

1 *Investigation of Extractable Organic Compounds in* 2 *Deep-sea Hydrothermal Vent Fluids along the Mid-Atlantic Ridge*

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11 **Abstract** – The possibility that deep-sea hydrothermal vents may contain organic compounds
12 produced by abiotic synthesis or by microbial communities living deep beneath the surface has
13 led to numerous studies of the organic composition of vent fluids. Most of these studies have
14 focused on methane and other light hydrocarbons, while the possible occurrence of more
15 complex organic compounds in the fluids has remained largely unstudied. To address this issue,
16 the presence of higher molecular weight organic compounds in deep-sea hydrothermal fluids was
17 assessed at three sites along the Mid-Atlantic Ridge that span a range of temperatures (51 to
18 >360 °C), fluid compositions, and host-rock lithologies (mafic to ultramafic). Samples were
19 obtained at several sites within the Lucky Strike, Rainbow, and Lost City hydrothermal fields.
20 Three methods were employed to extract organic compounds for analysis, including liquid:liquid
21 extraction, cold trapping on the walls of a coil of titanium tubing, and pumping fluids through
22 cartridges filled with solid phase extraction (SPE) sorbents. The only samples to consistently
23 yield high amounts of extractable organic compounds were the warm (51-91 °C), highly alkaline
24 fluids from Lost City, which contained elevated concentrations of C₈, C₁₀, and C₁₂ *n*-alkanoic
25 acids and, in some cases, trithiolane, hexadecanol, squalene, and cholesterol. Collectively, the
26 C₈-C₁₂ acids can account for about 15% of the total dissolved organic carbon in the Lost City
27 fluids. The even-carbon-number predominance of the alkanolic acids indicates a biological
28 origin, but it is unclear whether these compounds are derived from microbial activity occurring
29 within the hydrothermal chimney proximal to the site of fluid discharge or are transported from
30 deeper within the system. Hydrothermal fluids from the Lucky Strike and Rainbow fields were
31 characterized by an overall scarcity of extractable dissolved organic compounds. Trace amounts
32 of aromatic hydrocarbons including phenanthrenes and benzothiophene were the only
33 compounds that could be identified as indigenous components of these fluids. Although
34 hydrocarbons and fatty acids were observed in some samples, those compounds were likely
35 derived from particulate matter or biomass entrained during fluid collection. In addition, extracts
36 of some fluid samples from the Rainbow field were found to contain an unresolved complex
37 mixture (UCM) of organic compounds. This UCM shared some characteristics with organic
38 matter extracted from bottom seawater, suggesting that the organic matter observed in these
39 samples might represent seawater-derived compounds that had persisted, albeit with partial
40 alteration, during circulation through the hydrothermal system. While there is considerable
41 evidence that Rainbow and Lost City vent fluids contain methane and other light hydrocarbons
42 produced through abiotic reduction of inorganic carbon, we found no evidence for more complex
43 organic compounds with an abiotic origin in the same fluids.

44 1. INTRODUCTION

45 Ever since deep-sea hydrothermal systems first began to be explored in the late 1970s, the
46 occurrence of organic compounds in the hydrothermal fluids has been the subject of considerable
47 scientific study. One of the primary motivations behind these studies has been to determine the
48 source of the organic compounds and, in particular, to investigate whether some compounds
49 might be generated by abiotic organic synthesis within the hydrothermal system (e.g., Welhan
50 and Craig, 1983; Lilley et al., 1983, 1993; Berndt et al., 1996; Charlou et al., 2000, 2002, 2010;
51 Holm and Charlou, 2001; McCollom and Seewald, 2001, 2007; Foustoukos and Seyfried, 2004;
52 Proskurowski et al., 2008; Bradley and Summons, 2009; Lang et al., 2010, 2012). In addition,
53 some organic compounds in deep-sea hydrothermal fluids may be produced by biological
54 processes in subsurface environments, and can potentially provide insights into the activity of
55 deep-seated microbial communities (Deming and Baross, 1993; Bradley and Summons, 2009;
56 Reeves et al., 2014).

57 Field-based studies of organic compounds in deep-sea hydrothermal fluids have
58 predominantly focused on the abundance and isotopic composition of methane and light
59 hydrocarbons (e.g., Welhan and Craig, 1983; Lilley et al., 1983, 1993; Charlou et al., 1996,
60 2000, 2002; Proskurowski et al., 2008; Foustoukos et al., 2009). However, several studies have
61 analyzed deep-sea hydrothermal fluids for the presence of other dissolved compounds including
62 small carboxylic and amino acids (Haberstroh and Karl, 1989; Martens, 1990; Horiuchi et al.,
63 2004; Lang et al., 2010, 2013; Klevenz et al., 2010; Fuchida et al., 2014). Relatively few studies
64 have examined whether higher molecular weight hydrocarbons and other larger extractable
65 organic compounds are present in deep-sea hydrothermal fluids (Brault et al., 1988; Holm and
66 Charlou, 2001; Konn et al., 2009, 2012).

67 Sampling deep-sea hydrothermal fluids for higher hydrocarbons and other organic
68 compounds presents significant challenges. Based on the abundance of volatile light
69 hydrocarbons, higher molecular weight compounds might be expected to be present at
70 nanomolar or lower concentrations, requiring them to be concentrated prior to analysis for most
71 commonly used analytical methods. In addition, hydrocarbons and many other types of organic
72 compounds must be extracted from the aqueous phase prior to analysis for conventional
73 methods. While hydrocarbons and other low-polarity organic compounds have relatively high
74 aqueous solubilities at the elevated temperatures and pressures that occur within many deep-sea
75 hydrothermal systems, cooling of discharged fluids to ambient temperatures of the deep sea
76 (usually ~ 2 °C) may cause these compounds to exsolve and precipitate on the surfaces of the
77 sampling device. If only the fluid phase is analyzed, exsolved compounds may not be detected.
78 Furthermore, organic compounds from other sources, including seawater and hydrothermal vent
79 mineral deposits (e.g., Simoneit et al., 2004), can be inadvertently entrained into the sample
80 during fluid collection. In addition, hydrothermal vent chimneys are often inhabited by
81 biological communities, enhancing the potential for contamination from biomass if solids are
82 incorporated during sample collection. Oils, hydraulic fluid, and other organic compounds
83 leaking from the vehicle used to collect samples represent other potential sources of
84 contamination.

85 This study was undertaken to investigate the occurrence of extractable organic compounds in
86 hydrothermal systems along the Mid-Atlantic Ridge (MAR). Hydrothermal fluids were sampled
87 at three sites along the MAR representing a range of conditions (Table 1). Hot (up to 366 °C),
88 acidic fluids were sampled at systems hosted in ultramafic rocks at Rainbow and in basaltic
89 rocks at Lucky Strike (Von Damm et al., 1998; Charlou et al. 2000, 2002). Warm (~ 90 °C),

90 strongly alkaline fluids circulated through serpentinized ultramafic rocks were sampled at Lost
91 City (Table 1) (Kelley et al., 2005). The Rainbow and Lost City were targeted for study because
92 previous investigations have indicated that the methane and light hydrocarbons in the
93 hydrothermal fluids at these sites may have an abiotic origin (Charlou et al., 2002, 2010;
94 Proskurowski et al., 2008), and it has been suggested that other hydrocarbons with an abiotic
95 origin might also be present (Holm and Charlou, 2001; Konn et al., 2012). None of the systems
96 included in this study show any evidence for buried sediments that might contribute organic
97 compounds to circulating hydrothermal fluids.

98 Concurrent with our study, Konn et al. (2009, 2012) also assessed the presence of extractable
99 organic compounds in hydrothermal fluids from the Rainbow and Lost City sites, but used
100 somewhat different methods for extraction and analysis. They reported detection of a number of
101 extractable organic compounds in the fluids from both sites, including aliphatic, cyclic, and
102 aromatic hydrocarbons as well as fatty acids. Previous work by Holm and Charlou (2001) had
103 identified trace amounts of a homologous series of C₁₆-C₂₉ *n*-alkanes in hydrothermal fluids at
104 Rainbow. Other studies have identified formate, acetate, and amino acids in the fluids at Lost
105 City (Lang et al., 2010, 2013). Reeves et al. (2014) report concentrations of methanethiol in
106 hydrothermal fluids at many of the same sites included in this study, from samples collected on
107 the same expedition as those reported here.

108 2. METHODS

109 2.1. Fluid sampling

110 Hydrothermal fluid samples were acquired in July 2008 using the *Jason* remotely operated
111 vehicle (ROV) as part of the KNOX18RR expedition to the Mid-Atlantic Ridge on board the
112 R/V *Roger Revelle* (Fig. 1). Hydrothermal fluids were sampled at several vent sites across the
113 Lucky Strike, Rainbow, and Lost City hydrothermal fields, as summarized in Table 1. A single
114 vent fluid sample was also obtained at the TAG hydrothermal site before a shipboard equipment
115 failure prematurely terminated the cruise, but because no organic compounds were observed in
116 the sample it is not discussed further here. At Lucky Strike and Rainbow, fluids were collected
117 from a number of discrete vent locations distributed across the hydrothermal fields. Most
118 samples were obtained at natural orifices in chimney structures where there was vigorous
119 discharge of high-temperature (>250 °C) hydrothermal fluids (e.g., Fig. 1a). For the Ecurie site
120 at Rainbow, however, fluids were sampled from a cavity excavated into the side of a chimney
121 structure that had moderately hot fluids (~60 °C) slowly venting from its apex (Fig. 1b).
122 Theoretical studies have suggested that mixing zones within deep-sea hydrothermal systems
123 might be particularly favorable environments for abiotic organic synthesis (Shock and Schulte,
124 1998), and this site was chosen because mixing of high temperature fluids with seawater is a
125 likely reason for the low temperature of the vent fluids. Since the fluids venting at the apex of
126 the Ecurie structure were too diffuse to sample, a cavity was excavated in the side of the chimney
127 to gain access to more vigorous fluid flow (Fig. 1b). At Lost City, fluids were obtained at the
128 Beehive site on the side of a large structure (~60 m high) named Poseidon (Fig. 1c,d) and at an
129 unnamed orifice at the top of the same structure. For comparison with the vent fluids, samples of
130 deep seawater were also collected adjacent to the Lucky Strike, Lost City, and TAG sites using
131 the same methods employed to collect hydrothermal fluids.

132 Because it was not clear at the outset what approach might be most effective for extraction of
133 dissolved organic compounds from hydrothermal fluids, three different sampling strategies were
134 employed. First, organic compounds were extracted from hydrothermal fluids acquired using

135 isobaric gas-tight (IGT) syringe-type samplers (Seewald et al., 2002). A key feature of the IGT
136 samplers is that the fluid intake rate can be regulated to minimize the amount of seawater
137 entrained during sample collection. In an effort to minimize the contribution of organic
138 compounds from background sources, the original design of the samplers was modified by
139 replacement of the Viton O-rings on the sample chamber piston with Teflon O-rings to eliminate
140 the need for a lubricant. Nevertheless, it was still necessary to use a Viton O-ring and a lubricant
141 (Fluorolube) on the pressure seal for the sample chamber. Prior to each deployment, all wettable
142 surfaces in the samplers were washed with successive rinses of methanol, dichloromethane
143 (DCM), and hexane, and then dried in air. Despite these precautions, traces of fluorolube were
144 found in some samples, presumably derived from the lubricant used on the pressure seal or from
145 residual compounds in the sampler snorkels. The dead volume of the samplers (including the
146 snorkel; ~ 5 ml) was filled with deionized water (Fisher Scientific Optima, hereinafter referred to
147 as Fisher H₂O) prior to deployment rather than the usual practice of filling this volume with
148 bottom seawater. A Teflon cap was placed on the inlet of the sampler snorkel to limit seawater
149 infiltration during transport to the seafloor.

150 In the second approach, hydrothermal fluids on the seafloor were pumped through a 4 m long
151 coil of narrow titanium tubing (0.125" O.D. × 0.064" I.D.) to extract organic compounds (this
152 apparatus came to be known as the Peristaltic Organic Pump sampler or "POP gun") (Fig. 2).
153 The concept behind this device was that, because the Ti-coil is bathed in cold (~2 °C) seawater,
154 cooling of the hydrothermal fluids as they passed through the tubing might induce precipitation
155 of higher molecular weight organic compounds such as long-chain hydrocarbons and fatty acids
156 on the tubing walls, owing to the decreasing solubility of these compounds with decreasing
157 temperature and the large internal surface area of the narrow coiled tube. Prior to each
158 deployment, the Ti tubing was washed with DCM followed by MeOH, and then rinsed and filled
159 with Fisher H₂O.

160 The third approach used to extract organic compounds was to attach a purpose-built cartridge
161 containing a solid-phase extraction (SPE) sorbent to the outlet of the Ti tubing of the POP gun
162 (Fig. 2 inset). The SPE cartridges were intended to extract additional organic compounds that
163 remained dissolved in the fluid after passage through the Ti tubing. The SPE cartridges
164 contained several grams of SPE microbeads within a 2.54 cm outside diameter Teflon tube (2.5
165 cm O.D. × 8 cm long), capped with porous Teflon frits to allow flow of fluids through the
166 cartridge, and sealed with Swagelok fittings. Prior to deployment, the SPE sorbent was rinsed
167 with ~15 ml MeOH followed by ~30 ml of Fisher DI water while in the cartridge.

168 Two types of SPE sorbents targeted at different classes of organic compounds were used
169 separately during deployments of the POP gun (Table 1). For most deployments, the
170 "hydrophilic-lipophilic balanced" Oasis HLB sorbent (Waters Corporation) was used. This
171 sorbent had been used previously for extraction of organic compounds from fluids collected at
172 the Rainbow site (Holm and Charlou, 2001), and is designed to extract a broad spectrum of
173 acidic, basic, and neutral organic compounds from aqueous fluids. At two sample locations
174 (Rainbow and Lost City) fluids were extracted using the C8 phase from Grace Discovery
175 Sciences. This phase is designed to target smaller non-polar organic compounds in the octane
176 range, and was used in an attempt to isolate the type of alkylated monocyclic compounds that
177 had been reported in previous SPE extracts of hydrothermal fluids (Konn et al., 2009, 2012).

178 When fully assembled on *Jason*, the POP gun sampler consisted of a coiled 4 m length of
179 titanium tubing connected at the outlet end through Teflon tubing to the cartridge containing the
180 SPE sorbent, and then to a peristaltic pump (Fig. 2). At the seafloor, the inlet of the POP gun was

181 inserted into the orifice of a hydrothermal vent, and fluid was then pumped through the device
182 for a period of about an hour in most cases. During sampling, flow of hydrothermal fluid
183 through the POP gun was visually confirmed by the presence of shimmering (i.e., warm) fluid at
184 the outlet of the peristaltic pump. A benchtop test with the fully assembled POP gun indicated
185 that the flow rate was about 21 ml min^{-1} , although this flow rate may not have been achieved by
186 the peristaltic pump during operation at in situ pressures on the seafloor. Assuming the benchtop
187 flow rate to be representative, it is estimated that $\sim 1.2 \text{ L}$ of hydrothermal fluid would have been
188 pumped through the POP gun during each 60 minute sample. Owing to logistical considerations,
189 POP gun and SPE samples could not be obtained at every site where fluids were collected using
190 IGT samplers during the cruise (Table 1).

