Methane seepage intensities traced by biomarker patterns in authigenic carbonates
from the South China Sea

Hongxiang Guan ^a, Dong Feng ^{b*}, Nengyou Wu ^a, Duofu Chen ^b

^a *Key Laboratory of Gas Hydrate, Guangzhou Institute of Energy Conversion, Chinese
Academy of Sciences, Guangzhou 510640, China*

^b *Key Laboratory of Marginal Sea Geology, South China Sea Institute of Oceanology,
Chinese Academy of Sciences, Guangzhou 510301, China*

* Corresponding author. Tel.: +86 20 89022336; fax +86 20 84451672.

E-mail address: feng@scsio.ac.cn (D. Feng).

ABSTRACT

Authigenic carbonate rocks from an active seep (Site F) located in the South China Sea (SCS) at a water depth of 1120 m were studied using mineralogical and lipid biomarker analyses. Carbonate mineral compositions, in specific samples, were predominantly aragonite, high-Mg calcite (HMC), or a mixture of both. Abundant ^{13}C -depleted lipid biomarkers diagnostic for archaea provide evidence that anaerobic oxidation of methane (AOM), mediated by anaerobic methane oxidizing archaea (ANME) and their bacterial partners, are the major processes leading to formation of the carbonates. High Methane Index, dominance of ANME-2 assemblages and the predominant contribution of AOM-related biomarkers in the aragonitic carbonate point to the strong impact of AOM communities during its precipitation, which is likely caused by high seepage intensities. The relatively low Methane Index and abundant allochthonous input of the HMC carbonate are a result of the weak impact of AOM microbial communities under conditions of low methane seepage. Evidence for this interpretation also comes from relatively higher carbonate content, lesser contribution of allochthonous organic matter and lower pyrite content in the aragonitic carbonate than in the HMC carbonate. High concentrations of ^{13}C -depleted hopanoids were observed in the aragonitic sample which may be a result of the intermittent presence of oxic conditions in an overall anoxic condition, possibly induced by changing seepage intensities. Our data suggests that lipid biomarkers in authigenic carbonate from seep sites can be helpful for improving assessments of the fluid dynamics in such a complex system.

Keywords: Biomarkers; cold seep; authigenic carbonate; stable isotopes; methane seepage intensity; South China Sea

1. Introduction

Cold seeps are ubiquitously discovered along continental margins worldwide, accompanied by methane-loading fluids migrating to the seafloor and the shallow subsurface environments (e.g., Elvert et al., 2000; Pancost et al., 2000; Zhang et al., 2002; Formolo et al., 2004; Peckmann and Thiel, 2004; Suess, 2014). The key

biogeochemical process at seeps is the anaerobic oxidization of methane (AOM), which is performed by consortia of anaerobic methane-oxidizing archaea (ANME) and sulfate-reducing bacteria (SRB) (e.g., Boetius et al., 2000). The reaction of AOM occurs close to the seafloor and promotes the precipitation of carbonate minerals (e.g., Berner, 1980; Roberts and Aharon, 1994; Aloisi et al., 2000; Peckmann et al., 2001; Peckmann and Thiel, 2004; Sassen et al., 2004; Chevalier et al., 2014; Blumenberg et al., 2015). The biomarker patterns and mineral composition of authigenic carbonates have been found to vary in response to the ambient conditions such as flux of methane (e.g., Greinert et al., 2001; Luff and Wallmann, 2003; Haas et al., 2010; Guan et al., 2013; Blumenberg et al., 2015). Seep carbonates also have the potential to archive information of microbial assemblages involved during its formation and associated environmental parameters (Niemann and Elvert, 2008; Haas et al., 2010; Birgel et al., 2011; Guan et al., 2013; Feng et al., 2014). The great advantage of seep carbonates is that they record the biogeochemical processes, not only the composition at a discrete time but also throughout the long-term seepage history (cf. Feng et al., 2014). Therefore, biomarker patterns combined with compound-specific stable carbon isotopes allow for the identification of environments of formation, predominant consortia during the precipitation of carbonates as well as their carbon sources (e.g., Peckmann and Thiel, 2004; Niemann and Elvert, 2008; Haas et al., 2010; Birgel et al., 2011; Guan et al., 2013; Feng et al., 2014).

In the past decade, over thirty methane seeps have been discovered on the northern continental slope of the South China Sea (SCS), including Site F (Fig. 1; Feng et al., 2015). Our study site, Site F (also named Formosa Ridge by Liu et al., 2008), is the only site known to have active gas seepage (Feng and Chen, 2015). Feng and Chen (2015) reported that the carbon source for authigenic carbonates is exclusively

composed of biogenic methane as indicated by the extremely and consistently low $\delta^{13}\text{C}$ values of the carbonates. The same authors also proposed that the fluid intensities at this site varied dramatically during the carbonate precipitation (from at least 10,600 years ago to present). Here, we investigated mineralogy, biomarker patterns and their $\delta^{13}\text{C}$ values, as well as $\delta^{13}\text{C}$ values of carbonates from Site F of the SCS. Our objectives were to: (1) identify the environments in which the different types of carbonate formed and (2) assess the link among methane seepage intensities, biomarker patterns and the ANME consortia involved at this seep site.

