Eastern Lau Spreading and Valu Fa Ridge
2015
April 21-May14
R/V Roger Revelle, ROV Jason II

RR1507 Preliminary Cruise Report

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Summary of activities and sample collection

We departed from Auckland, 21 April 2015, and arrived on site April, 24. Unfortunately, due to mainly weather related issues, we lost almost 9 of the 15 planned sampling days with the Remotely Operated Vehicle (ROV), Jason. During the cruise, about 54 different vent deposits and about 27 (duplicate) hydrothermal fluid samples were collected for microbiological and geochemical investigations (Table 1).

Table 1. Overview of samples collected and processed during the RR1507 research expedition to the Eastern Lau Spreading Center.

Sample	Type	Sub-Type	DNA/RNA extracts	Culture	Chemistry	RNAlater
ABE						
J2-815-7-R1	Rock	Chimney	+	+		
J2-815-1-R1	Rock	Chimney	+	+	W1-IGT8, W2-IGT7	
J2-815-5-R1	Rock	Chimney	+	+	W1-IGT3, W2-IGT1	
J2-815-6-R2	Rock	Chimney	-	-		
J2-815-6-R1	Rock	Chimney	+	-		Yes
J2-815-11-R2	Rock	Chimney	+	+	W1-IGT5, W2-IGT6	
J2-815-9-R1	Rock	Chimney	+	+	W1-IGT4, W2-IGT2	
J2-815-16-R1	Rock	Flange	+	+		
J2-815-ABE-R1	Rock	Misc.	-	+		
J2-815-14-R1	Rock	Chimney	-	-		
J2-815-11-R1	Rock	Chimney	+	-		Yes
Mariner						
J2-816-3-R1	Rock	Flange(TB)	+	+		
J2-816-3-R3	Rock	Flange(TB)	+	+		
J2-816-3-B1	mat	Slurp	N/A	N/A		
J2-816-1-B1	mat	Slurp	N/A	N/A		
J2-816-3-R2	Rock	Flange(TB)	+	-	W1-IGT6, W2-IGT5	Yes
Mariner/Vai Lili						
J2-817-6-R1	Rock	Chimney - M	+	+		
J2-817-3-R1	Rock	Flange -M	+	+		
J2-817-2-R1	Rock	Chimney -M	+	+	W1-IGT6, W2-IGT5	
J2-817-10-R1	Rock	Flange - V	+	+	W1-IGT3, W2-IGT7	
J2-817-7-R1	Rock	Chimney - M	+	+	W1-IGT1, W2-IGT8	
J2-817-10-R3	Rock	Flange -V	+	-	W1-IGT3, W2-IGT7	Yes
J2-817-10-R2	Rock	Flange - V	+	+	W1-IGT3, W2-IGT7	
J2-817-8-B1	mat	Slurp	N/A	N/A		
J2-817-11-R1	Rock	Chimney -M	+	+	W1-IGT5B, W2-IGT6B	
J2-817-10-R4	Rock	Flange - V	-	+	W1-IGT3, W2-IGT7	
J2-817-4-R1	Rock	Chimney	-	-		
J2-817-5-B1	mat	Slurp	N/A	N/A		
J2-817-8-B2	mat	Slurp	N/A	N/A		

Mariner						
J2-818-1-R1	Rock	Chimney	-	+		
J2-818-4-R1	Rock	Chimney	+	+		
J2-818-3-R1	Rock	Chimney	+	+	W1-IGT4, W2-IGT5	
J2-818-2-R1	Rock	Chimney	+	+		
J2-818-6-R2	Rock	Flange (Toadstool)	+	+		
J2-818-10-R1	Rock	Chimney	+	+		
J2-818-6-R1	Rock	Flange (Toadstool)	+	-		Yes
J2-818-8-R1	Rock	Chimney	+	+	W1-IGT7, W2-M	
J2-818-11-R1	Rock	Chimney	+	-		
J2-818-4-R2	Rock	Chimney	+	-	W1-IGT2, W2-IGT3	Yes
J2-818-9-R1	Rock	Misc.	N/A	N/A		
J2-818-4-R3	Rock	Chimney	N/A	N/A		
Tui Malila						
J2-819-1-R1	Rock	Chimney	+	+		
J2-819-3-R1	Rock	Chimney	+	+	W1-IGT4, W2-IGT5	
J2-819-2-R1	Rock	Chimney	+	+	W1-IGT8, W2-IGT6	
J2-819-2-R2	Rock	Chimney	+	-	W1-IGT8, W2-IGT6	Yes
J2-819-6-R1	Rock	Chimney	+	+	W1-IGT7, W2-IGT3	
J2-819-5-R1	Rock	Chimney	+	+		
J2-819-6-R2	Rock	Chimney	+	-	W1-IGT7, W2-IGT3	Yes
J2-819-4-R1	Rock	Chimney	+	+	W1-IGT2	
J2-819-9-R1	Rock	Flange	+	+		
J2-819-7-R2	Rock	Flange-Mothership	+	+	W1-IGT6B, W2-IGT8B	
J2-819-7-R1	Rock	Flange-Mothership	+	-	W1-IGT6B, W2-IGT8B	Yes
J2-819-11-R1	Rock	Chimney	+	+	W1-IGT7B, W2-IGT2B	
J2-819-12-R2	Rock	Chimney	+	+	W1-IGT3B	
J2-819-10-R1	Rock	Chimney	+	+		
J2-819-12-R1	Rock	Chimney	+	-	W1-IGT3B	Yes
J2-819-8-R1	Rock	Chimney	+	+	W1-IGT5B, W2-IGT4B	

Additional Activities. In addition to the day-to-day operations, we had science meetings on a regular basis. During transits, participants volunteered to give science talks that were open to all the crew and scientific party. Talks were given by Gilberto Flores, Jeff Seewald and Guy Evans. We maintained a blog of the cruise at www.laugeomicro2015.blogspot.com. Data can be obtained from the Jason Virtual Van http://4dgeo.whoi.edu/webdata/virtualvan/html/VV-rr1507/index.html

Acknowledgements

The success of this cruise reflects the efforts of many people at shore and at sea. Much of the success of this cruise relied on the teamwork between the scientists, Jason team and

the crew of the RV *Roger Revelle*. We thank Captain Tom Desjardins and the officers and crew of the RV *Roger Revelle*, and 'Tito' Collasius and the Jason team for their dedication to the scientific objectives of the cruise. We gratefully acknowledge the efforts of Liz Brenner for pre-cruise logistics; the shipboard technician, Brett Hembrough for his assistance. Much of the science at sea could not have been done without the logistics and equipment support from Matt Stott at the GNS, New Zealand. We also thank the New Zealand and Tongan governments for providing us the permits to do the research. This work was funded by US-NSF grants, and provided a unique opportunity to collaborate with colleagues from Russia, Taiwan and France.

Cruise Objectives

Extreme environmental gradients exist at deep-sea hydrothermal vents where high temperature, low pH and reduced fluids mix with cold oxygenated seawater. This results in a plethora of microbes taking advantage of abundantly available microniches. From small subunit (16S) rRNA gene surveys and directed enrichment culturing of vent deposits from many sites, patterns in diversity are emerging that suggest that geochemical processes, particularly those that affect fluid pH, play a fundamental role in regulating microbial diversity and community composition. The ELSC was chosen to investigate the relationship between vent geochemistry and microbial community dynamics because it provides large and systematic changes in fluid and rock geochemistry, spreading rate, magmatic/tectonic processes, and proximity to the volcanic arc over its relatively short length of ~250 km. The individual vent fields therefore provide excellent natural laboratories for exploring, in depth, the factors that influence the diversity and relationships of microbial communities associated with actively forming deep-sea hydrothermal deposits**.**

We initially proposed to explore 3 geochemically different hydrothermal fields along the ELSC. We hypothesized that, given the extreme environmental characteristics (e.g., low fluid pH and high iron at Mariner), we will see distinct differences in the metagenomes and particularly in the metatranscriptomes among Kilo Moana, ABE and Mariner.

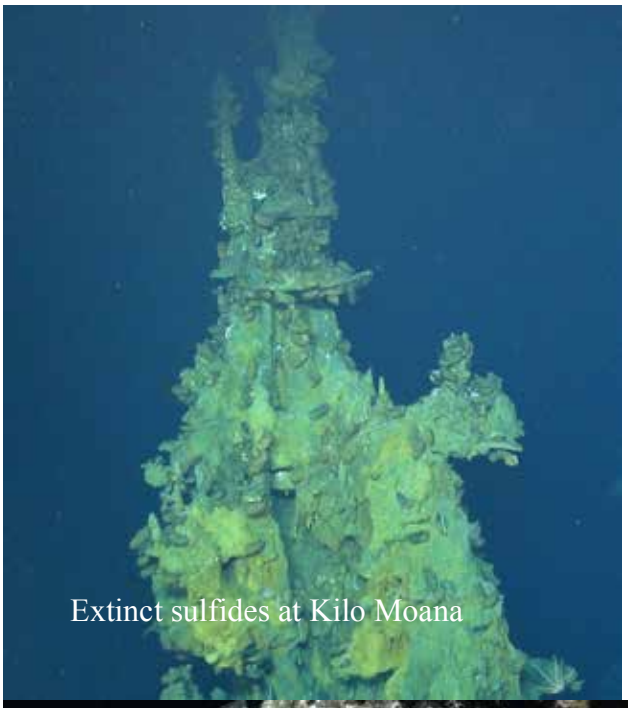
Therefore, the cruise goals were to collect samples to explore the following objectives:

1) Link geochemical and microbial dynamics along the ELSC
2) Use of metagenomic and transcriptomic data to explore biogeochemical cycles that are regulating the functional roles of the microbial communities in vent fields along the ELSC.
3) Use the metagenomic information to enrich for targeted novel Thermoprotei and acidophiles.

Summary of Jason lowerings and sites.

