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Assessment of apolar lipids in subseafloor rocks and potential contaminants from the Atlantis Massif (IODP Expedition 357)

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ABSTRACT

International Ocean Discovery Program Expedition 357 drilled 17 boreholes across the Atlantis Massif with the goals of investigating carbon cycling and the presence of life in a zone of active serpentinization. The expedition recovered multiple lithologies including gabbros, basalts, carbonate sands, and serpentinites. A subset of contrasting lithologies were analyzed for apolar lipid content to determine if non-volatile organic molecules can be detected in the oceanic subsurface. The definitive detection and identification of abiotic and biological lipids in the subsurface of an actively serpentinizing system would be a significant step towards understanding a variety of scientific processes, including the evolution of pre-biotic chemistry, microbial habitability, and the global carbon cycle. Given the high potential for contamination during drilling, a suite of materials used in sample collection and processing were also analyzed to characterize their signatures. An n-alkane series ranging from C_{18} to C_{30} with $\delta^{13}C$ isotopic values of $-30.9‰$ to $-28.8‰$ was present in lithologically diverse samples. Multiple lines of evidence point to the rock saw used to remove core exteriors during sample processing as the source of these compounds. Many of the other sample-handling procedures designed to reduce surface contamination were determined to be effective and could be implemented in future projects. This result highlights the value of careful prevention and characterization of contamination to allow for more accurate interpretations of complex and dynamic subsurface processes, and the importance that future reports of these compounds occurs in conjunction with thorough contamination assessments.

1. Introduction

The presence of non-volatile lipids in subsurface rocks can provide valuable insights into processes fundamental to pre-biotic chemistry, microbial habitability, and evolution, and oceanic carbon cycling. Such compounds are of particular interest in actively serpentinizing environment as they have the potential to be synthesized abiotically (McCollom and Seewald, 2006). Serpentinization is a series of spontaneous reactions that ensue when seawater hydrates ultramafic peridotites, ultimately producing serpentine, a variety of secondary minerals, and hydrogen (Moody, 1976). Environments where these reactions occur have the potential for the abiotic formation of organic molecules via Fischer-Tropsch Type and/or Sabatier synthesis from the resulting hydrogen (McCollom and Seewald, 2007). Volatile compounds such as methane, ethane, propane, butane, and formate have been identified in fluids from oceanic serpentinite-hosted hydrothermal systems and have been attributed to abiotic synthesis reactions (Kelley et al., 2001, 2005; Proskurowski et al., 2008; Lang et al., 2010, 2018; Charlou et al., 2010). Laboratory experiments have also demonstrated that long-chain saturated hydrocarbons and alcohols containing up to 32 carbon atoms can be synthesized under serpentinizing conditions. (McCollom and Seewald, 2006).

The hydrogen produced during serpentinization can also fuel chemolithoautotrophic microorganisms in the subsurface (Schrenk et al., 2013). These communities will generate lipid biomarkers that can be used to identify their metabolic pathways and carbon sources (Delacour et al, 2008; Bradley et al., 2009; Schrenk et al., 2013; Plümper et al., 2017). The chimney structures of the serpentinite-hosted Lost City Hydrothermal Field (30°N, Mid-Atlantic Ridge) harbor dense biofilms inhabited by methane- and sulfur-cycling archaea and bacteria (Schrenk et al., 2004; Bradley et al. 2009; Brazelton et al., 2006; Lang et al., 2012; Méhay et al., 2013). Lipid analyses of these carbonate-brucite chimneys have identified saturated and unsaturated 2, 6, 10, 15, 19-pentamethylicosane (PMI) compounds, which are highly specific apolar biomarkers produced by methanogenic and methanotrophic archaea (Peters et al., 2005; Bradley et al., 2009; Méhay

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et al., 2013). Similar microbial communities are hypothesized to inhabit the rocky subsurface adjacent to Lost City and would produce a similar suite of biomarkers.

International Ocean Discovery Program (IODP) Expedition 357 drilled into serpentinite rocks with the goal of, in part, identifying "the potential for a hydrogen-based deep biosphere, and hydrocarbon synthesis in an active serpentinite-hosted hydrothermal system" (Früh-Green et al., 2017b). Apolar alkanes were targeted as compounds of interest since they have the potential to be synthesized abiotically in actively serpentinizing environments (McCollom and Seewald, 2006) and because they have been previously identified as the products of biological synthesis in rocks and carbonate chimneys from the Atlantis Massif and Lost City Hydrothermal Field (Delacour et al., 2008; Bradley et al., 2009; Méhay et al., 2013). It is possible that some of these non-volatile lipids can exist in serpentinized rocks and be extracted and identified (Klein et al., 2015).

A major concern during these operations is verifying that detected organic molecules are inherent to the original sample and not an artifact from sample collection or processing procedures. Microbiologists recovering rock and sediment cores during oceanic drilling have been particularly focused on minimizing and characterizing contamination and have lead the development of chemical, microbiological, and particulate tracer methods to quantify microbial contamination throughout the process (Smith et al., 2000; Lever et al., 2006; D'Hondt et al., 2007; Santelli et al., 2010; Frise et al., 2017; Orcutt et al., 2017). For example, methods involving perfluoromethylcyclohexane (PFC) tracers and fluorescent microspheres have been extensively implemented to measure the intrusion of drilling fluids, often seawater, and nonindigenous microorganisms, respectively, into drilled cores (Smith et al., 2000; Lever et al., 2006; Orcutt et al., 2017).

Similar issues face studies of lipids and other organic compounds in low biomass subsurface environments. However, the techniques implemented to reduce microbial contamination by tracking cells are not necessarily well suited for quantifying organic geochemical contamination. Fluorescent microspheres and PFC have distinctly different mobilities than non-volatile lipids, and would not behave in a similar fashion. Recent studies have been devoted explicitly to identifying and remediating sources of potential contamination in low biomass rocks collected during continental drilling (e.g. Sherman et al., 2007; Brocks et al., 2008; Brocks 2011; French et al., 2015), but similar studies have not yet been performed for oceanic drilling. Seabed rock drills in particular have the potential for high recoveries of hard-rock samples and are being used with increasing frequency. The cooling fluid used with these drills is local seawater, making the addition of tracers somewhat more challenging (Orcutt et al., 2017). They also utilize a unique suite of oils and greases that could contaminate samples and overprint *in situ* signatures.

In addition to contamination considerations, sample collection and processing will also ideally treat samples in a manner appropriate for different types of analyses. In large, multi-disciplinary studies, multiple laboratories would optimally be able to carry out analyses on the same well homogenized sample, particularly when working with highly heterogeneous rocks. The simultaneous analysis of lipid biomarker isotopes, activity measurements, and metagenomic data, for example, can be particularly powerful in identifying active microbial communities. Since microbes often live in veins or fractures, adjacent samples may not have similar signatures.

IODP Expedition 357 took place from October to December 2015, and drilled a series of 17 boreholes across the Atlantis Massif (30° N/42° W) (Fig. 1; Früh-Green et al., 2017b). Two seabed rock drills were used, the MARUM Center for Marine Environmental Sciences Meeresboden-Bohrgerät 70 (MeBo) and the British Geological Survey RockDrill2 (RD2). Both were modified such that PFC tracer could be injected into the drilling fluid to track the intrusion of deep seawater into cores (Früh-Green et al., 2017b; Orcutt et al., 2017). The goals of the present work were to (1) design a sampling and processing scheme to minimize contamination of organics while remaining compatible with microbiological analyses, (2) characterize the lipid profile of materials that could introduce contamination to samples, and (3) determine if recovered samples contain biological and/or non-biological apolar lipids.

2. Materials and methods

2.1. Site description

The Atlantis Massif is an oceanic core complex uplifted to a depth of 700 m below sea level (mbsl) between 1.5 and 2 million years ago at the intersection of the Mid-Atlantic Ridge and the Atlantis Transform Fault (Fig. 1) (Kelley et al., 2001; Blackman et al., 2002; Früh-Green et al., 2004; Karson et al., 2006). The Massif has three separate lithological domains, including a gabbroic central dome, a peridotite-dominated southern wall, and a basaltic eastern block (Blackman et al., 2002; Greene et al., 2015; Früh-Green et al., 2017b). The Lost City Hydrothermal Field (LCHF) lies on the peridotite-dominated southern wall of the massif, which is one of the most studied regions of active serpentinization in the ocean (Kelley et al., 2001).

2.2. Sample collection

Three types of samples were collected for analysis. First, prior to IODP Exp. 357, materials that would be used for sample collection and storage were analyzed to determine which ones would be least likely to transfer unwanted lipids to samples. Second, oils, greases, and other potential contaminants that may have also come into contact with samples during the cruise and processing stages were collected. Third, hard rock samples were recovered from the subseafloor as part of the expedition.

Pre-cruise materials included materials that would be used for sample storage and gloves (Table 1). Escal Neo bags (Mitsubishi Gas Chemical P/N HT480100M), GX-P film (Toppan Co, Japan), and Whirlpak bags are regularly used to store microbiological samples since they are transparent and sterile. Escal Neo and GX-P are impermeable to gases and can therefore be flushed with nitrogen to maintain anoxic conditions for culturing. Teflon sheeting (McMaster Carr P/N 8569K75, 36″ wide × 0.003″ thickness × 20′ length) was acid washed and autoclaved before analysis. Nylon sheeting (VWR P/N 12243-186) is often used in clean rooms and was tested without additional cleaning procedures. Multiple types of laboratory gloves were tested, including Semperguard Xpert (VWR P/N SEMP816780635), VWR Nitrile (P/N 112-2373), VWR Nitrile (P/N 112-2371), and KC500 purple nitrile (VWR P/N 32934-082).

Potential contaminants included a range of oils and greases used on the rock drills as well as other materials the samples may have been exposed to during drilling or processing (Table 1). A glass fiber filter was dipped in HPLC grade methanol and swiped along the interior of the metal liners that were used when recovering the uppermost core material. Shards from the plastic liner that were used when recovering the lower core material were also analyzed.

Considerations made in determining the treatment of the rock samples is described in more detail below (Section 4.1) and also outlined elsewhere (Früh-Green et al., 2017c; Orcutt et al., 2017). In brief, upon arriving on deck, samples were transferred as quickly as possible to acid-washed and autoclaved Teflon sheeting, logged

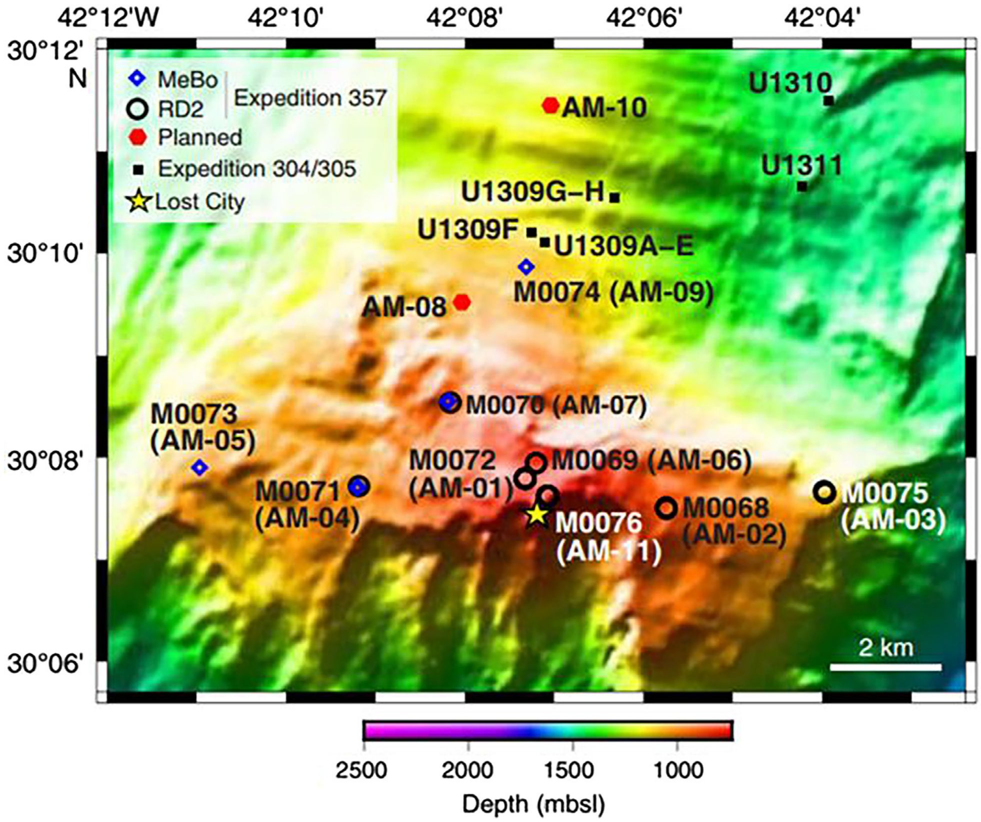


Fig. 1. Map of the Atlantis Massif. Locations of RD2 (black circle) and MeBo (blue diamond) cored sites overlain on multibeam bathymetry (50 m resolution) acquired during Expedition 357. Yellow star pinpoints the Lost City hydrothermal field, while the black square denotes locations of cored sites from Expedition from 304/305 and planned Expedition 357 sites (red circle) that were not drilled. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.) Figure reproduced from Früh-Green et al., 2017a.

and photographed, stored in Escal Neo bags flushed with N_2 to maintain anaerobic conditions, and stored at 4 °C until further processing. Whole round cores were subsampled for culturing experiments and representative geochemical analyses (e.g. XRD, gas analysis). Half the samples were flame sterilized while half were not. Samples were then wrapped in new acid-washed Teflon sheeting, placed in Whirlpak bags and stored at −80 °C until shore-based processing at the Kochi Core Center, Japan.