2. Geological settings and materials

The SCS is bordered to the north and west by passive continental margins and to the east by the eastward-dipping ancient subduction zone of the Manila Trench (Taylor and Hayes, 1980; Pautot et al., 1986). Normal faults provide migration pathways for fluids and gases from the deep hydrocarbon-generating subsurface to the modern seafloor (cf. Han et al., 2008). The study area is located at the transition between the passive northern margin and the accretionary eastern margin to the SW of Taiwan Island (Fig. 1). The geologic setting of this site has been described in detail by Liu et al. (2008). Briefly, underneath the southern summit of the ridge, seismic profiles identify a well-developed bottom simulating reflector (BSR) and a clear vertical migration pathway from the deep subsurface to the ocean bottom (Liu et al., 2008).

During the Dayang 31 cruise in the summer of 2013, deep submergence vehicle (DSV) Jiaolong was used to conduct sampling at Site F on the continental slope of the SCS (Fig. 1). The carbonate samples analyzed in this study were collected during dives from the subsurface of the seafloor using the manipulator arms of Jiaolong. Water depth at the site was approximately 1120 m and the bottom water temperature was

typically approximately 3.5 °C. Living mussels, crabs and corals were present during sampling activities. Detailed petrographical and geochemical characteristics of the carbonates are described elsewhere (Feng and Chen, 2015). Four seep carbonates, 54-1, 61-3, 61-6, and 55-4, collected from Site F of the SCS were chosen for biomarker analysis based on their occurrence, mineralogy, and stable carbon and oxygen isotopes (Table 1 and Fig. 2). The $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values of the samples vary from -55.4‰ to -36.8‰ and from 2.6‰ – 4.8‰ , respectively.

3. Methods

Mineral composition of the bulk samples was determined by X-ray diffraction (XRD; Rigaku DXR 3000) at the Guangzhou Institute of Geochemistry, Chinese Academy of Sciences (GIG, CAS). Sample preparation and measurements were described by Tong et al. (2013). Calcite with less than 5 mol% MgCO_3 is considered as low-Mg calcite (LMC), whereas calcite with 5–20 mol% MgCO_3 is referred to as high-Mg calcite (HMC; Burton and Walter, 1991). Carbonate phases with 30–40 mol% MgCO_3 are classified as protodolomite (Naehr et al., 2007). Carbonate contents [expressed as weight percent (wt%) CaCO_3] of the samples were determined by comparing sample weights before and after removing the volatile CaCO_3 fraction using 5% HNO_3 .

The method used for preparation and extraction of lipids is described in detail in Guan et al. (2013), and *n*-icosane (D_{42}), 19-methylarachidic acid and tridecyl alcohol were added as internal standards. The conditions used for GC–MS and GC–IRMS detection were identical to those published in Guan et al. (2013). The carbon isotope ratios are given as δ values ($\delta^{13}\text{C}$ in ‰) relative to Vienna-PeeDee Belemnite (V-PDB). The fatty acid methyl esters and trimethylsiloxyl derivatives were corrected for the

addition of carbon during preparation. Each sample was analyzed at least in duplicates. The standard deviation of the compound-specific carbon isotope measurements was < 0.7‰.

For analysis of glycerol dibiphytanyl glycerol tetraethers (GDGTs), an aliquot of the dried neutral fraction was re-dissolved in hexane/isopropanol (99:1, v:v) using high performance liquid chromatography (HPLC) and atmospheric pressure positive ion chemical ionization mass spectrometry (APCI-MS; Agilent 6410 QQQ LC-MA) at the GIG, CAS. Separation of GDGTs was achieved with an Alltech Prevail Cyano column (2.1×150 mm, 3 μ m, Alltech, USA) maintained at 30 °C (Hu et al., 2012). The elution gradient was 99% hexane and 1% isopropanol isocratic for the first 5 min, followed by a linear gradient to 1.8% isopropanol in 40 min with a constant flow rate of 0.2 ml/min. The column was then cleaned with a mixture of hexane (60%) and isopropanol (40%) for 15 min. Finally, isopropanol was reduced to 1% for 15 min to re-equilibrate the column. The conditions for APCI-MS were: nebulizer pressure 60 psi, vaporizer temperature 300 °C, drying gas (N₂) flow 5 L/min and temperature 200 °C, capillary voltage -2.5 kV, and corona 5 μ A. GDGTs were ionized under APCI conditions and detected with single ion monitoring (SIM) to enhance sensitivity at m/z 1302, 1300, 1298, 1296, 1294 and 1292. The GDGTs were identified based on accurate mass, retention time, and diagnostic fragments (Liu et al., 2012a). No internal standard was added for the quantification of GDGTs. To test the stable carbon isotopes of GDGT-cleaved biphytanes, isoprenoidal tetraethers were subjected to ether cleavage as described by Liu et al. (2012b). An aliquot of the neutral fraction was reacted with 200 μ l BBr₃ (1 M in DCM; Aldrich) for 2 h at 60 °C. After reaction, the sample was dried with argon, 200 μ l superhydride (LiEt₃BH in THF, Aldrich) was then added to reduce the bromides to hydrocarbons at 60 °C for 2 h. A few drops of water were added to

quench the reaction. The hydrocarbons were extracted (3×) with *n*-hexane and the extractions were combined for GC–MS and GC–IRMS analyses. Identification of individual compounds was based on published mass spectra and GC retention times.

