depleted at −66‰ (the average of 1000 m [−67‰]), 1500 m [−64‰]), and 2000 m [−66‰] samples). Phytane and acyclic biphytane **a** showed slight enrichments relative to biphytane **b** (−51‰ and −54‰, respectively), while bicyclic biphytane **c** showed the least ^{13}C-depletion (−41‰). Biphytane and phytane isotope compositions in the sediments were similar to δ^{13}C-values in the upper water column, without any of the strong isotope depletions

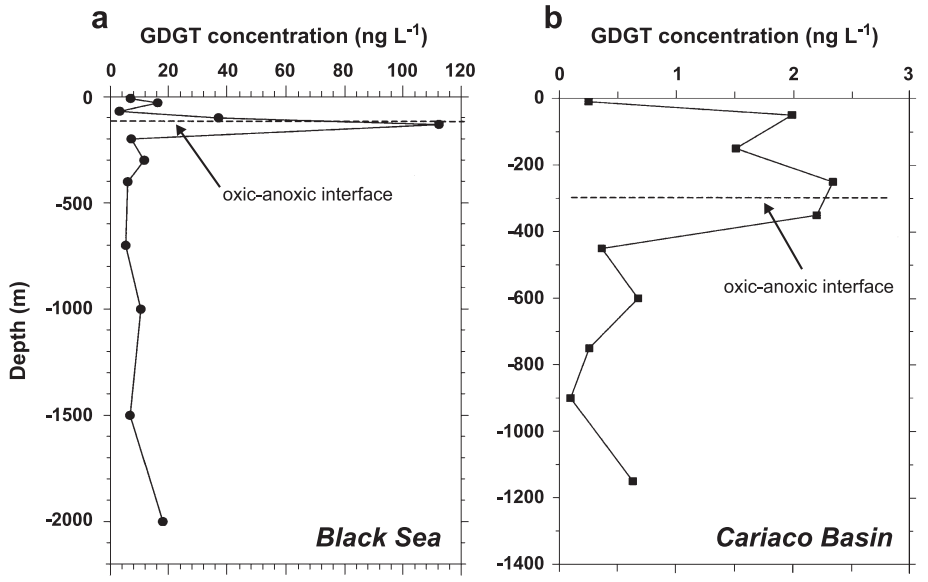


Fig. 2. Volume-normalized concentrations (ng l^{-1}) of total GDGTs in the water column of the Black Sea and Cariaco Basin.