At the Kochi Core Center, the frozen whole rock cores were further processed, homogenized, and distributed as described elsewhere (Orcutt et al., 2017). These activities took place within a class 10,000 clean room to minimize the contribution of cells and particles through air transfer (Masui et al., 2009). If the rock cores were large enough, the exterior was removed using a specialized diamond-tipped rock saw that had a chilled cooling plate. This design eliminated the need for cooling water and thus minimized the introduction of microbial or organic surficial contaminants. The rock saw was isolated within a clean booth under HEPA-filter units (Masui et al., 2009). All rock saw surfaces, including the entirety of the band saw blade, were cleaned with steam, RNase AWAY, methanol, and dicholoromethane between each sample. If the rock cores were too small for cutting or composed of rubble, they were placed into combusted (5 hr, 500 °C) glass beakers and rinsed 10 times with Milli-Q water. After being cut or rinsed, the samples were crushed and homogenized into a fine powder with stainless steel impact mortars and pestles, which were autoclaved then cleaned with RNase AWAY and methanol between each sample. Homogenization with impact mortars was performed while working between a KOACH benchtop laminar flow system. Due to time constraints at the Kochi Core Center, eleven samples were processed at the Bigelow Laboratory for Ocean Sciences following the same rinsing and cleaning procedure. The same homogenized powders were subsampled and distributed to multiple principal investigators so that samples would be subjected to multiple geochemical and microbiological analyses. Samples for organic geochemical analysis were stored in combusted (500 °C, 5 hrs) glass jars with acid washed (10% HCl, overnight) Teflon-lined caps. For this study, 19 rocks and sands were analyzed, representing a range of lithologies and sample processing strategies (Table 2).

2.3. Solvent extraction

Gloves and bags were cut into 2 g pieces then ultrasonically extracted three times with 9:1 mixture of dichloromethane (DCM, OmniSolv HPLC grade) to methanol (MeOH, Omnisolv HPLC grade). Grease and filter samples were ultrasonically extracted three times with 9:1 DCM:MeOH. The oil samples were dissolved by adding 10 µL of oil into 1 mL of hexane. The 19 rock and sand samples were freeze-dried prior to extraction with a VirTis Bench-Top Pro with Omnitronics Lyophilizer with 8 port acrylic manifold using a Visi Trap connected to an oil-free scroll pump. Aliquots (17–88 g, Table 3) of each core was ultrasonically extracted three times with MeOH, three times in a 3:1 mixture of DCM: MeOH, and two times with DCM; the amount of sample extracted is listed in Table 2. Multiple samples of freshly combusted (5 hrs, 500 °C) sand were treated identically to samples to assess any contamination introduced during extraction and processing.

Extracts were combined and concentrated by rotary evaporation. If needed, samples were filtered through combusted glass wool to remove particles then passed over a column of Na_2SO_4 to remove water. The remaining total lipid extract was separated over

Table 1
Potential contaminants analyzed for apolar lipid content.

Name	Description and use
Acid-washed Teflon sheeting	Used to wrap samples (McMaster Carr P/N 8569K75)
Nylon sheeting	Plastic sheeting; tested but not used (VWR P/N 12243-186)
GX-P film	Sterile bag for maintaining anoxic conditions; tested but not used
Escal Neo bag	Sterile bag for maintaining anoxic conditions; used for sample storage
Whirpak bag	Sterile bag; used for sample storage
Gloves, Semperguard Xpert	Gloves on the ship and at the KCC (VWR P/N SEMP816780635)
Gloves, VWR P/N 112-2371	Gloves tested but not used for sample handling
Gloves, VWR P/N 112-2373	Gloves tested but not used for sample handling
Gloves, KC500 purple nitrile	Gloves used when handling and processing samples during in house procedures (VWR P/N 32934-082)
B30 transformer oil	Used on the RD2 rock drill
VHVi 2 Compensation oil	Used as transformer oil on the MeBo rock drill
Panolin 22 hydraulic oil	Used on the MeBo rock drill
Atlantis 22 hydraulic oil	Used on both the RD2 and the MeBo rock drills
Umbilical cord grease	Grease used to lubricate umbilical cord that remotely powered and controlled the RD2 rock drill
MeBo seawater grease	Grease used on the MeBo drill
Saphire Aqua 2 grease	Multi-purpose water resistant grease; RD2 drill
Brit-Lube Load Guard Extreme grease	Calcium complex grease fortified with PTFE; used on the RD2 drill
Core barrel top grease	Grease from the top of the core barrel
K-nate grease	Calcium sulphonate grease used on the RD2 drill
Loctite 638 adhesive	Adhesive used on the threads of the drill rods
Finkox GC Meisselpaste	Adhesive
Anti-seize lubricant	Lubricant used on the MeBo drill
Spray paint	Spray paint used on the RD2 drill
Methylated spirits	Solvent used to clean metal core barrel liners
Wipe of stainless steel core liner	Glass fiber filter dipped in methanol and swiped along the interior of stainless steel metal core liners
Plastic core liner shards	Shards plastic core liners
RNase AWAY	Solution used to remove ribonuclease contamination from plastics, glassware, and the chilled rock saw
Rock saw	Specialized diamond-tipped rock saw with chilled cooling plate used to remove exteriors from WRC

a Sepra™ NH_2 (Sigma-Aldrich P/N 57212-U) bulk packing column into multiple fractions by eluting with solvents of increasing polarity: F1: apolar (eluted with hexane), F2: esters and ketones (eluted with 3:1 hexane: DCM), F3: alcohols and sterols (eluted with 9:1 DCM: acetone), and F4: fatty acids (eluted with 4% formic acid in DCM). Elemental sulfur was removed from the n-alkane (F1) fraction using activated copper. The F1 fraction was dissolved in a known volume of hexane prior to gas chromatography- mass spectrometry (GC-MS) and gas chromatography- combustion- isotope ratio mass spectrometry (GC-C-IRMS) analyses.

2.4. GC-MS and compound-specific isotope analysis

The n-alkane (F1) fractions were analyzed by GC-MS on an Agilent Technologies 5975 Inert XL Mass Selective Detector with an Agilent J&W GC HP-5MS UI capillary column capillary column (30 m × 0.25 mm; 0.25 μm film thickness) using He as a carrier gas. Samples were injected in pulsed splitless mode. The temperature program was 70–150 °C (15 °C/min), 150–200 °C (5 °C/min), 200–300 °C (5 °C/min), and held at 300 °C for 10 min. Concentrations of each peak were quantified by comparison to a four- point standard curve (injection of 15 to 90 ng of each compound) of a C_7-C_{30} alkane series (P/N 49451-U, Sigma Aldrich).

The compound-specific carbon isotope ratios for each n-alkane (F1) fraction were analyzed by GC-C-IRMS using a Thermo Trace 1310 gas chromatograph with a Thermo GC IsoLink II with an Agilent DB-5 capillary column (30 m × 0.25 mm, 0.25 μm film thickness) with helium as the carrier gas. A Gerstel CIS-6 inlet was held in splitless mode during injection then set to a 10:1 split after three minutes. The column inlet temperature program was initially at 40 °C then ramped to 150 °C at 16 °C/min, and then ramped to 300 °C at 12 °C/min. The temperature program of the GC was initially held at 45 °C for one minute then ramped to 130 °C at 40 °C/min, then ramped to 250 °C at 6 °C/min, then ramped to 290 °C at 2 °C/min, then ramped to 320 °C at 6 °C/min, and then held at 320 °C for six minutes. Each sample was injected in duplicate and a standard mixture of n-alkanes (A6 Standard, Arndt Schimmellman, Indiana University) with known isotopic composition was analyzed every third injection as an external isotope standard and to monitor system performance. The standard deviation of duplicate injections ranged from 0.0 to 0.4‰. The carbon isotope ($\delta^{13}C$) ratios of each compound are expressed as per mil (‰) relative to the Vienna Pee Dee Belemnite (VPDB).

3. Results

3.1. Characterization of potential contaminants in sample storage, handling, and processing

Twenty-eight grease, oil, and plastic samples that could have come into contact with the cores were characterized for their apolar lipid composition and distribution for comparison to the core samples (Table 1). Chromatograms of these potential contaminants can be found in Appendix A. The Escal Neo bag and GX-P film F1 chromatograms contained a series of even-numbered alkanes ranging from C_{14} to C_{30}, but lacked bell-shaped humps, also known as unresolved complex mixtures (UCMs), caused by the coelution of many cyclic and branched compounds that the gas chromatograph cannot fully resolve and could obscure compounds of interest. Apolar lipids were below detection in the Whirlpak bag, nylon sheeting, and acid-washed Teflon sheeting F1 chromatograms. The hydrocarbon (F1) fraction of the four glove types used during sample processing and handling all showed large UCMs. The two VWR gloves and the KC500 gloves also contained elevated concentrations of benzene hydrocarbons, while the KC500 gloves also exhibited an n-alkane series ranging from C_{22} to C_{27}. The hydrocarbon (F1) chromatograms of the transformer oils (B30, VHVi 2 Compensation), hydraulic oils (Atlantis 22, Panolin 22), and a seawater grease used on the MeBo drill exhibited large UCMs without any identifiable apolar lipids. The multiple greases used on the RD2 drill contained detectable concentrations of apolar compounds among prominent UCMs. Specifically, the K-nate and Brit-Lube Load Guard Extreme greases both contained benzene hydrocarbons and alkane series ranging from C_{12} to C_{22}

Table 2
Samples extracted and analyzed and their processing methods.

Sample name	Depth (mbsf)	Amount processed (g)	Generalized lithology	Detailed description	Processing method[a]		
					Flame sterilized	Exterior removal	Alkanes
357-69A 4R1 U 75–85 cm	5.91	3.99	Carbonate sand, lithics	Carbonate ooze with lithic fragments	no	Syringe	
357-69A 4R1 U 121–131 cm	6.37	20.35	Carbonate sand, lithics	Carbonate ooze with lithic fragments	no	Cut and Rinsed	yes
357-69A 5R1 F? 137–151 cm	8.25	30.78	Gabbro	Metadolerite	unknown	Cut	yes
357-69A 10R1 F 104–124 cm	15.76	43.93	Serpentinite	Serpentinized harzburgite cut by carbonate vein	yes	Cut	yes
357-70A 2R1 U 87–97 cm	2.65	68.48	Basalt	Carbonate-cemented basaltic breccia	no	Cut	yes
357-70A 3R1 F 19–29 cm	3.05	31.52	Basalt	Basalt breccia in a carbonate matrix	yes	Cut	yes
357-70A 3R1 U 29–38 cm	3.15	65.40	Basalt	Basalt breccia in a carbonate matrix	no	Rinsed	
357-71A 1R2 F 0–11 cm	0.58	48.70	Serpentinite	Serpentinized dunite	yes	Cut	yes
357-71A 2R1 F 67–77 cm	3.39	88.41	Gabbro	Metagabbro with clear leucocratic domains	yes	Cut	yes
357-71B 3R1 U 0–10 cm	3.44	76.99	Serpentinite	Serpentinized harzburgite with metagabbro interval	no	Rinsed	
357-71C 3R1 U 0–2,4–10 cm	5.02	22.84	Serpentinite	Fragments of serpentinised harzburgite	no	Rinsed	
357-72B 5R1 U 40–43 cm	6.39	40.20	Schist	Talc-amphibole-chlorite schist	no	Rinsed	
357-72B 8RCC F 5–20 cm	12.33	87.90	Serpentinite	Serpentinized harzburgite	yes	Cut	yes
357-74A 1R1 U 10–20 cm	0.10	4.01	Carbonate sand	Fossiliferous carbonate sand	no	Syringe	
357-76B 5R1 U 14–28 cm	6.87	41.30	Serpentinite	Serpentinized dunite	no	Rinsed	
357-76B 5R1 F 28–36 cm	7.01	37.59	Serpentinite	Serpentinized dunite	yes	Rinsed	
357-76B 9R1 F 34–41 cm	13.21	20.30	Serpentinite	Oxidized porphyroclastic serpentinized harzburgite	yes	Cut	yes
357-76B 9R1 U 43–52 cm	13.30	17.02	Serpentinite	Oxidized porphyroclastic serpentinized harzburgite	no	Cut	yes
357-76B 10R1 U 91–111 cm	15.50	67.75	Serpentinite	Fractured serpentinized dunite	no	Cut	yes

[a] Syringe: Interior sampled by syringe; Cut: Exterior removed by chilled rock saw; Rinsed: Rinsed 10 times with Milli-Q water.