191 2.2. Sample processing

192 The three sampling strategies employed required use of different protocols to extract organic
193 compounds (Fig. 3). Following deployment and return to the surface, all sampling devices were
194 immediately placed in a refrigerator at $4 \text{ }^{\circ}\text{C}$ until they were processed (usually within ~ 2 hours).
195 All solvents used to extract organic compounds were Fisher Scientific Optima grade.

196 For the IGT samplers, the first two aliquots of fluid from each sample were collected in pre-
197 cleaned vials and stored at -20°C for later analysis of inorganic chemistry (5 ml; Table 1) and
198 dissolved free amino acids plus other organic compounds ($\sim 20 \text{ ml}$). The remaining fluid (~ 120
199 ml) was then transferred to a pre-cleaned glass bottle, where organic compounds were extracted
200 by adding $\sim 8 \text{ ml}$ of dichloromethane (DCM) and then shaking vigorously for 10 minutes. The
201 DCM phase was then removed by pipette into a separate vial for further processing. Because the
202 Lost City hydrothermal fluids are strongly alkaline, a second liquid-liquid extraction was
203 performed to extract any organic acid anions that may have been present following acidification
204 to $\text{pH} < 3$ by addition of concentrated HCl. The $\text{pH}_{25^{\circ}\text{C}}$ values of the Rainbow and Lucky Strike
205 fluids were well below the $\text{p}K_{\text{a}}$ of alkanolic acids, so these samples were not acidified.

206 The DCM extracts were concentrated by evaporation under a gentle stream of N_2 at room
207 temperature. When the extract had been concentrated to $\sim 2 \text{ ml}$, it was capped and transferred to
208 a freezer at -20°C , which in most cases resulted in exsolution of residual water to form a ring of
209 ice within the sample vial at the top of the solvent. When this occurred, the solvent phase was
210 transferred to a new vial, concentrated further, and then stored at -20°C until analysis. During
211 concentration of these and all other extracts, care was taken to prevent the solvent from
212 evaporating to dryness to limit the loss of semi-volatile organic compounds. Prior to analysis,
213 the concentrated extracts were treated with Cu powder to remove native sulfur. The Cu for this
214 treatment was activated by soaking in concentrated HCl and then rinsed with organic solvents.

215 After removal of the hydrothermal fluid, the interior of the IGT sampler was rinsed with
216 successive aliquots of methanol (MeOH) and DCM to collect organic compounds that may have
217 precipitated on the walls of the sampler. Both rinses were combined into a single glass vial. In
218 practice, a small amount of residual hydrothermal fluid remained in the sampler that was
219 collected along with the solvents. The combined solvents were evaporated to approximately half
220 their original volume with a stream of N_2 , at which point the extract usually separated into an
221 $\text{H}_2\text{O}/\text{MeOH}$ -rich phase underlain by a DCM-rich phase. Additional DCM and acidified Fisher
222 H_2O were added to the vial to enhance transfer of organic compounds to the DCM phase. To
223 reduce the possibility of contaminating the samples with organic compounds, the H_2O used for
224 this step was extracted with DCM before adding it to the solvent extract. After shaking, the
225 DCM-rich layer was pipetted into a separate vial and processed using the same methods
226 described for the fluid extract.

227 To extract organic compounds precipitated on the walls of the Ti tubing of the POP gun,
228 remaining fluid was removed by suction with a syringe and the tubing was then rinsed with ~5
229 ml aliquots of MeOH and DCM in succession. The solvents were pushed through the tubing
230 using a solvent-cleaned glass syringe with Teflon plunger. The MeOH used for this procedure
231 was mildly acidified with HCl to ensure extraction of carboxylic acids and other weakly acidic
232 compounds present as anions. In some cases, this had the unforeseen consequence of converting
233 organic acids to methyl esters. While most of the MeOH extracts from the POP gun samples
234 were found to contain small amounts of organic compounds, very few of the DCM extracts
235 contained detectable organic compounds, indicating that most soluble organic compounds were
236 removed from the tubing by the MeOH rinse.

237 Because the MeOH extracts from the POP gun inevitably contained a small amount of
238 hydrothermal fluid that remained in the tubing, the extracts were partially concentrated under a
239 stream of N₂, and DCM added. The combined MeOH-DCM extract was then placed in a -20 °C
240 freezer to induce phase separation, and the DCM-rich (lower) phase was removed to a clean vial
241 for further processing (note that although DCM was added to these samples, they are still
242 referred to as “MeOH extracts” to reflect the solvent used in the initial extraction step). These
243 extracts as well as the original DCM rinses from the POP gun samples were further concentrated
244 using the same methods described for the IGT fluid samples.

245 To process the SPE cartridges, hydrothermal fluids remaining in the cartridges was first
246 removed by suction using a large, gas-tight syringe. A glass/Teflon syringe was then used to
247 pass ~10 ml aliquots of MeOH and DCM through the cartridges in succession. To ensure
248 extraction of organic acid anions, the solvents for this step were mildly acidified with HCl. The
249 MeOH and DCM rinses were collected in separate pre-cleaned glass vials and processed
250 separately using the methods described above for the IGT and POP gun extracts.

251 In addition to the fluid samples, organic compounds were extracted from a portion of
252 carbonate chimney from Lost City. The extracted chimney sample was a large fragment (36 g,
253 wet weight) of a small, active flange collected from the Beehive/EXOMAR-12 site adjacent to
254 where the vent fluid samples were collected. Because the flange crumbled into pieces during
255 collection by the ROV, it is not possible to precisely determine what part of the structure the
256 fragment represents, but it was mostly composed of material from the flange interior. The
257 chimney fragment was crushed in a slurry of 80% DCM/20% MeOH, transferred to a pre-
258 cleaned glass bottle, and sonicated twice for 10 minutes with vigorous shaking of the sample in
259 between. Following removal of the extract to a separate bottle, the chimney was extracted two
260 additional times using 80% DCM/20% MeOH followed by 100% DCM. When all of the
261 extracts were combined, the solvents separated into two phases, presumably because of the
262 presence of seawater or vent fluid in the chimney sample. At this stage, the solvent was acidified
263 with HCl to ensure that alkanolic acids would enter the organic solvent, and the lower, DCM-rich
264 phase was then removed to a separate bottle. In order to remove inorganic salts, the solvent
265 extract was rinsed twice with Fisher DI water that had been pre-extracted with DCM to remove
266 organic compounds. The resulting solvent extract was concentrated under a stream of N₂ and
267 prepared for analysis using procedures similar to those used for the fluid samples.

268 **2.3. Analytical methods**

269 The solvent extracts were analyzed onshore using gas chromatography-mass spectroscopy
270 (GC-MS) on an Agilent 6890 interfaced to a 5973 mass selective detector. Routine analyses
271 were performed with an Alltech AT-5ms column (60 m length, 320 µm internal diameter, 0.25
272 µm film thickness) using a temperature program of 50 °C initial temperature for 5 min, 10

273 °C/min to 325 °C, and hold at this temperature for 30 min. For chimney samples, organic acids
274 in an aliquot of the solvent extract were converted to fatty acid methyl esters (FAME) prior to
275 analysis by reaction with BF₃ in methanol using kits from Supelco. Compounds were routinely
276 identified by comparison of mass fragment spectra with those in the NIST02 reference library.
277 In a few cases where the spectral identifications were ambiguous (e.g., phenanthrenes,
278 hexadecanol), retention times and mass spectra were confirmed by comparison with analyses of
279 known compounds.

280 During analysis of extracts by GC-MS, a number of compounds were observed at short
281 retention times (<24 min) that could be attributed to trace contaminants from the solvents which
282 had been concentrated in the extracts during sample preparation (note that these relatively
283 volatile compounds were lost in some samples that were inadvertently evaporated to dryness).
284 Prominent among these were undecane and several methyldecane isomers that was found to be a
285 trace contaminant in the MeOH solvent, and several small methylcycloalkanes present in the
286 DCM. Owing to interference from these compounds, low molecular weight organic compounds
287 that may have been present in the hydrothermal fluid samples with short retention times could
288 not be confidently identified during this study. In addition, the sorbents used in the SPE
289 cartridges were found to release relatively large amounts of a spectrum of organic compounds
290 during sample processing, most of which did not match any compounds in the reference library
291 and could not be identified. These contaminants are discussed more fully below, and example
292 mass fragmentograms for the unknown compounds are provided as Supplemental Figure S1.

293 Compound specific carbon isotope analyses were performed on select samples with an
294 Agilent 6890 coupled to a Delta plus XL mass spectrometer via a combustion interface operated
295 at 1080 °C using a column and temperature program similar to the GC-MS analyses. Instrument
296 error was estimated using an external standard containing 15 *n*-alkanes with known δ¹³C values
297 and was measured to be 0.2‰ during the period when these samples were analyzed. All extracts
298 were derivatized using BSTFA +1% TMCS and pyridine and heated at 70 °C for one hour.
299 Results were corrected for the addition of methyl carbon by derivatizing phthalic acid with a
300 known isotopic value using the same BSTFA and subtracting the contribution of methyl carbons
301 by mass balance.

302 Quantification of fatty acids in selected samples was performed by conversion of the acids to
303 methyl esters and comparison of peak areas for reconstructed ion chromatograms with reference
304 standards for octanoic and dodecanoic acids. Analysis of hydrothermal fluid samples for the
305 presence of dissolved free amino acids was performed by gas chromatography with flame ionization
306 detection following extraction and derivatization of the amino acids with EZ:faast kits from
307 Phenomenex Inc. (Torrance, CA), using the column and analytical parameters specified by the
308 manufacturer. Although an effort was made to detect highly polar compounds by direct injection of
309 small aliquots (1-2 μl) of hydrothermal fluid into the GC-MS using an AquaWax column (Alltech),
310 no additional organic compounds were identified by this method.

311 **2.4. Assessment of potential sources of background contaminants**

312 In an effort to evaluate potential exogenous sources of organic compounds that might
313 contaminate the hydrothermal fluid samples, samples of bottom seawater were extracted and
314 analyzed using the same procedures utilized for the hydrothermal samples (Fig. 4). A sample of
315 bottom seawater was obtained with the IGT sampler in the vicinity of the TAG field (J2-364-
316 IGT6), but no organic compounds were found in either the DCM extract of the fluid or in the
317 solvent rinse of the sampler. Similarly, no organic compounds were observed in a DCM extract
318 of the Fisher H₂O used in sample processing. Samples of bottom seawater were obtained with

319 the POP gun in the vicinity of the Lucky Strike and Lost City vent fields (J2-356-POP1, J2-362-
320 POP1). The MeOH extracts of both seawater samples exhibited a large, unresolved complex
321 mixture (UCM) of organic compounds that were dominated by aliphatic components (Fig. 4a;
322 see Supplemental Fig. S2 for an example mass fragmentogram of the UCM). The UCM
323 displayed three distinct peaks centered at retention times of ~26, ~29.5 and ~36 min. Analyses
324 of the oils used for pressure compensation and hydraulic parts on the Jason submersible are also
325 characterized by broad UCM humps (Figs. 4b and 4c). However, the UCM humps for the oils
326 show very different distribution patterns than those observed for the seawater samples, indicating
327 that the UCM observed in the seawater samples included little or no contribution from the
328 submersible. No organic compounds were found in the DCM extracts of the seawater POP gun
329 samples.

330 Solvent extracts of the SPE cartridges that were attached to the POP gun during sampling of
331 bottom seawater [(J2-356-SPE(HLB), J2-362-SPE(C8))] contained numerous organic
332 compounds, most of which did not match any compounds in the spectral library used in the GC-
333 MS analysis (Figs. 4d and 4e). Similar suites of compounds were observed in all other samples
334 processed through the SPE cartridges, including the hydrothermal fluids and 1 L samples of
335 Fisher H₂O pumped through the cartridges and processed using the same procedures.
336 Consequently, the compounds seen in the extracts of the seawater samples appear to be derived
337 predominantly from the HLB and C8 SPE phases rather than the fluid samples, and likely
338 represent fragments of the polymers used to coat the extraction beads. In the case of the C8
339 phase, the suite of compounds seen in the extracts for all samples included small amounts of C₁₆
340 and C₁₈ alkenes and alkenones, which also appeared to be derived from the SPE beads. While
341 trace amounts of organic compounds derived from seawater may be present in the SPE samples,
342 they could not be distinguished from the pervasive background of compounds from the SPE
343 phases.

344 3. RESULTS

345 A summary of the organic compounds that were positively identified in the hydrothermal
346 vent samples is provided in Table 2, with more detailed discussion in the following sections.
347 Note that some compounds in this table were identified using methods that were not applied at
348 all sites, so results for different sites may not be directly comparable. For brevity, only the most
349 salient results of the GC-MS analyses are shown in the figures displayed here; figures showing
350 additional analyses of samples are provided as Supplemental Materials.

351 The concentration of Mg in fluid samples collected with the IGT samplers are listed in Table
352 1. Magnesium concentrations of fluids sampled from deep-sea hydrothermal vents are routinely
353 used to estimate the amount of seawater entrained into the fluid during sampling, based on the
354 presumption that the vent fluids have Mg concentration approaching zero as a result of fluid-rock
355 interactions in the subsurface while seawater has a concentration of ~52.2 mmol/kg (German and
356 Seyfried, 2014). However, analysis of IGT samples obtained for this study indicates that
357 endmember vent fluids at the Rainbow field may have Mg concentrations of 1.5-2 mmol/kg (see
358 Seyfried et al., 2011). Only two of the samples collected with the IGT samplers had elevated Mg
359 contents (US4, Kremlin) indicating a substantial seawater component. The remaining samples
360 have Mg concentrations <8 mmol/kg and most have concentrations of 2.6 mmol/kg or less,
361 indicating nearly pure hydrothermal fluids were obtained. Also listed in Table 1 for reference
362 are measured pH values (at 25 °C) and endmember concentrations of dissolved H₂, CO₂, and

363 CH₄ in the fluids determined from separate IGT samples obtained at the same chimney orifices
364 as the organic samples (Reeves et al., 2014).

365 3.1. Lost City

366 Lost City was the only vent field where significant amounts of extractable organic
367 compounds were consistently observed in the hydrothermal fluid samples. The initial liquid-
368 liquid extracts of hydrothermal fluids from the Beehive site at Lost City collected with the IGT
369 samplers yielded no detectable organic compounds (Fig. 5a), but several compounds were
370 present in the sample from the top of Poseidon, including trithiolane, hexadecanol, and squalene
371 (Fig. 5e). Furthermore, hexadecanol, squalene, and cholesterol were observed in the solvent rinse
372 of the IGT sampler used to collect fluids at the top of Poseidon (Fig. 5f), indicating that a
373 fraction of these compounds may have precipitated onto the walls of the sampler. No organic
374 compounds were observed in solvent rinses of the IGT samplers for the Beehive site.

375 Following acidification and re-extraction of the Beehive and Poseidon fluids, a suite of three
376 alkanolic acids was observed that included *n*-octanoic, *n*-decanoic and *n*-dodecanoic acids (C₈,
377 C₁₀, and C₁₂, respectively) (Fig. 5b & 5f). These compounds were particularly abundant in the
378 samples from the Beehive site (Fig. 5b), with relatively low levels observed in the sample from
379 the top of Poseidon (Fig. 5f). Both fluid samples from Beehive yielded similar alkanolic acid
380 concentrations that decreased with increasing carbon number (C₈ > C₁₀ > C₁₂) from values of
381 0.72 to 0.23 μmol/L (Table 3). In both samples, acid concentrations decreased with increasing
382 carbon number (C₈ > C₁₀ > C₁₂). Since it is not clear that the extraction methods completely
383 removed the acids from these fluid samples, the reported concentrations should probably be
384 regarded as minimum values. The alkanolic acids are characterized by relatively heavy carbon
385 isotope compositions, with values between -2.8‰ and -7.6‰ (Table 3). The extract of the
386 acidified fluid for the Poseidon sample also included trithiolane, hexadecanol, and squalene as
387 well as trace amounts of cholesterol, indicating that the initial liquid-liquid extraction prior to
388 acidification did not completely remove these compounds (Fig. 5f).