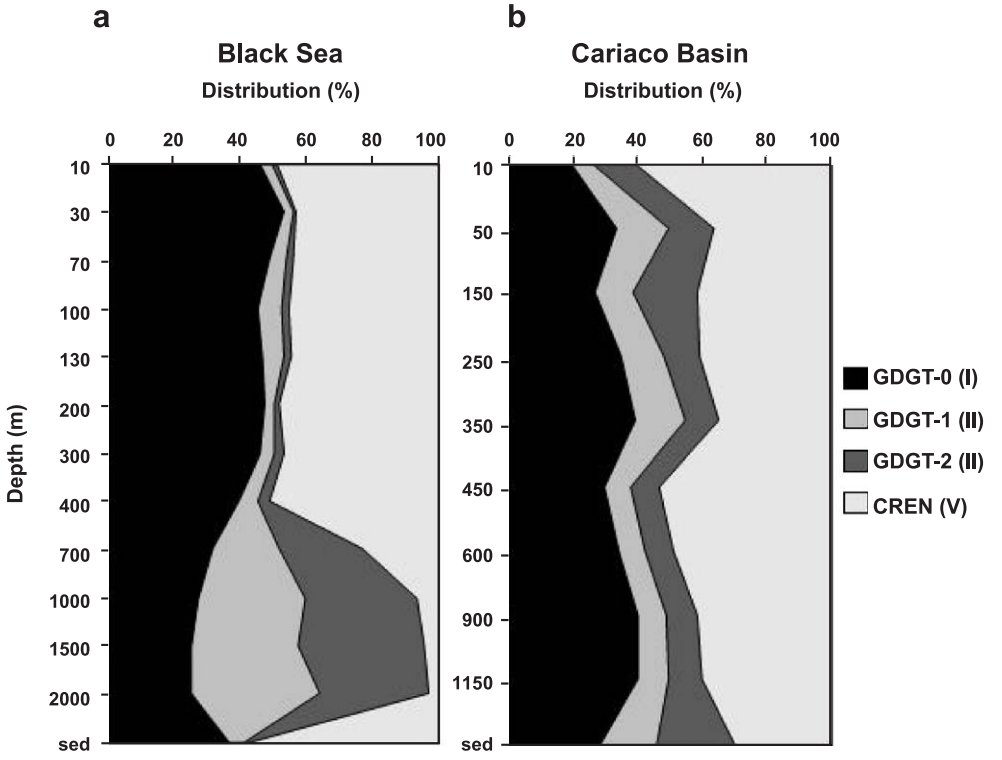


Fig. 3. Relative distributions of the major GDGTs in suspended particulate matter and surface sediments of (**a**) the Black Sea and (**b**) the Cariaco Basin.