We departed Auckland at 1600 21 April. Arrived at the Mariner vent field about 3.5 days later. Due to it being too winding we proceed North to Kilo Moana in the hopes of there being less wind or swell. Still windy (averaging 15-20 knots, swell 5-9), but deployed (J814) after weather delays on 26th (swell 5-8, 11 knots). Relatively short dive as no visible high temperature activity. We then proceed to ABE, and had another weather delay for about 29.5 hours, and deployed (J815) Jason at 1800, 27th. Ended this dive after about 24 hours, as we had filled all samplers. We then proceeded to Mariner, with the intent of returning to ABE to obtain more samples from the Southern area, and to retrieve the chimney scaffold. Unfortunately, because of continued weather and related issues, we never managed to return to ABE. We had 3 dives at Mariner/Vai Lilli areas. J816 was a relatively short dive as it had to be terminated because of a hydraulic leak (Jason in

around 7:00, out at 14:35). A quick turnaround of Jason enabled us to have a long diver with 2 elevators on J817. Although we planned to terminate the dive at midnight anyway, this dive was ended with an issue with one of the manipulators. Our final Mariner dive, J818, was relatively short, and used to collect samples that were needed to make our collections more complete from this vent field. We then used the remainder of the time that we had in the ELSC to do a single long dive (2 elevators) at Tui Malila.



Extinct sulfides at Kilo Moana

Kilo Moana (J2-814)

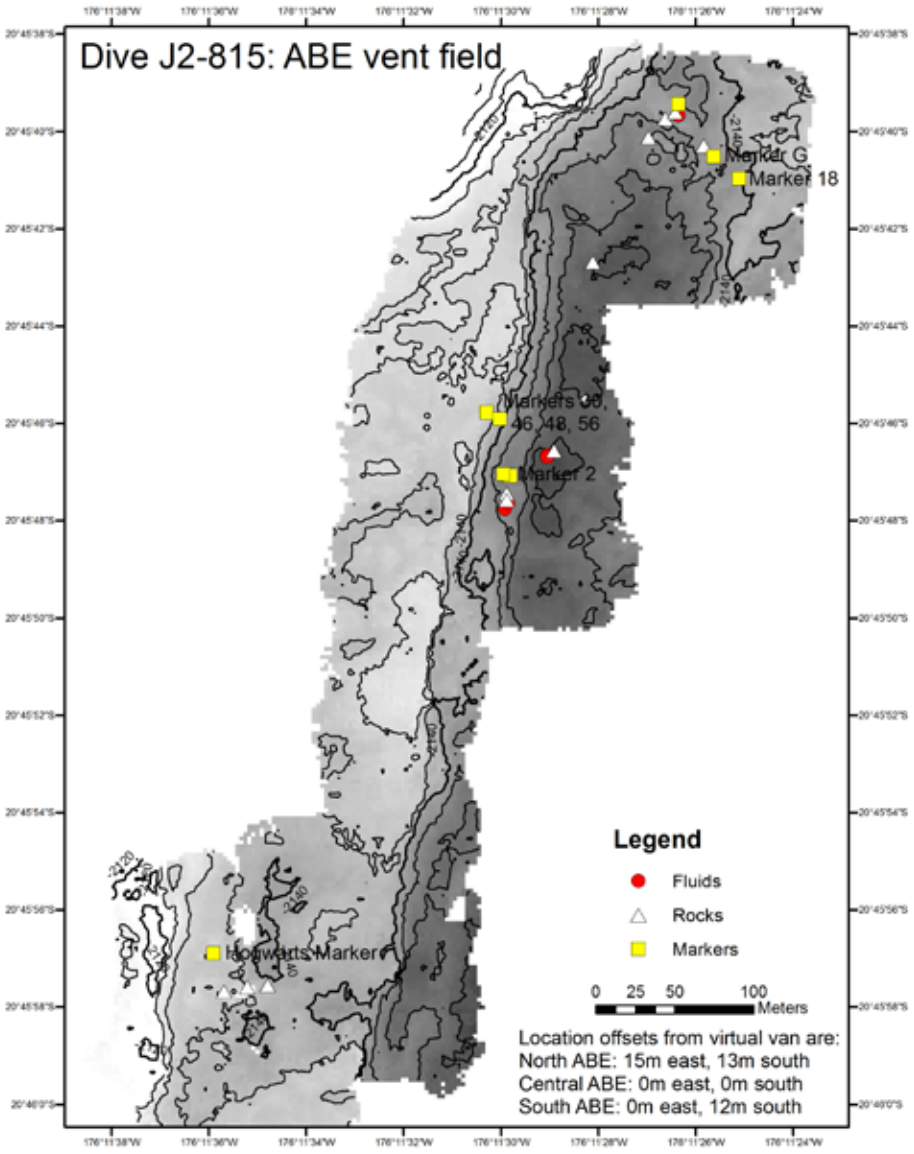
After waiting for weather/swell conditions to improve, we had our first dive (J814) at Kilo Moana. During this dive we visited all the 3 areas that have previously been visited at this site in 2005 and 2009, and did not detect any diffuse or high temperature flow. We did see many mussel shells, and some accumulations of live mussels. Additionally, barnacles were abundant. The mussels were typically covering the extinct sulfides, almost as if they were trying to be more in the water column and between the rocks. One thought is that they were potentially filter feeding, and hence better positioned for that task in higher in the water column. A few mussels were collected, chucked and frozen. In discussions with Chuck Fisher, it is possible that the mussels were 'starving' as their gills appeared watery, reduced and the membranes very thin.

Mussels at Kilo Moana

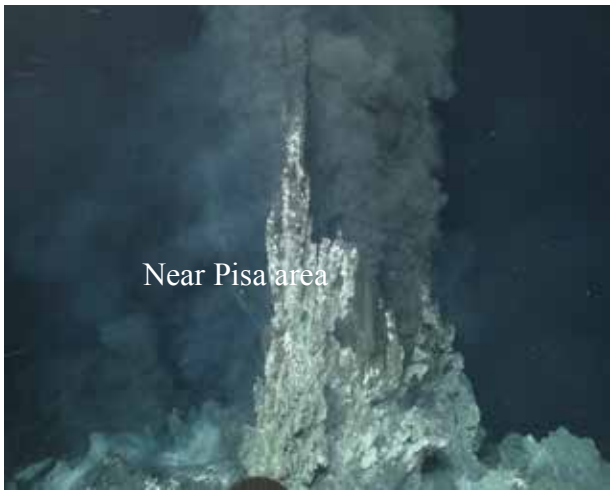
Deployment of chimney scaffold at ABE

ABE (J2-815)

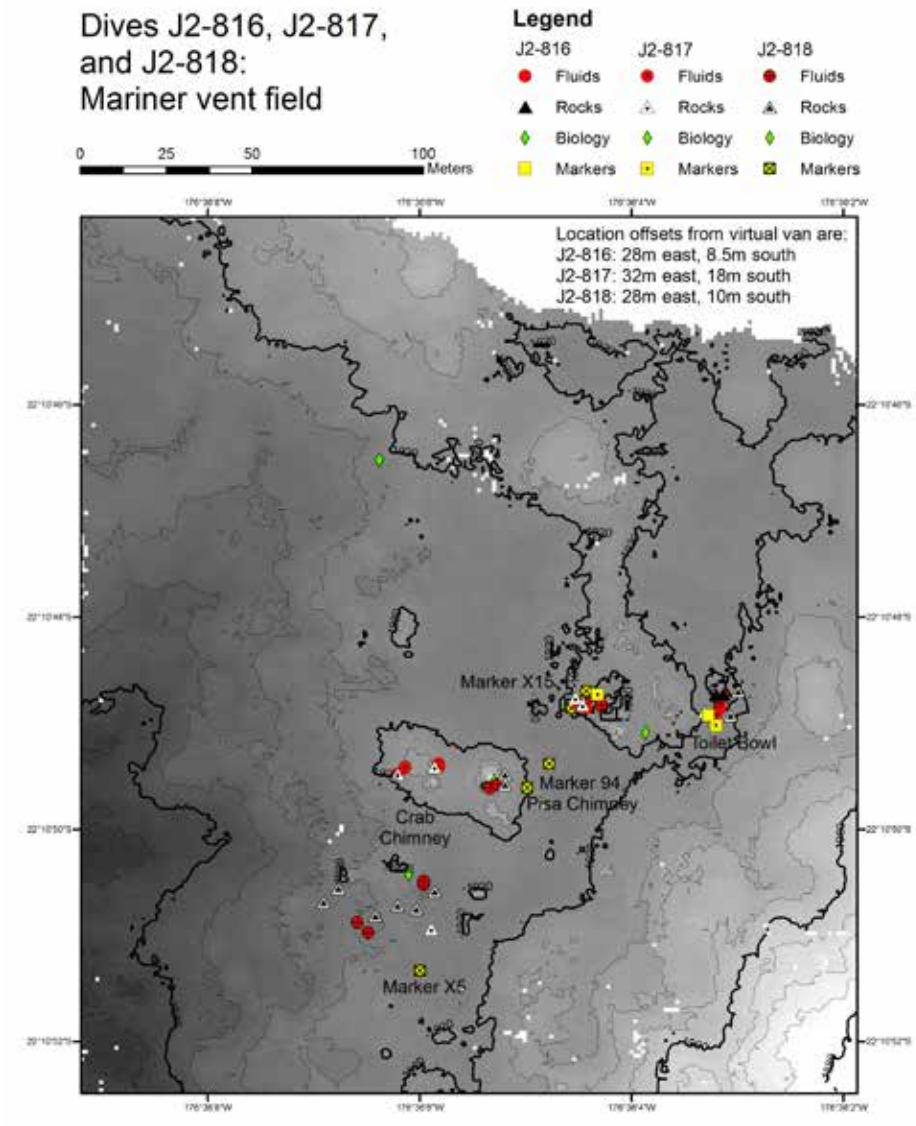
We had only one dive at ABE. We had planned to come back, but because of the weather issues, we were unable to return. We started the dive in the Northern area,



where we also deployed a chimney scaffold. We continued to sample towards the South. We took note of any mosaic sites Chuck Fisher was interested in, and also tried to locate a settlement experiment that Beth Orcutt asked us to retrieve. We were not successful in the latter. The southern area looked a lot more active, and since we had filled all our bioboxes, elevators and water samplers, we decided to end the dive, and planned to return to ABE at a later time. Unfortunately, due to time constraints during the cruise, we were unable to return to ABE. We did obtain several good sulfide chimneys that yielded preliminary thermophilic enrichment cultures shipboard.