389 Conspicuously absent from the Lost City IGT sample extracts were alkanolic acids with fewer
390 than 8 or greater than 12 carbon atoms (e.g., C₆, C₁₄). To evaluate whether the absence of these
391 compounds may have been an artifact of the analytical protocols, an artificial seawater solution
392 with pH adjusted to 9.5 containing ~200 μg/L each of C₆, C₁₂, and C₁₄ alkanolic acids was
393 prepared and processed with the same methods used for the natural samples. Quantitative
394 analyses of the resulting extracts showed that recovery of the C₁₄ acid was essentially identical to
395 the C₁₂ acid, implying that the absence of the C₁₄ acid in the Lost City extracts accurately reflects
396 its abundance in these samples. On the other hand, recovery of the C₆ acid was only about 40%
397 relative to the C₁₂ acid, perhaps because extraction of the C₆ acid from the water phase by the
398 solvent was less efficient owing to its relatively higher polarity or because this compound was
399 partially volatilized during sample concentration. Nevertheless, even partial recovery of the C₆
400 acid suggests that it would have been detected in the samples if it had been present at levels
401 comparable to the other acids. Accordingly, it appears that levels of both the C₆ and C₁₄ acids in
402 the Lost City hydrothermal fluids were significantly lower than those of the C₈–C₁₂ acids.

403 The two POP gun samples at Lost City (J2-361-POP1, J2-361-POP2) were both obtained at
404 the Beehive site, and the MeOH extracts were found to contain C₁₀ and C₁₂ alkanolic acids
405 similar to those observed in the IGT extracts from this location (Fig. 5c). However, the relative
406 proportions of these compounds were reversed from the IGT samples, with the C₁₀ acid present
407 in much lower amounts than C₁₂. The C₈ alkanolic acid was not observed in either MeOH extract

408 of the POP gun samples. No organic compounds were detected in DCM extracts of the POP
409 guns.

410 Despite the presence of contaminants from the extraction phase, organic compounds that
411 could be confidently attributed to the hydrothermal fluid were identified in the Beehive sample
412 that was pumped through the HLB SPE phase [J2-361-SPE(HLB)] and extracted with MeOH
413 (Fig. 5d). This sample contained large amounts of the same suite of C₈, C₁₀, and C₁₂ *n*-alkanoic
414 acids that were seen in the IGT samples, and these compounds were present at levels far above
415 those of any background contaminants. Estimates of the concentrations of the acids in the
416 hydrothermal fluid were calculated from the measured abundance of the compounds in the
417 extract and the amount of fluid pumped through the SPE cartridge (~1.2 L). The resulting
418 concentrations are similar to those measured for the Beehive IGT samples, and show the same
419 decrease in concentration with increasing carbon number (Table 3). No organic compounds
420 were identified in the DCM extract of the HLB phase other than those that could be attributed to
421 background sources. Fluids from the Beehive site were also pumped through a cartridge
422 containing the C8 SPE microbeads [J2-361-SPE(C8)]. However, no identifiable compounds that
423 could be attributed to the hydrothermal fluid were found in solvent extracts of this phase (see
424 Supplemental Fig. S3).

425 To aid in evaluation of potential sources for alkanolic acids observed in the Lost City vent
426 fluids, organic compounds were extracted and analyzed from a piece of carbonate chimney
427 collected from the Beehive site where the IGT and POP gun samples were obtained. As shown
428 in Figure 6a, analysis of the total extract by GC-MS was dominated by a UCM, although several
429 individual hydrocarbons could be identified including *n*-hexadecane and squalene. Also
430 prominent in the total extract are a number of wax esters (Fig. 6a). These compounds apparently
431 formed through condensation of alkanolic acids with alkanols and alkenols in the extracts induced
432 by acidification of the solvents.

433 In order to determine the distribution and diversity of alkanolic acids in the chimney, the acids
434 in an aliquot of the total extract were converted to fatty acid methyl esters (FAME) (Figs. 6b and
435 6c). The FAME analysis revealed an assortment of alkanolic and alkenolic acids ranging in
436 carbon number from C₈ to C₂₈, with a strong predominance for compounds with an even number
437 of carbon atoms. The C₁₄, C₁₆, and C₁₈ compounds are substantially more abundant than
438 compounds with other numbers of carbon atoms, and occur in approximately equal proportions.
439 There is a sharp drop in abundance of the alkanolic acids with fewer than 14 carbon atoms.
440 While it is possible that this drop off could be partially explained by loss of the relatively volatile
441 shorter-chain acids during concentration of the sample, the same procedures used to minimize
442 loss of semi-volatile compounds in the hydrothermal fluid extracts were employed in sample
443 preparation of the chimney extract, suggesting that the relative amounts of these compounds are
444 likely to reflect their in situ abundance. The C₁₄-C₁₈ carboxylic acids in the chimney extract had
445 carbon isotopic compositions between -13.5‰ and -20‰, substantially lighter than the acids in
446 the fluid (see Supplemental Table S1 for a complete listing of isotopic analyses for the chimney
447 extract).

448 **3.2. Lucky Strike and Rainbow**

449 Most solvent extracts of the fluids collected at Lucky Strike and Rainbow using the IGT
450 samplers were found to contain either no detectable organic compounds or trace amounts of
451 compounds that appeared to be mostly derived from sources other than the hydrothermal fluid
452 (Fig. 7). Several of the IGT extracts contained small peaks whose mass spectra did not provide a

453 close match to any compounds in the spectral library, and were inferred to be contaminants,
454 possibly fragments of plastic polymers (see Supplemental Fig. S1 for an example). The source
455 of these compounds could not be determined. Several of the fluid extracts were also found to
456 contain small amounts of Fluorolube lubricant, but these compounds could be readily identified
457 as contaminants by their mass fragmentation patterns during GC-MS analysis. An example is
458 provided in Figure 7b, which shows the extract for a fluid sample from the Lucky Strike
459 hydrothermal field (J2-359-IGT6). The only identifiable compound in this sample that could not
460 be attributed to Fluorolube or other contaminants was a small peak for dibenzothiophene.
461 Dibenzothiophene was present in one other sample, also from Lucky Strike (J2-358-IGT6), but
462 was not found in any other samples analyzed for this study. Analyses of the solvent rinses of the
463 IGT samplers following removal of the fluid did not reveal the presence of any compounds that
464 could be confidently attributed to a hydrothermal fluid source. Most of these rinse samples,
465 however, did include small amounts of phthalates from an unidentified source as well as traces of
466 Fluorolube.

467 In contrast to the scarcity of organic compounds in the IGT samples, organic compounds
468 were observed in several of the POP gun samples from sites at Rainbow and Lucky Strike.
469 Extracts from all sites at Rainbow sampled with the POP gun contained small amounts of PAH
470 that included phenanthrene, methylphenanthrenes, and C₂-phenanthrenes (e.g., Figs. 8a-c). In
471 addition, samples from the Guillaume vent contained hexadecanol and a C₁₈ alkenoic acid (Fig.
472 8c). Because the solvents used in the extracts were mildly acidified, the organic acids in this and
473 other samples were converted to methyl esters during sample processing. While it is possible
474 that these compounds represent membrane components of microorganisms entrained into the
475 POP gun during sampling, essentially identical results were obtained from both POP gun
476 samples at this site, indicating it is more likely that the compounds were indigenous to the
477 hydrothermal fluid.

478 Extracts of the POP gun samples from the high temperature Stylo 2 and Padraig sites at
479 Rainbow also contained a small, narrow UCM hump centered at a retention time of ~36 min
480 (Fig. 8b). A UCM hump was also evident in both samples from the lower temperature (191 °C)
481 Ecurie site at Rainbow, but in this case the UCM was much broader and extended from 26 to 38
482 min retention time (Fig. 8a). In all cases, the UCM was dominated by aliphatic components as
483 indicated by a predominance of mass fragments with mass/charge ratios (*m/z*) of 55, 57, 69, 71,
484 83, and 85 (see Supplemental Fig. S2). The single POP gun sample obtained at Lucky Strike did
485 not contain a prominent UCM, but did include several even-carbon-numbered alkenoic and
486 alkanolic acids ranging in carbon number from C₁₄ to C₂₂, with C₁₆ and C₁₈ alkenoic acids
487 particularly abundant (Fig. 8d). Since these compounds are predominant components of
488 bacterial membranes, it appears likely that in this case the alkenoic and alkanolic acids must have
489 been extracted from biomass entrained into the POP gun during sampling rather than being
490 dissolved in the hydrothermal fluid.

491 Among the DCM extracts of the POP gun samples, organic compounds were observed in
492 only some of the Rainbow samples and were not found in any samples from Lucky Strike.
493 Extracts of samples from the high temperature Guillaume and Padraig sites at Rainbow all
494 contained phenanthrene and methylfluorenes present in trace amounts that were just barely
495 detectable (Fig. 9a). However, these compounds were not observed at the other high temperature
496 site sampled, Stylo 1. One of the DCM extracts for the POP gun samples from the lower
497 temperature Ecurie site at Rainbow contained a homologous series of *n*-alkanes ranging in
498 carbon number from 23 to 33 (Fig. 9b). The alkanes exhibited a bell-shaped distribution in

499 abundance centered around $\sim C_{28}$, with no apparent even- or odd-carbon-number preference.
500 These hydrocarbons were not observed in any other POP gun extract.

501 None of the MeOH extracts of the HLB cartridges from the Rainbow and Lucky Strike
502 systems contained organic compounds that could be differentiated from the background of
503 compounds derived from the SPE sorbent (see Supplemental Fig. S4). The same suite of
504 background peaks observed in the MeOH extracts were also evident in the DCM extracts of the
505 HLB phase (Supplemental Fig. S4). In addition, the DCM extracts also included C_{16} and C_{18}
506 alkenes that were not present in the MeOH extracts, but which also appeared to be derived from
507 the extraction phase since they were present in all samples processed through the SPE cartridges.

508 Despite the ubiquitous presence of background contaminants, organic compounds that could
509 be attributed to the hydrothermal fluids were identified in some DCM extracts of the HLB phase.
510 The DCM extract of sample J2-359-SPE(HLB) from the Medea site at Lucky Strike (Fig. 10b)
511 was found to contain methyl esters of C_{16} and C_{18} alkanolic and alkenolic acids similar to those
512 observed in the MeOH extract of the POP gun at this same sample location (Fig. 8d). The DCM
513 extract of the HLB phase obtained from the Ecurie site at Rainbow (Fig. 10a) contained a
514 homologous series of C_{23} - C_{33} *n*-alkanes with a distribution identical to that observed in the DCM
515 extract of the POP gun sample for this site (Fig. 9b). In both cases, the compounds identified
516 were not observed in DCM extracts of the SPE cartridges from other sites.

517 The only high-temperature sample processed through a SPE cartridge containing the C8
518 sorbent was from the Stylo 2 site at Rainbow [J2-355-SPE(C8)]. Extracts of this sample
519 contained the same suite of compounds observed in all other C8 extracts that appeared to be
520 attributable to background from the sorbent phase (Supplemental Fig. S3). Although there is a
521 broad “window” during the GC-MS analysis between 16 and 30 min retention time where there
522 is little or no background, no organic compounds indigenous to the sample were observed in this
523 interval.

524 **3.3. Amino acid analyses**

525 Preserved fluid samples from the IGT samples from all sites were analyzed for the presence
526 of free amino acids by GC-MS following extraction from the fluid and derivatization. However,
527 no free amino acids were detected in any of the hydrothermal fluids or in bottom seawater
528 samples at a detection limit of 5 nmol kg^{-1} . This result is consistent with the absence of
529 detectable free amino acids at similar levels in 319 °C hydrothermal vent fluids from the
530 Guaymas Basin (Haberstroh and Karl, 1989) and >200 °C hydrothermal vent fluids from the
531 Marianas Trough (Fuchida et al., 2014). Conversely, Klevenz et al. (2010) reported several
532 dissolved amino acids present above the 5 nmol kg^{-1} threshold from high-temperature
533 hydrothermal sites further south on the Mid-Atlantic Ridge, with total free amino acid
534 concentrations up to 377 nmol kg^{-1} . Lang et al. (2013) reported total hydrolysable amino acids
535 (THAA) for hydrothermal fluids from the Lost City system ranging from 736 to 2300 nmol kg^{-1} ,
536 but did not report free amino acid concentrations. The apparent absence of detectable free amino
537 acids in the Lost City fluids suggests that the amino acids reported by Lang et al. (2013) are
538 predominantly present as peptides or proteins.

539 **4. DISCUSSION**

540 **4.1. Organic compounds in Lost City hydrothermal fluids**

541 Lost City was the only sampling location in this study where significant amounts of dissolved
542 organic compounds were consistently observed in the hydrothermal fluids. At that site,

543 substantial concentrations of C₈-C₁₂ alkanolic acids as well as several other organic compounds
544 (squalene, hexadecanol, trithiolane, and cholesterol) were observed in multiple samples and, in
545 the case of the alkanolic acids, were identified in all samples regardless of the collection and
546 processing methods used (Fig. 5). Although several of the compounds found in the Lost City
547 fluids were also present at low abundance in extracts of the carbonate chimney recovered from
548 that site, the fluid extracts lacked numerous other compounds that were abundantly present in the
549 chimney extract. We therefore conclude that the organic compounds observed in the Lost City
550 samples were indigenous to the hydrothermal fluid and not derived from chimney particles
551 entrained during sampling.

552 Measured concentrations of the C₈-C₁₂ acids for the two IGT samples and the SPE sample at
553 the Beehive are in good agreement, and indicate concentrations for individual acids in the 0.23 to
554 0.77 μmol/L range, which is equivalent to 2.7-6.2 μmol C/L (Table 3). For comparison,
555 concentrations of dissolved formate and acetate for fluids venting at the Beehive site in 2005
556 were found to be ~144 μmol/L and 8.5 μmol/L, respectively (Lang et al., 2010). The measured
557 concentration of dissolved organic carbon (DOC) in the fluids sampled in 2005 was ~102 μmol
558 C/L, of which ~50 μmol C/L can be accounted for as formate and acetate (note that some of the
559 formate and acetate are lost during sample preparation for DOC analysis) (Lang et al., 2010).
560 Collectively, the C₈-C₁₂ acids can account for ~11-15 μmol C/L, or 11-15% of the DOC (Table
561 3). Taken together, it appears that the carboxylic acids that have been measured so far (including
562 formate) can account for >60% of the DOC in the hydrothermal fluids at the Beehive site. Total
563 hydrolysable amino acids can account for an additional 3-5% of DOC (Lang et al., 2013),
564 leaving about one third of the total DOC still unaccounted for at this point. A number of
565 alkylated monocyclic compounds have been identified in the Lost City fluids using other
566 methods (Konn et al., 2009) that might contribute to the remaining third, or it could be composed
567 of highly polar molecules (e.g., pyruvate, alkanols) not detectable by the methods employed to
568 date. Alternatively, the sampling methods used may have underestimated the amounts of
569 carboxylic acids present.