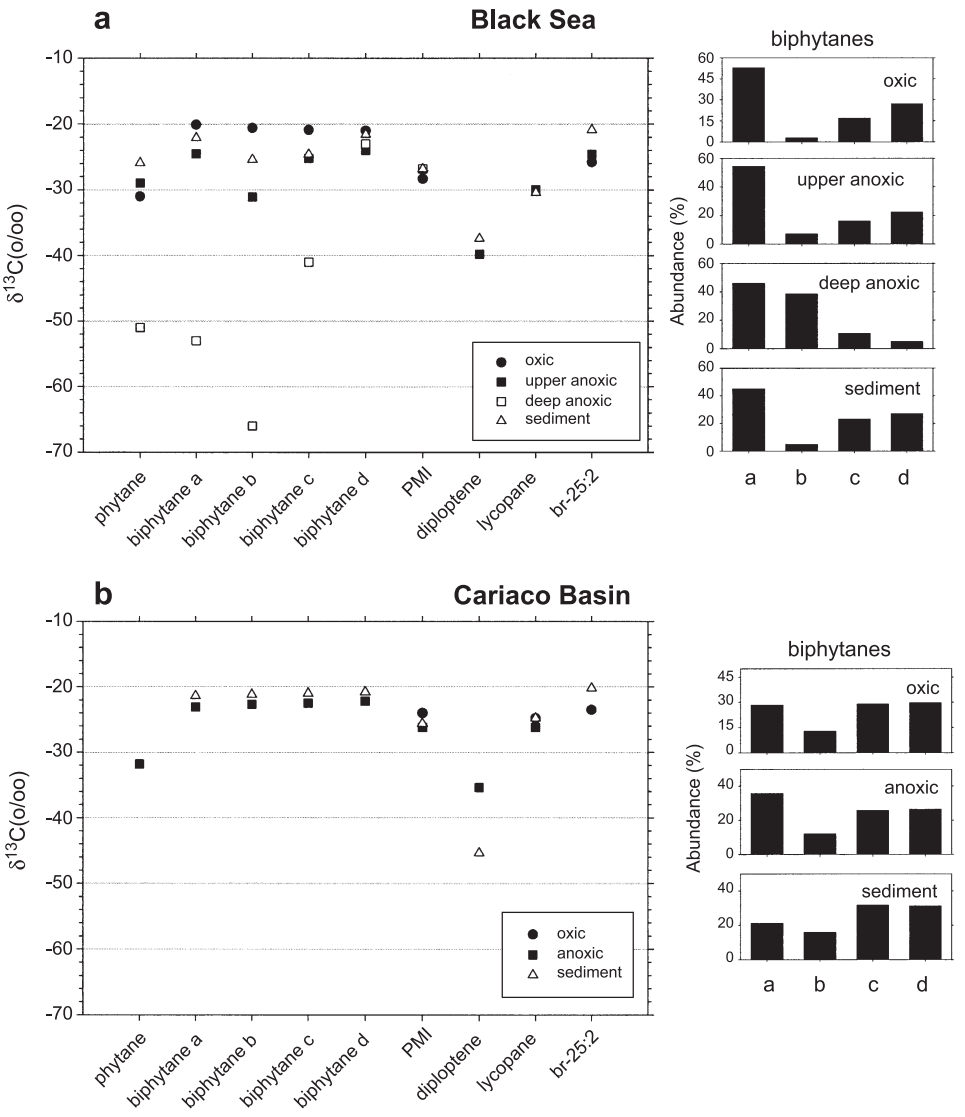


Fig. 4. (**a**) Mean δ^{13}C-values (‰) and biphytane distributions for composite samples of SPM and surface sediments in the Black Sea. Oxic = 10 – 100 m; upper anoxic = 130 – 400 m; deep anoxic = 1000 – 2000 m; sediment = 0 – 1 cm. (**b**) Mean δ^{13}C-values and biphytane distributions for composite samples from SPM and surface sediments in the Cariaco Basin. Oxic = 10 – 250 m; anoxic = 350 – 1150 m; sediment = 0 – 2 cm. δ^{13}C-values for 2,6,10,15,19-pentamethylicosane (PMI), diploptene [hop-22(29)-ene], lycopane (2,6,10,14,19,23,27,31-octamethyldotriacontane) and $C_{25:3}$ highly branched isoprenoid alkadiene (br-25:3) are from Freeman et al. (1994); **a** – **d** refer to biphytanes shown in Appendix A.

characteristic of biphytanes **a**, **b**, and **c** that characterized the deep anoxic zone.

The uniform GDGT distributions in the Cariaco Basin are supported by distributions of the biphytanes and their isotopic compositions (Fig. 4). High abundances of biphytanes **c** and **d** are consistent with the dominance of crenarchaeol, while high levels of biphytane **a** follow from high abundances of GDGT-0. Isotope compositions of all four biphytanes in the anoxic zone and sediment of the Cariaco Basin were virtually indistinguishable at −20 ‰ to −23 ‰. Although δ^{13}C measurements were not made on upper

water column SPM, we assume that they would be similar to those of the sediments, based on the Black Sea results. Thus unlike the Black Sea, there is no ^{13}C-depletion for archaeal biomarkers in the deep Cariaco Basin.

The stable isotope presented here are for a relatively limited set of biomarkers. We recognize that additional data for other biomarkers would help constrain our interpretations, but to do so requires further sampling in both the Black Sea and the Cariaco Basin, as is planned for the future.

4. Discussion

4.1. GDGTs derived from marine crenarchaeota

GDGTs are major lipid components of archaeal membranes (De Rosa and Gambacorta, 1988; Koga et al., 1993) and thus, along with their constituent biphytanes, are biomarkers for archaea in the environment. Crenarchaeol (**V**) and biphytane **d** are considered indicators of the non-thermophilic planktonic crenarchaeotes that constitute an important component of the picoplankton in the ocean (DeLong, 1992; Fuhrman et al., 1992, 1993; Fuhrman and Davis, 1997; Massana et al., 2000; Karner et al., 2001), since these compounds are common in particulate matter and sediments from diverse low-temperature marine environments (Schouten et al., 2000; Pancost et al., 2001; Sinninghe Damsté et al., 2002a,c; Wakeham et al., 2003; Wuchter et al., unpublished results). Uniform isotope compositions of biphytane **d** at −21 ‰ to −25 ‰ throughout the water columns of the Black Sea and Cariaco Basin support a predominantly planktonic crenarchaeal source for crenarchaeol.