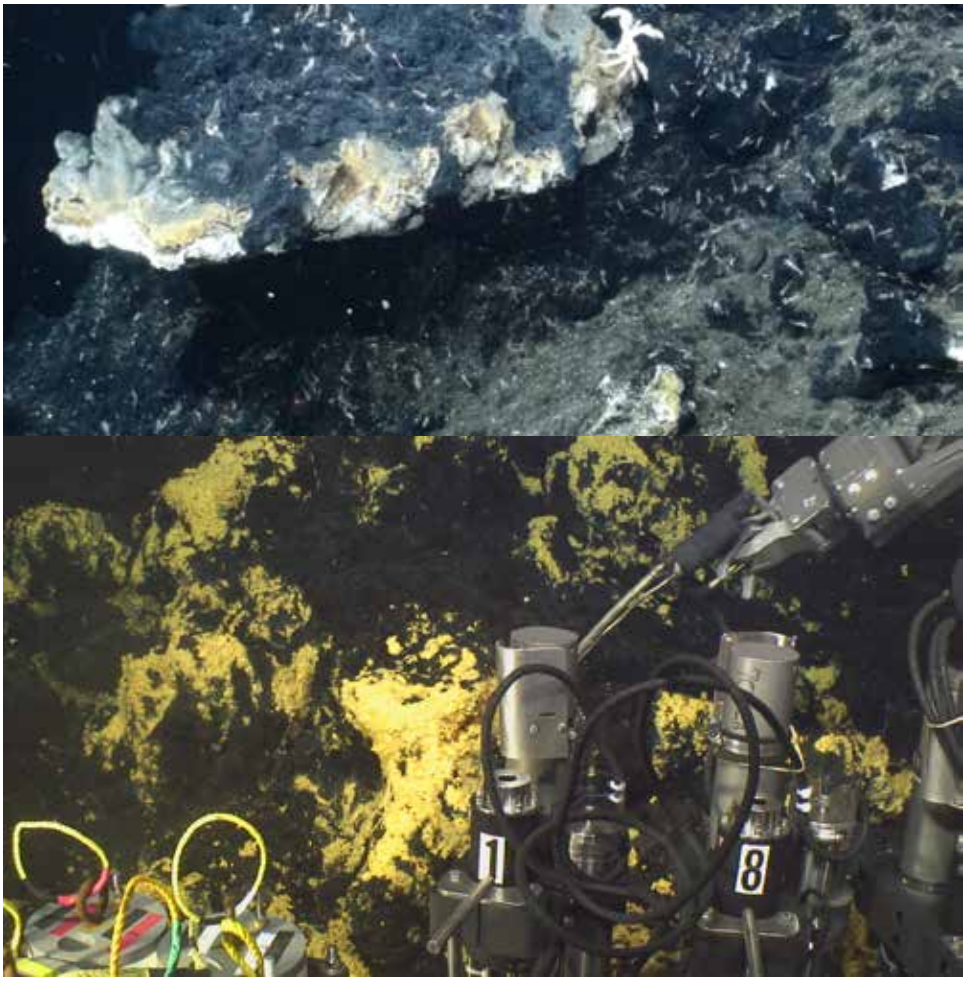


Mariner and Vai Lili (J2-816, 817, 818). Our focus on Mariner was based on our past experience with the microbial community diversity as this vent field. In particular, the one area, "Toilet Bowl"/"Bench top" (photo, title page) has yielded DNA sequences never before detected at deep-sea vents (Reysenbach et al. 2006). Additionally, the Vai Lili site has extensive microbial mats bathed in 60°C fluids, and poorly sampled but limited sulfide chimneys. We successfully sampled the major areas, the western and eastern, and southern areas. We obtained the most comprehensive sample collection from the Mariner vent field, and thus our future studies from samples collected on this research cruise will



most likely focus on this site, as we did not obtain a good statistical sampling from the other sites to produce a robust statistical comparative study. Much of our sampling was focused on the eastern area of Mariner where the unusual 'Toilet Bowl" structures are. The geochemistry of the hydrothermal fluids emanating from these structures suggest that the fluids are not mixed with seawater, and cooled (conductively) during accent. This is significant, as in all other low temperature fluids collected globally from deep-sea vents is always cool, because it has mixed with colder seawater.

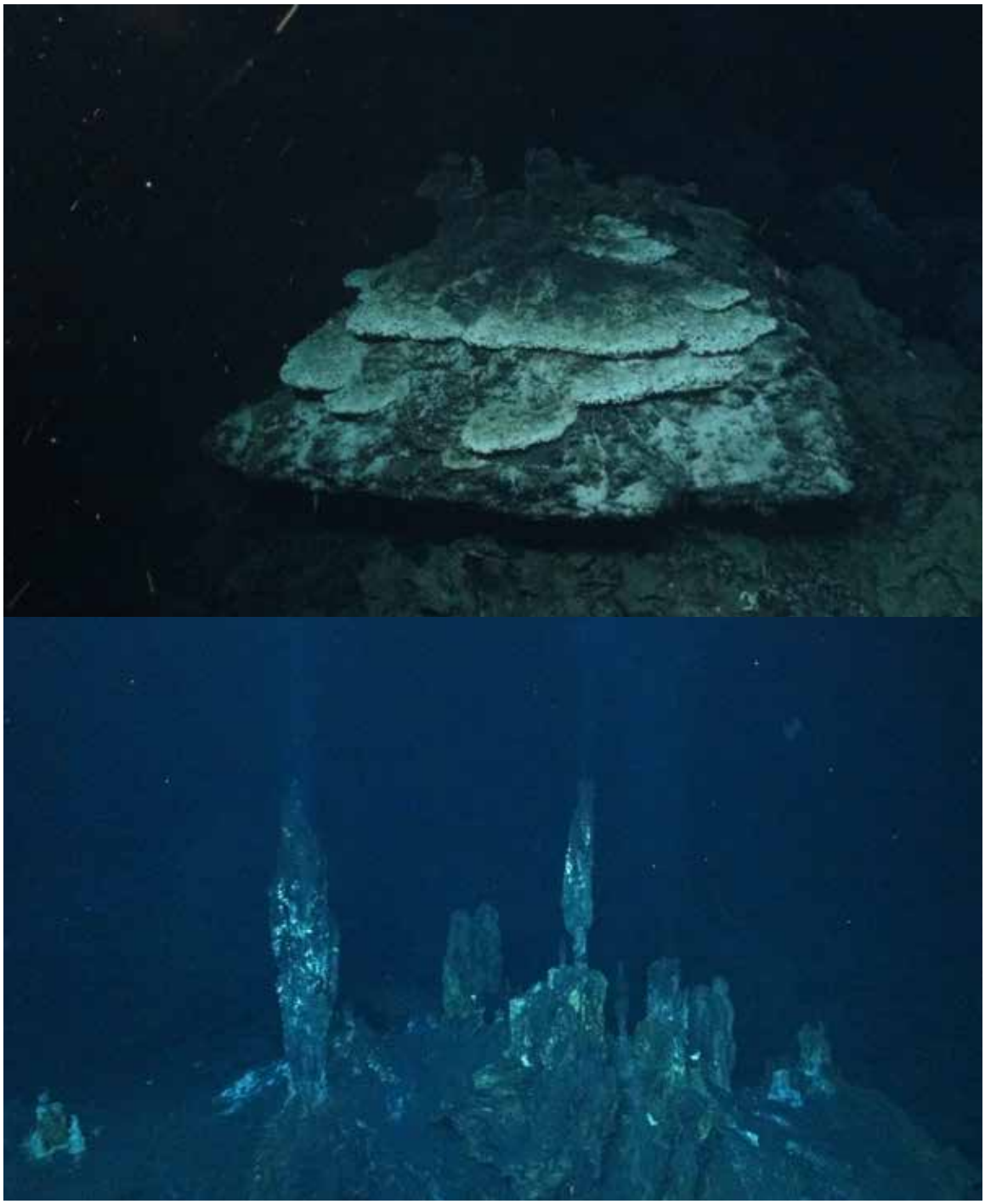
We obtained the most comprehensive sample set from Mariner vent field during this