4. Results

4.1. Mineralogy

The carbonate content and carbonate mineralogy of the samples analyzed are summarized in Table 1. The carbonate content was 41–68 wt%. The predominance of aragonite in sample 54-1 and HMC in sample 61-6 is apparent. Samples 61-3 and 55-4 had similar mineral compositions, containing about half aragonite and half HMC. Sample 61-6 contained a small amount of dolomite.

4.2. Hydrocarbons

The aragonitic carbonate contained the highest concentration of hydrocarbons (1032 ng/g rock). The mixed mineral carbonates and HMC carbonate had concentrations of hydrocarbons ranging from 387–467 ng/g rock (Supplementary Table S1). Aragonitic carbonate was predominated by the tail-to-tail linked isoprenoids crocetane and PMI (78% of all hydrocarbons), whereas HMC carbonate and the mixed mineral carbonate 55-4 were dominated by the long-chain *n*-alkanes from *n*-C₂₃ to *n*-C₃₁ (53–62% of total hydrocarbons) and the short-chain *n*-alkanes from *n*-C₁₅ to *n*-C₂₂ (14–23% of total hydrocarbons) (Fig. 3 and Supplementary Fig. S1). In the mixed mineral carbonate 61-3, the contribution of crocetane and PMI was nearly equal to that of *n*-alkanes, 47% and 49% of total hydrocarbons, respectively. In the mixed mineral carbonate 55-4, the most abundant isoprenoids were PMI (15% of total hydrocarbons) and crocetane/phytane (13%, ~7:3 mixture).

Most $\delta^{13}\text{C}$ values of *n*-alkanes varied from -33‰ to -29‰ , but *n*-C₂₉ in the mixed mineral carbonate 61-3 showed the most depleted $\delta^{13}\text{C}$ value of -35‰ . The most ^{13}C -depleted isoprenoid was crocetane observed in aragonitic carbonate with a $\delta^{13}\text{C}$ value of -139‰ . The mixed crocetane/phytane peaks showed depleted ^{13}C signatures in the mixed mineral carbonates ($\delta^{13}\text{C}$: -123‰ and -94‰ , respectively). The $\delta^{13}\text{C}$ values of PMI fell in the range of -128‰ to -100‰ (Table 2).

4.3. Carboxylic acids

The lowest content of carboxylic acids was found in the mixed mineral carbonate 55-4 with concentrations of 786 ng/g rock. The concentrations of carboxylic acids in aragonitic, HMC and the mixed mineral carbonate 61-3 ranged from 1353–2355 ng/g rock (Supplementary Table S2). In all samples, the short-chain *n*-fatty acids from *n*-C_{12:0} to *n*-C_{22:0} were predominant, accounting for 51% to 76% of all carboxylic acids and peaking at *n*-C_{16:0} (29–40% of total carboxylic acids) (Fig. 4 and Supplementary Fig. S2). The terminally branched *iso*-/*anteiso*-C₁₃, *iso*-/*anteiso*-C₁₅ and -C₁₇ fatty acids, as well as *iso*-C₁₄ and *iso*-C₁₆ fatty acids, represented 33% of the total carboxylic acids in the aragonitic carbonate and accounted for 14–25% of all carboxylic acids in the HMC carbonate and the mixed mineral carbonates. The most abundant hopanoic acid preserved in aragonitic carbonate was C₃₂ 17 β (H),21 β (H)-hopanoic acid (6%), followed by C₃₁ 17 β (H),21 β (H)-hopanoic acid (3%), and C₃₂ 17 α (H),21 β (H)-hopanoic acid (1%).

The *n*-fatty acids had $\delta^{13}\text{C}$ values ranging from -77‰ to -30‰ . The most depleted value measured was -122‰ for phytanic acid in the mixed mineral carbonate 61-3. The terminally branched *iso*-/*anteiso*-C₁₃, C₁₅ and C₁₇ fatty acids, as well as *iso*-C₁₄ and C₁₆ fatty acids were ^{13}C -depleted (-111‰ to -67‰) in aragonitic carbonate and

the mixed mineral carbonates. Intermittent $\delta^{13}\text{C}$ values (from -47‰ to -36‰) were observed in the HMC carbonate. Furthermore, the $\delta^{13}\text{C}$ values of hopanoic acids ranged from -84‰ to -72‰ (Table 2).