GDGT-0 (**I**), comprised of two acyclic biphytanes (**a**), is also widely distributed in archaea (Kates, 1993; Koga et al., 1993) and marine environments (Schouten et al., 2000; Pancost et al., 2001; Sinninghe Damsté et al., 2002a,b,c; Wakeham et al., 2003). In contrast to crenarchaeol, concentrations of GDGT-0 in the Black Sea increase in the deep anoxic zone (Table 1), and acyclic biphytane **a** is strongly depleted in ^{13}C in the deep anoxic zone (−53 ‰ at depths >1000 m vs. −23 ‰ at depths shallower than 1000 m; Fig. 4). These concentration and isotopic patterns indicate multiple origins for GDGT-0 in the Black Sea, including archaea living in both the upper and deep zones in the water column. On the other hand, GDGT-0 relative abundances and isotope values for biphytane **a** in the Cariaco Basin are uniform over the water column, suggesting a more homogeneous source.

GDGT concentration profiles would suggest that archaea are considerably more abundant in the water column of the Black Sea than in the Cariaco Basin, perhaps by nearly 10-fold. Using the content of GDGT in archaeal cell membranes (1.0×10^{-3} pg of GDGT cell^{-1}) in a calculation procedure described by Sinninghe Damsté et al. (2002a), we can estimate cell densities for archaea in the Black Sea and Cariaco Basin. For the Black Sea, average total concentrations of 15 ng l^{-1} for crenarchaeol at 30 m, and 100 ng l^{-1} at the concentration maximum at 130 m would correspond to 1.5×10^4 and 10×10^4 planktonic archaeal cells ml^{-1} at 30 and 130 m, respectively, in the upper part of the Black Sea water column. For the Cariaco Basin, GDGT concentrations of 2 ng l^{-1} for the interval 50–350 m and 0.4 ng l^{-1} for the other depths gives 0.2×10^4 and 0.04×10^4 cells ml^{-1}.

Our estimates of archaeal cells in the Black Sea and Cariaco Basin generally compare favorably with estimates made elsewhere (Karner et al., 2001; Sinninghe Damsté et al., 2002a), although our value for the deep anoxic zone of the Cariaco Basin is low. Crenarchaeal cell densities at the open ocean Hawai'i Ocean Time-series site (ALOHA) have been reported by Karner et al. (2001). Ribosomal RNA gene surveys were used to determine that there are 4×10^4 archaeal cells ml^{-1} in the epipelagic zone (<150 m depth) and 0.3×10^4 cells ml^{-1} in deep waters (>150 m) at ALOHA. In the Arabian Sea, Sinninghe Damsté et al. (2002a) used concentrations (7–15 ng l^{-1}) of crenarchaeol, the dominant GDGT in Arabian Sea particulate matter, to estimate that there might be 0.7×10^4 to 1.5×10^4 crenarchaeal cells ml^{-1} in waters <500 and >500 m, respectively.

It is uncertain why cell density estimates in the deep Cariaco Basin are an order of magnitude lower than at these other sites, although the data set for comparison is certainly limited. There are currently no independent estimates of archaeal cell densities or morphologies, via for example molecular genetic probing or fluorescent probe visualization, in either the Black Sea or the Cariaco Basin with which to compare our GDGT-based cell densities. Our filtering

procedure may have under-sampled archaeal cells in general, since the glass fiber filters of nominal 0.7 μm pore size we used discriminate against picoplankton-sized material (Lee et al., 1995), and although the effective pore size of glass fiber filters decreases as material clogs the ''pores'', the size of particles collected is poorly constrained. Nonetheless, we did observe strong bacterial biomarker signals, including for sulfate-reducing bacteria, in the anoxic zone (Wakeham and Ertel, 1988; Wakeham, 1990; Wakeham and Beier, 1991; Wakeham et al., 1991). The same filtering procedure was used in both the Black Sea and Cariaco Basin, and we can only assume that we collected a similar particle-size distribution at both locations. Finally, initial studies in our lab using 0.2 μm cellulose acetate filters show only slightly elevated GDGT concentrations compared to 0.7 μm filters (Wuchter et al., unpublished results). Thus, there may be a significant difference in the types and morphologies of crenarchaeal cells in the two basins or growth conditions are unfavourable for the crenarchaeota. This question needs to be resolved by improved sampling techniques in conjunction with determination of microbial community structure.