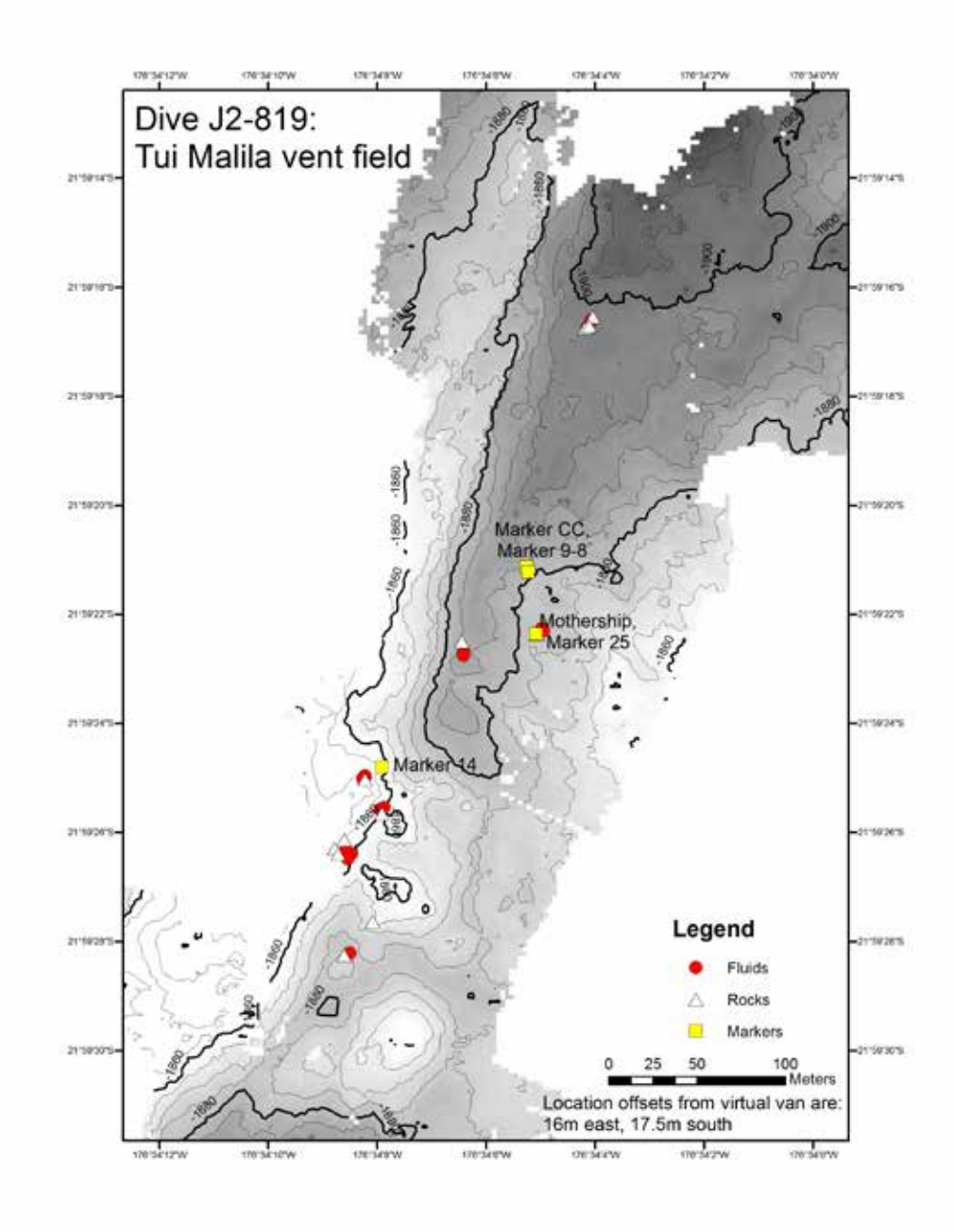
Vai Lili samples. Upper panel, hydrothermal 'flange' with black manganese. Bottom panel, manganese-iron microbial mats

cruise. Additionally, numerous samples were fixed in situ to preserve RNA. We also obtained samples from the Vai Lili vent field and microbial mats. These samples looked much richer in manganese (black deposits) than in previous visits to the site in 2005 and 2009. One last surprise is that we saw tubeworms at Mariner (below). This is the first time that the tubeworm, *Arcovestia ivanovi*, has been detected at Mariner. It has been detected in other vent fields in the ELSC.

Tui Malila (J2-819). We had one dive to explore the previously sampled Tui Malila chimneys. The western most sites seemed to be very active and areas we had not previously sampled. The large flange "Mothership" was still active, and was also sampled again. The northern area did not appear very active. We took note of sites we thought were previous mosaic sites that Chuck Fisher's group were interested in.



Tu'i Malila: Upper panel, "The Mothership", lower panel, the western area of Tu'i Malila, "The Secret Garden"



Individual team shipboard activities and preliminary observations.

DNA extractions. Sulfide samples were extracted at sea using the MoBio soil DNA kit following the modified protocol of the Reysenbach laboratory. These DNAs were used for shipboard quantitative PCR (qPCR).

Quantitative PCR of phylogenetic and functional gene markers (Table 2). Quantification of archaeal and bacterial 16S rRNA genes, functional genes for methanogenesis - *mcrA* (encoding the α subunit of the methyl-coenzyme M reductase (MCR), which catalyzes the last step in methanogenesis and is present in all methanogens), *dsrB* (encoding the β subunit of DsrAB-type dissimilatory

(bi)sulfite reductase which either catalyzes the reduction of sulfite to sulfide during anaerobic respiration of sulfate, sulfite and organosulfonates, or acts in reverse during sulfur oxidation) in DNA samples was performed according to a previously described method (Kubista et al., 2006). We used a MiniOpticon Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA) using SYBR Green I as a dye to monitor amplification. Quantification of bacterial and archaeal 16S rRNA genes was estimated using primers 515F-907R and 338F-915R , respectively. qPCR thermal programmes were adjusted experimentally according to the standard protocol. Standards and negative controls were run in duplicate. Specificity of each reaction was confirmed by melting curve analysis from 72°C to 95°C with a plate read every 0.5°C.

We determined relative abundance of Archaea and Bacteria (Fig 1). Archaea were more prevalent in samples 818-4-R1-1, 818-6-R2-1, 818-10-R1-1, 818-8-R1-1, 819-1-R1-1. Methanogens (*mcrA* gene) were only detected in one sample (815-16-R1-2). This result confirms previous observations (Flores et al., 2011) that methanogens are not prevalent at hydrothermal sites in the ELSC. End point PCR using primers for specific amplification of 16S rRNA genes of anaerobic methanotrophic Archaea of ANME-1 cluster was negative for all samples. *dsrB* genes were detected and quantified for 16 samples. In some cases (for example sample 819-1-R1-1) copy number of *dsrB* genes was nearly equal to the copy number of bacterial 16S rRNA genes indicating the prevalence of the biogeochemical sulfur cycle in microbial communities of hydrothermal dpeosits.

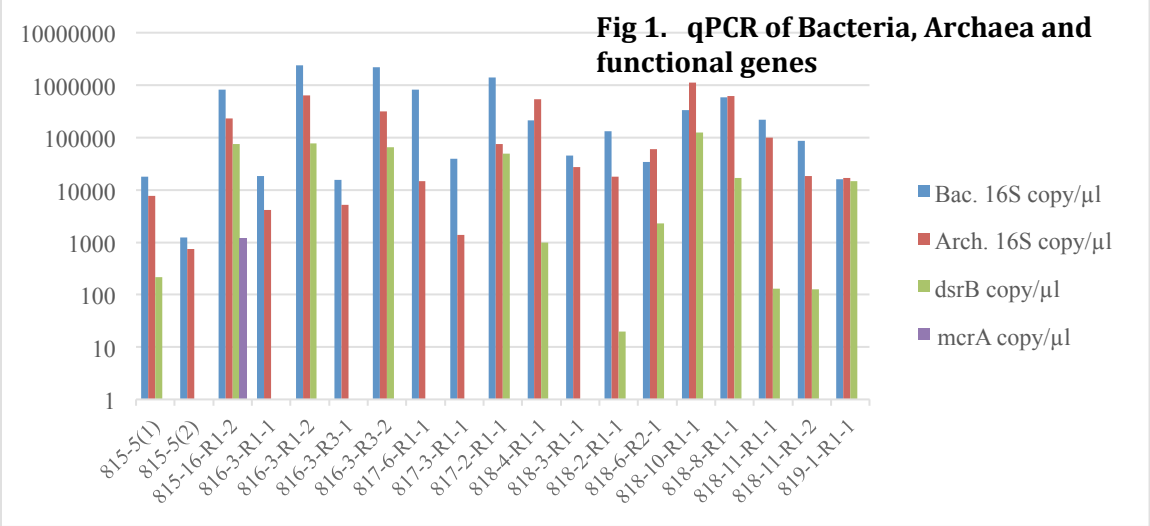


Fig 1. qPCR of Bacteria, Archaea and functional genes

Shipboard enrichment culturing efforts

Gilbert Flores and Nick Rhoades. Eleven sulfide samples were used to enrich for thermoacidophiles at 60 °C and 90 °C. The anaerobic medium used for these enrichments contained organics (yeast extract and peptone), elemental sulfur, and was at pH 4.5-5. It is the same medium used to isolate *Aciduliprofundum boonei* and was used in our previous expeditions to the Mid-Atlantic Ridge, East Pacific Rise, Guaymas Basin, and

Eastern Lau Spreading Center. Cultures were monitored daily for growth by looking for changes in turbidity and dark-field microscopy. Most of the cultures exhibited growth at both temperatures (Tables 2 & 3). Cocci of various sizes and uniformity were observed in most cultures. Small, short rods were also observed in some cultures. We observed long skinny rods in VL-336-15 and were able to make one transfer of this culture. The culture did not transfer a second time. For all cultures that exhibited growth, we have preserved the initial enrichment, the 1st transfer, and the last transfer to continue isolation at home. At this point, none of the cultures appear pure even though some were diluted up to 10^{-6}.

Table 2. Results of thermoacidophilic enrichments at 60 °C. TBD=to be determined.

Sample ID	Culture ID	Vent Field	Type of Structure	60 °C
J2-815-1-R1-2-32	A-32-15	ABE	Chimney	Little to no growth
J2-815-5-R1-2-52	A-52-15	ABE	Chimney	Mix of large and small cocci, with short rods after transfer and dilution.
J2-815-16-R1-2-117	A-117-15	ABE	Flange	Short rods and large cocci after transfer and dilution
J2-816-3-R1-3-137	M-137-15	Mariner	Flange (Toilet Bowl)	Mostly short fat rods with few cocci. After transfer and dilution
J2-816-3-B1-1-198	M-198-15	Mariner	Flange (Toilet Bowl – slurp)	Dense growth of diplococci and short rods, after transfer and dilution
J2-817-3-R1-1-241	M-241-15	Mariner	Flange	Short and medium rods with very few cocci, after transfer and dilution
J2-818-6-R2-2-443	M-443-15	Mariner	Flange (Toadstool)	TBD
J2-817-8-B1-1-317	VL-317-15	Vail Lili	Mn-Fe Mat	Few large rods after week long incubation. Did not transfer.
J2-817-10-R4-1-336	VL-336-15	Vai Lili	Flange	Long Skinny rods, Not yet able to transfer
J2-819-9-R1-2-728	TuM-728-15	Tui Malila	Flange	TBD
J2-819-7-R2-2-746	TuM-746-15	Tui Malila	Flange (Mother Ship)	TBD

Table 3. Results of thermoacidophilic enrichments at 90 °C. TBD=to be determined.