Table 3
Concentrations (ng compound/g rock) and $\delta^{13}C$ signatures of n-alkanes extracted from IODP Expedition 357 rocks. Samples without reported $\delta^{13}C$ were below detection for isotopic analysis. "–" is used to indicate that compounds were below detection on the GC/MS. "b.d.l." is below detection limit for isotopic analysis.

Name	69A 4R1 U 121–131		69A 5R1 F? 137–151	69A 10R1 F 104–124		70A 2R1 U 87–97		70A 3R1 F 19–29	71A 1R2 F 0–11	71A 2R1 F 67–77	76B 5R1 U 14–28		76B 9R1 F 34–41		76B 9R1 U 43–52		76B 10R1 U 91–111
Amount (g)	20.4		30.8	43.9		68.5		31.5	48.7	88.4	41.3		20.3		17.02		67.8
	Conc.	$\delta^{13}C$ (‰)	Conc.	Conc.	$\delta^{13}C$ (‰)	Conc.	$\delta^{13}C$ (‰)	Conc.	Conc.	Conc.	Conc.	$\delta^{13}C$ (‰)	Conc.	$\delta^{13}C$ (‰)	Conc.	$\delta^{13}C$ (‰)	Conc.
Alkanes																	
C_{18}	21	b.d.l.	–	–	–	6	b.d.l.	–	–	–	14	b.d.l.	–	–	–	–	–
C_{19}	22	b.d.l.	–	–	–	7	b.d.l.	–	–	–	15	b.d.l.	21	b.d.l.	–	–	–
C_{20}	30	b.d.l.	–	–	–	7	b.d.l.	–	–	–	16	b.d.l.	36	−30.2	–	–	–
C_{21}	48	−30.4	14	–	–	10	b.d.l.	13	20	–	24	−30.4	68	−30.7	32	b.d.l.	–
C_{22}	86	−30.7	16	10	b.d.l.	16	−30.3	13	21	–	34	−30.4	104	−30.6	43	−30.6	–
C_{23}	104	−30.4	17	11	b.d.l.	20	−30.3	14	21	5	41	−30.5	139	−30.7	47	−30.4	7
C_{24}	113	−30.6	17	12	b.d.l.	22	−30.2	15	22	5	44	−30.3	139	−30.8	45	−30.2	7
C_{25}	103	−30.3	19	14	−30.2	22	−30.2	15	22	6	44	−30.3	180	−30.8	45	b.d.l.	9
C_{26}	96	−30.6	19	14	−30.0	20	−30.4	15	22	6	42	−30.4	154	−30.7	42	b.d.l.	9
C_{27}	81	−30.3	17	coelute	−29.2	18	−30.3	12	21	6	38	−30.1	130	−30.7	37	b.d.l.	9
C_{28}	75	−30.7	19	15	−28.8	16	−30.3	13	22	–	37	−30.6	113	−30.9	39	b.d.l.	9
C_{29}	55	−30.5	19	15	b.d.l.	15	−30.3	13	21	–	34	−30.5	97	−30.7	39	b.d.l.	9
C_{30}	60	b.d.l.	–	14	b.d.l.	12	b.d.l.	13	–	–	32	−30.7	82	−30.6	–	–	9

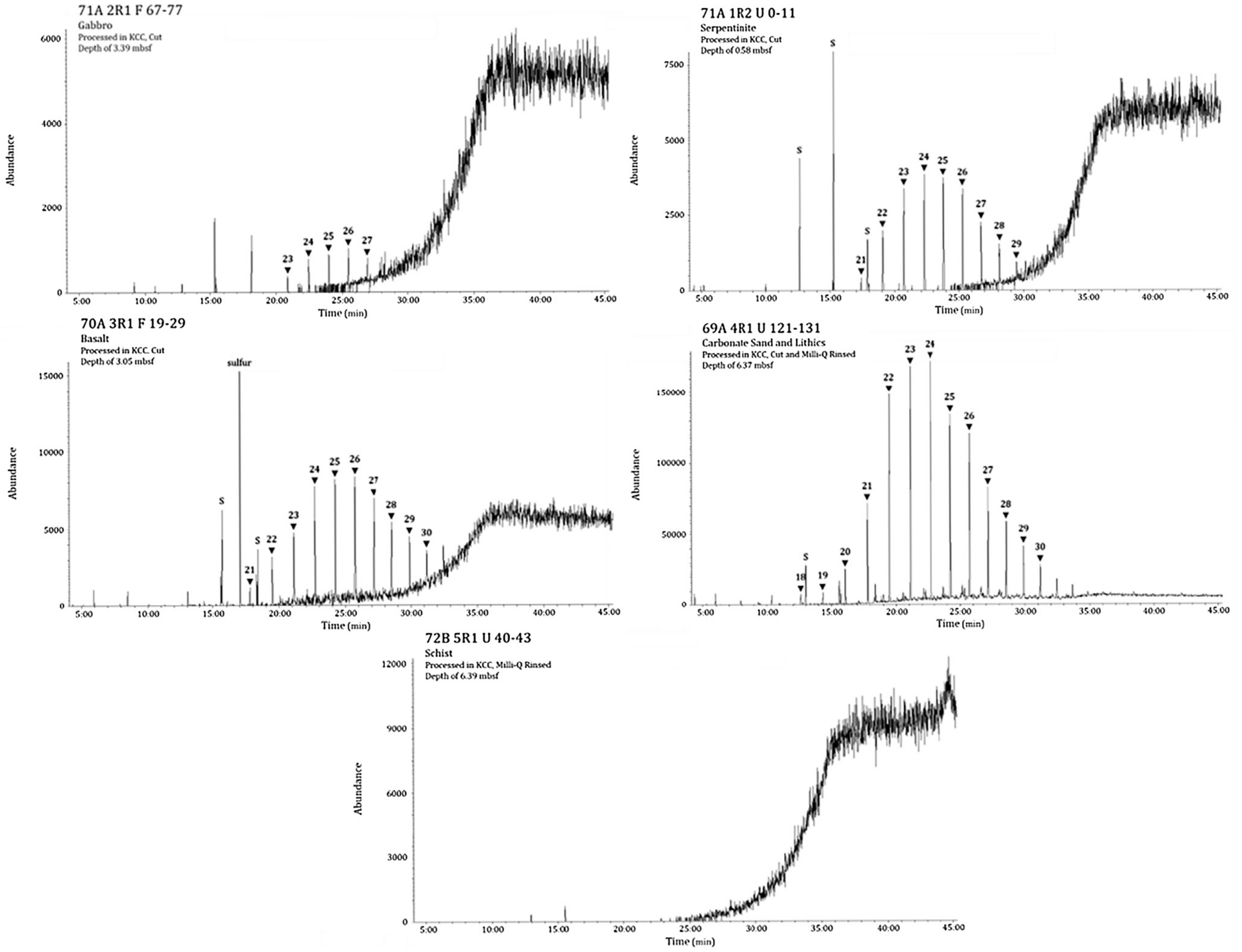


Fig. 2. Expedition 357 sample chromatograms. GC/MS chromatograms displaying the distribution of n-alkanes (marked with a ▼ with the corresponding number indicating the carbon number of the specific alkane, S indicates siloxanes) in samples with various lithologies and processing types. Cut samples exhibited a characteristic n-alkane series ranging from C_{18} to C_{30} without the presence of any isoprenoidal biomarkers. The Milli-Q rinsed sample contained no detectable compounds.

and C_{14} to C_{19}, respectively. The Sapphire Aqua 2 grease exhibited a minor alkane series ranging from C_{14} to C_{18}, isopropyl palmitate, and isopropyl stearate. The umbilical cord grease F1 chromatogram was comprised of a series of alkanes ranging from C_{16} to C_{32} with an average isotopic signature of $-31.2 \pm 0.3‰$ (Fig. 2). A minor alkane series ranging from C_{15} to C_{17} was also identified in the grease collected from the top of the core barrel.

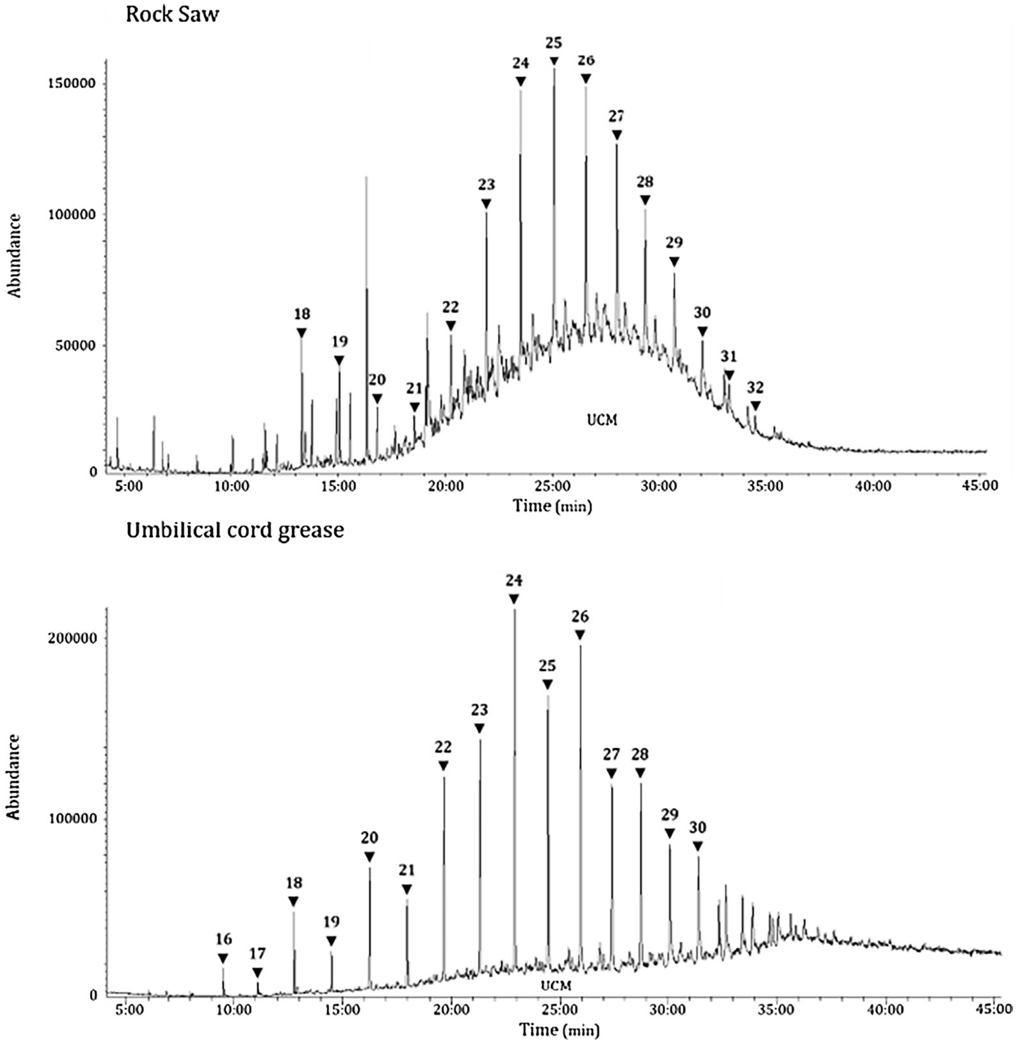
Two types of core liners were used during drilling. Stainless steel liners were used in the upper portion of cores and plastic liners were used for deeper samples. The interior of the stainless steel liners were cleaned with methylated spirits before being loaded on the rock drill. Potential contamination from the core liners were assessed by swiping the stainless steel core liner with a methanol-soaked filter. Shards of the plastic core liner were directly extracted. Apolar material was mostly below detection in the filter with exception of minor concentrations of phthalates and C_{19} alkane. Apolar lipids were not identified in the shards of the plastic core liner.

The F1 chromatogram for Loctite 638 adhesive contained elevated concentrations of cyclohexanols, while another adhesive, Finkox CG Meisselpaste, contained an alkane series ranging from C_{12} to C_{22} with isopropyl palmitate and isopropyl stearate peaks emerging from a large UCM. Apolar compounds were observed in the Anti-seize lubricant in the form of a series of alkanes increasing in abundance from C_{15} to C_{26} before becoming obscured by a UCM. A minor even-numbered alkane series ranging from C_{18} to C_{22} was observed in the tube F1 chromatogram. The spray paint used on the RD2 rock drill contained only ethyl oleate. The methylated spirts, used to clean the stainless steel core liners, contained a minor abundance of phthalates. The RNase AWAY solution, used to clean surfaces of extraneous RNA during sample processing, lacked apolar lipids.