570 The detection of only even-carbon-numbered alkanolic acids in the Lost City samples
571 indicates that the acids have a biological source. Although abiotic organic synthesis reactions
572 such as the Fischer-Tropsch process are known to produce long-chain carboxylic acids (e.g.,
573 McCollom et al., 1999, 2010), these reactions typically produce a mixture of even- and odd-
574 carbon-numbered compounds in roughly equal amounts as the result of sequential addition of
575 single carbon units (McCollom and Seewald, 2007). There is currently no known mechanism for
576 abiotic synthesis of organic compounds in two-carbon units that might produce exclusively even-
577 carbon-numbered compounds, nor is there any evidence from natural organic products thought to
578 have an abiotic origin for an even-number predominance. For instance, abiotic organic
579 compounds in meteorites have approximately equal proportions of even- and odd-carbon-
580 numbers (Yuen and Kvenvolden, 1973; Huang et al., 2005). Furthermore, the absence of other
581 types of even-carbon-numbered compounds (e.g., hydrocarbons) makes it unlikely that the acids
582 come from an abiotic source.

583 Since the C₈-C₁₂ alkanolic acids are too small to be components of lipid membranes and are
584 not known as common metabolic products of microbial metabolism, the biological source for the
585 compounds is unclear. However, these compounds are known to be inhibitors of some metabolic
586 pathways such as fermentation (e.g. Legras et al., 2010), and perhaps they play a similar role in
587 the Lost City microbial community. These compounds are probably produced by bacteria, since
588 archaea do not appear to synthesize linear alkanolic acids. Studies of the microbial communities

589 at the hottest Lost City vents (70-91 °C) have found that the interiors of the carbonate chimneys
590 are dominated by a single species of archaea related to the Methanosarcinales, but the chimney
591 exteriors and hydrothermal fluids contain bacteria that could be the source of the alkanolic acids
592 (Schrenk et al., 2004; Brazelton et al., 2006). Alternatively, the compounds might be produced
593 in the subsurface. In either case, the presence of C₈-C₁₂ alkanolic acids at relatively high
594 abundance may reflect novel metabolic pathways taking place in this high pH ecosystem.
595 Another possibility is that the compounds are formed through thermal decomposition of
596 bioorganic matter in the subsurface. In this respect, it may be notable that recent studies of
597 insoluble carbonaceous matter from seafloor serpentinites have found that they contain a
598 large component of aliphatic compounds up to C₁₂ associated with carboxylate functional groups
599 (Pasini et al., 2013).

600 The alkanolic acids in the fluid and chimney samples from Lost City form complementary
601 sets, with the fluid containing C₈, C₁₀, and C₁₂ acids but none of the longer chain acids, while the
602 chimney contained substantial amounts of C₁₄, C₁₆, and C₁₈ acids with only small amounts of the
603 shorter chain acids (Figs. 5 and 6). This result suggests two possible origins for the acids
604 dissolved in the Lost City hydrothermal fluids. The first possibility is that a complete suite of C₈
605 to C₁₈ acids is produced by biological processes occurring within the chimney walls, but the
606 shorter acids are preferentially dissolved by the circulating hydrothermal fluids owing to their
607 relatively higher aqueous solubility. Alternatively, all of the acids may be produced by
608 biological activity deeper in the system that are mobilized by the hydrothermal fluids, but only
609 the longer-chain acids are subsequently removed by precipitation as the fluids cool during
610 mixing with seawater within the walls of the chimney (a mechanism that would be directly
611 comparable to that used as the basis for the design of our POP gun sampling device). In the
612 former case, mobilization of the shorter acids would represent a significant loss of fixed organic
613 carbon from the microbial communities living in the chimney walls, while the latter case would
614 represent a significant net transfer of organic carbon from deep within the system to the seafloor
615 chimney environment.

616 The carbon isotopic compositions of the C₈-C₁₂ alkanolic acids do not allow for clear
617 discrimination between these two possibilities. The C₈-C₁₂ acids in the fluid are relatively heavy
618 compared to the C₁₄-C₁₈ acids in the adjacent chimney (-2.8‰ to -7.6‰ vs. -13.5‰ to -20‰)
619 (Fig. 11). However, the C₁₆ and C₁₈ acids in other chimney samples from Lost City analyzed by
620 Bradley et al. (2009) overlap with the heavier values observed in the fluids, with some values as
621 low as -1.1‰ (Fig. 11). Thus, it appears possible, and maybe even likely, that the C₁₄-C₁₈ acids
622 in the Lost City chimneys are composed of a mixture of heavier compounds from the same
623 source as the acids observed in the hydrothermal fluid and lighter compounds derived from in
624 situ microbial activity, with the relative contributions from these sources varying locally. Within
625 this scenario, however, the ultimate source of the heavier acids could either be local to the
626 chimney deposits or somewhere deeper within the system.

627 The IGT fluid sample collected at the top of the Poseidon structure contained substantially
628 lower amounts of the C₈-C₁₂ alkanolic acids than the samples collected from the Beehive site on
629 the side of Poseidon (Fig. 5), and also contained a number of compounds not observed in the
630 other samples including squalene, trithiolane, hexadecanol, and cholesterol. The fluid from the
631 Poseidon summit also had a substantially lower temperature (51 °C vs. 91 °C), indicating the
632 fluid had conductively cooled or mixed with cold seawater during passage through the length of
633 the structure. Squalene is a common product of the microbial community living within Lost City
634 chimney structures (Méhay et al., 2013), and microbial activity within the chimney structure is

635 presumably the source of this compound as well as the hexadecanol and cholesterol. Although
636 O₂ is required for biosynthesis of cholesterol, influx of seawater into chimney mixing zones
637 could supply O₂ for aerobic organisms, and the cholesterol was likely entrained into circulating
638 fluids from these zones. Microbial or other abiotic processes occurring within the chimney may
639 have also led to partial loss of alkanolic acids in the fluid. To our knowledge, trithiolane has not
640 previously been reported to occur in deep-sea hydrothermal systems. However, we have
641 observed trithiolane and several other cyclic carbon-sulfur compounds in strongly alkaline (pH >
642 11) fluids discharged from serpentinite-hosted springs at Aqua de Ney in northern California
643 (Feth et al., 1961; Barnes et al., 1972), suggesting these compounds may be common in fluids
644 discharged from serpentinites (Fig. 5g). Whether the trithiolane observed in the Lost City fluid
645 is a biological or abiotic product is presently unclear.

646 **4.2. Organic compounds in high-temperature hydrothermal fluids**

647 The high-temperature (≥ 190 °C) hydrothermal fluids examined for this study were
648 characterized by an overall scarcity of extractable dissolved organic compounds. Most samples
649 were found to contain either no detectable organic compounds or only trace amounts of organic
650 compounds composed primarily of polycyclic aromatic hydrocarbons. More substantial amounts
651 of organic compounds were observed in a few of the POP gun and SPE samples but, as discussed
652 further below, these compounds appeared to represent particulate matter or biomass entrained
653 during sample collection rather than dissolved components present in the hydrothermal fluids.

654 Several of the MeOH extracts from the POP gun samples at Rainbow were found to contain
655 unresolved complex mixtures of organic compounds (e.g., Figs. 8a and 8b). The UCMs
656 observed for the hydrothermal samples have some similarities to the UCM found in the POP gun
657 samples of deep seawater, but there are also some substantial differences. The UCM observed
658 for bottom seawater displayed a broad hump extending from ~25 to ~37 min retention time, with
659 three distinct peaks centered at ~26, ~29.5 and ~36 min (Fig. 4a). The UCM for the moderately
660 hot fluids (191 °C) from the Ecurie site also displayed a broad hump extending from ~25 to ~37
661 min (Fig. 8a). However, while the Ecurie samples display a distinct peak at 36 min similar to
662 that for seawater, there is only a slight indication of a peak at 29.5 min and none at 26 min.
663 Conversely, the UCM humps evident in the samples from the ≥ 350 °C Stylo 2 and Padraig sites
664 are much narrower, with a single peak at 36 min that coincides closely with the final peak in the
665 seawater UCM (Fig. 8b). For both seawater and hydrothermal fluid samples, mass fragmentation
666 patterns from GC-MS analyses show that the UCMs are dominated by saturated alkyl ($m/z = 57,$
667 $71, 85,$ etc.) and monounsaturated alkenyl ($m/z = 55, 69, 83,$ etc.) fragments, indicating the
668 compounds that comprise the UCMs have a large aliphatic component (see Supplemental Fig.
669 S2). This observation appears to be consistent with studies indicating that dissolved organic
670 matter (DOM) in deep Atlantic Ocean water contains a large aliphatic component (Hertkorn et
671 al., 2013), although the actual molecular structures of the compounds making up the UCM
672 remain to be determined.

673 The similarities between the hydrothermal and seawater UCMs suggest that the mixture of
674 compounds that comprise the hydrothermal UCMs may represent seawater organic matter that
675 has been modified by high-temperature processes or biological activity during circulation though
676 the hydrothermal system. If this is the case, the hydrothermal samples appear to have
677 preferentially lost the lower molecular weight fraction of the UCM, with more extensive loss
678 occurring at the higher temperature Stylo 2 and Padraig sites than at the lower temperature
679 Ecurie site. Regardless, differences between the seawater and hydrothermal UCMs indicate that
680 the organic compounds found in the hydrothermal samples do not simply represent seawater

681 organic matter entrained during sample processing or shallow mixing of seawater into the fluids.
682 In contrast, the fluids from Lucky Strike and Lost City contained no apparent UCM, suggesting
683 that the compounds that constitute seawater UCM are quantitatively removed during circulation
684 through these systems.

685 Although the UCMs observed in the POP gun samples from Ecurie represent the largest
686 amount of organic matter observed in any of the high-temperature fluid samples in this study,
687 these compounds appear to be derived from particulate materials entrained during sampling
688 rather than compounds dissolved in the hydrothermal fluid. Extracts of hydrothermal chimneys
689 from the Rainbow field also contain substantial UCM humps (Simoneit et al., 2004). While
690 direct comparisons are complicated by differences in analytical protocols, the UCM humps for
691 many chimney samples reported by Simoneit et al. (2004) are broad with a peak towards the
692 higher molecular weight fraction, and appear to be very similar to the UCMs observed for the
693 Ecurie samples. In addition, many of the chimney samples display a suite of linear alkanes
694 ranging in carbon number from $\sim C_{22}$ to $\sim C_{33}$. This suite of alkanes displays a bell-shaped
695 distribution in abundance centered at about C_{28} , with no even-odd carbon number preference.
696 The carbon number range and abundance distribution of these compounds is nearly identical to
697 the suite of *n*-alkanes observed in the DCM extracts of the POP gun and SPE samples at the
698 Ecurie site (Figs. 9b and 10a). Although long-chain alkanes are usually associated with plant
699 waxes, Simoneit et al. (2004) suggested that their occurrence in the chimney deposits may reflect
700 thermal processing of organic matter within the Rainbow hydrothermal environment.
701 Conversely, a direct microbial source for these longer-chain compounds cannot be precluded.

702 Combined, the UCMs and alkanes observed in the Ecurie fluids provide a very close match
703 to the organic compounds extracted from chimneys at Rainbow, suggesting a common source for
704 the organic compounds observed in the chimney deposits and the POP gun and SPE samples.
705 While most of the fluids collected for this study were sampled directly from natural orifices, at
706 Ecurie the fluids were sampled from a cavity excavated into the side of a chimney structure that
707 was weakly venting lower temperature fluids (~ 60 °C) at its apex (Fig. 1b). The tip of the POP
708 gun was embedded in loose chimney materials during sampling, and it is likely that some
709 chimney particles were entrained into the POP gun and SPE cartridge during sampling, and are
710 the source of the UCM and alkanes observed in the POP gun and SPE extracts. If this is the
711 case, it suggests that a substantial fraction of the UCM found in the hydrothermal chimneys
712 could be seawater-derived organic matter that has undergone alteration to remove some of the
713 lower molecular weight fraction. Whether this alteration is primarily chemical or biological
714 remains to be determined.

715 The source of smaller UCMs observed in POP gun samples from the higher temperature
716 Stylo 2 and Padraig sites at Rainbow is more difficult to evaluate (Fig. 8b). The POP gun
717 extracts for these sites also contain several PAHs, and the narrow UCM peak and lack of alkanes
718 observed in the POP gun samples distinguishes them from the suite of organic compounds
719 reported for chimney samples. These observations suggest that the UCM and PAH found in the
720 extracts were present as dissolved compounds in the fluids.

721 Extracts of the two POP gun samples from the Guillaume site at Rainbow contained
722 hexadecanol and a C_{18} alkenoic acid in addition to several PAH (Fig. 8c). Hexadecanol was not
723 observed in other samples from Rainbow, but it was observed in the fluid sampled from the top
724 of Poseidon at Lost City (Fig. 5e-g). While hexadecanol is a plausible microbial product, it is
725 difficult to explain how this compound would occur in isolation without being accompanied by a
726 suite of other alkanols or alkenoic acids if derived from a biological source. On the other hand,

727 there is no obvious source of contamination that would explain the occurrence of this compound
728 in the samples. Thus, the hexadecanol appears to be indigenous to the samples, but the ultimate
729 source for this compound remains obscure. Similarly, the C₁₈ alkenoic acid found at the
730 Guillaume site appears to be an indigenous biological product, but its source is uncertain.

731 Overall, several PAH, particularly phenanthrene together with its methyl and dimethyl
732 homologs, were the only organic compounds consistently found in the high-temperature fluids
733 from the Rainbow field that appeared to be indigenous to the fluids (e.g., Figs. 8 and 9).
734 Although the PAHs were not quantified, they were present at very low levels that were barely
735 detectable in the extracts. Phenanthrene and other PAHs are prominent constituents of organic
736 matter that has been exposed to high-temperature alteration in hydrothermal environments
737 (Simoneit, 1988; Kawka and Simoneit, 1994; Ventura et al., 2012), suggesting that the PAH
738 observed in the fluids at Rainbow may represent organic matter that has undergone thermal
739 processing during circulation through the hydrothermal system. Because this process obscures
740 the original composition of the organic matter, it is difficult to identify the ultimate source of
741 these compounds.

742 At the Lucky Strike site, the hydrothermal fluid extracts were largely devoid of organic
743 compounds that could be attributed to the fluids with a high degree of confidence, with the
744 possible exception of trace dibenzothiophene that was observed in a couple of samples (e.g., Fig.
745 7b). The only POP gun sample obtained at Lucky Strike (J2-359-POP1) was found to contain a
746 number of fatty acid methyl esters, dominated by C₁₆ and C₁₈ *n*-alkanoic and *n*-alkenoic acids
747 (Fig. 8d) (note that fatty acids present in the sample were probably converted to methyl esters
748 during sample processing). These compounds were also observed in the SPE sample attached to
749 the POP gun (Fig. 10b), but not in the IGT fluid sample obtained at the same vent site (Fig. 7b).
750 The similarity of the fatty acids found in the POP gun and SPE samples to the components of
751 bacterial membranes strongly suggests that these compounds were derived from microbial
752 biomass entrained during sample collection with the POP gun rather than representing dissolved
753 organic compounds from the hydrothermal fluid.