4.4. Alcohols

The concentrations of total alcohols were 3983 ng/g rock and 3703 ng/g rock in aragonitic carbonate and the mixed mineral carbonate 61-3, respectively. In contrast, HMC carbonate and the mixed mineral carbonate 55-4 had much lower contents of total alcohols from 475–500 ng/g rock (Supplementary Table S3). The isoprenoidal glycerol diethers archaeol and *sn* 2-hydroxyarchaeol were the most abundant compounds in aragonitic carbonate and the mixed mineral carbonate 61-3, making up 55–65% of total alcohols (Fig. 5). In the mixed mineral carbonate 55-4 and HMC carbonate, the major compounds were *n*-alcohols, accounting for 72–88% of total alcohols, with long-chain *n*-alcohols being predominant (54% and 69%; Supplementary Fig. S3). The hopanols, represented by C_{32} 17 β (H),21 β (H)-hopanol and C_{32} 17 α (H),21 β (H)-hopanol, accounted for 11% and 1% of alcohols in aragonitic carbonate and the mixed mineral carbonate 55-4, respectively.

Most of the *n*-alcohols showed $\delta^{13}\text{C}$ values from -37‰ to -32‰ , while *n*- $\text{C}_{14:0}$, *n*- $\text{C}_{15:0}$, and *n*- $\text{C}_{16:0}$ alcohols showed more negative $\delta^{13}\text{C}$ signatures in aragonitic carbonate and the mixed mineral carbonate 61-3, and no ^{13}C -depleted values in HMC carbonate (Table 2 and Supplementary Table S3). The most depleted $\delta^{13}\text{C}$ value was -133‰ , observed for *sn*2-hydroxyarchaeol in the mixed mineral carbonate 55-4. The other two isoprenoidal glycerol diethers, archaeol and *di*-hydroxyarchaeol, and phytanol exhibited $\delta^{13}\text{C}$ values from -132‰ to -112‰ . The hopanols had $\delta^{13}\text{C}$ values varying from -101‰ to -47‰ in all samples.

4.5. GDGTs

The most prominent GDGT in aragonitic carbonate and the mixed mineral carbonate 61-3 was GDGT-2 (46% and 52% of all GDGTs, respectively; Table 3 and Supplementary Fig. S4), followed by GDGT-1 (30% and 31% of all GDGTs, respectively), whereas crenarchaeol and GDGT-3 had the lowest percentages (less than 3%) of all GDGTs. In HMC carbonate, the most predominant GDGT was also GDGT-2, followed by similar contents of GDGT-0 and GDGT-1 (22% and 23% of all GDGTs), and crenarchaeol was found to contribute to 15% of all GDGTs. The mixed mineral carbonate 55-4 showed almost uniform contributions of GDGT-0, GDGT-1, and GDGT-2 (29–34% of all GDGTs), and the lowest contents of GDGT-3 and crenarchaeol (between 2–4% of all GDGTs) (Table 3 and Fig. 6). In aragonitic carbonate and the mixed mineral carbonates, $\delta^{13}\text{C}$ values of acyclic, monocyclic and bicyclic biphytanes derived from ether-cleavage of GDGTs were in the same range (av. -119‰) as values found for archaeol and *sn*2-hydroxyarchaeol (see Table 3).

5. Discussion

Extremely negative $\delta^{13}\text{C}$ values (as low as -55‰ ; Table 1) of the authigenic carbonates from this site reveal that biogenic methane is the main carbon source. This interpretation is also supported by the measured tissue $\delta^{13}\text{C}$ values of approximately -70‰ in *Bathymodiolus platifrons* from the same site that largely reflect the isotope composition of methane in local environments (Feng et al., 2015). Further evidence comes from characteristic lipid biomarkers of AOM and their stable carbon isotopic compositions of the seep carbonates. The carbon isotopic composition of diagnostic lipids for archaea and bacteria (with $\delta^{13}\text{C}$ values as low as -139‰ for archaeal

biomarkers and -121‰ for bacterial biomarkers) is typical of carbonates formed at methane seeps and clearly shows precipitation from oxidation of extremely ^{13}C -depleted biogenic methane (Pancost et al., 2000; Blumenberg et al., 2004; Elvert et al., 2005).