The rock saw used at the Kochi Core Center was tested by swiping a filter dipped in methanol across the blade and chilled saw plate. The extracted filter contained a series of n-alkanes ranging from C_{18} to C_{32} (Fig. 2). Compound-specific isotope analysis of these compounds range from $-31.0‰$ to $-30.1‰$ with an average value of $-30.6 \pm 0.4‰$. Non-volatile compounds were below detection in the laboratory processing blank of combusted sand that was treated identically as samples during freeze drying, solvent extraction, and analysis.

3.2. Characterization of samples

The saturated hydrocarbon (F1) fraction of extracted core samples contained either a characteristic series of n-alkanes ranging from C_{18} to C_{30} and up to C_{32} or contained no detectable n-alkanes (Table 3; Figs. 2, A.7, A.8, A.9). Concentrations of measurable saturated hydrocarbons ranged from 6 to 180 ng compound/g rock across 11 lithologically variable samples (Table 3). Notably,


Rock Saw

Umbilical cord grease

Fig. 3. Rock saw and umbilical cord grease chromatograms. GC/MS chromatograms displaying the distribution of n-alkanes (marked with a ▼ with the corresponding number indicating the carbon number of the specific alkane) for the rock saw and umbilical cord grease contaminants.

only samples that were cut with the rock saw had detectable alkanes, whereas samples that were not cut but instead serially rinsed with MilliQ water did not contain detectable alkanes (Table 2). When detected, higher n-alkane concentrations were weakly but significantly inversely correlated to the amount of sample extracted (Fig. 4; $r^2 = 0.38$; $p = 0.04$). Alkane concentrations were not significantly different among basalt, gabbro, and serpentinite lithologies (ANOVA one-way analysis; $F(2,7) = 65$, $p = 0.65$). The chromatogram of the n-alkane series showed no odd or even carbon number predominance (odd-even predominance (OEP; Scalan and Smith, 1970) of 1.0 ± 0.1), and abundances decreased as carbon number increases. Compound-specific isotope analysis of the saturated hydrocarbons ranged from −30.9‰ to −28.8‰ with an average value of −30.3 ± 0.4‰ for samples that contained enough material for isotopic analysis. The isotopic values did not increase or decrease with chain length and they were consistent in samples of diverse lithologies.

The n-alkane series was not observed in several samples (Table 2). For these samples, the F1 fraction was dissolved in 5 μL and 1 μL was injected. The lowest standard had 15 ng of each compound on column and, therefore, compound abundances in extracts below 75 ng were below detection. The detection limit for the original samples depended on the amount that was extracted with the highest detection limit in the smallest sample (3.99 g of sample; 19 ng compound/g rock detection limit) and the lowest detection limit in the largest sample (76.99 g of sample; 1 ng compound/g rock detection limit).

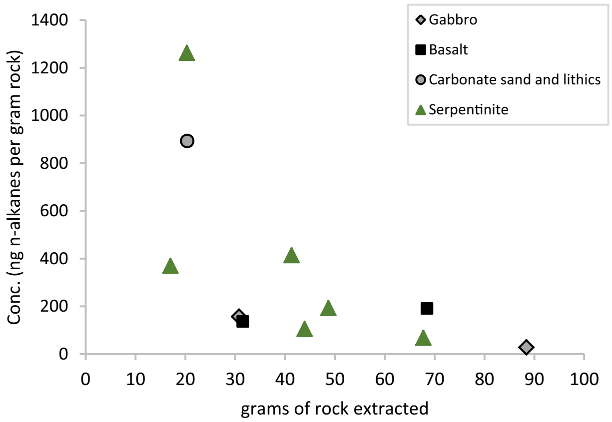


Fig. 4. Concentrations of n-alkanes per grams of rock extracted vs grams of rock extracted for samples with detectable compounds.

4. Discussion

4.1. Considerations for sample handling

Treatment of the samples was balanced between the needs of multiple investigators and the logging requirements of IODP. The following constraints were considered during sample processing and storage, also described in Früh-Green et al., 2017c. Samples would ideally be:

(1) processed as quickly as possible upon arrival on deck.
(2) photographed and logged for a permanent record.
(3) stored under anaerobic conditions until they could be fully processed, to preserve material for culturing.
(4) treated in ways that would minimize introduction of biological and organic materials.

Flame sterilization is a widespread technique implemented by microbiologists to remove surficial microbial contamination (Lever et al., 2006). While this process is extremely effective at destroying cells, it has the potential to alter the signatures of any organic compounds present in the cores via oxidation or recombination into different molecules, which would impede the identification of indigenous lipids. In the interests of accommodating multiple PIs, the exterior of approximately half of the samples were flame sterilized while half were not. The potential effects of flame sterilization on the organic geochemistry of the cores were not addressed as part of this study.

4.2. Optimizing sample storage and handling

Prior to sampling, storage and handling materials were tested for possible interference with lipid analysis. The Escal Neo and GX-P film bags used regularly to store core microbiological samples on the IODP vessel DV Chikyu contained relatively high abundances of even n-alkanes and were deemed problematic for organic geochemical analyses. Nylon sheeting had only minor contributions of apolar lipids, but contained high abundances of amino acids that could interfere with other future analyses (data not shown). Acid-washed and autoclaved Teflon sheeting had only minimal contributions of lipid biomarkers. To meet the needs of both minimizing the introduction of organic contaminants and keeping anaerobic conditions until further processing, samples were wrapped in Teflon sheeting upon arrival on deck then placed inside Escal Neo that were flushed with N_2 and sealed.

While all attempts were made to only handle the cores with the Teflon sheeting or solvent-cleaned metal instruments, it was at times necessary to touch the samples with gloved hands shipboard. Several different glove types were tested to identify those that would minimize the contribution of undesirable compounds. Three glove types were available at sea: Semperguard Xpert, VWR Nitrile 112-2373, and VWR Nitrile 112-2371. Semperguard Xpert gloves contained a large UCM peak but no identifiable alkanes, while the two VWR gloves containing elevated concentrations of benzene hydrocarbons along with a UCM. Therefore, the Semperguard Xpert gloves were used at sea and during later processing. The KC500 purple nitrile gloves were used during all in-house extraction and separation procedures and contained a UCM and an identifiable n-alkane series ranging from C_{22} to C_{27}, though at this time it was never necessary to touch the sample directly and great care was taken to avoid contact between the gloves and the sample.

4.3. Source of n-alkane in rock samples

An n-alkane series was identified in 11 out of 19 core samples and, when present, had a similar distribution and similar isotopic compositions between samples (Fig. 3, Table 3). As described below, these n-alkanes are likely introduced contamination rather than indigenous to the samples. The odd-to-even predominance index (Scalan and Smith, 1970) had an average value of 1.0 ± 0.1 across all 11 rock samples, indicating a high degree of thermal maturity.

It is unlikely these were introduced during drilling as the majority of drill-related materials did not contain n-alkanes or identifiable apolar biomarkers (Fig. 2, Table 3). One exception was the umbilical cord grease that used on the winch and cable of the RD2 rock drill. This grease was not used on the MeBo rock drill and, on the RD2 rock drill, was approximately 25 m away from the point of drilling. The n-alkane distribution of this grease had a minor even-over-odd carbon number predominance (Fig. 2) and had a $\delta^{13}C$ value of −31.7‰ to −30.8‰, which was slightly more negative than the isotopic value for the alkanes in the rock cores. It was therefore unlikely to be responsible for the n-alkanes observed in the core samples.

The alkane concentrations were compared to a complementary study that used a chemical tracer to assess sample contamination by drilling fluids. PFC was used as a tracer to qualify core contamination during seabed drilling on IODP Expedition 357 (Früh-Green et al., 2017a; Orcutt et al., 2017). The PFC was introduced into the drilling fluid and, subsequently, concentrations were measured in rock and fluid samples (Früh-Green et al., 2017a; Orcutt et al., 2017). The presence of PFC and, therefore, the intrusion of drilling fluid into the interior of the core, depended on core coherency (intact or rubbly), lithology, and processing style (Orcutt et al., 2017). Regardless of borehole location, PFC concentrations were lower in the interiors of cores that were intact, did not contain veins, were not metasomatically overprinted (no talc), and were rinsed with Milli-Q water (Orcutt et al., 2017). The samples with the highest PFC abundances were not the same as the samples with highest alkane abundances. This implies that the drilling fluid, as identified by tracer intrusion, did not substantially contribute to the n-alkanes in the rock samples.

The eleven samples processed by rock cutting at Kochi Core Center contained the n-alkane series while the eight samples that were rinsed with Milli-Q water did not (Fig. 3). One possibility is that the rinse may have washed away loose phyllosilicate minerals which are expected to contain the highest concentrations of adsorbed organic molecules or removed cells that reside in fractures or carbonate veins. Clays have a stronger affinity for organic molecules (Hedges, 1977) and as a secondary product of serpentinization, are associated with locations with higher fluid flow (Moody, 1976) where microorganisms are more likely to reside. Alternatively, the n-alkane series could have been introduced to samples during cutting, despite extensive cleaning of the rock saw. Samples that were rinsed with Milli-Q were not cut with the rock saw.

To test this possibility, a combusted filter dipped in methanol was swiped across the saw blade and chilled plate and analyzed for lipid content. The extracts of these filters contained a series of n-alkanes that were identical to the distribution observed in our samples (Figs. 2 and 3). The $\delta^{13}C$ values of the rock saw hydrocarbons ranged from −31.0‰ to −30.1‰, indistinguishable from what was observed in the rock samples (−30.9‰ to −28.8‰). For example, the C_{24} alkane in the carbonate sand and lithics sample 69A 4R1 U 121-13 had a $\delta^{13}C$ isotopic signature of −30.6‰ with a corresponding $\delta^{13}C$ isotopic value of −30.9‰ in the rock saw. The hydrocarbon compounds found in the Atlantis Massif samples were therefore most likely sourced from the rock saw that was used to remove the core exteriors.

Previous research has advocated for the physical removal of core exteriors over rinsing with solvent to more effectively eliminate surficial contamination of anthropogenic petroleum products (Brocks et al., 2008), thereby inadvertently supporting the continued use of a rock saw as an effective method to remove these exteriors. More recently, minor contamination from a rock saw was implicated as the source of sterols in Archean rocks (French et al., 2015) that had previously been held as evidence for the rise of eukaryotes at ~2.7 Ga (Brocks et al., 1999). The Kochi Core Center rock saw has been optimized to prevent microbiological contamination and was enclosed in a filtered air clean booth and extensive cleaned with multiple solvents. These steps do not appear to have been entirely sufficient for the complete removal of lipid contamination. This result is consistent with the observations of French et al. (2015) who found that solvent rinsing was insufficient to fully remove biomarker contamination, including alkanes, steranes, and hopanes. Complete elimination of hydrocarbon contamination required heating the blade to 300 °C for 1 h and then ultrasonicating it in a combusted aluminum foil envelope containing solvent (French et al., 2015). Another study identified micro-ablation as a viable method to remove surficial hydrocarbon contamination of small and irregularly-shaped rock samples (Jarrett et al., 2013). Such steps may have been successful in preventing the introduction of the n-alkane series in the current study.

5. Conclusions

The advent of new drilling techniques will greatly expand the access to subsurface rocks on the continent and in the ocean. In many cases, recovered samples will have low biomass and compounds of interest will be present at extremely low abundances, if at all. Sample analysis must therefore be carried out with a thorough assessment of potential contamination to avoid the possible misrepresentation of *in situ* compounds and processes. Recent studies devoted to identifying and remediating sources of potential contamination in low biomass continental rocks have identified a number of procedural recommendations, including employing stainless steel crushing tools, proper choice of system blanks, and more stringent cleaning methods (Sherman et al., 2007; Brocks et al., 2008; Brocks, 2011; French et al., 2015; Leider et al., 2016). While several of these approaches were employed in the current study, insufficient cleaning methods on our part likely introduced contamination from the rock saw. While it is possible that abiotically- or biotically- derived compounds could have been present in the cores, it was impossible to identify any due to contamination overprinting.

Despite this result, it must be noted that the lack of a UCM hump in cut and Milli-Q rinsed sample chromatograms suggests that the sample processing and handling procedures implemented during this expedition were effective at eliminating certain types of surface or background contamination. Additionally, the Milli-Q rinsed samples did not contain the same alkane contamination. This provides some optimism for detecting compounds in future drilling projects despite the difficulties inherent in completely eliminating contamination during drilling.