754 In addition to an aliphatic component, deep seawater DOM has also been found to contain a
755 significant component of polycyclic aromatic compounds with attached carboxyls and other
756 functional groups (e.g., Dittmar and Koch, 2006). It has been hypothesized that some of these
757 compounds could originate in submarine hydrothermal systems. Our results do not show
758 evidence for a substantial input of aromatic compounds into the deep sea from high temperature
759 hydrothermal systems, since such compounds were only detected in trace amounts. However,
760 more substantial amounts of PAH could be contributed from hydrothermal systems that are
761 buried by organic-rich sediments where fluids may discharge much higher amounts of aromatic
762 compounds (Simoneit, 1988).

763 **4.3. Comparison with previous studies**

764 The results of this study stand in sharp contrast with previous studies that have reported the
765 presence of diverse organic compounds in fluids from the Rainbow and Lost City vent fields, as
766 well as for a hydrothermal system on the East Pacific Rise (Brault et al., 1988; Konn et al., 2009,
767 2012). Konn et al. (2012) extracted small aliquots (10 ml) of hydrothermal fluids from the
768 Rainbow field using a stir-bar solid phase extraction approach, and reported detection of a
769 diverse suite of organic compounds that included alkanes, alkanolic acids, cycloalkanes, and
770 aromatic hydrocarbons. The alkanolic acids observed ranged from C₉ to C₁₈, with a
771 predominance of even carbon number and particularly high abundances of C₁₂, C₁₄, and C₁₆
772 acids. In contrast, we observed no alkanolic acids in the Rainbow samples, despite extraction of

773 much larger sample volumes (>10× larger volumes for the IGT fluid samples, ~100× larger for
774 the POP gun and SPE samples). Although Konn et al. (2012) did not quantify the amount of
775 acids in their samples, the relatively strong GC-MS response they observed for the C₁₂-C₁₆ acids
776 suggests that these compounds would have been readily detectable in our samples if they had
777 been present at comparable levels (or even at substantially lower levels).

778 Konn et al. (2012) also reported observation of a homologous series of *n*-alkanes ranging
779 from C₉ to C₁₉, with especially high abundances of C₁₀, C₁₁, and C₁₂ alkanes at Rainbow. We
780 observed *n*-alkanes in only one Rainbow sample (Ecurie), and these compounds had a much
781 higher range of carbon numbers (C₂₃-C₃₃). Alkanes in the carbon number range reported by
782 Konn et al. (2012) were not found in any of the hydrothermal fluids we sampled at Rainbow
783 (note that undecane and several methyldecanes *were* observed in all of our samples wherever
784 MeOH was used as a solvent, but these compounds were identified as contaminants from the
785 solvent and were not derived from the fluids). In agreement with our results, Konn et al. (2012)
786 also reported the observation of trace amounts of PAHs, but found a much broader spectrum of
787 compounds than the limited number observed during this study (primarily phenanthrene and
788 alkylated homologs; Table 2). Although monocyclic alkylated aromatic compounds and
789 cycloalkanes were prominent components of the suite of organic compounds reported by Konn et
790 al. (2012), the methods employed for this study were not suited for identification of compounds
791 of that type.

792 In an earlier study, Konn et al. (2009) had reported detection of a suite of compounds at Lost
793 City that were very similar to those they identified at Rainbow. Again, their results are very
794 different from those found in the present study. While Konn et al. (2009) reported alkanolic acids
795 to be present in the Lost City fluid, these compounds were only present at low levels and were
796 dominated by the C₁₂, C₁₄, and C₁₆ acids rather than the C₈, C₁₀, and C₁₂ acids found to be
797 dominant in this study. Konn et al. (2009) report carbon isotopic compositions for the C₈-C₁₂
798 alkanolic acids, but their reported values (C₈ = -31.1‰, C₁₀ = -19.6‰, and C₁₂ = -25.5‰) are
799 considerably lighter than the compositions measured for the same compounds in this study
800 (Table 3), although the values reported by Konn et al. are similar to the longer-chain alkanolic
801 acids in the Lost City chimney extract. Konn et al. (2009) also report detection of a number of
802 alkanes and PAH at Lost City, but none of those were observed in the samples analyzed for this
803 study.

804 Some key aspects of the organic compounds identified in the Rainbow and Lost City fluids
805 by Konn et al. (2009, 2012) are particularly noteworthy. First, nearly identical suites of organic
806 compounds were reported for hydrothermal fluids in both systems, even though the fluids have
807 radically different chemistries and have experienced very different hydrothermal circulation
808 histories (see, for example, Fig. 9 of Konn et al., 2009). End-member hydrothermal fluids at
809 Rainbow attain temperatures well in excess of 365 °C and are among the most acidic and metal-
810 rich observed in mid-ocean ridge hydrothermal systems worldwide (Table 1; Charlou et al.,
811 2002). In contrast, the hydrothermal fluids discharged from the Lost City system have
812 apparently experienced maximum temperatures of about 150-250 °C during circulation through
813 the subsurface and have become strongly alkaline through the process of serpentinization (Kelley
814 et al., 2005; Proskurowski et al., 2006; Foustoukos et al., 2008). There is no obvious reason to
815 expect that the organic composition of the Rainbow and Lost City fluids should be similar, and it
816 would be truly remarkable if fluids experiencing such radically different conditions were
817 characterized by the same organic content.

818 Second, the suite of organic compounds reported by Konn et al. has some highly unusual
819 characteristics that would be difficult to explain by natural processes. For instance, the *n*-alkanes
820 are dominated by C₁₀ and C₁₁ compounds in both systems, with much lower amounts of other *n*-
821 alkanes. Typically, natural sources of organic compounds display much broader and more
822 regular distributions of *n*-alkanes, with peaks in distribution at higher carbon number (e.g.,
823 Simoneit et al., 2004; Delacour et al., 2008). Conversely, abiotic organic synthesis pathways
824 produce mixtures of compounds that display a regular decrease in alkane abundance with
825 increasing carbon number rather than having a peak at a discrete number of carbons (e.g.,
826 McCollom et al., 2010). A natural source for high abundances of C₁₀ and C₁₁ *n*-alkanes to the
827 exclusion of lower and higher carbon number compounds is difficult to explain. Also, while
828 homologous series of alkanes and alkanolic acids in geologic samples tend to exhibit regular
829 patterns of abundance with carbon number (e.g., Brault et al., 1988; Simoneit et al., 2004), the
830 homologues reported by Konn et al. (2009, 2012) exhibit no regular or consistent pattern. For
831 example, the alkanolic acids show no regular abundance with carbon number, and the C₁₀, C₁₁,
832 and C₁₂ *n*-alkanes appear to be present in roughly equal amounts while those with both fewer
833 (C₉) and greater (C₁₃-C₁₉) are present in substantially lower amounts (see Fig. 5 in Konn et al.,
834 2012). It is somewhat difficult to conceive of a natural process that could explain the irregular
835 distributions observed for these compounds.

836 It is not clear how to reconcile the discrepancies between the results of the present study and
837 those reported for the same sites by Konn et al. (2009, 2012). The discrepancies may reflect real
838 temporal or spatial differences in the organic composition of the hydrothermal fluids that were
839 sampled, or differences in the effectiveness of the protocols to extract and detect organic
840 compounds from the fluids. However, the successful detection of organic compounds in the
841 samples from Lost City and other sites indicates that the methods used in the present study were
842 effective in extracting dissolved organic compounds when they are present, suggesting that it is
843 unlikely that our study would have missed the compounds reported by Konn et al. (2009, 2012)
844 if they had been present. Alternatively, the unusual distribution of compounds and close
845 similarities between different sites observed by Konn et al. (2009, 2012) may be an indication
846 that many of the compounds they reported are derived from an unrecognized background source
847 of contamination rather than the hydrothermal fluids. Further comparative tests of the extraction
848 and analytical protocols employed in these various studies as well as analysis of additional
849 samples of hydrothermal fluids using multiple methods are needed to resolve this issue.

850 In an earlier study, Holm and Charlou (2001) reported observation of trace amounts of C₁₆ to
851 C₂₉ *n*-alkanes in samples of high-temperature (364 °C) hydrothermal fluids from the Rainbow
852 field. These compounds were extracted from 50 ml aliquots of hydrothermal fluids that had been
853 stored in a cold room for a considerable length of time prior to analysis. Organic compounds
854 were extracted from the fluid with SPE units containing the same HLB sorbent used in this
855 study. In the present study, alkanes were not identified in any of the high-temperature (326-366
856 °C) hydrothermal fluids sampled at Rainbow, even in the sample from Stylo2 where more than a
857 liter of fluid was pumped through the SPE cartridge containing HLB. The only site where
858 alkanes were observed at Rainbow was at the lower temperature Ecurie site (Figs. 9b and 10a),
859 but the alkanes in those samples have a substantially different carbon number distribution (C₂₂ to
860 C₃₃) from those reported by Holm and Charlou (2001), and are most likely derived from chimney
861 particulates entrained during sampling. Consequently, we are unable to reproduce or confirm the
862 results of Holm and Charlou (2001).

863 The only other study to provide a detailed analysis of organic matter extracted from deep-sea
864 hydrothermal vent fluids is Brault et al. (1988). Those authors analyzed dissolved organic
865 compounds in >250 °C fluid venting from a basalt-hosted hydrothermal system at 13°N on the
866 East Pacific Rise collected with a 350 ml Ti syringe sampler and extracted with a liquid-liquid
867 method using chloroform as the solvent. The extract contained a suite of *n*-alkanes ranging in
868 size from C₁₇ to C₃₅ along with an unconsolidated complex mixture (UCM) of unidentified
869 compounds dominated by aliphatic components. The UCM hump in the extract was broad with a
870 peak toward the higher molecular weight end centered at about C₂₉. The alkanes exhibited a
871 smooth distribution with a peak in abundance at about C₂₈, with a very slight preference for even
872 carbon number compounds. The total amount of aliphatic hydrocarbons in their hydrothermal
873 fluid sample was ~56 µg l⁻¹, which was ~100 times greater than levels in the local seawater.

874 The suite of organic compounds observed by Brault et al. (1988) were not observed in any of
875 the hydrothermal fluids examined in the present study using comparable liquid-liquid extraction
876 methods. Although Brault et al. (1988) extracted a larger volume of hydrothermal fluid than was
877 included in the IGT samples (350 ml vs. ~120 ml) analyzed here, if aliphatic hydrocarbons had
878 been present in comparable amounts to those reported by Brault et al. they should have been
879 readily detectable in the IGT extracts. Samples from the Ecurie site at Rainbow were the only
880 samples in this study where a suite of compounds similar to those reported by Brault et al. (1988)
881 were identified. These samples contained both a suite of alkanes peaking at ~C₂₈ and a broad
882 UCM with a peak towards the higher molecular weight end (Figs. 9b and 10a). In the case of the
883 Ecurie sample, however, the organic compounds in the extract appear to be derived from
884 particles of chimney sulfides included in the sample rather than the hydrothermal fluid itself.
885 These observations suggest that the compounds reported by Brault et al. (1988) may also have
886 been derived from chimney particulates entrained into the sampler during fluid collection, which
887 common occurrence for the large syringe samplers used in that study.

888 4.4. Abiotic organic compounds

889 One of the primary reasons that deep-sea hydrothermal systems have become strong
890 candidates as the site of the origin of life is that conditions within these systems appear to be
891 highly favorable for the abiotic synthesis of organic compounds (e.g., Shock, 1990, 1992; Shock
892 and Schulte, 1998; McCollom and Seewald, 2007). Ultramafic-hosted hydrothermal systems are
893 viewed as particularly favorable environments for abiotic synthesis owing to the strongly
894 reducing conditions and highly elevated concentrations of H₂ that develop during
895 serpentinization of ultramafic rocks (Berndt et al., 1996; McCollom and Seewald, 2001, 2013).
896 Indeed, a substantial body of evidence has accumulated that the methane and other light
897 hydrocarbons found in fluids discharging from the ultramafic-hosted deep-sea hydrothermal
898 vents at Lost City and Rainbow have a predominantly abiotic origin (Prokurowski et al., 2008;
899 Charlou et al., 2002, 2010). Formate has also been identified as a product of abiotic carbon
900 reduction at Lost City (Lang et al., 2010).

901 Whether other, more-complex organic compounds with an abiotic origin can be identified in
902 deep-sea hydrothermal fluids remains an open issue. Holm and Charlou (2001) reported
903 detection of C₁₆-C₂₉ *n*-alkanes in hydrothermal fluids from the Rainbow system that they
904 attributed to abiotic formation through Fischer-Tropsch-type synthesis in the hydrothermal
905 system. Konn et al. (2009) also speculated that some of the *n*-alkanes and *n*-alkanoic acids they
906 observed in extracts of Lost City and Rainbow hydrothermal fluids might have an abiotic origin.

907 In the present study, no evidence was found for the presence of abiotic organic compounds in
908 any of the hydrothermal fluid extracts. Although linear alkanes are the primary products of

909 Fischer-Tropsch-type abiotic organic synthesis (e.g., McCollom and Seewald, 2007; McCollom
910 et al., 2010), alkanes were observed at only one site during this study (Ecurie at Rainbow) and
911 those compounds were likely derived from particulate matter rather than the fluid. Furthermore,
912 while Fischer-Tropsch products characteristically exhibit a log-linear decrease in abundance with
913 increasing carbon number (e.g., McCollom and Seewald, 2006; McCollom et al. 2010), the
914 alkanes observed at the Ecurie site show a bell-shaped distribution centered at $\sim C_{28}$ with no
915 compounds below C_{23} (Figs. 9b and 10a). Thus, the distribution of these hydrocarbons does not
916 resemble the distribution that would be expected for abiotically formed compounds. As
917 suggested by Simoneit et al. (2004), thermal alteration of organic precursors within the
918 hydrothermal system is the most likely source for these compounds. Fischer-Tropsch synthesis
919 also produces alkanolic acids, but the abundance of those compounds would also be expected to
920 follow a regular log-linear decrease with carbon number with no even-odd carbon number
921 preference (e.g., McCollom et al., 1999; McCollom et al. 2010). While alkanolic acids were
922 found in the Lost City fluids and chimney extracts, the strong even-carbon-number preference
923 for these compounds indicates they are biological products.

924 The absence of extractable abiotic hydrocarbons in the hydrothermal fluids should not
925 necessarily be completely unexpected. Assuming that abiotic hydrocarbons in deep-sea
926 hydrothermal fluids would follow a distribution similar to typical Fischer-Tropsch products,
927 extrapolation from the concentrations of light hydrocarbons observed in the Lost City and
928 Rainbow systems (which are in the nmol kg^{-1} range) to predict the expected concentrations of the
929 extractable higher hydrocarbons ($\geq C_{10}$) suggests that these compounds should be present at
930 levels of 1 pmol kg^{-1} or less (Fig. 12). At such low concentrations, it would be very difficult to
931 detect abiotic hydrocarbons even if they could be concentrated significantly during extraction by
932 SPE or other methods. Other abiotic high-molecular-weight organic compounds (alkanoic acids,
933 etc.) might be expected to have similarly low concentrations, suggesting that it may be extremely
934 challenging to detect the presence of these compounds in deep-sea hydrothermal fluids.