4.2. GDGTs as biomarkers for AOM in euxinic waters

High abundances of GDGT-1 (**II**) and GDGT-2 (**III**) and strong isotopic depletions of biphytanes **a**, **b**, and **c** (−78‰ to −54‰) have been reported in sediments associated with cold methane seeps in the Mediterranean (Pancost et al., 2001). That GDGT-1 and GDGT-2 are also significantly enriched in the deep anoxic zone of the Black Sea and biphytanes **a**, **b**, and **c** are strongly depleted in ^{13}C support the transfer of isotopically depleted carbon into archaeal lipids via anaerobic oxidation of methane. $\delta^{13}C$-values for methane in the Black Sea were uniform at −48‰ between 1500 and 2200 m (Reeburgh et al., 1996); the $\delta^{13}C$-value of CH_4 in the Cariaco water column is not known. Thus for the most strongly depleted biphytane, **b** (−66‰), that is derived only from GDGT-1 and GDGT-2, an isotope fractionation during AOM in the deep anoxic zone of the Black Sea of at least ~20‰ is possible. Isotope fractionations in sediments can be as great as 95‰ (Whiticar, 1999). Intermediate $\delta^{13}C$-values for biphytanes **a** and **c** (−53‰ and −41‰, respectively) as well as for phytane (−51‰) show that ether lipids from which these compounds are derived have origins from multiple groups of archaea, at least one of which is involved in AOM in the anoxic zone.

There are multiple lines of evidence that archaea play a prominent role in anaerobic oxidation of methane, including specific biomarkers and strongly depleted isotopic compositions (Hinrichs et al., 2000; Elvert et al., 2000, 2001; Pancost et al., 2000, 2001; Thiel et al., 2001; Bian et al., 2001; Schouten et al., 2001; Orphan et al., 2001a,b; Michaelis et al., 2002; Wakeham et al., 2003) and molecular gene sequencing (Hinrichs et al., 1999; Orphan et al., 2001a,b, 2002; Teske et al., 2002). Although these reports are primarily derived from methane-rich sediments, including sediments proximate to methane vents and hydrates, our initial working hypothesis in investigating euxinic water columns of the Black Sea and Cariaco Basin was that enrichments in dissolved methane and its anaerobic oxidation in both basins would produce characteristic GDGTs and isotopically depleted biphytanes in both basins. Our hypothesis has been proven only partially correct. We do indeed observe AOM-derived GDGTs (GDGT-1 and GDGT-2) and strong ^{13}C-depletions for biphytanes (**a**, **b**, and **c**), but then only in the deep part of the Black Sea and in neither the upper anoxic zone of the Black Sea nor at any depths of the Cariaco.

Since there is evidence from other sites that AOM can be carried out by a consortium of methanogenic archaea and sulfate-reducing bacteria (Hoehler and Alperin, 1996; Valentine and Reeburgh, 2000; Hinrichs and Boetius, 2002), we tested for such a syntrophy in the Black Sea by measuring the isotopic composition of fatty acids specific to sulfate-reducing bacteria (SRBs), in addition to the biphytanes. While SRB-derived fatty acids (e.g., branched-chain C_{15} and C_{17}) were abundant (Wakeham and Beier, 1991), we did not observe them to be depleted in ^{13}C anywhere in the anoxic water column (Wakeham et al., 2003). This negative result does not rule out such a syntrophy if only a small fraction of SRBs present in the anoxic zone are involved in a methane-oxidizing consortium and their isotopic signal is diluted by that of the more abundant non-AOM-SRBs. Recent experiments by Nauhaus et al. (2002) show that, at least in the laboratory, anaerobic oxidation of CH_4 may be accompanied by sulfate