Sample ID	Culture ID	Vent Field	Type of Structure	90 °C
J2-815-1-R1-2-32	A-32-15	ABE	Chimney	Large diplo cocci and plieomorphic smaller cocci after transfer and dilution.
J2-815-5-R1-2-52	A-52-15	ABE	Chimney	Produces H_2 Gas production throughout. From small motile cocci and a few larger cocci
J2-815-16-R1-2-117	A-117-15	ABE	Flange	Mostly large cocci after transfer and dilution.

J2-816-3-R1-3-137	M-137-15	Mariner	Flange (Toilet Bowl)	Dense growth of small and large cocci after transfer and dilution.
J2-816-3-B1-1-198	M-198-15	Mariner	Flange (Toilet Bowl – slurp)	Dense growth of irregular cocci after transfer and dillution
J2-817-3-R1-1-241	M-241-15	Mariner	Flange	Very dense growth of mostly small cocci after transfer and dilution.
J2-818-6-R2-2-443	M-443-15	Mariner	Flange (Toadstool)	TBD
J2-817-8-B1-1-317	VL-317-15	Vail Lili	Mn-Fe Mat	No inoculation
J2-817-10-R4-1-336	VL-336-15	Vai Lili	Flange	Little to no growth at 90 °C
J2-819-9-R1-2-728	TuM-728-15	Tui Malila	Flange	TBD
J2-819-7-R2-2-746	TuM-746-15	Tui Malila	Flange (Mother Ship)	TBD

Stephane L'Haridon (UOB, France). Primary objectives were to enrich for (i) methanogenic microorganisms from sulfides at different temperatures (60 and 80°C); (ii) thermoacidophilic lithauthotrophic and organotrophic anaerobic microorganisms on synthetic media and natural sea water media at different temperatures, (iii) microorganisms belonging to the genus *Ignicoccus* in order to look for the presence of symbiotic Nanoarchaeota, (iv) isolate microorganisms using High Throughput method from the pelagic Tongan Water.

A total of 42 chimney samples were preserved under anaerobic conditions and stored at 4°C. 12 chimney samples from Tu'i Malila, 13 from ABE and 17 from Mariner vent field. First enrichments were performed on board on selected samples collected to target the metabolism of the microorganisms mentioned above. Additionally, pelagic seawater was sampled at 5 different depth (5 m, 100 m, 200 m, 300 m and 500 m deep). Using a high throughput method, 34 microplates of 96 wells were inoculated and incubated at room temperature. The detection of the growth in the wells will be performed back to the lab in Brest, France, after 2 months of incubation.

Positive enrichments (Table 4) were obtained shipboard for the lithoautotrophic and organotrophic thermoacidophiles microorganisms. Serial dilutions were performed directly on board for isolation of these microbes. Methanogenic enrichments will be analyzed back to the lab with an appropriate microscope to detect the presence of fluorescence cells under UV light which could indicate the presence of methanogenic microorganisms. Positive enrichments on lithoautotrophic medium to target the enrichment of members of the *Ignicoccu*s genus were obtained. DNA extraction and PCR amplification with the primers designed to amplify specifically the Nanoarchaeota will be performed to detect their presence in the enrichments.

Table 4. Preliminary enrichments performed under different conditions.

Aliquot Number Samples Code	Thermoacidophiles				*Ignicoccus* Medium
	Autotrophic		Organotrophic		80°C
	60°C	80°C	60°C	80°C	
19 J2-815-7-R1	+++ Short highly motile rods Curved rods, spiriilla	+ Few long rods			
38 J2-815-1-R1		++ Long rods		++ Rods	
80 J2-815-11-R2					
93 J2-815-9-R1					++ Cocci with small cells attached
138 J2-816-3-R1		+ Some rods		+ Few rods	
224 J2-817-6-R1					
334 J2-817-11-R1			++ Rods		++ Cocci with small cells attached
723 J2-819-9-R1-2	Still under incubation				

Houghton and Carrere (GNS, NZ). Microbial enrichments targeting the cultivation of aerobic methanotrophs, thermoacidophiles, anaerobic methanotrophs and anaerobic acidophiles were prepared from 28 samples recovered from 4 vent fields across the Eastern Lau Spreading Centre (ELSC). 68 methanotroph enrichment cultures (pH 3-9) were incubated at 60 °C and vented every 48 hours to ensure sufficient oxygen (O_2) within headspaces, after which, methane gas (CH_4) was added to 10 % (v/v). Methanotroph media contained (g/L): Sea salt, 25; B vitamin mix, 0.1; $SrCl_2$-$6H_2O$, 0.02; yeast extract, 0.01; FeEDTA solution, 1 mL; Wolin trace metals solution, 1 mL; trace elements solution, 1 mL; and 1 mL 500 mM $CeSO_4$. Future efforts will probe enrichment cultures for the methane monooxygenase (*pmoA and mmoX)* genes; signatures of methanotrophy. Samples positive for *pmoA* or *mmoX* will be inoculated into enrichment bioreactors and subsequently isolated on solid media. Thermoacidophilic enrichment cultures (44 samples; pH 2-5; containing yeast extract and S^0) were incubated at 80 °C.

Anaerobic acidophilic enrichment cultures were prepared by sparging acidophilic media with 80:20% N_2:CO_2 and adding Na_2S. Samples were incubated at either 60 °C or 80 °C and monitored daily for growth by observing culture turbidity and via microscopy. Putative growth, characterized by the presence of small cocci, was observed in several acidophilic and anaerobic acidophilic enrichments. Significant biomass was observed in sample AN-49 J2-815-5-R1-49 (pH 5, 60 °C). Both large and small cocci were observed. This culture was transferred into fresh media (10 % v/v) and is being incubated at 60 °C.

Olga Podosokorskaya (Russian Academy of Science). Enrichment cultures of methanogens were obtained in artificial modified MJ media (Table 5). It contained different substrates (20 mM) and in some cases yeast extract (0.05 g/l, Helicon, Russia) as a source of growth factors. The media were inoculated with 0.5 - 1 ml of samples (10-20% of total volume) and incubated at different temperatures (60, 80 and 90 ^{o}C).

The following methanogenic substrates and combinations were used:
Methanol, Methanol + yeast extract, 3-methylamine, 3-methylamine + yeast extract, Methanol + H_2, Methanol + H_2 + yeast extract, 3-methylamine + H_2, 3-methylamine + H_2 + yeast extract, Acetate, Acetate + yeast extract, Methanol + formate, Methanol + formate + yeast extract, H_2/CO_2 , H_2/CO_2 + yeast extract, Formate, Formate + yeast extract.

In addition, some enrichments were obtained using a 'substrate stimulation' method. In this case, samples of water (diffuse fluid mixed with sea water) and sediments were placed directly into small bottles (volume of bottles - 20 ml or 60 ml). Then oxygen traces were deleted during 3 consistent heating-degas-pumping cycles. Substrates were added. No additional source of growth factors (yeast extract, vitamins, tungstate etc) was used. Using these approaches about 126 enrichment cultures were obtained.
Growth of microorganisms was evaluated by means of observation of bubble formation and positive or negative (in case of H_2/CO_2 as a substrate) pressure in cultivated tubes or bottles. After 6-9 days of incubation a few number of enrichments demonstrated apparent turbidity and one of features mentioned above. These were transferred into fresh media.

TABLE 5. Positive enrichment cultures that were transferred.

Sample	Time and temperature of Incubation	Substrate	Characteristic
J2-817-5-B1 -2	60 ^{o}C, 6 days	Formate+methanol	Turbidity
	60 ^{o}C, 6 days	Acetate	Turbidity
	60 ^{o}C, 6 days	Methanol+H2	Turbidity, - pressure
J2-817-11-R1 -2	60 ^{o}C, 6 days	Methanol	Turbidity
	60 ^{o}C, 6 days	Formate+methanol	Turbidity
J2-815-11-R2-1	60 ^{o}C, 9 days	Formate + YE	Turbidity

J2-815-7-R1-1	60 ^{o}C, 9 days	Methanol+sulfur+YE	Turbidity, bubbles
J2-817-5-B1 -2	80 ^{o}C, 8 days	Formate+methanol	Turbidity
	80 ^{o}C, 8 days	Acetate	Turbidity, + pressure
	80 ^{o}C, 8 days	Methanol+H2	Turbidity, - pressure, > 8% of methane
J2-817-11-R1 -2	80 ^{o}C, 6 days	H2+CO2+YE	- pressure, > 6% of methane in 1 and 2 transfers
J2-816-3-B1-1	90 ^{o}C, 8 days	Formate+methanol	Turbidity

Enrichment cultures of hydrolytics were obtained using modified MJ media (g/l): KCl 0.325; $MgCl_2 \cdot 6H_20$ 2.75; MgSO4 $\cdot$ $7H_20$ 3.45 3.45; NH_4Cl 0.25; $CaCl_2 \cdot 2H_20$ 0.15; NaCl 20; K_2HPO_4 0.12; $Fe(NH_4)_2(SO_4)_2 \cdot 6H_20$ 0.01 (Sako *et al*., 1996); trace element solution (1 ml l^{-1}; Kevbrin & Zavarzin, 1992), vitamin solution (1 ml l^{-1}; Wolin *et al*., 1963) and $Na_2S \cdot 9H_20$ 0.5. Media also contained different substrates (2 g/l) and yeast extract (0.02 g/l) as a source of growth factors. The media were inoculated with 0.2 – 0.5 ml of sample (2-5% of total volume) and incubated at different temperatures (60, 80 and 90 ^{o}C).