935 5. CONCLUDING REMARKS

936 The results of this study illustrate many of the difficulties faced in trying to detect solvent
937 extractable hydrocarbons and other higher molecular weight dissolved organic compounds in
938 end-member deep-sea hydrothermal fluids. These compounds, if they are present at all, appear
939 to occur at dissolved concentrations that are at or below the detection limit of commonly used
940 extraction and analysis methods. Additional detection of organic compounds may require use of
941 methods to concentrate the compounds from large amounts of fluid prior to analysis.
942 Furthermore, because of the very low levels at which these compounds occur, it is essential to
943 fully evaluate potential background sources of organic compounds that could easily be mistaken
944 for components dissolved in the fluids, including those from sources such as chimney
945 particulates, biomass, and contaminants introduced during sample handling. The POP gun and
946 SPE sorbent approaches used in this study show some promise for concentrating organic
947 compounds and lowering detection limits, but clearly the level of background compounds from
948 the sorbent phases needs to be significantly reduced to maximize the utility of this method. If
949 this can be accomplished, the ability to concentrate organic compounds from several liters or
950 more of hydrothermal fluids may allow even trace compounds to be detected and characterized,
951 and may ultimately reveal additional details of the sources and fate of organic matter in deep-sea
952 hydrothermal environments.

953

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Table 1. Summary of samples collected for analysis of organic compounds, with selected chemical information.

Site/Vent	Temp. (°C)	Lat. (N)	Long. (W)	Sample ID			pH (25°C)	Mg	H ₂	ΣCO ₂	CH ₄
				IGT	POP gun	SPE(<i>phase</i>)					
<i>Rainbow (ultramafic-hosted)</i>											
Guillaume	359	36° 13.752'	33° 54.222'	J2-352-IGT7	J2-352-POP1, J2-352-POP2		3.24	1.9	15.8	21.2	2.03
Regner	326	36° 13.751'	33° 54.232'	J2-353-IGT7			3.45	8.0	13.5	21.1	1.86
Padraig	350	36° 13.766'	33° 54.173'	J2-353-IGT6	J2-353-POP1, J2-353-POP2		3.43	5.4	16.1	19.6	1.79
Stylo 1	366	36° 13.781'	33° 54.070'	J2-354-IGT7			3.28	2.1	15.0	24.6	2.10
Ecurie	191	36° 13.748'	33° 54.188'	J2-354-IGT6	J2-354-POP1, J2-354-POP2	J2-354-SPE(HLB)	2.99	7.4	9.4	18.7	1.79
Stylo 2	359	36° 13.777'	33° 54.072'	J2-355-IGT7	J2-355-POP1, J2-355-POP2	J2-355-SPE(C8), J2-355-SPE(HLB)	3.27	2.6	16.5	20.9	2.10
<i>Lucky Strike (basalt-hosted)</i>											
2608(top)	323	37° 17.508'	32° 16.675'	J2-356-IGT6			3.86	0.9	0.048	34.7	1.01
US4	299	37° 17.276'	32° 16.529'	J2-357-IGT6			4.75	29.5	0.053	133	0.74
Crystal	306	37° 17.450'	32° 16.920'	J2-358-IGT6			3.64	1.5	0.041	117	0.82
Medea	251	37° 17.659'	32° 16.900'	J2-359-IGT6	J2-359-POP1 [#]	J2-359-SPE(HLB) [#]	3.68	2.2	0.063	98	0.89
<i>Lost City (ultramafic-hosted)</i>											
Beehive	91	30° 7.431'	42° 7.203'	J2-360-IGT6			10.6	0.1	10.4	0.18	1.9
Beehive	90	30° 7.434'	42° 7.202'	J2-361-IGT6	J2-361-POP1, J2-361-POP2	J2-361-SPE(HLB), J2-361-SPE(C8)	10.6	1.0	10.4	0.18	1.9
Top of Poseidon	51	30° 7.430'	42° 7.203'	J2-362-IGT6			10.8	2.6	-	-	-
Seawater (Lucky Strike)					J2-356-POP1	J2-356-SPE(HLB)	-	-	-	-	-
Seawater (Lost City)					J2-362-POP1	J2-362-SPE(C8)	-	-	-	-	-
Seawater (TAG)				J2-364-IGT6			-	-	-	-	-

Dissolved concentrations of Mg, H₂, ΣCO₂, and CH₄ in mmol kg⁻¹. ΣCO₂ is total dissolved CO₂ (=CO_{2(aq)} + HCO₃⁻ + CO₃²⁻). “-“ = not measured. [#]For this sample, fluid was processed through the POP gun for only 20 minutes; for all other POP gun samples, fluids were pumped for about 60 minutes.

Table 2. Summary of organic compounds detected in hydrothermal vent fluid samples.

Site: Sample types:	Rainbow						Lucky Strike				Lost City	
	Guillaume (I,P,S)	Regner (I)	Padraig (I,P,S)	Stylo1 (I)	Ecurie (I,P,S)	Stylo2 (I,P,S)	2608 (I)	US4 (I)	Crystal (I)	Medea (I,P,S)	Beehive (I,P,S)	Poseidon (I)
<i>Carboxylic acids</i>												
Octanoic acid	-	-	-	-	-	-	-	-	-	-	P	P
Decanoic acid	-	-	-	-	-	-	-	-	-	-	P	P
Dodecanoic acid	-	-	-	-	-	-	-	-	-	-	P	P
Tetradecanoic acid	-	-	-	-	-	-	-	-	-	B	-	-
Hexadecanoic acid	-	-	-	-	-	-	-	-	-	B	-	-
Hexadecenoic acid	-	-	-	-	-	-	-	-	-	B	-	-
Octadecanoic acid	-	-	-	-	-	-	-	-	-	B	-	-
Octadecenoic acid	P	-	-	-	-	-	-	-	-	B	-	-
Doicosanoic acid	-	-	-	-	-	-	-	-	-	B	-	-
<i>Aliphatic hydrocarbons</i>												
<i>n</i> -Alkanes [range]	-	-	-	-	CS [C ₂₃ -C ₃₃]	-	-	-	-	-	-	-
Squalene	-	-	-	-	-	-	-	-	-	-	-	P
<i>Aromatic hydrocarbons</i>												
Phenanthrene	P	-	P	-	P	P	-	-	-	-	-	-
Methylphenanthrenes	P	-	P	-	P	P	-	-	-	-	-	-
C ₂ -phenanthrenes	-	-	P	-	P	P	-	-	-	-	-	-
Methylfluorene	P	-	-	-	-	-	-	-	-	-	-	-
Dibenzothiophene	-	-	-	-	-	-	-	-	P	P	-	-
<i>Other compounds</i>												
Cholesterol	-	-	-	-	-	-	-	-	-	-	-	P
Hexadecanol	P	-	-	-	-	-	-	-	-	-	-	P
Trithiolane	-	-	-	-	-	-	-	-	-	-	-	P
UCM	-	-	-	-	CS	P	-	-	-	-	-	-

Compounds that were positively identified and inferred to be indigenous to the fluid are designated with a “P”, while compounds that were detected but interpreted to come from other sources are identified designated with a “CS” (chimney sulfide source) or “B” (biomass source). “-“ = compound not detected in fluid sample. Sample types: I = IGT sampler, P = POP gun, S = SPE cartridge.

Table 3. Abundances[†] and isotopic composition of *n*-alkanoic acids in Lost City hydrothermal fluids samples.

Sample	J2-360- IGT6	J2-361- IGT6	J2-361- SPE(HLB)
	————— $\mu\text{mol acid/L}$ —————		
C ₈	0.72	0.53	0.77
C ₁₀	0.47	0.37	0.54
C ₁₂	0.28	0.23	0.31
	————— $\mu\text{mol C/L}$ —————		
C ₈	5.7	4.2	6.2
C ₁₀	4.7	3.7	5.4
C ₁₂	3.4	2.7	3.7
Total	13.8	10.6	15.3
	————— $\delta^{13}\text{C}$ (‰) —————		
C ₈	-5.9	-7.6	
C ₁₀	-4.3	-2.8	
C ₁₂	-4.8	-3.6	

[†]Concentrations are given for the acids and for the equivalent number of carbon atoms.

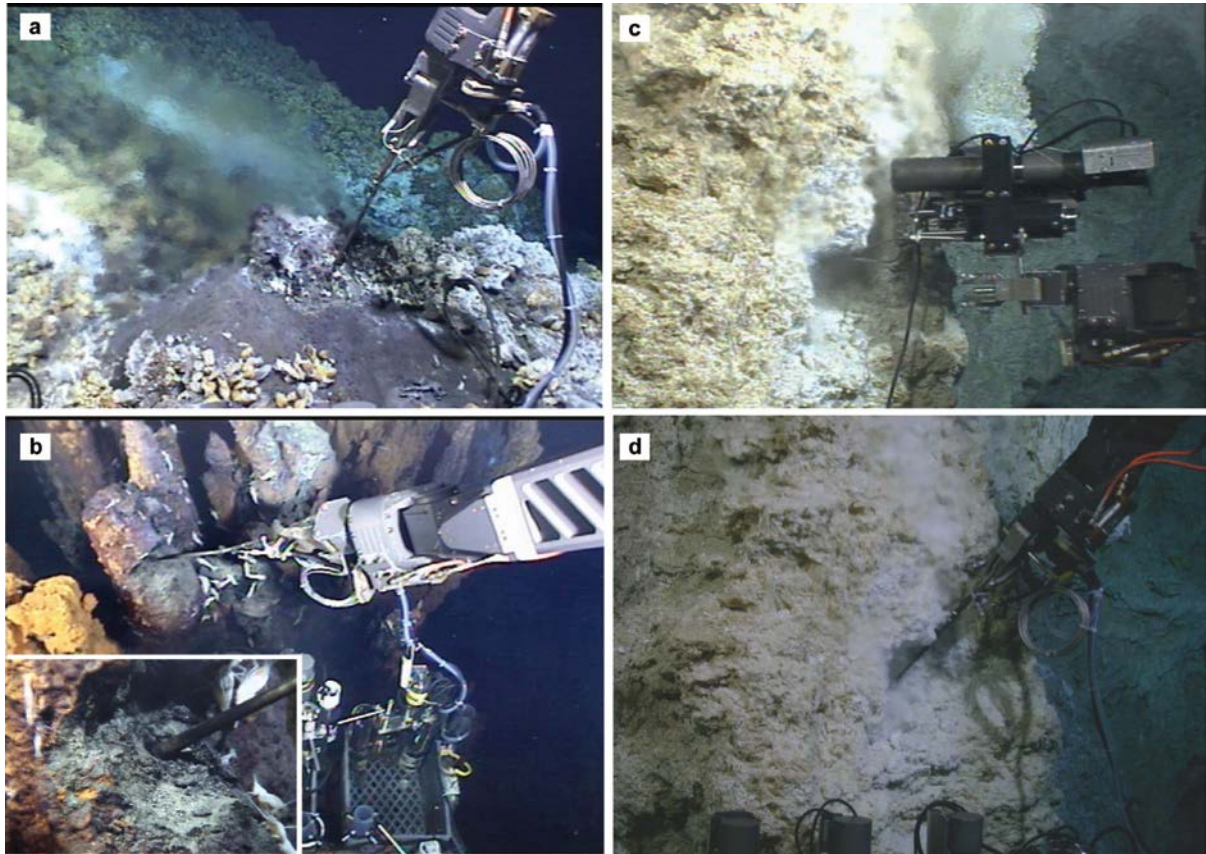


Figure 1. Representative images of fluid sampling at the deep-sea hydrothermal vent sites. (a) Sampling with the POP gun at the Medea site at Lucky Strike. (b) Sampling with the POP gun at the Ecurie site at Rainbow. The fluid at this site was obtained from a cavity excavated into the side of an older, less active chimney structure. Inset shows closeup of tip of POP gun in side of chimney. (c, d) Fluid sampling at the Beehive site at Lost City with the isobaric gas-tight sampler (c) and POP gun (d).

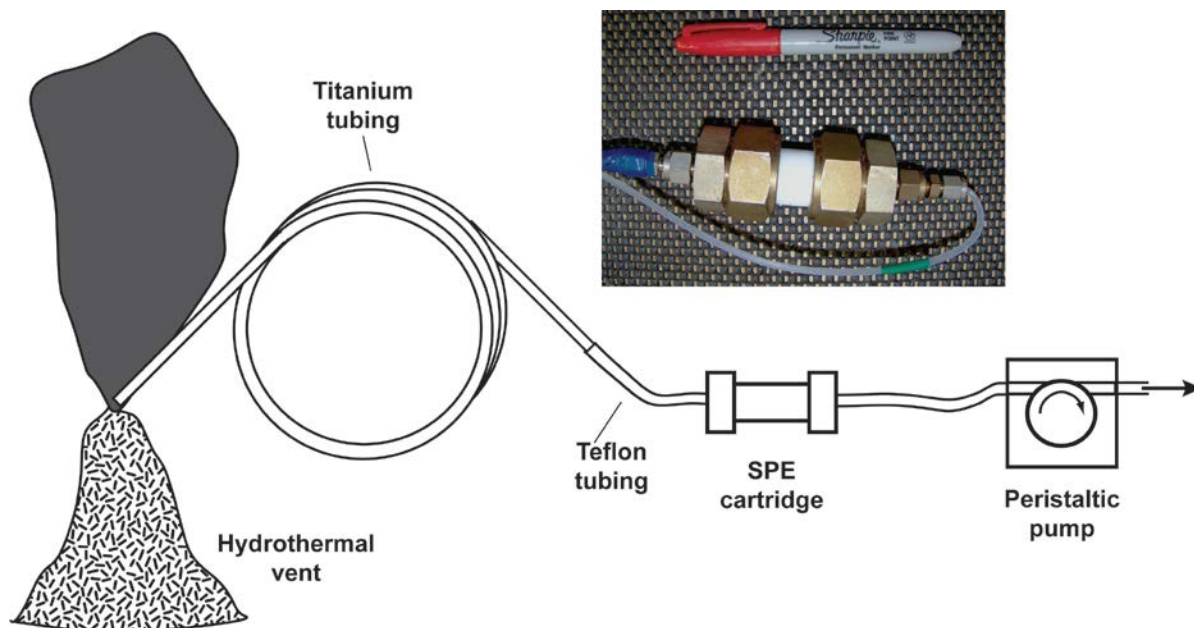


Figure 2. Schematic drawing of the peristaltic organic pump fluid sampling device (POP gun). The sampler consists of a coil of titanium tubing (4 m long) connected through Teflon tubing to a cartridge filled with solid phase extraction (SPE) microbeads, which is then connected to a peristaltic pump with silicon tubing. During operation, the tip of the sampler is placed in a hydrothermal vent, and the peristaltic pump draws fluid through the tubing and SPE cartridge. The inset shows an image of the SPE cartridge, consisting of SPE microbeads within a 2.54 cm diameter Teflon tube capped with porous Teflon frits and sealed at the ends with stainless steel Swagelok fittings.