reduction at a molar ratio of ~1:1. *n*-Alkyl and methyl-branched *n*-alkyl fatty acids such as *iso*- and *anteiso*-C_{15} derived from sulfate-reducing bacteria are depleted in ^{13}C in methane hydrate-containing sediments (Hinrichs et al., 2000) and cold methane-seep sediments (Pancost et al., 2000) having ^{13}C-depleted-archaeal lipids. In the Black Sea, the annual, basin-wide rate for AOM at ~0.7 mol C m^{-2} $year^{-1}$ (Reeburgh et al., 1991) is roughly comparable to the sulfate reduction rate of. ~1.5 mol C m^{-2} $year^{-1}$ (Albert et al., 1995), so it is unclear why we did not find ^{13}C-depleted SRB fatty acids. Alternately, AOM could be carried out by obligately methanotrophic archaea operating without a bacterial partner (Hinrichs et al., 1999; Orphan et al., 2002; Valentine, 2002). In either case, coordinated geochemical and microbiological measurements are needed to better characterize the biogeochemistry of AOM.

Fig. 4 shows, in addition to the biphytanes, $\delta^{13}C$-values for several other biomarkers that have been implicated in methane oxidation in marine systems. PMI has often been attributed to methanogenic archaea (Brassell et al., 1981; Tornabene and Langworthy, 1979; Holzer et al., 1979; Risatti et al., 1984; Schouten et al., 1997), but strong ^{13}C-depletions in methane-rich sediments (e.g., Hinrichs et al., 2000; Elvert et al., 2000, 2001; Pancost et al., 2000) are also consistent with methanotrophy. However, in neither the Black Sea nor in the Cariaco Basin are $\delta^{13}C$-values of PMI (−27‰ to −29‰) depleted enough to reflect AOM as its source (or one of its sources). Lycopane (2,6,10,14,19,23,27,31-octamethyldotriacontane) has also been attributed to methanogens as might fit with its isotope value (−25‰ to 30‰), but Wakeham et al. (1993) used ^{13}C data and the presence of lycopane in oxic seawater to suggest a photoautotrophic source. Clearly, the sources of PMI and lycopane are complex and poorly constrained. On the other hand, diploptene [hop-22(29)-ene] is a general biomarker for various prokaryotes (Rohmer et al., 1984; Ourisson et al., 1987) and has been associated with aerobic oxidation of methane. Diploptene concentrations peak in the suboxic zone and upper anoxic zone in both the Black Sea and the Cariaco Basin (Wakeham, 1988; Wakeham et al., 1991), and with ^{13}C-values between −38‰ and −45‰ in the Black Sea and Cariaco Basin (Fig. 4), diploptene could derive from chemoautotrophy and/or aerobic methanotrophy.

Why are molecular and isotopic indicators of AOM only present in the deep Black Sea even though the process occurs throughout the anoxic water columns of both the Black Sea and the Cariaco Basin? At present, we can only speculate and additional data are needed to resolve the question(s). In the Black Sea, rates of AOM in the upper (<700 m) anoxic region of the Black Sea are, on average, as high as in the deep (>700 m) anoxic zone, so rate differences alone cannot explain the different biomarker distributions. Does the absence of AOM-associated biomarkers in the upper anoxic zone imply that archaea such as those studied in CH_4-rich sediments and apparently present in the deep anoxic zone are not major oxidizers of the methane in the upper anoxic zone? How important are bacterial methanotrophs? So far, we have only an equivocal story from diploptene. Does the shallow anoxic zone of the Black Sea and the Cariaco Basin harbor a distinct CH_4-oxidizing archaeal community and biomarker distribution from that of the deep anoxic zone and seep sediments? If so, then we may have not yet identified the correct biomarkers. Since AOM rates in the Cariaco Basin are roughly 10-fold lower than in the Black Sea, it is possible that AOM-derived biomarkers are masked by other archaeal biomarkers. However, this scenario would not explain why the euxinic Black Sea appears to be partitioned into two functionally different environments with respect to AOM.