Future research will undoubtedly focus on the detection of similar suites of compounds, particularly as drilling technology improves and increases recoveries in hard rock settings. Detecting lipids and other organic molecules in the oceanic subsurface will continue to be a goal for researchers interested in understanding processes essential to pre-biotic chemistry, microbial habitability and evolution, and oceanic carbon cycling. As this research progresses, methodological improvements to the minimization and characterization of potential contamination should be simultaneously developed and future publications should include the results of these assessments. This will be necessary to lend credence to future reports of *in situ*, deep biosphere organic compounds.

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Competing interests

The authors declare no competing financial interests.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.orggeochem.2018.05.003.

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References

Blackman, D.K., Karson, J.A., Kelley, D.S., Cann, J.R., Früh-Green, G.L., Gee, J.S., Hurst, S.D., John, B.E., Morgan, J., Nooner, S.L., Ross, D.k., Schroeder, T.J., Williams, E.A., 2002. Geology of the Atlantis Massif (Mid-Atlantic Ridge, 30°N): Implications for the evolution of an ultramafic ocean core complex. Marine Geophysical Research 23, 443–469.

Bradley, A.S., Hayes, J.M., Summons, R.E., 2009. Extraordinary ^{13}C enrichment of diether lipids at the lost city hydrothermal field indicates a carbon-limited system. Geochimica et Cosmochimica Acta 73, 102–118.

Brazelton, W.J., Schrenk, M.O., Kelley, D.S., Baross, J.A., 2006. Methane- and Sulfur-Metabolizing Microbial Communities Dominate the Lost City Hydrothermal Ecosystem. Appl. Environ. Microbiol., 6257–6270.

Brocks, J.J., Logan, G.A., Buick, R., Summons, R.E., 1999. Archean molecular fossils and the early rise of eukaryotes. Science 285, 1033–1036.

Brocks, J.J., Grosjean, E., Logan, G.A., 2008. Assessing biomarker syngeneity using branched alkanes with quaternary carbons (BAQCs) and other plastic contaminants. Geochimica et Cosmochimica Acta 72, 871–888.

Brocks, J.J., 2011. Millimeter-scale concentration gradients of hydrocarbons in Archean shales: live-oil escape or fingerprint of contamination? Geochimica et Cosmochimica Acta 75, 3196–3213.

Charlou, J.L., Donval J.P., Konn, C., Ondreas, H., Fouquet, Y., Jean-Baptiste P., Fourre E., (2010). High production of H2 and CH4 and abiotic hydrocarbons in ultramafic-hosted hydrothermal systems on the Mid-Atlantic Ridge. In: Rona P. A., Devey C.W., Dyment J., Murton B.J. (Eds.) Diversity of hydrothermal systems on slow spreading ocean ridges, vol 188, Geophysics monograph series. AGU, Washington, DC, pp. 265–296.

D'Hondt, S., Inagaki, F., Ferdelman, T., JØrgensen, B.B., Kato, K., Kemp, P., Sobecky, P., Sogin, M., and Takai, K., 2007. Workshop report: Exploring subseafloor life with the Integrated Ocean Drilling program. Scientific Drilling 5, 26-37.

Delacour, A., Früh-Green, G.L., Bernasconi, S.M., Schaeffer, P., Kelley, D.S., 2008. Carbon geochemistry of serpentinites in the Lost City Hydrothermal System (30°N, MAR). Geochimica et Cosmochimica Acta 72, 3681–3702.

French, K.L., Hallmann, C., Hope, J.M., Schoon, P.L., Zumberge, J.A., Hoshino, Y., Peters, C.,A., George, S.C., Love, G.D., Brocks, J.J., Buick, R., Summons, R.E., 2015. Reappraisal of hydrocarbon biomarkers in Archean rocks. PNAS 112, 5915–5920.

Frise, A., Kallmeyer, J., Kitte, J.A., Martinez, I.M., Bijaksana, S., Wagner, D., Lake Chalco Drilling Science Team, I.C.P., Towuti Drilling Science Team, I.C.D.P., 2017. A simple inexpensive technique for assessing contamination during drilling operations. Limnology and Oceanography Methods 15, 200–211.

Früh-Green, G.L., Connolly, J.A.D., Plas, A., Kelley, D.S., Groberty, B., 2004. Serpentinization of oceanic peridotites: implications for geochemical cycles and biological activity. In: Wilcock, W.D., DeLong, E.F., Kelley, D.S., Baross, J.A., Cary, S.C. (Eds.), Geophysical Monograph Series 144. American Geophysical Union, Washington, DC, pp. 119–136.

Früh-Green, G., Orcutt, B.N., Green, S.L., Cotterill, C., Morgan, S., Akizawa, N., Bayrakci, G., Behrmann, J.-H., Boschi, C., Brazelton, W.J., Cannat, M., Dunkel, K.G., Escartín, J., Harris, M., Herrero-Bervera, E., Hesse, K., John, B.E., Lang, S.Q., Lilley, M.D., Liu, H.Q., Mayhew, L.E., McCaig, A.M., Menez, B., Morono, Y., Quéméneur, M., Rouméjon, S., Sandaruwan Ratnayake, A., Schrenk, M.O., Schwarzenbach, E. M., Twing, K., Weis, D., Whattham, S.A., Williams, M., Zhao, R., 2017a. Expedition 357 summary, In: Früh-Green, G.L., Orcutt, B.N., Green, S.L., Cotterill, C., and Scientists, E. (Eds.), Atlantis Massif Serpentinization and Life. Proceedings of the International Ocean Discovery Program, 357, International Ocean Discovery Program, College Station, TX.

Früh-Green, G., Orcutt, B.N., Green, S.L., Cotterill, C., Morgan, S., Akizawa, N., Bayrakci, G., Behrmann, J.-H., Boschi, C., Brazelton, W.J., Cannat, M., Dunkel, K.G., Escartín, J., Harris, M., Herrero-Bervera, E., Hesse, K., John, B.E., Lang, S.Q., Lilley, M.D., Liu, H.Q., Mayhew, L.E., McCaig, A.M., Menez, B., Morono, Y., Quéméneur, M., Rouméjon, S., Sandaruwan Ratnayake, A., Schrenk, M.O., Schwarzenbach, E. M., Twing, K., Weis, D., Whattham, S.A., Williams, M., Zhao, R., 2017b. Eastern sites, In: Früh-Green, G.L., Orcutt, B.N., Green, S.L., Cotterill, C., and Scientists, E. (Eds.), Atlantis Massif Serpentinization and Life. Proceedings of the International Ocean Discovery Program, 357, International Ocean Discovery Program, College Station, TX.

Früh-Green, G., Orcutt, B.N., Green, S.L., Cotterill, C., Morgan, S., Akizawa, N., Bayrakci, G., Behrmann, J.-H., Boschi, C., Brazelton, W.J., Cannat, M., Dunkel, K.G., Escartín, J., Harris, M., Herrero-Bervera, E., Hesse, K., John, B.E., Lang, S.Q., Lilley, M.D., Liu, H.Q., Mayhew, L.E., McCaig, A.M., Menez, B., Morono, Y., Quéméneur, M., Rouméjon, S., Sandaruwan Ratnayake, A., Schrenk, M.O., Schwarzenbach, E. M., Twing, K., Weis, D., Whattham, S.A., Williams, M., Zhao, R., 2017c. Expedition 357 methods, In: Früh-Green, G.L., Orcutt, B.N., Green, S.L., Cotterill, C., and Scientists, E. (Eds.), Atlantis Massif Serpentinization and Life. Proceedings of the International Ocean Discovery Program, 357, International Ocean Discovery Program, College Station, TX.

Greene, J., Tominaga, M., Blackman, D., 2015. Geologic implications of seafloor character and carbonate lithification imaged on the domal core of the Atlantis Massif. Deep-Sea Research Part II 121, 246–255.

Hedges, J.I., 1977. The association of organic molecules with clay minerals in aqueous solutions. Geochimica et Cosmochimica Acta 41 (1119–1123), 1.

Jarrett, A.J.M., Schinteie, R., Hope, J.M., Brocks, J.J., 2013. Micro-ablation, a new technique to remove drilling fluids and other contaminants from fragmented and fissile rock material. Organic Geochemistry 61, 57–65.

Karson, J.A., Früh-Green, G.L., Kelley, D.S., William, E.A., Yoerger, D.R., Jakuba, M., 2006. Detachment shear zone of the Atlantis Massif core complex, Mid-Atlantic Ridge, 30°N. Geochemistry, Geophysics, Geosystems 7, Q06016.

Kelley, D.S., Karson, J.A., Blackman, D.K., Früh-Green, G.L., Butterfield, D.A., Lilley, M. D., Olson, E.J., Schrenk, M.O., Roe, K.K., Lebon, G.T., Rivizzigno, P., AT3-60 Shipboard Party, 2001. An off-axis hydrothermal vent field near the Mid-Atlantic Ridge at 30°N. Nature 412, 145–149.

Kelley, D.S., Karson, J.A., Früh-Green, G.L., Yoerger, D.R., Shank, T.M., Butterfield, D. A., Hayes, J.M., Schrenk, M.O., Olson, E.J., Proskurowski, G., Jakuba, M., Bradley, A., Larson, B., Ludwig, K., Glickson, D., Buckman, K., Bradley, A.S., Brazelton, W.J., Roe, K., Elend, M.J., Delacour, A., Bernasconi, S.M., Lilley, M.D., Baross, J.A., Summons, R.E., Sylva, S.P., 2005. A serpentinite-hosted ecosystem: the lost city hydrothermal field. Science 307, 1428–1434.

Klein, F., Humphris, S.E., Guo, W., Schubotz, F., Schwarzenbach, E.M., Orsi, W.D., 2015. Fluid mixing and the deep biosphere of a fossil Lost-City type hydrothermal system at the Iberia Margin. PNAS Early Edition 112, 12036–12041.

Lang, S.Q., Butterfield, D.A., Schulte, M., Kelley, D.S., Lilley, M.D., 2010. Elevated concentrations of formate, acetate and dissolved organic carbon found at the Lost City hydrothermal field. Geochimica et Cosmochimica Acta 74, 941–952.

Lang, S.Q., Früh-Green, G.L., Bernasconi, S.M., Lilley, M.D., Proskurowski, G., Méhay, S., Butterfield, D.A., 2012. Microbial utilization of abiogenic carbon and hydrogen in a serpentinite-hosted system. Geochimica et Cosmochimica Acta 92, 82–99.

Lang, S.Q., Früh-Green, G.L., Bernasconi, S.M., Brazelton, W., Schrenk, M., McGonigle, J., 2018. Deeply-sourced formate fuels sulfate reducers but not methanogens at Lost City hydrothermal field. Scientific Reports 8, 755.

Leider, A., Schumacher, T.C., Hallmann, C., 2016. Enhanced procedural blank control for organic geochemical studies of critical sample material. Geobiology 14, 469–482.

Lever, M.A., Alperin, M., Engelen, B., Inagaki, F., Nakagawa, S., Steinsbu, B.O., Teske, A., 2006. Trends in basalt and sediment core contamination during IODP Expedition 301. Geomicrobiology Journal 23, 517–530.

Masui, N., Morono, Y., Inagaki, F., 2009. Bio-archive core storage and subsampling procedure for subseafloor molecular biological research. Scientific Drilling 8, 35–37.

McCollom, T.M., Seewald, J.S., 2006. Carbon isotope composition of organic compounds produced by abiotic synthesis under hydrothermal conditions. Earth and Planetary Science Letters 243, 74–84.

McCollom, T.M., Seewald, J.S., 2007. Abiotic synthesis of organic compounds in deep-sea hydrothermal environments. Chemical Reviews 107, 382–401.

Méhay, S., Früh-Green, G.L., Lang, S.Q., Bernasconi, S.M., Brazelton, W.J., Schrenk, M O., Schaeffer, P., Adam, P., 2013. Record of archaeal activity at the serpentinite-hosted lost city hydrothermal field. Geobiology 11, 570–592.

Moody, J.B., 1976. Serpentinization: a review. Lithos 9, 125–138.

Orcutt, B.N., Bergenthal, M., Freudenthal, T., Smith, D., Lilley, M.D., Schneiders, L., Früh-Green, G.L., 2017. Contamination tracer testing with seabed drills: IODP Expedition 357. Scientific Drilling 23, 39–46.

Peters, K.E., Walters, C.C., Moldowan, J.M., 2005. The Biomarker Guide II 2nd Edition: Biomarkers and Isotopes in Petroleum Systems and Earth History. Cambridge University Press, UK.

Plümper, O., King, H.E., Geisler, T., Liu, Y., Pabst, S., Savov, I.P., Rost, D., Zack, T., 2017. Subduction zone forearc serpentinites as incubators for deep microbial life. PNAS 114, 4324–4329.

Proskurowski, G., Lilley, M.D., Seewald, J.S., Früh-Green, G.L., Olson, E.J., Lupton, J.E., Sylva, S.P., Kelley, D.S., 2008. Abiogenic hydrocarbon production at lost city hydrothermal field. Science 319, 604–607.