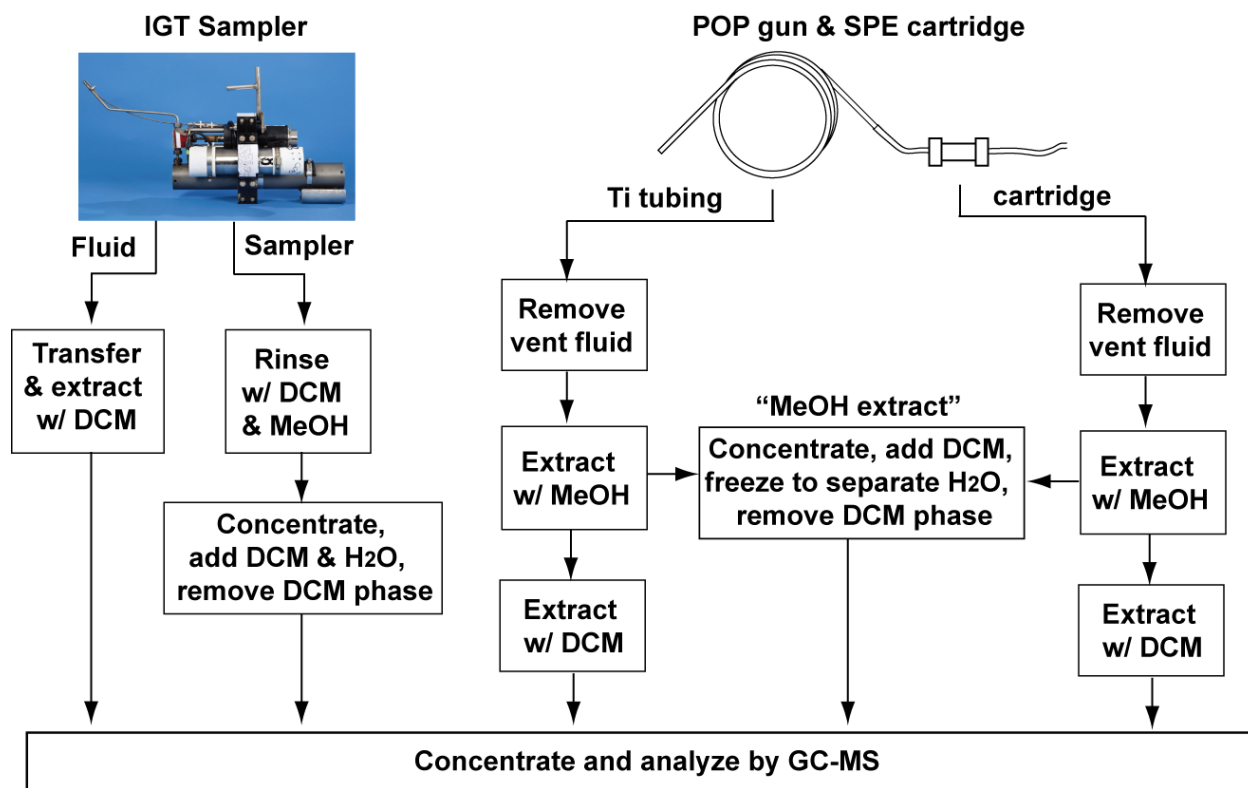


Figure 3. Flow diagram summarizing methods used to extract and prepare organic compounds for analysis from the different sampling methods used in this study.

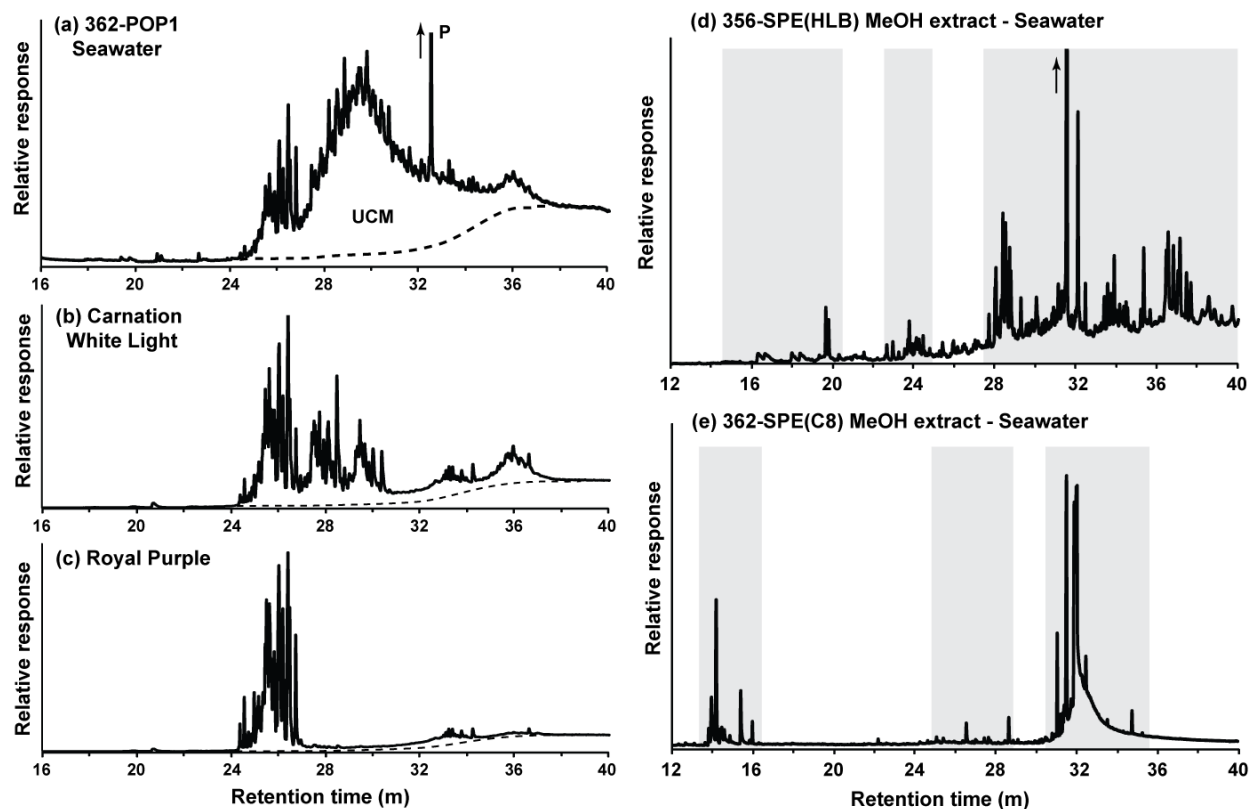


Figure 4. Total ion chromatograms from GC-MS analysis of potential sources of background organic compounds that could have contaminated the hydrothermal fluid samples. (a) MeOH extract of seawater sample from the vicinity of the TAG hydrothermal field obtained with the POP gun, showing a large unresolved complex mixture of compounds (UCM). Results for a seawater sample taken in the vicinity of the Lucky Strike hydrothermal vent field (J2-356-POP1) were very similar. (b,c) Oils used for lubrication and pressure compensation on ROV *Jason*. These oils also display a UCM, but they are distinctly different from the seawater UCM. (d,e) MeOH extracts of deep seawater samples processed using the HLB and C8 SPE cartridges. Gray areas highlight prominent peaks for organic compounds that were present in all samples processed through these cartridges, including Fisher H₂O blanks, and are inferred to be contaminants extracted from the SPE microbeads. In all but a few cases, the mass fragmentation patterns for these compounds did not match any compounds in the reference database and could not be identified.

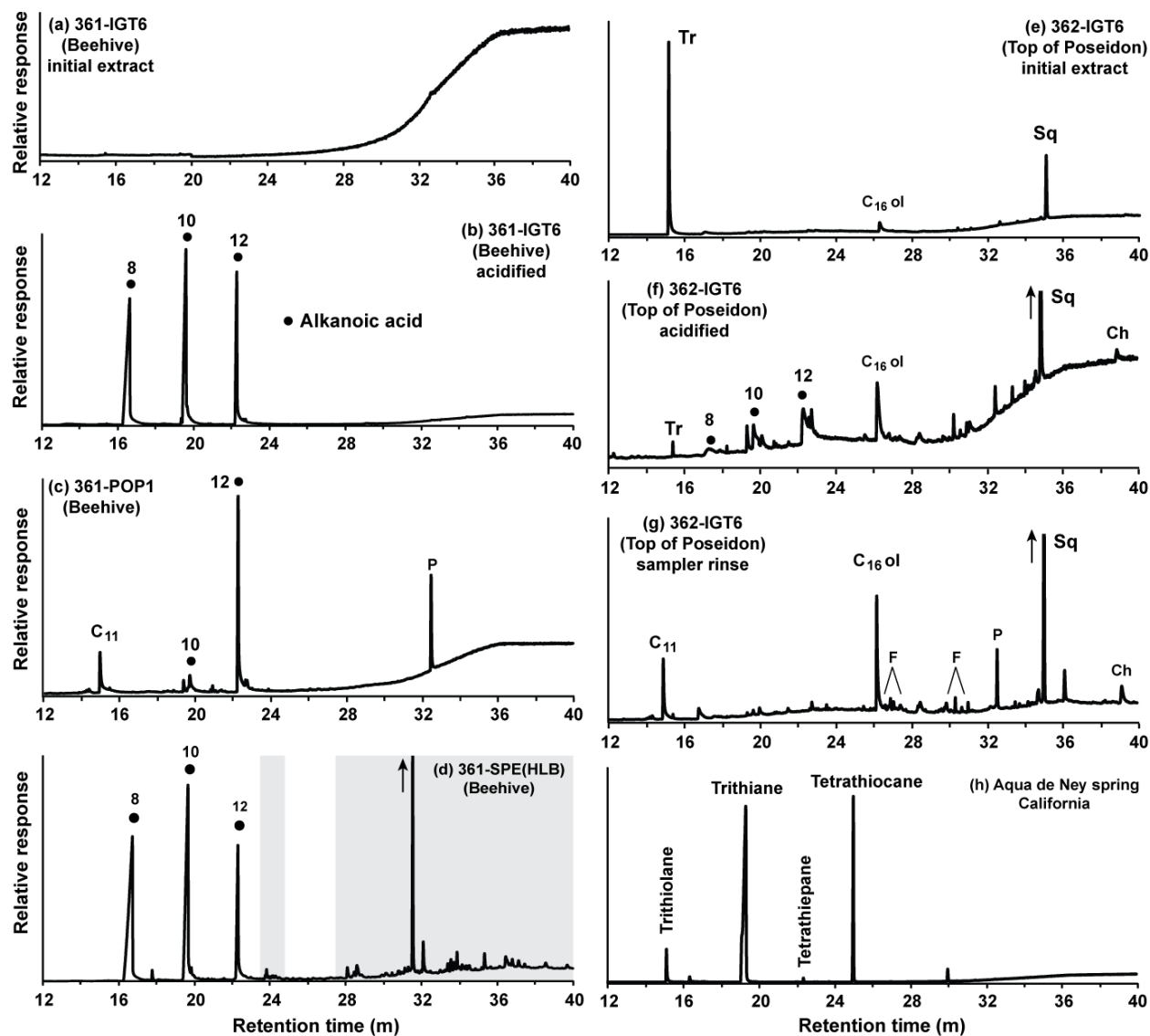


Figure 5. Total ion chromatograms for samples from the Lost City hydrothermal system. (a,e) Initial DCM extracts for samples from Beehive and Top of Poseidon (ToP). The Beehive sample shows no peaks above the instrumental baseline, while the ToP sample has peaks for trithiolane (Tr), hexadecanol ($C_{16}ol$), and squalene (Sq). (b,f) Second DCM extracts from Beehive and ToP samples following acidification of the fluid. The Beehive sample exhibits prominent peaks for octanoic, decanoic and dodecanoic acids (C_8 , C_{10} and C_{12} , respectively), which are also evident in smaller amounts in the ToP sample along with cholesterol (Ch). Sample J2-360-IGT6 produced results identical to the Beehive sample shown. (c) Analysis of POP gun MeOH extract from Beehive site. The other POP gun sample for this site produced the same result. (d) Sample from Beehive vent processed with the HLB SPE cartridge and extracted with MeOH. Peaks outlined by gray areas are inferred to be contaminants because they were present in all MeOH extracts of the HLB cartridges (see Fig. 4d and Supplemental Fig. S1). (g) Solvent rinse of IGT sampler from ToP site. (h) Analysis of DCM extract of highly alkaline fluid (pH = 11.8) discharged from Aqua de Ney spring, northern California. The spring fluid includes trithiolane and several other cyclic C-S compounds (trithiane, tetrathiepane, tetrathiocane). The sample was

collected in a glass bottle and extracted using the same procedures as the IGT samples. Undecane (C₁₁) comes from the methanol solvent, compounds labeled “F” are from the Fluorolube lubricant, and “P” is dioctyl phthalate. Unlabeled peaks could not be identified.

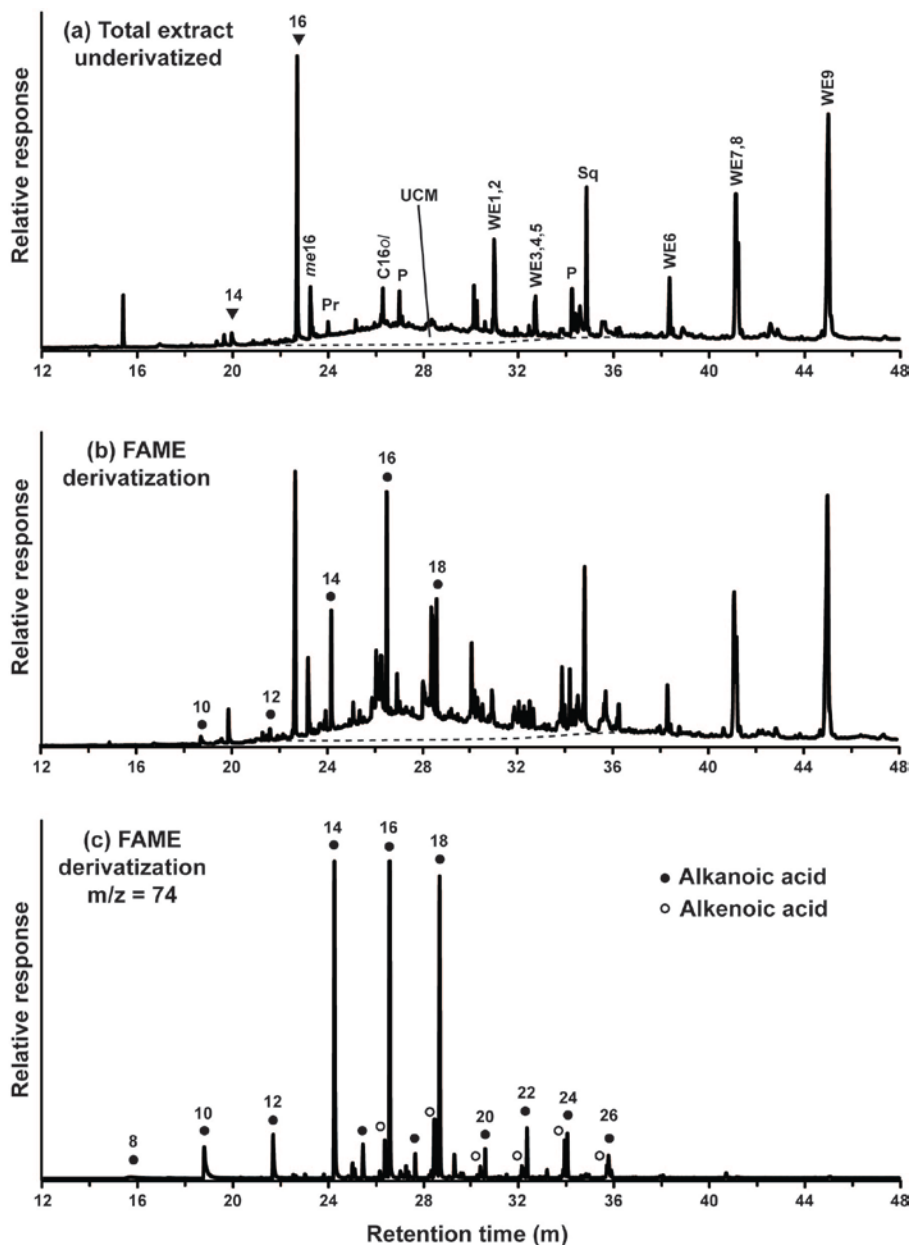


Figure 6. Chromatograms of Lost City chimney extracts. (a) Total ion chromatogram for analysis of total extract. Dashed line shows instrument baseline, and area marked UCM is an unresolved complex mixture of compounds. Peaks labeled “WE” are wax esters, and other labeled peaks are *n*-alkanes (inverted triangles, labeled with carbon number), methylhexadecane (*me16*), pristane (Pr), hexadecaol (C16ol), and squalene (Sq). (b) Total ion chromatogram of total extract derivatized to convert fatty acids to methyl esters (FAME). Only peaks for methyl esters of the C₁₀-C₁₈ alkanic acids are labeled; other peaks correspond to those labeled in (a) and (c). (c) Mass fragmentogram for mass-to-charge ratio (*m/z*) of 74, characteristic for FAME.