4.3. Water column AOM and the sediment record

Anaerobic oxidation of methane in sediments is an important controlling factor in the cycling of methane in the ocean, and AOM consumes most of the CH_4 in the euxinic waters. Yet in neither the Black Sea nor the Cariaco Basin is there the kind of evidence for AOM in the sediments we studied as has been observed in seep sediments in the Black Sea (e.g., Thiel et al., 2001; Michaelis et al., 2002) and elsewhere. Neither GDGT nor biphytane distributions in Black Sea and Cariaco sediments nor biphytane $\delta^{13}C$-values fit the consensus pattern for AOM. Essentially, the sediments are not recording AOM either in-situ or in the water column.

Several factors may be involved in the absence of a sedimentary record of euxinic water column AOM. AOM may be highly localized so that a "global" record cannot develop. Even though most methane in

anoxic water columns is consumed by AOM, high turnover rates may not produce high biomass of the AOM-mediating organisms relative to the total organic matter pool, such that AOM-derived biomarkers would not constitute a significant or even measurable fraction of the overall biomarker content. This "masking" explanation may help to account for the absence of an AOM signal in the upper anoxic zone of the Black Sea and throughout the anoxic Cariaco Basin. Even if a signal for water column AOM were detectable, as in the deep anoxic zone of the Black Sea, the pertinent biomarkers apparently are preferentially associated with the fine suspended (slowly sinking) particle pool rather than the larger (fast-sinking) aggregates that are the source of most sedimentary material. Measurements of GDGTs and isotopes of biphytanes in sediment trap samples from the anoxic zone of the Black Sea (Wakeham et al., 2003) support this suggestion. The GDGTs indicative of AOM (GDGT-1 and GDGT-2) were not enriched in trap material like they are in suspended material, and δ^{13}C-values of biphytanes **a** and **c** are indistinguishable from that of crenarchaeol's biphytane **d** that is derived from non-methane-oxidizing archaea. We hypothesize that vertical transport of archaeal lipids in euxinic basins requires an aggregation process, such as grazing, that packages those compounds produced in the small-sized, suspended particle pool in the upper water column into the sinking particle pool. In the absence of such a packaging process in the anoxic zone, the distinctive biomarkers produced by AOM and associated with suspended particles are not efficiently incorporated into the sinking particle pool and thus are not transferred to the sediments. In contrast, grazing in oxic surface waters will imprint the sinking particle pool with archaeal lipids produced in the upper water column, and sediments will preferentially reflect this upper-water archaeal source. Therefore, there would be at best only a weak link between water column AOM and accumulation/preservation of the pertinent biomarker indicators in underlying sediments.

4.4. Surface water GDGT distributions—a temperature effect?

Distributions of GDGTs in surface water particulate matter are clearly different between the Black Sea and the Cariaco Basin (Fig. 3). GDGT-1 and GDGT-2 comprise a relatively uniform 20–30% of total GDGTs throughout the Cariaco Basin's water column, even in surface waters. It is tempting to speculate that this difference might be reflecting variability in archaeal community structure in the two basins. Although crenarchaeota are widely distributed throughout surface and deeper waters of the ocean (DeLong, 1992; Fuhrman and Davis, 1997; Fuhrman et al., 1992, 1993; Massana et al., 2000; Karner et al., 2001; Sinninghe Damsté et al., 2002a), they may be highly diverse and may well biosynthesize variable GDGT distributions. To date, no cultured pelagic crenarchaeota and few upper ocean particulate matter samples have been investigated for GDGTs (Sinninghe Damsté et al., 2002a; Wakeham et al., 2002) or biphytanes (Hoefs et al., 1997; DeLong et al., 1998; King et al., 1998; Wakeham et al., 2003), although a more comprehensive survey is currently underway by our group. Only one marine psychrophilic crenarchaeon, the sponge symbiont *Cenarchaeum symbiosum*, has been analyzed (DeLong et al., 1998; Sinninghe Damsté et al., 2002b), and its GDGTs are dominated by crenarchaeol and GDGT-0.