5.1. Constraints on formation conditions of seep carbonates

The aragonitic carbonate contained nearly pure suites of AOM biomarkers, and the concentration of isoprenoids derived from ANME including crocetane, PMI, squalene, phytanic acid, phytanol, *sn2*-O-/*sn3*-O-phytanyl glycerol monoethers, diether-bound isoprenoids archaeol, *sn2*-hydroxyarchaeol and dihydroxyarchaeol was up to 3500 ng/g. In this aragonitic carbonate and the mixed mineral carbonate 61-3, the high ratios of *sn2*-hydroxyarchaeol over archaeol (*sn2*-OH/Ar-ratio, both approximately 3) and high contents of ^{13}C -depleted crocetane are in accordance with the dominance of ANME-2 assemblage (Hinrichs et al., 2000; Nauhaus et al., 2005; Niemann and Elvert, 2008; Chevalier et al., 2010, 2011; Birgel et al., 2011; Ziegenbalg et al., 2012). Both samples have low ratios of *anteiso*-/*iso*- $\text{C}_{15:0}$ (0.6 and 0.8, respectively), indicating a dominance of Seep-SRB1 eco-types associated with ANME-2 (Elvert et al., 2003; Blumenberg et al., 2004; Niemann and Elvert, 2008). In the mixed mineral carbonate 55-4, the relatively high abundance of crocetane, low ratio of *sn2*-OH/Ar-ratio (0.6) and the intermediate ratio of *anteiso*-/*iso*- $\text{C}_{15:0}$ (1.0) imply a mixture of ANME-1 and ANME-2 consortia during its precipitation. On the other hand, the biomarker patterns in the HMC carbonate were completely different from that of the aragonitic carbonate, which was mainly composed of odd-even predominant long-chain *n*-alkanes and even-odd predominant long-chain *n*-alcohols, indicating the incorporation of abundant terrestrial organic matter (Eglinton and Hamilton, 1963; Simoneit, 1977;

Hu et al., 2009a; 2009b; Blumenberg et al., 2015a). Although the typical biomarkers (*anteiso-/iso-C_{15:0}*) for SRB were present, they show no ¹³C-depleted values and only one biomarker (PMI) with strong ¹³C-depletion could be attributed to ANME. It is suggested that the HMC carbonate most likely precipitated within sediments of high allochthonous input (Blumenberg et al., 2015a).

The ANME-1 phylogenetic cluster synthesizes GDGTs 0-3 in carbonate reef and microbial mats (Blumenberg et al., 2004; Thiel et al., 2007; Stadnitskaia et al., 2008; Rossel et al., 2008; 2010; Zhang et al., 2011). In the studied aragonitic and the mixed mineral carbonates, the GDGTs 0–2 account for more than 90% of all GDGTs. Their extremely low $\delta^{13}\text{C}$ values based on the ether-cleaved biphytanes are close to that of the archaeol and *sn*2-hydroxyarchaeol, indicating that GDGTs are primarily derived from ANME (Birgel et al., 2008). However, AOM-derived carbon may not be the only carbon source for carbonate authigenesis at the Site F cold seep. High percentage of crenarchaeol (up to 15%) and a high ratio of GDGT-0/Cren (1.5) of the HMC carbonate are similar to GDGTs pattern previously reported in marine Thaumarchaea (Schouten et al., 2000; 2002; 2013; Weijers et al., 2011; Feng et al., 2014). It is well-known that crenarchaeol and GDGT-0 are two most abundant GDGTs in shallow subsurface sediment of the northern South China Sea (e.g., Jia et al., 2012). The total contribution of crenarchaeol and GDGT-0 can be up to 70%, and crenarchaeol accounts for > 50% of total GDGTs (Jia et al., 2012; Ge et al., 2013). In terms of the HMC carbonate, the relatively high abundance of crenarchaeol suggested that GDGTs in the carbonate are largely sourced from marine Thaumarchaea. The molecular indicator “Methane Index” has been used to evaluate the impact of methane in paleoenvironments (Zhang et al., 2011). The Methane Index values of the aragonitic and the mixed mineral carbonates are much higher (0.97–0.99) than that of the HMC

carbonate (0.81). This relation suggests a stronger impact of AOM on the aragonitic and the mixed mineral carbonates over the HMC carbonate (cf. Zhang et al., 2011).

In the aragonitic carbonate and the mixed mineral carbonate 61-3, the dominance of ANME-2 and favored precipitation conditions for aragonite suggest that the carbonates formed in seafloor subsurface environments with high methane flux (e.g., Luff and Wallmann, 2003; Blumenberg et al., 2004; Stadnitskaia et al., 2008; Haas et al., 2010; Rossel et al., 2010; Guan et al., 2013). The high contributions of the AOM-related biomarkers (Table 2), represent the strong impact of AOM microbial communities and accordingly high seepage intensities, which are in line with the predominant consortia of ANME-2. Blumenberg et al. (2015a) reported a considerable difference between the top and the basement plate of chemohermes in the northwestern Black Sea. The nearly pure suite of AOM biomarker patterns in the aragonitic carbonate and the mixed mineral carbonate 61-3 resemble the top part of chemohermes biomarker characteristics, suggesting their formation in anoxic bottom water even in a water column and a paleoenvironment with low organic matter input (Blumenberg et al., 2015a). On the contrary, in the mixed mineral carbonate 55-4, a mixture of ANME-1 and ANME-2 consortia were dominant during its precipitation. The abundant allochthonous biomarkers consisted of terrestrial organic matter and marine Thaumarchaea, which was most likely originally within sediments, probably resulting from low methane supply and high allochthonous input (Blumenberg et al., 2015a). This is clearly the case for the favored environment conditions of ANME-1 and precipitation of HMC. (e.g., Luff and Wallmann, 2003; Blumenberg et al., 2004; 2015; Stadnitskaia et al., 2008; Haas et al., 2010; Rossel et al., 2010; Guan et al., 2013). The HMC carbonate has a relatively low Methane Index value and abundant allochthonous

input that point to the precipitation under weak impact of AOM communities, which is most likely to occur at low seepage intensities.