Santelli, C.M., Banerjee, N., Bach, W., Edwards, K.J., 2010. Tapping the subsurface ocean crust biosphere: low biomass and drilling-related contamination calls for improved quality controls. Geomicrobiology Journal 27, 158–169.

Scalan, E.S., Smith, J.E., 1970. An improved measure of the odd-even predominance in the normal alkanes of sediment extracts and petroleum. Geochimica et Cosmochimica Acta 34, 611–620.

Schrenk, M.O., Brazelton, W.J., Lang, S.Q., 2013. Serpentinization, carbon, and deep life. Reviews in Mineralogy & Geochemistry 75, 575–606.

Schrenk, M.O., Kelley, D.S., Bolton, S.A., Baross, J.A., 2004. Low archaeal diversity linked to subseafloor geochemical processes at the Lost City Hydrothermal Field, Mid-Atlantic Ridge. Environ. Microbiol. 6, 1086–1095.

Sherman, L.S., Waldbauer, J.R., Summons, R.E., 2007. Improved methods for isolating and validating indigenous biomarkers in Precambrian rocks. Organic Geochemistry 38, 1987–2000.

Smith, D.C., Spivack, A.J., Fisk, M.R., Haveman, S.A., Staudigel, H., 2000. Tracer-based estimates of drilling-induced microbial contamination of deep sea crust. Geomicrobiology Journal 17, 207–219.

APPENDIX A

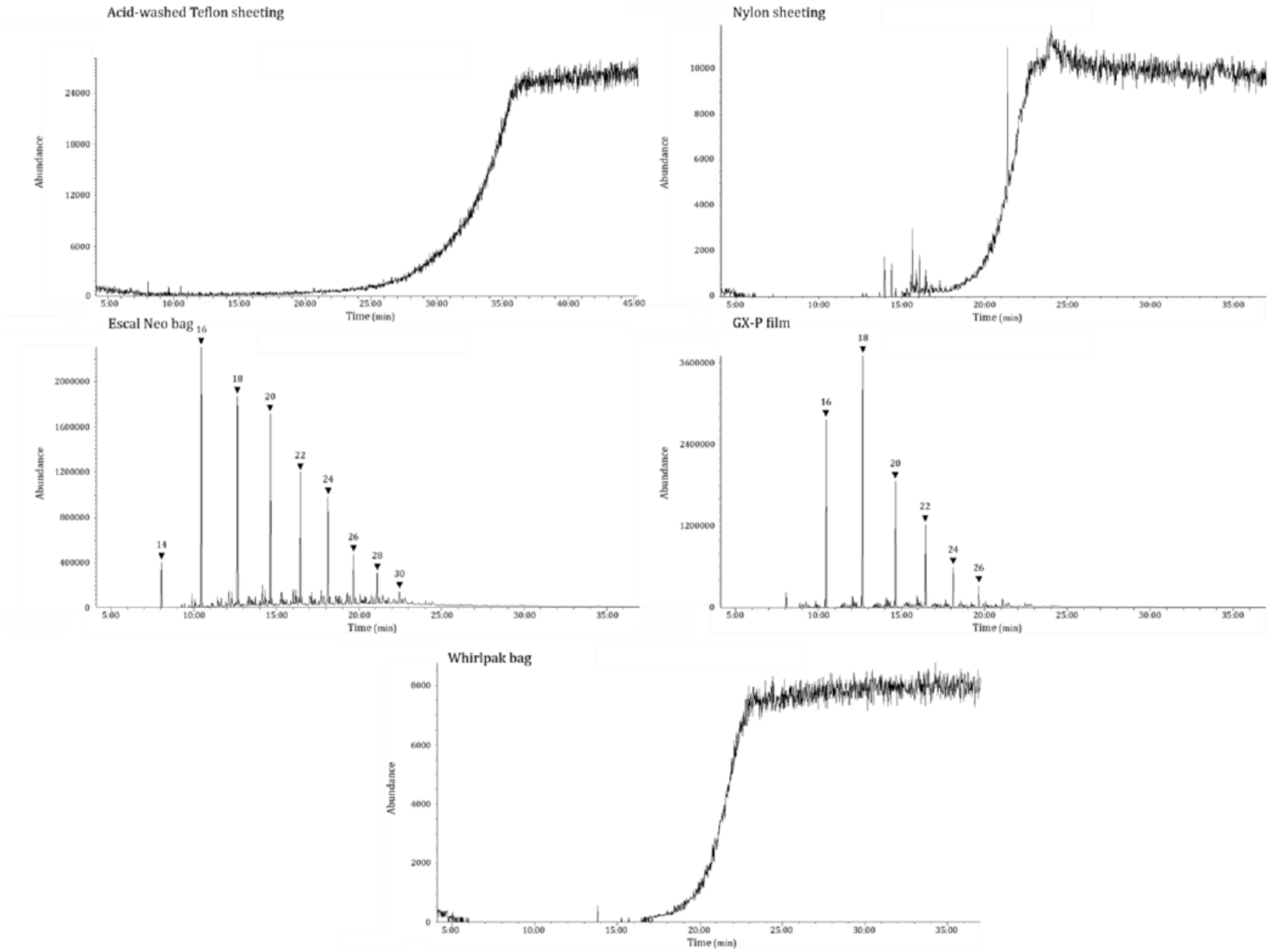

Figure A.1 Contaminant chromatograms: bags. GC/MS chromatograms displaying the distribution of n-alkanes (marked with a ▼ with the corresponding number indicating the carbon number of the specific alkane) in sheeting and bags tested for sample storage.

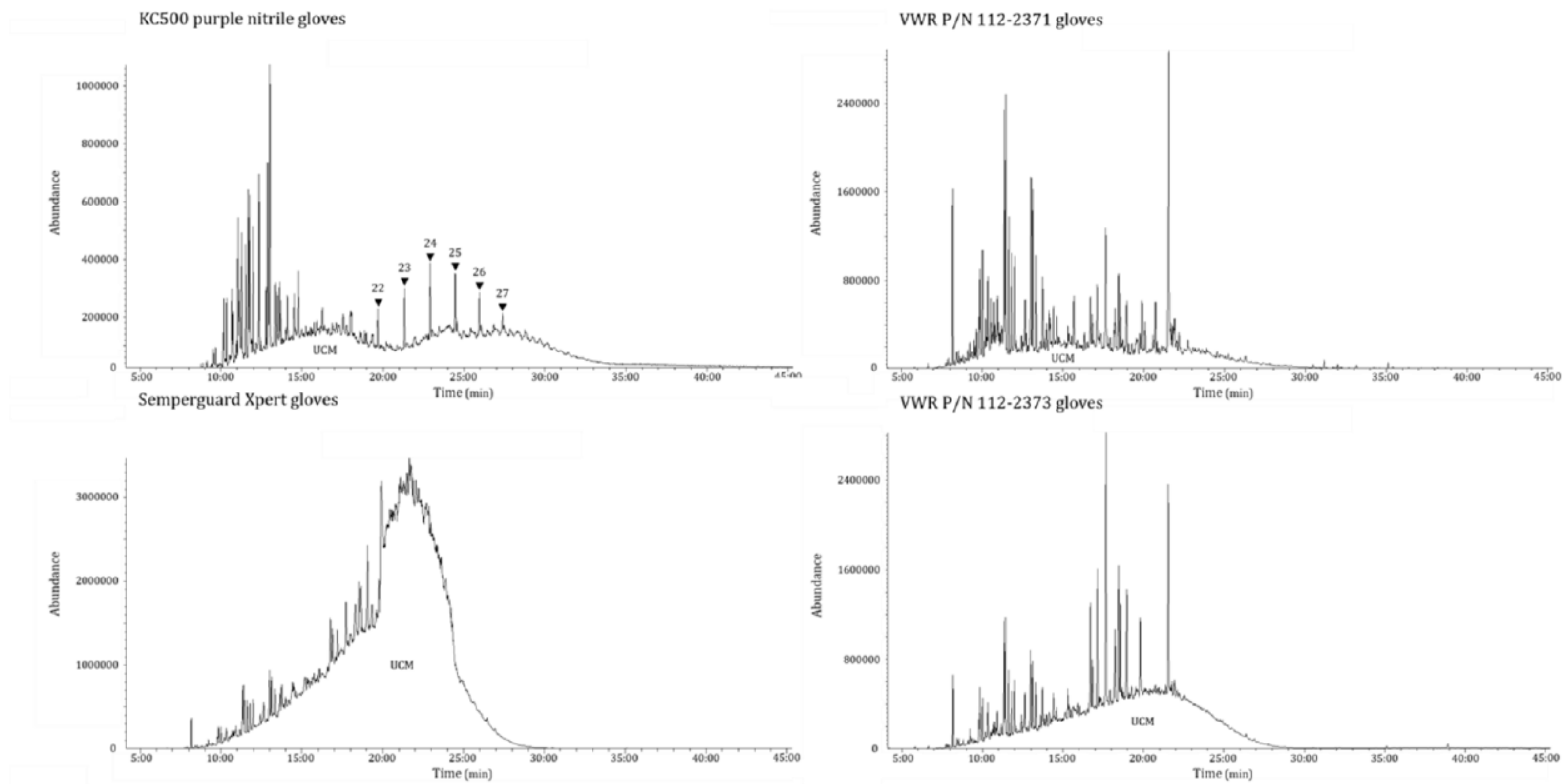

Figure A.2 Contaminant chromatograms: gloves**.** GC/MS chromatograms displaying the saturated hydrocarbon distribution (marked with a ▼ with the corresponding number indicating the carbon number of the specific alkane, UCM denotes unresolved complex mixture) in 4 glove samples. Two grams of each glove type underwent the extraction procedure and out of 25 μL of sample dissolved in hexane, 1 μL was injected into the GC-MS and GC-C-IRMS.

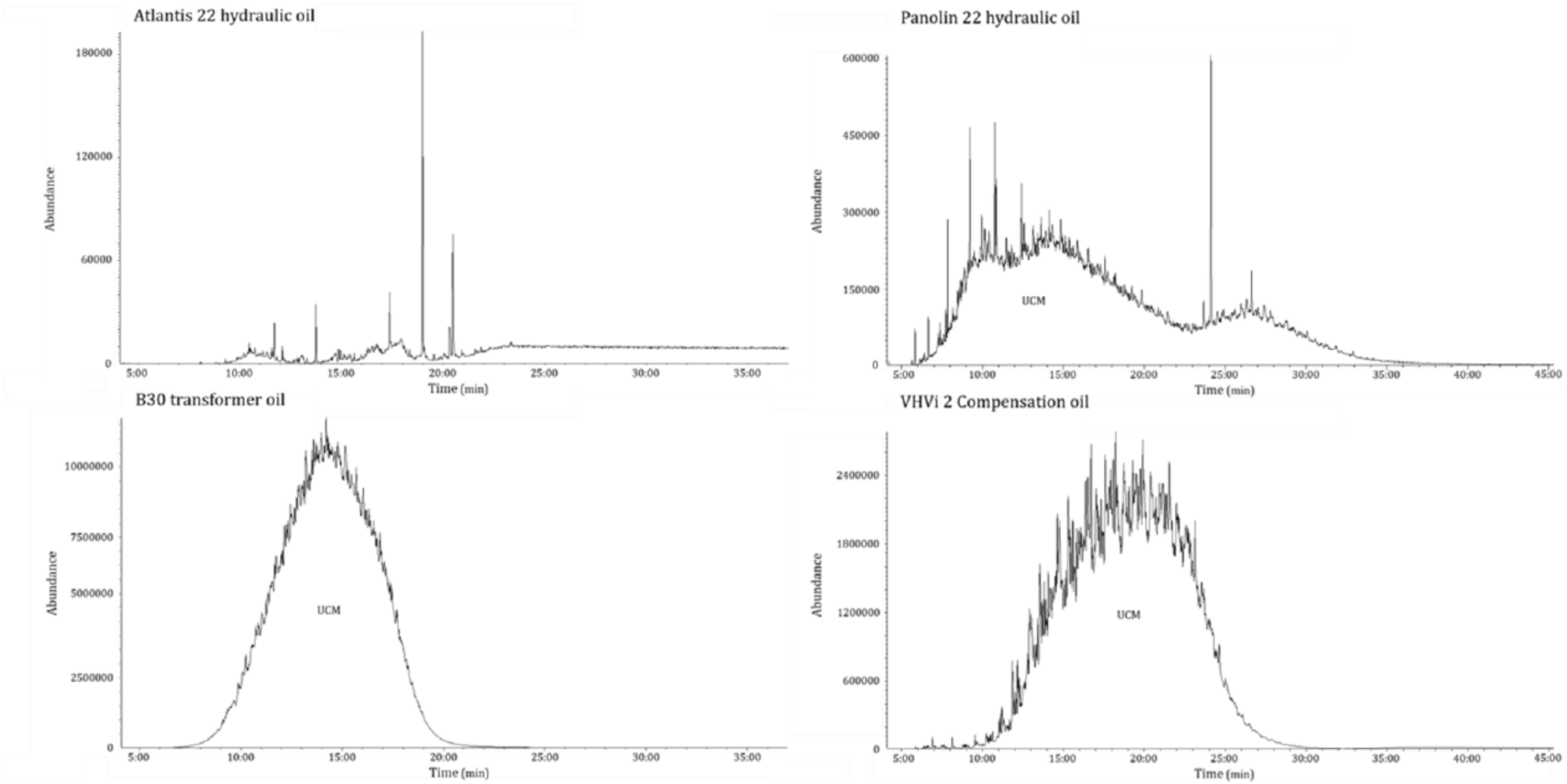

Figure A.3 Contaminant chromatograms: oils. GC/MS chromatograms displaying the saturated hydrocarbon distribution (UCM denotes unresolved complex mixture) in oil samples.