We used following polysaccharides as substrates for hydrolytic microorganisms:
Pectin, Mannan, Chitin, Chitosan, Alginate, Xanthan gum. In addition, enrichments was obtained with peptone or yeast extract (in low concentration) as a sole carbon and energy source. Growth of hydrolytics was evaluated microscopically and by turbidity observation. In all, 23 preliminary enrichments for hydrolytic microorganisms were obtained.

Summary of Fluid Chemistry; Jeff Seewald and Sean Sylva, Woods Hole Oceanographic Institution.

Sample Inventory: 39 gastight fluid samples, 1 Major sample

Objectives: The primary objective of this cruise was to characterize the composition of hydrothermal fluids along the Eastern Lau Spreading Center to provide a geochemical context for the activity of vent associated microbial communities. In addition, the composition of vent fluids will be used to assess the temporal and spatial variability of the vent field chemistry at a back-arc spreading center.

Collection Methods. Fluids were collected primarily using titanium isobaric gastight (IGT) fluid samplers and a titanium "major" sampler. In, general, two gas-tight samples were collected at each edifice. The IGT samplers were equipped with thermocouples that

allowed for real-time temperature measurement during collection of fluids. Communication with the IGT samplers was achieved via an inductively coupled link (ICL) that allowed RS-232 communication. Reported temperature for each IGT fluid sampler represents the maximum temperature recorded while the thermocouple/snorkel tip was inserted in the vent orifice prior to, during, or after sampling.

Sample Processing. Fluid samples were processed within 4 hours of vehicle recovery. Subsamples were extracted from the IGT bottles for shipboard measurement of pH (25°C) and aqueous H_2S, CH_4, H_2, and CO concentrations. Separate aliquots were archived for shore-based analysis of major anions and cations , trace elements, organic acids, abundance and isotopic composition of CO_2 and CH_4, abundance, and isotopic composition of NH_3 and NO_3, and hydrogen and oxygen isotopes of water. The aliquot for trace element analysis (~20 ml) was acidified with 25 µl of ultra-pure HNO_3. An aliquot of this acidified sample was then diluted 100X (v/v) for shore based determination of aqueous SiO_2. Samples for NH_3 and NO_3 concentration and isotopic analysis were filtered through a 0.2 µm cellulose acetate syringe filter and stored frozen. Samples for hydrogen and oxygen isotope analysis of water were filtered through a 0.2 µm cellulose acetate syringe filter and stored frozen. The aliquots for measurement of CO_2 and CH_4 concentrations and isotopic composition were archived in evacuated serum vials with butyl-rubber stoppers. Vials for CH_4 measurements used stoppers that were boiled in 2N NaOH. Vials for CO_2 measurements used unboiled stoppers. Following complete removal of the fluid from the samplers, solid precipitates were removed from the bottle by sequentially rinsing with water and acetone and collected on a 0.45 µm nylon filter.

Shipboard Analytical Methods. A Ross combination electrode was used for measuring pH (25°C). To minimize loss of acid volatiles, samples were not sparged with an inert gas during measurement. Attainment of stable pH values indicated that significant sulfide oxidation was not occurring during measurement, the absence of an inert gas overlying the sample, notwithstanding. Aqueous H_2S concentrations are also determined gravimetrically by acidifying a separate fluid aliquot with 25 wt. % phosphoric acid and precipitating the evolved H_2S as Ag_2S in a 3 wt % $AgNO_3$ solution. The precipitated Ag_2S will be weighed in a shore based laboratory to determine H_2S concentrations. For low level H_2S samples, concentrations were determined by ion specific electrode. Dissolved H_2, CH_4, and CO were measured by gas chromatography (GC) following a headspace extraction in a purpose-built inlet system. For samples with relatively high concentrations of H_2 and CH_4, the a 5Å molecular sieve column, nitrogen carrier gas, and a thermal conductivity and flame ionization detector connected in series were used. Concentrations of CO were measured using a 5Å molecular sieve column, helium carrier gas, and a helium ionization detector.

APPENDIX

Preliminary Watch Schedule and microbio chores

TIME	SCIENCE	DATA*	DVD*
4-8	Anna-Louise	Karen	Olga
		Stephane	Morgan
8-12	Gilbert	John	Nick
		Carlo	Wan-Yen
12-4	Jeff	Guy	Alex
	Rick	Niya	Jessica

*self organize (split watches, backup etc.)

Best Of Video:
Rick, Gilbert, John, Nick, Jessica and volunteers.

Weblog:
Rick , Carlo, others

CTD
Stephane

Datasheets
Guy and MICRO---John and Nick and GILBERT master sheet and sulfide sheets

Dive plan, Dive notebook, Jason basket
Jessica

Transcribe dive notebook
Jessica

RNA later
Gilbert and Karen and Carlo

DNA extractions
Rick, Karen and Gilbert

qPCR
Alex and Olga (ALR and Gilbert)

Biobox cleaning and readiness for samples
Jessica and Nick

Please offer to help.. even if you are not assigned a specific task.

Scientific personnel/responsibilities

Chief Scientist Anna-Louise Reysenbach
Jason Group Alberto Collasius, Expedition Leader

Watches – Pilot/Engineer
Scott Hansen/Casey Agee
Akel Kevis-Stirling/ Chris Lathan
Korey Verhein/Scott McCue
Jimmy Varnum/ James Pelowski

Watch leaders/ Virtual Van/ DVD
Anna-Louise Reysenbach/Karen Houghton or Stephane L'Haridon/ Olga Podosokorskaya or Morgan Haldeman
Gilbert Flores/John Kelley or Carlo Carere/Nick Rhoades or Wan-Yen Cheng
Jeff Seewald or Rick Davis/Niya Grozeva or Guy Evans/ Alexander Merkel or Jessica Hardwicke

Data Managers
Geology: Guy Evans
Micro: John and Nick, Gilbert – master sheet and sulfide sheets

Microbiology Anna-Louise Reysenbach, Gilbert Flores, Stephane L'Haridon, Rick Davis, Carlo Carere, John Kelley, Karen Houghton, Olga Podosokorskaya, Alexander Merkel, Nick Rhoades, Jessica Hardwicke

Geology Jeff Seewald, Sean Sylva, Wan-Yen Cheng, Niya Grozeva, Guy Evans

Scientific Marine Technician Brett Hembrough

Ship's personnel/responsibilities
Captain Tom Desjardins
1st Officer Eric Wakeman, *2nd Officer* Heather Galiher, *3rd Officer* Todd Crump

Chief Engineer Jack Healy , *1st Asst Engr* Matthew Peer, *2nd Asst Engr* John Clifford, *3rd Asst Engr* Sue Swader

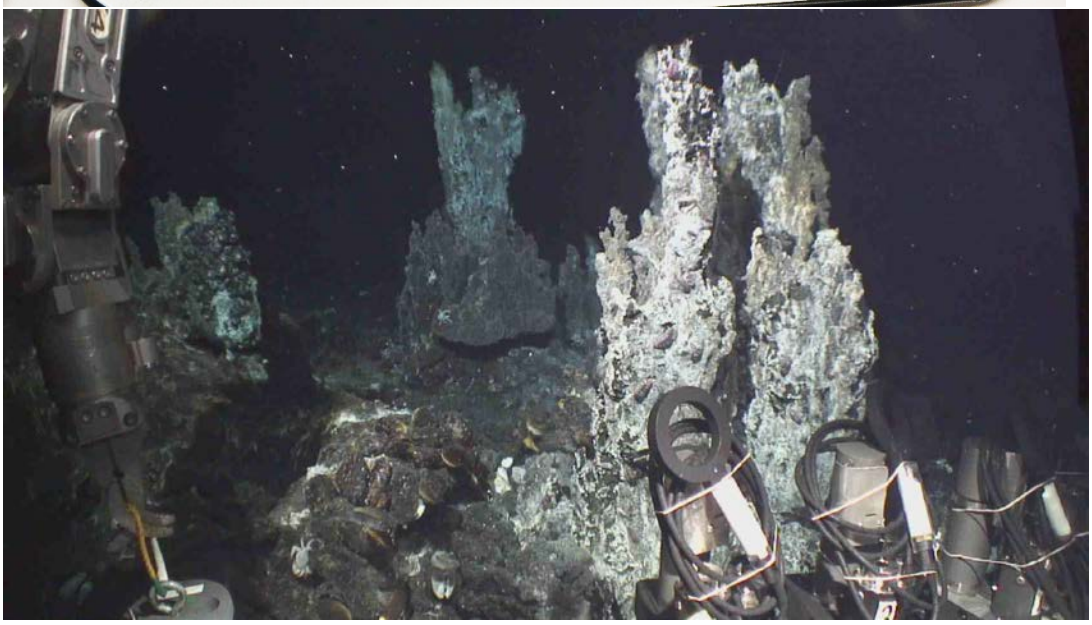
Able Seaman Sandor Vinkovits, Derek Haddon, Steve Lewis