5.2. The influence of changing methane seepage on hopanoids

The influence of methane seepage intensities is reflected not only in the biomarker patterns as discussed above but also in the occurrence of hopanoids. The hopanoids, including diplopterol, hopanoic acids and hopanols, have been recognized in diverse environments but also in seep settings (Pancost et al., 2000; Birgel and Peckmann., 2008; Birgel et al., 2011; Ziegenbalg et al., 2012; Guan et al., 2013; Blumenberg et al., 2015b). These hopanoids revealed similar $\delta^{13}\text{C}$ values varying from -81‰ to -72‰ in the aragonitic carbonate (Table 2), suggesting the direct incorporation of methane-derived carbon. Although the co-occurrence of ^{13}C -depletion hopanoids with *iso-/anteiso*- C_{15} and C_{17} fatty acids recognized in seep samples were ascribed to SRB which involved in AOM (Pancost et al., 2000; Thiel et al., 2003), those ^{13}C -depleted diplopterol, hopanoic acids and hopanols recognized here were probably sourced from aerobic methanotrophic bacteria (cf. Birgel et al., 2011). Based on the presence of ^{13}C -depleted hopanoids that originated from aerobic methanotrophic bacteria and the occurrence of negative Ce anomalies in seep carbonate of the Gulf of Mexico, Birgel et al. (2011) concluded that these carbonates formed under at least temporarily oxic conditions. Indeed, the aragonitic carbonate from Site F, with slightly negative Ce anomalies ($\text{Ce}/\text{Ce}^* = 0.78$) (cf. Feng and Chen, 2015), suggests at least an intermittent presence of oxic conditions during its formation. Moreover, the presence of abundant cemented shell debris in the aragonitic carbonate is an additional argument in favor of precipitation under oxic conditions (see Fig. 4D of Feng and Chen, 2015). Evidence for this correlation is also based on the pyrite content of the carbonates (Table 1) that

shows the lowest pyrite content in the aragonitic carbonate as opposed to the mixed mineral carbonates and the HMC carbonates.

At cold seep sites, authigenic carbonates precipitate in the sulfate methane transition zone (SMTZ), which is controlled largely by the flux of methane (Borowski et al., 1996). Recently, by integrating seafloor observations, mineralogical and geochemical data and ^{14}C ages of the Site F carbonates, Feng and Chen (2015) suggested that the intensity of fluid at this seep site was changing from at least 10.6 ka ago until today. The SMTZ tends to be shallower within the sediments at sites of high methane flux than at sites of relatively low methane flux (Luff and Wallmann, 2003). Moreover, relatively high sulfate concentration and porosity on or near the seafloor surface favors precipitation of aragonite over HMC (Greinert et al., 2001). Accordingly, the occurrence of ^{13}C -depleted hopanoids in aragonitic carbonates often accompanies the dominance of ANME-2/DSS consortia and agrees with the formation under conditions of high methane seepage intensities (cf. Birgel et al., 2011; Guan et al., 2013). However, this does not mean that aerobic methanotrophs only occur in high seepage intensities. Natalicchio et al. (2015), on the other hand, reported the presence of ^{13}C -depleted hopanoids at ancient seeps that were typified by diffusive fluid seepage. The preservation of lipids of aerobic methanotrophs is favored in authigenic carbonates which formed under changing seepage intensities by either an abrupt increase or sudden decrease in seepage intensities, but is not likely to be preserved in seep carbonates forming at seeps with seepage continuing for extended periods of time (cf. Feng et al., 2009; Natalicchio et al., 2015).

6. Conclusions

Seep carbonates from Site F of the South China Sea formed as a consequence of

anaerobic oxidation of methane (AOM) as revealed by the presence of ^{13}C -depleted biomarkers of anaerobic methane oxidizing archaea (ANME) and associated sulfate-reducing bacteria (SRB). The occurrence of different biomarker patterns, carbonate minerals and dominant groups of AOM consortia appears to be closely related to the intensity of methane seepage. The nearly pure suite of AOM biomarkers and the predominance of ANME-2 group in aragonitic carbonate and the mixed mineral carbonate 61-3, indicate precipitation under conditions indicating a strong impact of AOM microbial communities and accordingly high methane flux. High-Mg calcite (HMC) carbonate appears to be formed at weak impacts of AOM microbial communities in the presence of low AOM-related biomarkers and a low Methane Index. Evidence for this interpretation also comes from the relatively higher carbonate content and lesser contributions of long-chain *n*-alkanes and *n*-alcohols in the aragonitic carbonate and the mixed mineral carbonate 61-3 than in the HMC carbonate. Abundant ^{13}C -depleted hopanoids in the aragonite confirm the episodic occurrence of oxic conditions, which was possibly induced by changing seepage intensities.