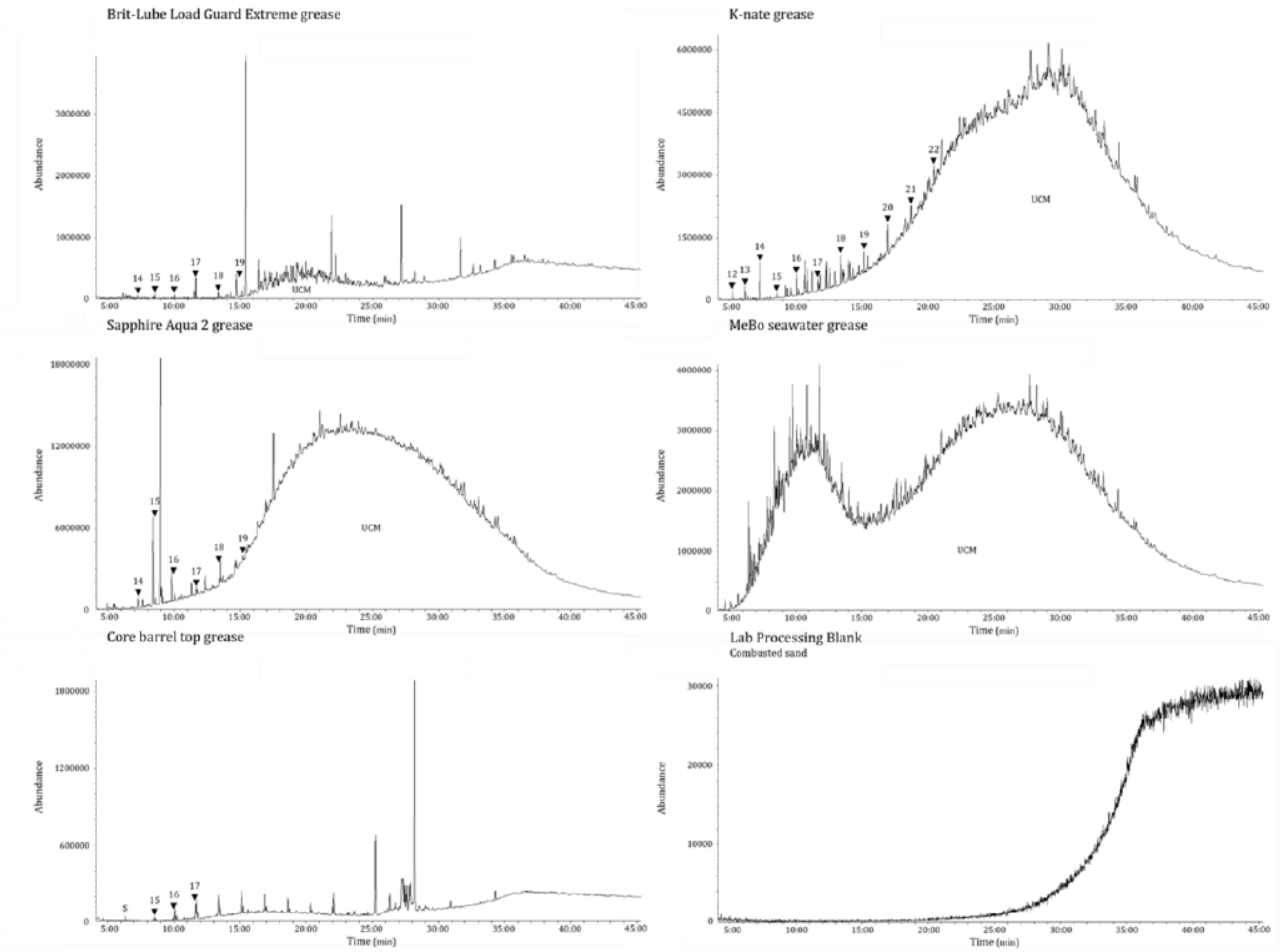

Figure A.4 Contaminant chromatograms: greases. GC/MS chromatograms displaying the distribution of n-alkanes (marked with a ▼ with the corresponding number indicating the carbon number of the specific alkane, S indicates siloxanes, UCM denotes unresolved complex mixture) in grease samples and the laboratory processing blank.

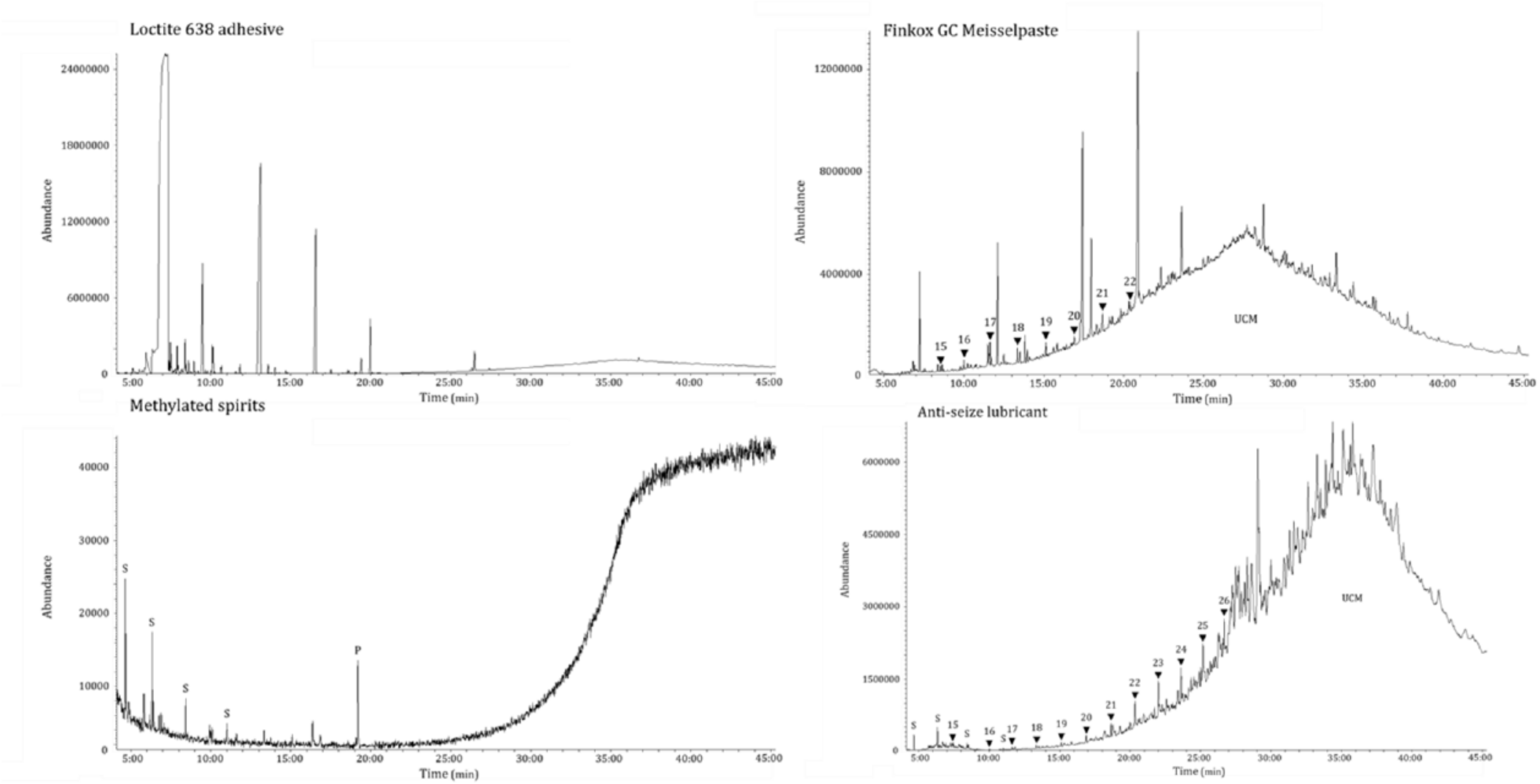

Figure A.5 Contaminant chromatograms: adhesives, solvents, and lubricants. GC/MS chromatograms displaying the distribution of n-alkanes (marked with a ▼ with the corresponding number indicating the carbon number of the specific alkane, S indicates siloxanes, P indicates phthalates, UCM denotes unresolved complex mixture) in the Loctite 638 adhesive, Finkox GC Meisselpaste adhesive, methylated spirits, and Anti-seize lubricant.

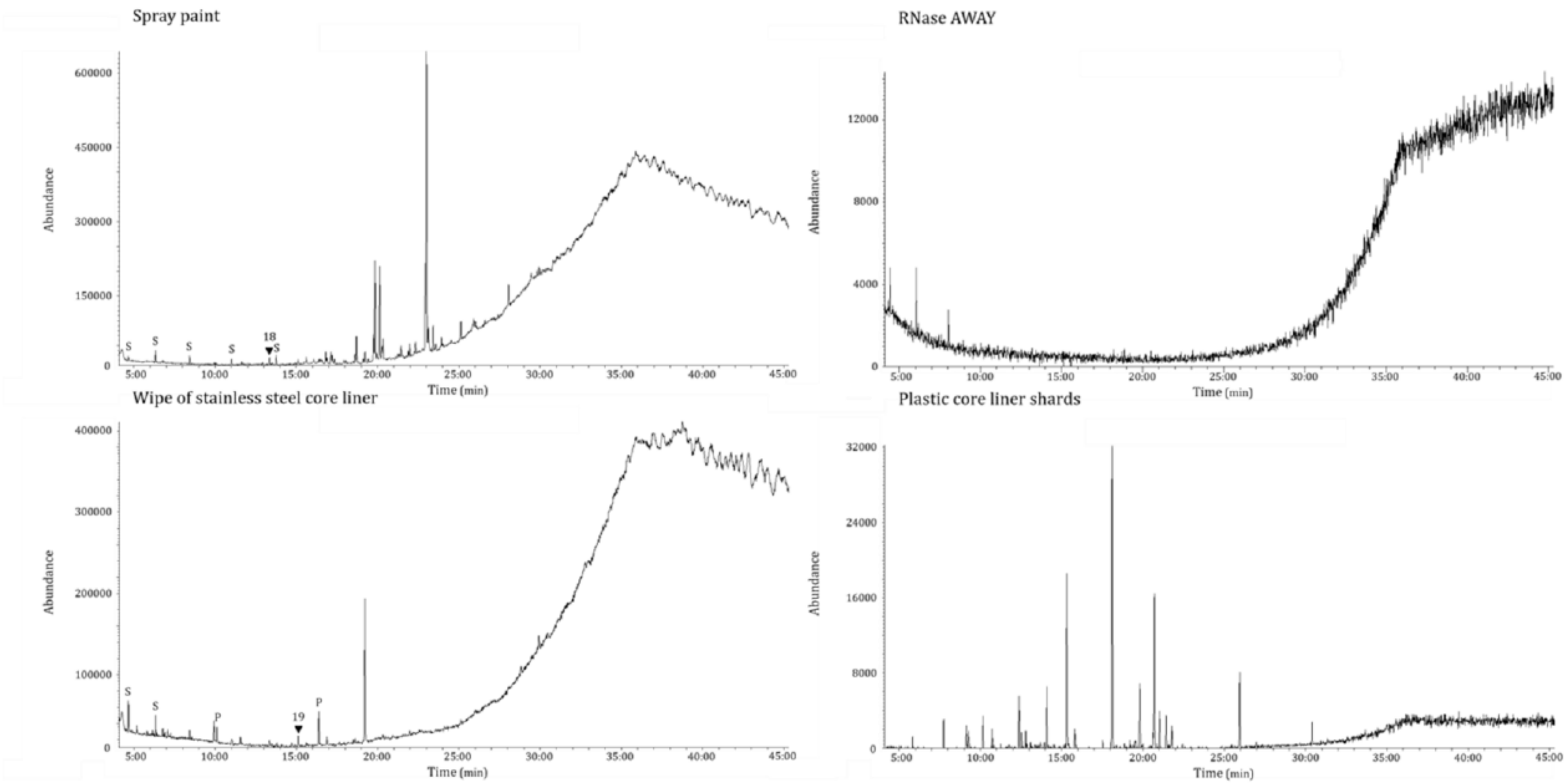

Figure A.6 Contaminant chromatograms: miscellaneous. GC/MS chromatograms displaying the distribution of n-alkanes (marked with a ▼ with the corresponding number indicating the carbon number of the specific alkane, S indicates siloxanes, P indicates pthalates) in the spray paint used on RD2, RNase AWAY, wipe of stainless steel core liner, and plastic core liner shards.