Oilers Tony Sullivan, Bob Juhasz, Mike Gaylord, Scott Myers
Wiper Joey Brown

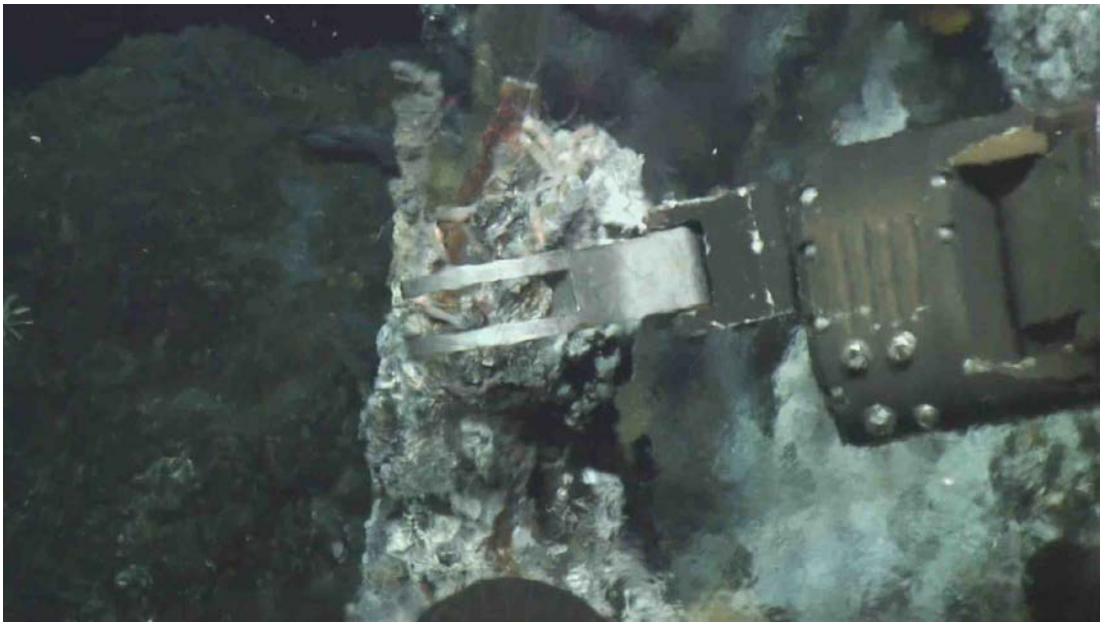
Chief Steward Mark, *Second Cook* Mark

Boatswain Gary Curry
OS Dominique Cummings
Electrician Manny Elliot
SCG Tech Bud Hale

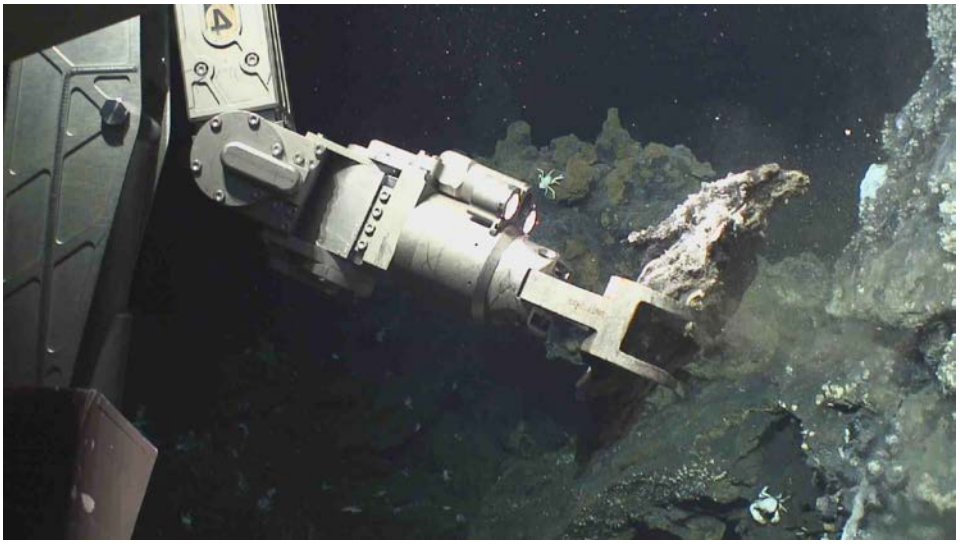
Lowering number	Site number	Sample name	Aliquots	Preliminary Temp. (°C)	RNA Later
J2-815	J2-815-1	J2-815-1-R1	21-38	284	
Event #	**Chemistry**	**Notes:**			
332	W1-IGT8 W2-IGT7	Lat: 20 45.661689 S Long: 176 11.458083 W Head: 32.66			
Description: Removed 2-3mm from the outer structure. White crust and little alvinellid worms. Two samples taken for DNA and culturing					



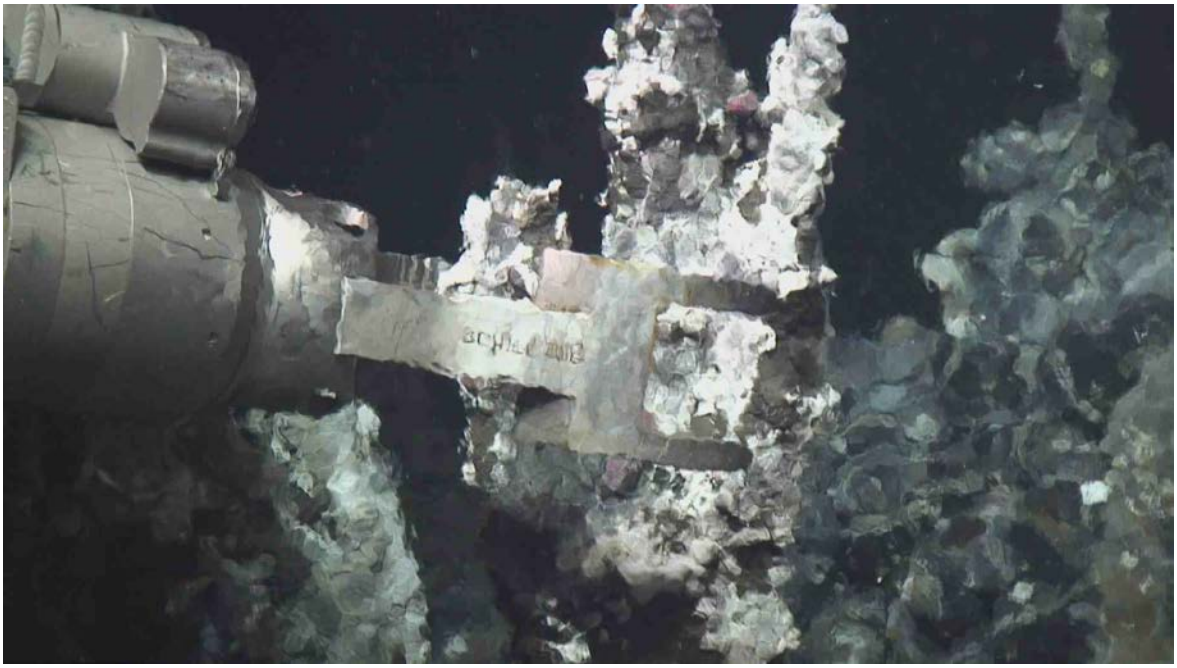
Lowering number	Site number	Sample name	Aliquots	Preliminary Temp. (°C)	RNA Later
J2-815	J2-815-5	J2-815-5-R1	39-52	298.87	-
Event #	**Chemistry**	**Notes:**			
668	W1-IGT3 W2-IGT1	Lat: 20 45.655299 S Long: 176 11.452701 W Heading: 230.31			
Description: Taking 2-3mm outer layer: DHEV2 layer. Hard Chalcopyrite lined interior and smelly stinky sulfide.					



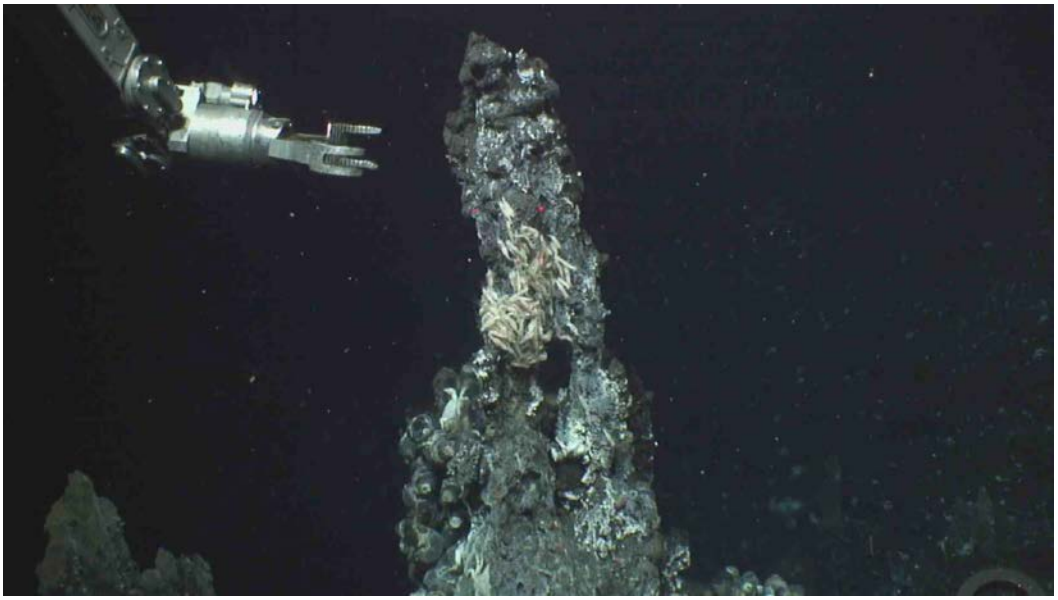
Lowering number	Site number	Sample name	Aliquots	Preliminary Temp. (°C)	RNA Later
J2-815	J2-815-6	J2-815-6-R1	60-62	303 C	Yes
Event #	**Chemistry**	**Notes:**			
781	-	Lat: 20 45.652784 S Long: 176 11.448998 W Heading: 32.71			
Description: RNA Later sample photographed and placed in fresh RNA later. Stored in 4C					