Labels identify methyl esters of *n*-alkanoic and *n*-alkenoic acids, with number of carbon atoms specified for even-numbered compounds. Alkenoic acids elute just before alkanolic acid of same carbon number (positions of double bonds were not determined). "P" = phthalates. Other abbreviations: WE1,2 = C₁₀-C₁₂ ester + C₈-C₁₄ ester; WE3,4,5 = C₁₂-C₁₂ ester + C₁₀-C₁₄ ester + C₈-C₁₄ ester; WE6 = C₁₄-C₁₆ ester; WE7,8 = C₁₄-C₁₈ ester + C₁₆-C₁₆ ester; WE9 = C₁₆-C₁₈ ester.

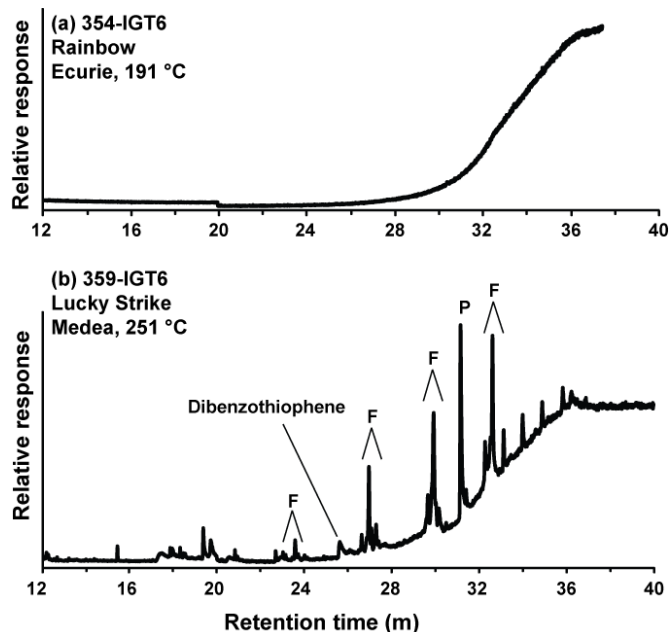


Figure 7. Total ion chromatograms for selected GC-MS analysis of extracts of IGT fluid samples. Families of peaks marked with an “F” represent contamination from the Fluorolube lubricant used in the samplers, and the peak labeled “P” is a phthalate. (a) Extract of fluid from the Ecurie site at Rainbow, showing an absence of peaks above the instrumental baseline. Most other fluid extracts were similar. (b) Extract of fluid from the Medea site at Lucky Strike. Unlabeled peaks in the chromatograms did not match any compounds in the spectral library, and are presumably additional contaminants introduced during sample processing.

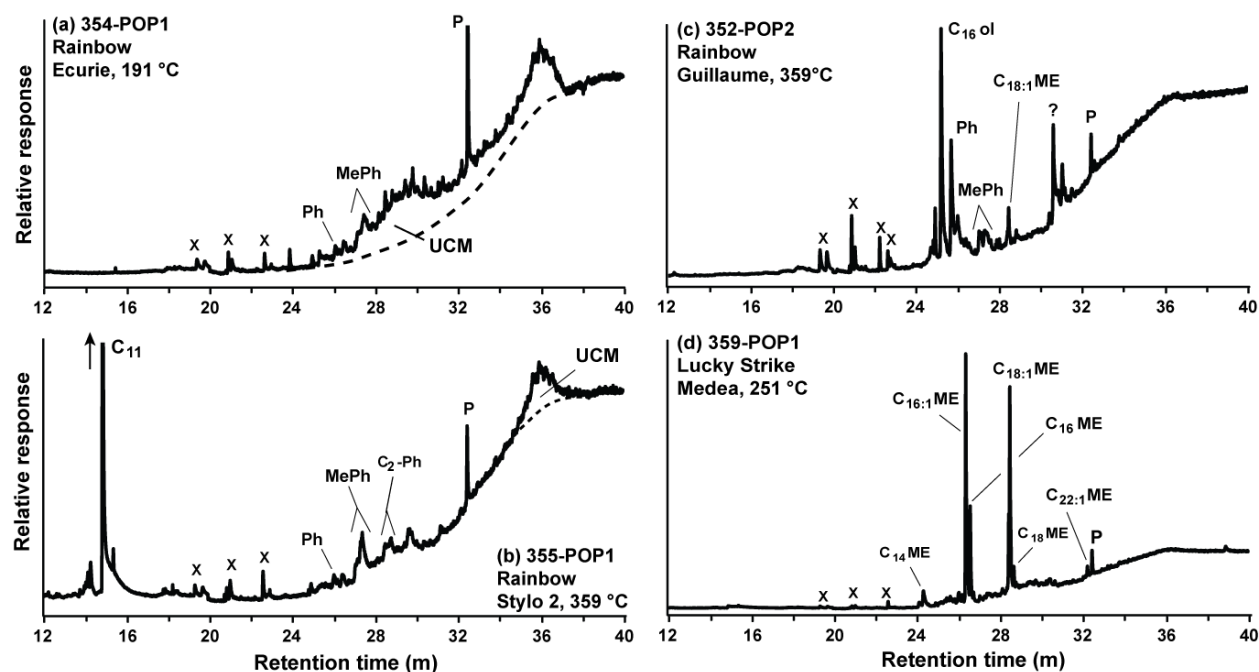


Figure 8. Total ion chromatograms for representative MeOH extracts of POP gun samples. The dashed lines in the chromatograms represent the instrument baseline. (a) Sample from the Ecurie vent at Rainbow (191 °C; J2-354-POP1) showing a small, broad UCM extending from ~25 to 38 min and peaks for phenanthrene (Ph) and methylphenanthenes (MePh). The sample also contains several C₂-phenanthenes (C₂-Ph), but peaks for these compounds are obscured by the UCM. The other POP gun sample at this site (J2-354-POP2) produced very similar results. (b) Sample from the Stylo 2 site at Rainbow (359 °C; J2-355-POP1) showing small peaks for several PAH including Ph, MePh, and C₂-Ph, as well as a small UCM centered at about 36 min. Extracts for other POP gun samples at the high-temperature Stylo 2 and Padraig sites at Rainbow showed similar trace levels of aromatic compounds. (c) Sample from the 359 °C Guillaume site at Rainbow. In addition to Ph and MePh, peaks corresponding to hexadecanol (C₁₆ol) and octadecenoic acid methyl ester (C_{18:1}ME) are present. The other POP gun sample at this site produced identical results. (d) Sample from the Medea vent at Lucky Strike (251 °C), including methyl esters of a number of alkanolic and alkenolic acids (C_xME and C_{x:1}ME, respectively, where *x* is the carbon number). Undecane (C₁₁) comes from the methanol solvent. Peaks marked with an “X” were unidentifiable compounds present in all samples and are presumably contaminants introduced during sample processing (see Supplemental Fig. S1). “P” = dioctyl phthalate.

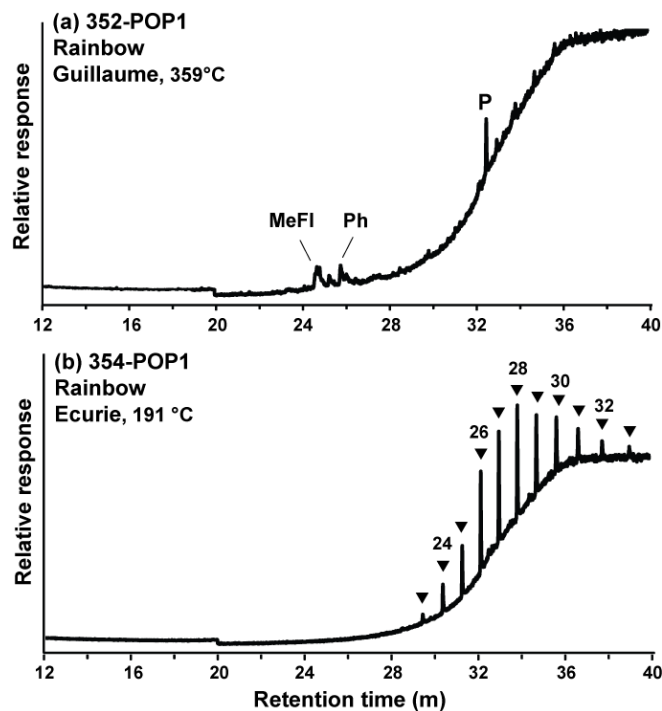


Figure 9. Total ion chromatograms for selected DCM extracts of POP gun samples. (a) Sample from the 359 °C Guillaume site at Rainbow, showing trace amounts of methylfluorene (MeFl) and phenanthrene (Ph). (b) Sample from the Ecurie site at Rainbow, showing peaks for a homologous series of *n*-alkanes with 23-33 carbon atoms (inverted triangles; labels show carbon chain length). Peak labeled “P” is dioctylphthalate. DCM extracts for other POP gun samples showed no organic compounds attributable to the hydrothermal fluids.

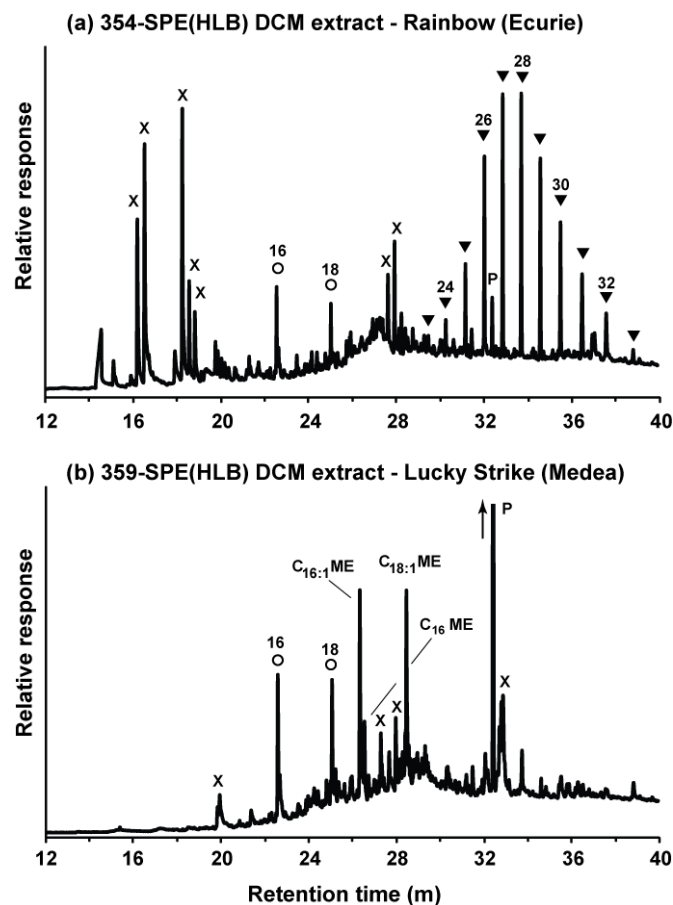


Figure 10. Total ion chromatograms for selected HLB SPE cartridge samples extracted with DCM. (a) Sample from the Ecurie site at Rainbow, showing peaks for a homologous series of *n*-alkanes with 23-33 carbon atoms (inverted triangles; numbers show carbon chain length). (c) Sample from Medea vent at Lucky Strike. Included in the extract are methyl esters of several alkanolic and alkenolic acids (C_x ME and $C_{x:1}$ ME, respectively, where x is the carbon number). Prominent peaks labeled with “X” as well as the broad hump of compounds centered at ~27.5 m are present in all DCM extracts of the HLB cartridges and are inferred to be contaminants from the SPE microbeads (see Supplemental Fig. S1 for examples). Note that the sample shown in (b) was inadvertently allowed to evaporate to dryness, resulting in loss of compounds at <19 m. Also identified as ubiquitous contaminants from the SPE microbeads are C_{16} and C_{18} *n*-alkenes (labeled with open circles). Peak labeled “P” is dioctylphthalate.

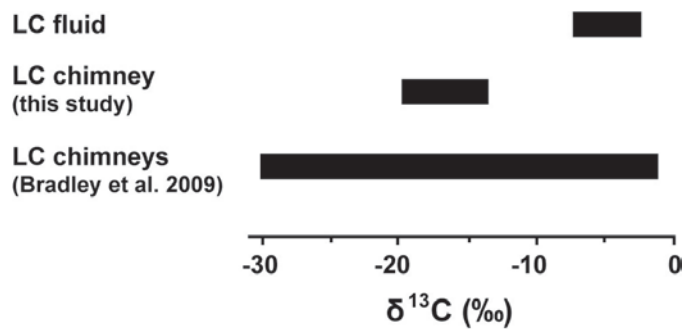


Figure 11. Range of carbon isotopic compositions for *n*-alkanoic acids measured for fluid and chimney samples from Lost City in this study, and reported for multiple active chimney samples from Lost City by Bradley et al. (2009).

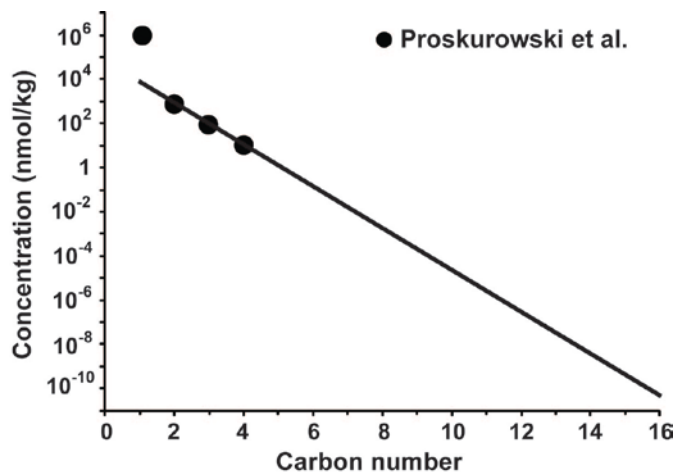


Figure 12. Predicted concentrations of linear alkanes as function of carbon number (line) based on extrapolation of measurements of C₂-C₄ alkanes measured at the Beehive vent at Lost City by Proskurowski et al. (2008) (circles). Line calculated assuming a log-linear decrease in alkane abundance with increasing carbon number typical of Fischer-Tropsch-type organic synthesis reactions.