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Figure captions

Fig. 1. Map showing the geographic location of Site F. Bathymetry map on the right courtesy of Prof. Chi-Yue Huang (National Cheng Kung University). The main sampling area is delineated by the black box.

Fig. 2. Morphologies of the seep carbonate samples analyzed in this study. (54-1): Carbonate slab with live mussels on the surface. (61-3): Nodules incorporated in composite authigenic carbonate aggregate. (61-6): Carbonate crust. (55-4): Carbonate concretion.

Fig. 3. Partial gas chromatograms (FID) of hydrocarbon fraction from samples 54-1 (above) and 61-6 (below); Istd: internal standard (*n*-icosane (D₄₂)); Cr/Ph: crocetane/phytane; PMI: pentamethylicosane; Pr: pristane; Sq: squalane. Gray dots: *n*-alkanes.

Fig. 4. Partial gas chromatograms (FID) of carboxylic acid fraction from samples 54-1 (above) and 61-6 (below); Istd: internal standard (19-methylarachidic acid); Ph: Phytanic acid; 10 me: 10 meC_{16:0}; αβ-32-acid: C₃₂ 17α(H),21β(H)- hopanoic acid; ββ-32-acid: C₃₂ 17β(H),21β(H)- hopanoic acid; cont. contamination. White dots: *n*-fatty acids. ω (c/t): the position of the double bond from the methyl end of the fatty acid, the suffixes c for *cis* and t for *trans* refer to geometric isomers.

Fig. 5. Partial gas chromatograms (FID) of alcohol fraction from samples 54-1 (above) and 61-6 (below). Istd: internal standard (tridecyl alcohol); *sn2*: *sn2*-O-phytanyl-glycerolether; *sn3*: *sn3*-O-phytanyl-glycerolether; DAGE: dialkyl glycerolether; Ar: archaeol; *sn2*-OH-Ar: *sn2*-hydroxyarchaeol; di-OH-Ar, dihydroxyarchaeol; THM: tetrahymanol; Black diamonds: *n*-alcohols.

Fig. 6. Partial HPLC-APCI-MS base peak chromatograms of GDGTs (a), and partial gas chromatograms of ether-cleaved lipids extracted (b) of the Site F carbonates. bp,

649 biphytanes, numbers indicate the number of cyclopentane moieties in the biphytane
650 hydrocarbon chain.

651

652

Figure 1

Figure 2

54-1

61-3

61-6

55-4

Figure 3

Figure 4

Figure 5

Figure 6

Table 1

Sample ID	Carb. Cont.	Qtz	LMC	Mg in LMC	HMC	Mg in HMC	Arag.	Protodol./dolo.	Mg in protodol./dolo.	Pyrite content ^a	Sample vs. VPDB ^b		Comments
	(wt%)	(%)	(%)	(mol%)	(%)	(mol%)	(%)	(%)	(mol%)	(wt.%)	δ ¹³ C (‰)	δ ¹⁸ O (‰)	
54-1	68	17	6				94			< 0.05	-42.2	2.6	matrix
											-36.8	3.6	matrix
											-46.7	3.2	cement
											-44.9	3.6	cement
											-42.4	3.4	cement
61-3	58	18			46	16	54			0.27	-44.4	3.6	cement
											-50.2	4.4	clast
											-48.5	3.6	matrix
											-49.3	3.6	cement
											-49.6	3.6	matrix
61-6	41	16			95	8	5		37	0.90	-50.2	4.4	clast
											-47.2	3.5	matrix
											-51.5	4.8	matrix
											-49.8	3.8	clast
											-46.7	3.7	matrix
55-4	50	14			56	7	44			0.32	-53.1	3.9	matrix
											-55.3	3.7	clast
											-53.4	4.6	clast

Carb. Cont.=Carbonate content, Qtz=Quartz, HMC=High-Mg calcite, LMC=Low-Mg calcite, Arag.=Aragonite, Protodol/dolo.=Protodolomite/dolomite.

^a D. Feng, unpublished data.

^b Data are from published source (Feng and Chen, 2015)