An alternate explanation for the different surface water GDGT distributions between the Black Sea and the Cariaco Basin may be related to environmental conditions. Notably, salinities in surface waters of the Black Sea are considerably lower than in the Cariaco Basin (16–18‰ vs. 36‰) and temperatures were different when our samples were collected (10–20 vs. 20–25 °C, respectively, in the upper 100 m). Salinity effects on biosynthesis of archaeal lipids have not been investigated, but salinity differences could lead to variations in archaeal species composition. In the case of temperature, there are documented cases where the composition of cellular lipids increases in unsaturation to compensate for reduced environmental temperature [e.g., membrane fatty acids (Russell, 1989) and alkenones (Prahl and Wakeham, 1987)]. In cultured thermophilic archaea there is an increase in the relative number of cyclopentane rings with temperature (Gliozzi et al., 1983; Uda et al., 2001), possibly a physical adaptation resulting from the higher thermal transition points of archaeal cell membranes composed of GDGTs containing greater numbers of cyclopentane rings. There is some evi-

dence that a GDGT-temperature effect may also recorded in some sediments (Schouten et al., 2002, 2003). Whether in fact temperature affects the abundance of GDGT-1 and GDGT-2 relative to total GDGTs in psychrophilic archaea in nature, as suggested by the Cariaco Basin and Black Sea results, remains to be demonstrated.

5. Overview and future work

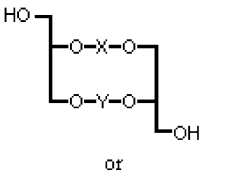
Euxinic waters of the Black Sea and Cariaco Basin contain high concentrations of methane and high rates of anaerobic oxidation of methane (AOM) in which archaea likely play a role. However, abundant CH_4 and high rates of AOM apparently do not a priori guarantee that diagnostic and strongly ^{13}C-depleted archaeal biomarkers commonly attributed to AOM will be found. Rather, distributions of archaeal biomarkers in the two basins are different, with AOM-specific biomarkers only being detected in the deep anoxic zone of the Black Sea. At present, and with the limited sample and data set available, we can only speculate as to why high rates of AOM in the upper anoxic zone of the Black Sea and throughout the Cariaco Basin did not yield the expected biomarker signals. Since AOM and AOM-derived lipids have been most often studied in methane-rich sediments, it is possible that the community structure of methanotrophs is not uniform in all euxinic basins, or even varies within a single basin depending on biogeochemical characteristics we presently do not understand. Perhaps not all methanotrophs produce the biomarkers that are common in CH_4-rich sediments, and we have not yet searched for the appropriate compounds. Alternately, biomarkers derived from AOM in the shallow anoxic portion of Black Sea and in the Cariaco Basin are masked by lipids produced by non-methanotrophic archaea. Our observations clearly emphasize the need to better understand the diversity of archaeal community structure and function in euxinic marine waters. Progress in this understanding will only come through coupled organic geochemical–microbial ecological sampling and analyses in the future.

Acknowledgements

We thank the Captains and crews of *R/V Iselin* and *R/V Knorr* for making their cruises successes, and to O.C. Zafiriou and J.W. Murray for their leadership on the Cariaco and Black Sea cruises, respectively. C. Shu and C.M. Lewis assisted in the laboratory at Skidaway Institute of Oceanography. We thank M. Elvert, Y. Huang, and K. Arzayus for critical reviews of the manuscript. This Research was supported by the U.S. Office of Naval Research, the U.S. National Science Foundation, and The Netherlands Organization for Scientific Research. **[LW]**

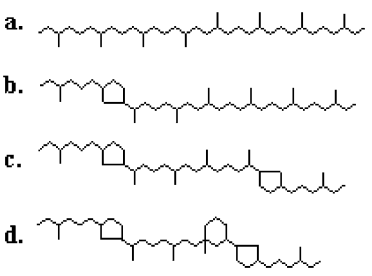
Appendix A

Structures of archaeal tetraether membrane lipids (GDGTs), composed of two glycerol head moieties containing linked two ether-linked isoprenoid chains (biphytanes **a**–**d**).



GDGT basic structure

GDGT structure	GDGT no.	X	Y
I	0	a	a
II	1	a	b
III	2	b	b
IV	3	b	c
V	cren	c	d



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