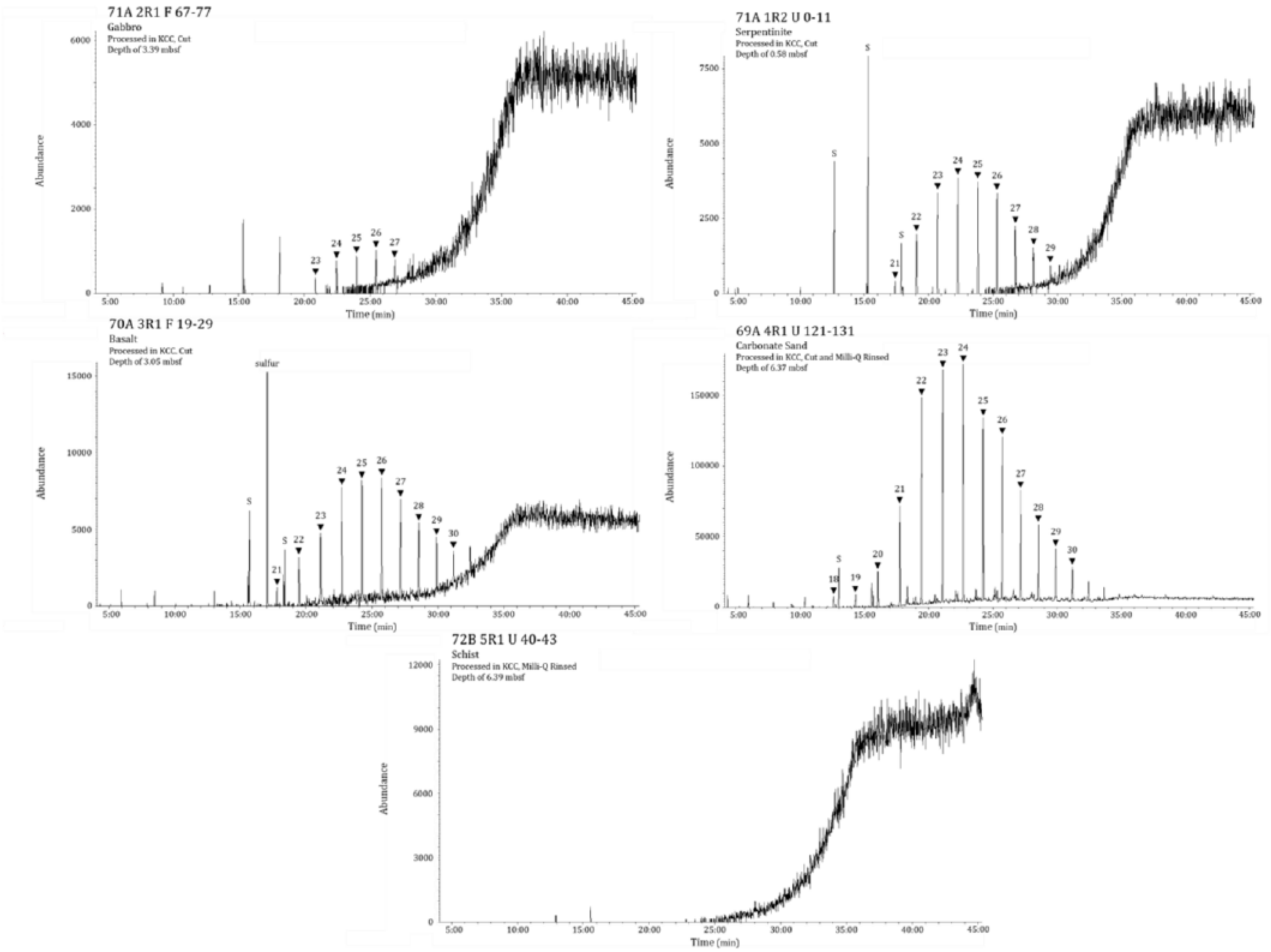

Figure A.7 Expedition 357 chromatograms: cut serpentinites. GC/MS chromatograms displaying the distribution of n-alkanes (marked with a ▼ with the corresponding number indicating the carbon number of the specific alkane, S indicates siloxanes) in cut serpentinites samples.

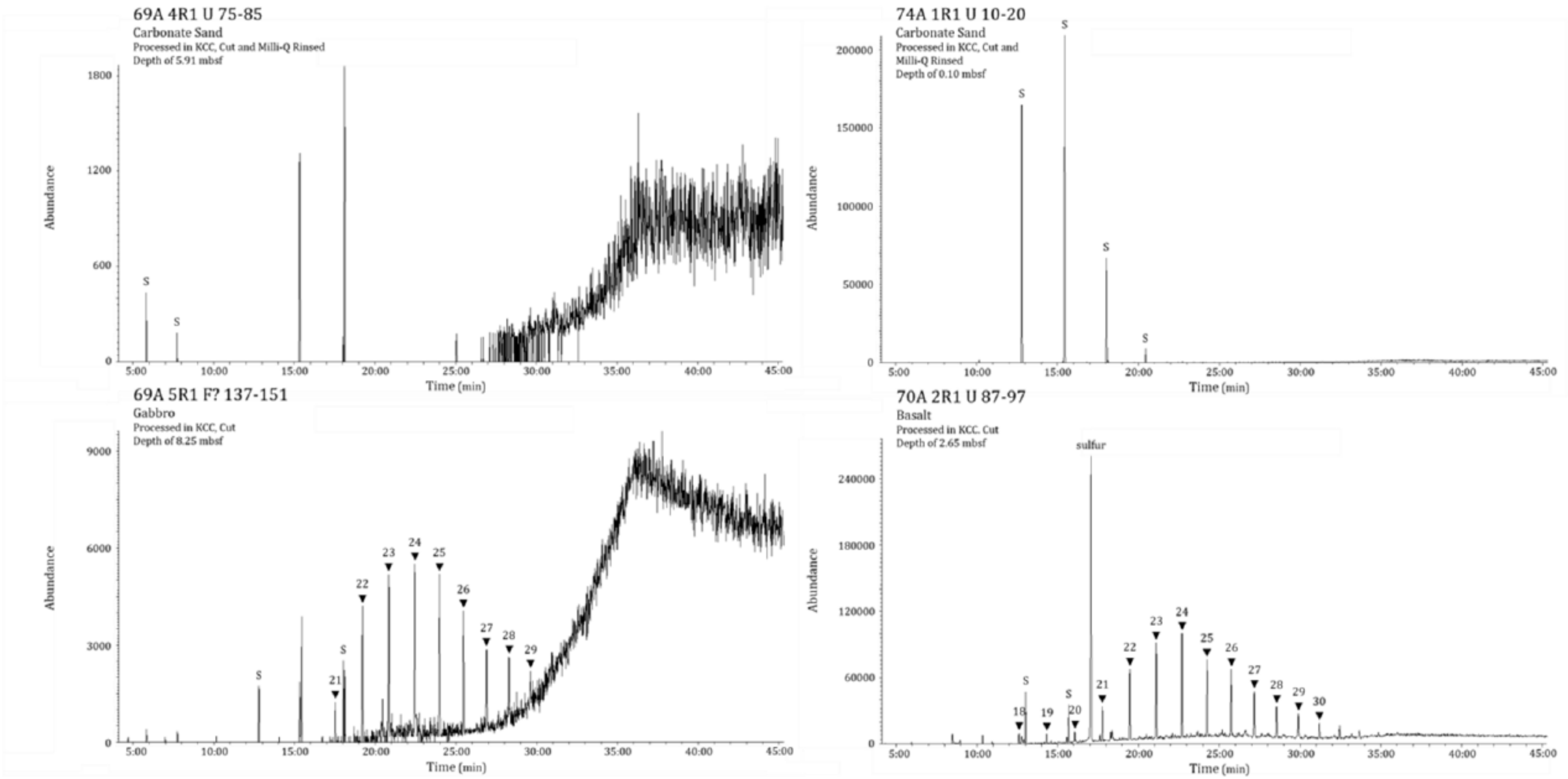

Figure A.8 Expedition 357 chromatograms: cut samples. GC/MS chromatograms displaying the distribution of n-alkanes (marked with a ▼ with the corresponding number indicating the carbon number of the specific alkane, S indicates siloxanes) in cut samples with various lithologies.

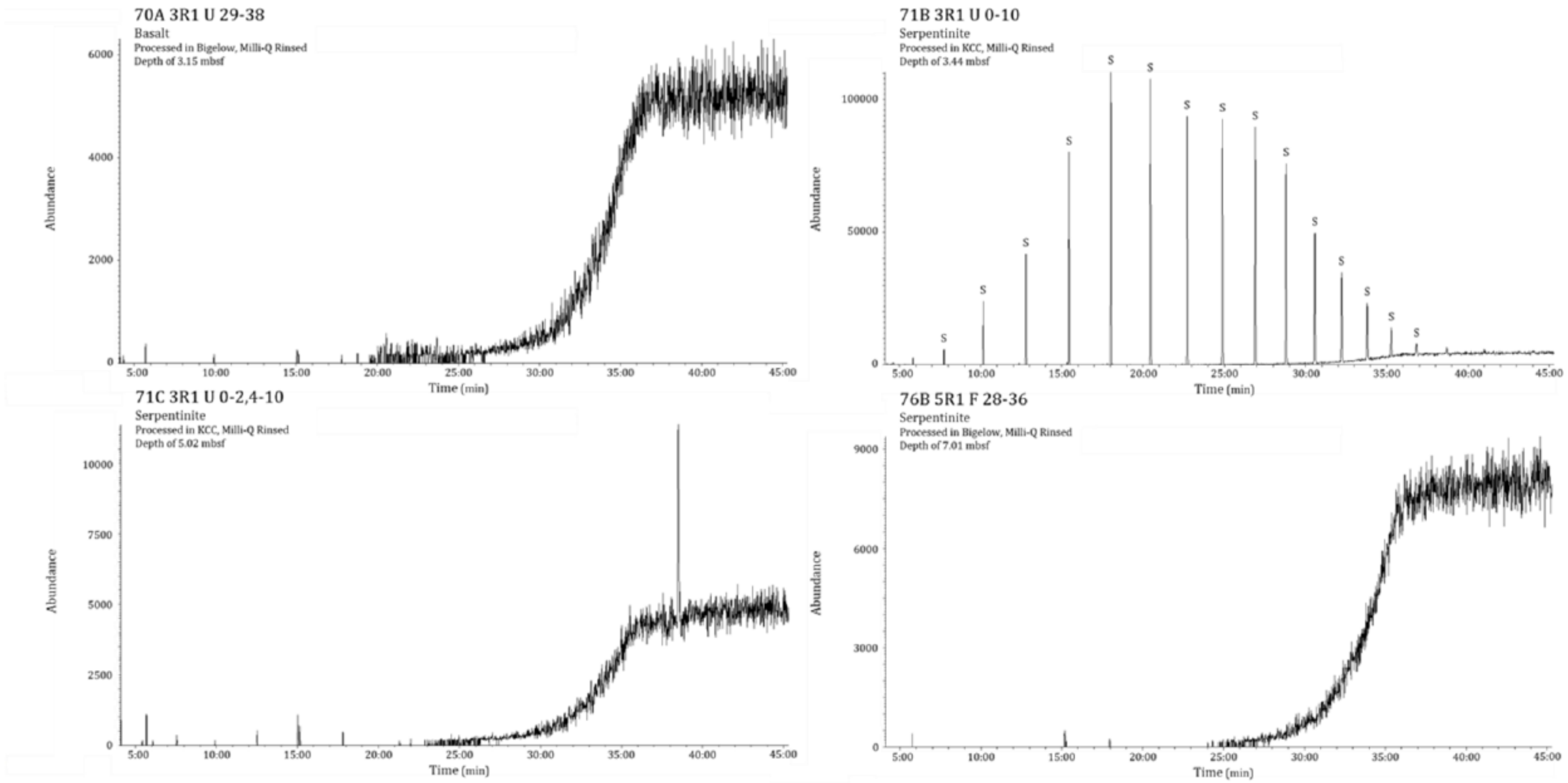

Figure A.9 Expedition 357 chromatograms: Milli-Q rinsed samples. GC/MS chromatograms displaying the distribution of n-alkanes (S indicates siloxanes) in Milli-Q rinsed samples with various lithologies.