Table 2

Sample ID	54-1		61-3		61-6		55-4	
	Content (ng/g rock)	$\delta^{13}\text{C}$ (‰) V-PDB	Content (ng/g rock)	$\delta^{13}\text{C}$ (‰) V-PDB	Content (ng/g rock)	$\delta^{13}\text{C}$ (‰) V-PDB	Content (ng/g rock)	$\delta^{13}\text{C}$ (‰) V-PDB
<i>archaea biomarkers</i>								
crocetane*/ph	690	-139	128	-123	40	-33	61	-94
PMI	112	-120	63	-125	3	-100	71	-128
squalane	4	-84	3	-94	2	n.d.	3	-97
phytanic acid	42	-121	41	-122	n.d.	n.d.	16	-113
phytanol	163	-127	325	-130	n.d.	n.d.	48	-132
<i>sn</i> 2- <i>O</i> -phytanyl-glycerolether	32	-120	28	-125	n.d.	n.d.	4	n.d.
<i>sn</i> 3- <i>O</i> -phytanyl-glycerolether	25	-118	34	-129	n.d.	n.d.	3	n.d.
archaeol	563	-123	565	-128	n.d.	n.d.	4	-127
<i>sn</i> 2-OH-Ar	1644	-125	1856	-129	n.d.	n.d.	3	-133
<i>di</i> -hydroxyarchaeol	244	-122	116	-124	n.d.	n.d.	n.d.	n.d.
OH-Ar/Ar	2.9		3.3		n.d.		0.6	
<i>SRB biomarkers</i>								
<i>Iso</i> -C ₁₃ fatty acid	8	-99	6	n.d.	n.d.	n.d.	n.d.	n.d.
<i>Anteiso</i> -C ₁₃ fatty acid	4	-86	4	n.d.	3	n.d.	n.d.	n.d.
<i>Iso</i> -C ₁₅ fatty acid	185	-108	198	-111	237	-40	33	-91
<i>Anteiso</i> -C ₁₅ fatty acid	144	-97	109	-104	304	-36	34	-85
<i>Iso</i> -C ₁₇ fatty acid	13	-87	21	-91	9	n.d.	5	-67
<i>Anteiso</i> -C ₁₇ fatty acid	34	-99	33	-107	9	-47	6	-96
<i>10Me</i> -C _{16:0}	n.d.	n.d.	45	-53	32	-47	2	n.d.
<i>Ai</i> -/ <i>iso</i> -C _{15:0} fatty acid ratios	0.78		0.55		1.28		1.0	

<i>Other bacterial biomarkers</i>								
<i>Iso</i> -C ₁₅ alcohol	15	-103	16	-102	4	-38	4	n.d.
<i>Anteiso</i> -C ₁₅ alcohol	24	-73	25	-72	5	-46	8	n.d.
DAGE C _{30(ai-C15:0/ai-C15:0)}	57	-108	70	-116	n.d.	n.d.	16	-113
DAGE C _{31:1}	n.d.	n.d.	28	-99	n.d.	n.d.	24	-84
DAGE C _{32:1}	n.d.	n.d.	29	-121	n.d.	n.d.	n.d.	n.d.
cholesterol	60	-60	21	n.d.	30	n.d.	n.d.	n.d.
cholestanol	171	-72	33	-52	17	n.d.	11	n.d.
diplopterol	142	-79	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
tetrahymanol	46	-107	n.d.	n.d.	n.d.	n.d.	12	-70
<i>n</i> -C ₁₄ alcohol	44	-104	17	n.d.	5	-37	1	n.d.
<i>n</i> -C ₁₅ alcohol	16	-99	8	-88	2	-37	6	n.d.
<i>n</i> -C ₁₆ alcohol	81	-104	42	-93	11	-37	14	n.d.
C ₃₂ 17 α (H),21 β (H)-hopanol	145	-79	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C ₃₂ 17 β (H),21 β (H)-hopanol	271	-81	2	-89	n.d.	-47	4	-101
C ₃₂ 17 α (H),21 β (H)-hopanoic acid	39	-80	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C ₃₁ 17 β (H),21 β (H)-hopanoic acid	16	-72	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C ₃₂ 17 β (H),21 β (H)-hopanoic acid	78	-74	n.d.	n.d.	20	n.d.	4	-84

1 n.d.: not detected.

Table 3

Sample ID	54-1		61-3		61-6		55-4	
	Content (ng/g rock)	$\delta^{13}\text{C}$ (‰) V-PDB	Content (ng/g rock)	$\delta^{13}\text{C}$ (‰) V-PDB	Content (ng/g rock)	$\delta^{13}\text{C}$ (‰) V-PDB	Content (ng/g rock)	$\delta^{13}\text{C}$ (‰) V-PDB
GDGT-0	9%		14%		23%		29%	
GDGT-1	30%		31%		22%		34%	
GDGT-2	52%		46%		35%		31%	
GDGT-3	6%		8%		5%		4%	
crenarchaeol	3%		1%		15%		2%	
bp-0		-116		-121		n.d.		-126
bp-1		-120		-122		n.d.		-127
bp-2		-110		-116		n.d.		-116
bp-3		n.d.		n.d.		n.d.		n.d.
GDGT-0/Cren	2.9		15.7		1.5		19.2	
GDGT-2/Cren	28.5		51.0		2.3		20.6	
MI	0.97		0.99		0.81		0.97	

1

n.d.: not detected.

2

MI: Methane Index, $\text{MI} = \{ [\text{GDGT-1}] + [\text{GDGT-2}] + [\text{GDGT-3}] \} / \{ [\text{GDGT-1}] + [\text{GDGT-2}] + [\text{GDGT-3}] + [\text{Crenarchaeol}] + [\text{Cren_isomer}] \}$