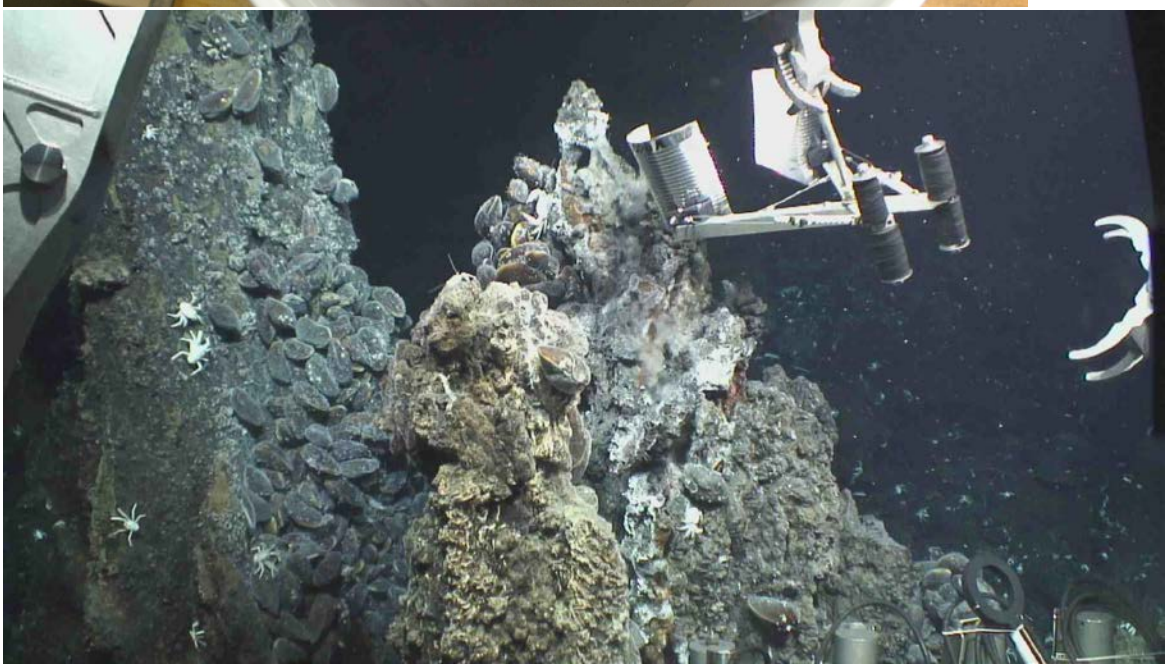
Lowering number	Site number	Sample name	Aliquots	Preliminary Temp. In	RNA Later
J2-815	J2-815-7	J2-815-7-R1	3-20	268.1C	-
Event #	**Chemistry**	**Notes:**			
833	-	Lat: 20 45.664469 S Long: 176 11.439024 W Heading: 32.66			
Description: Very amorphous minerals, little turrets, anhydrite, very friable. Whole structure was homogenized. Walls were 2-3mm thick.					



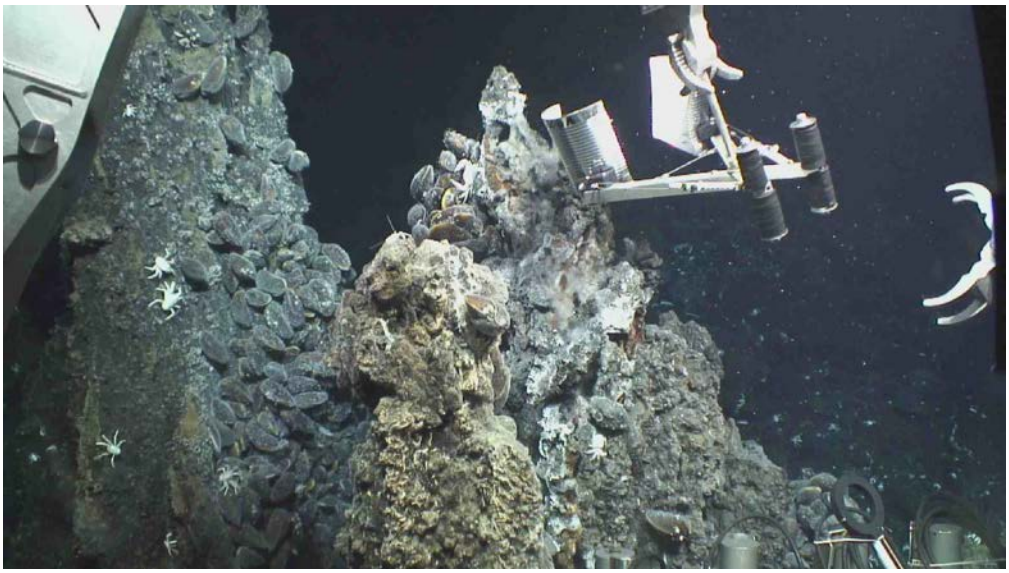
Lowering number	Site number	Sample name	Aliquots	Preliminary Temp. (°C)	RNA Later
J2-815	J2-815-9	J2-815-9-R1	81-93	289	-
Event #	**Chemistry**	**Notes:**			
1019	W1-IGT4 W2-IGT2	Lat: 20 45.775893 S Long: 176 11.482082 W Heading: 155.99			
Description: Thin walled with lots of anhydrite. A hard structure with sphalerite in the main conduit. Sampled outer crust where possible.					



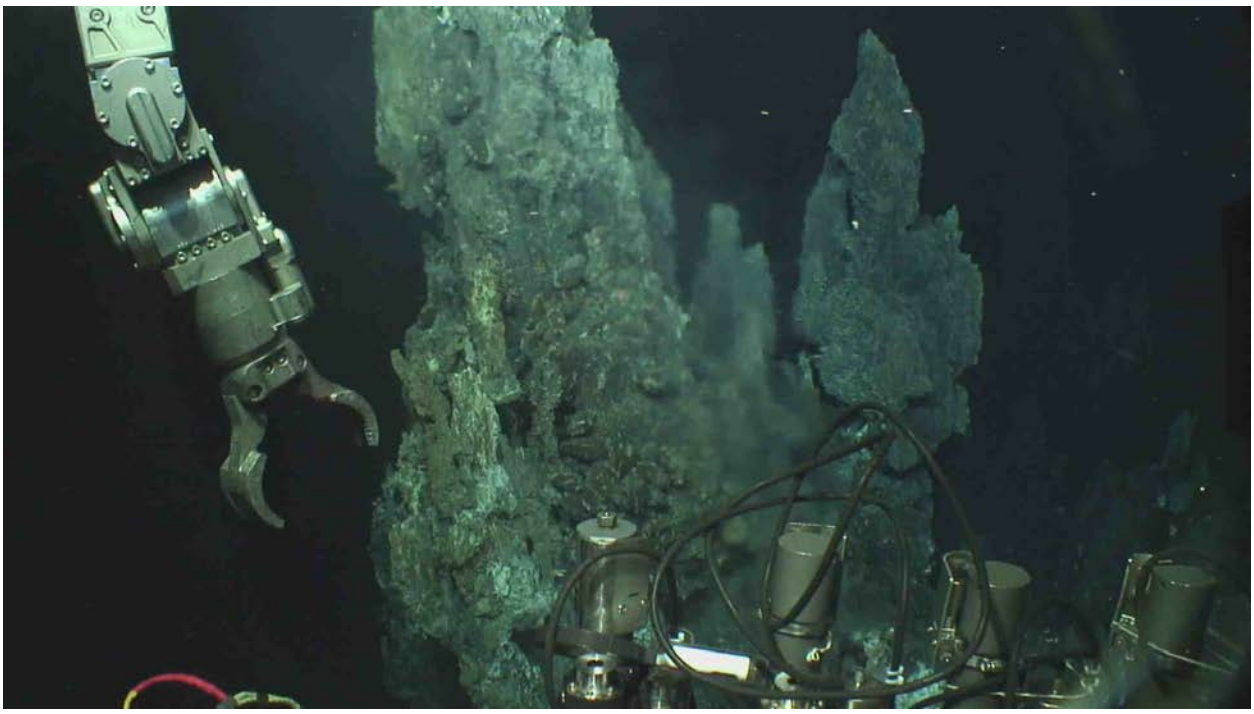
Lowering number	Site number	Sample name	Aliquots	Preliminary Temp. (°C)	RNA Later
J2-815	J2-815-11	J2-815-11-R1	120-124	-	Yes
Event #	**Chemistry**	**Notes:**			
1171	-	Lat: 20 45.791651 S Long: 176 11.497201 W Heading: 232.96			
Description: Outer wall of structure contained lots of anhydrite and very little biomass. Sample 120 was a white biofilm that was floating in the RNA Later container.					



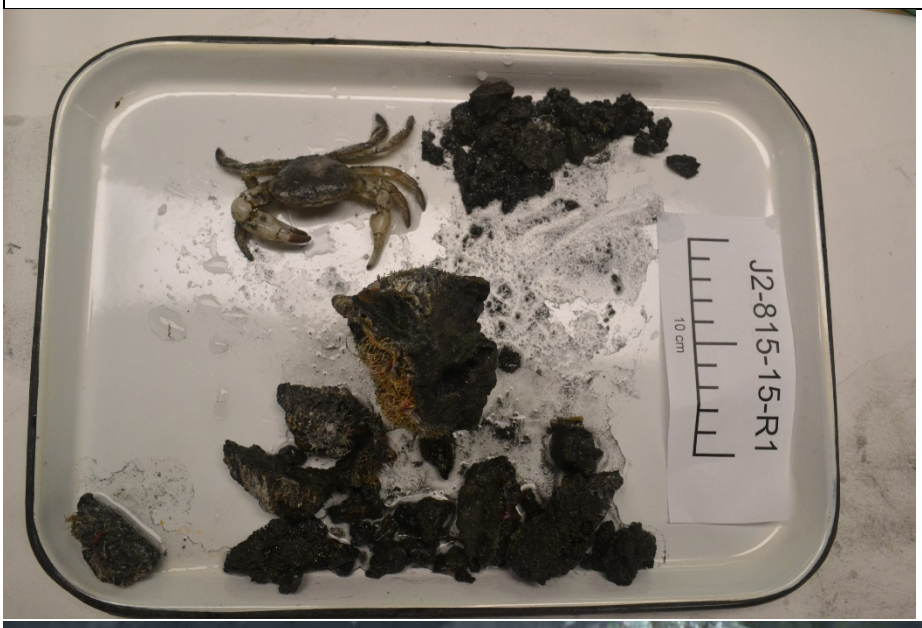
Lowering number	Site number	Sample name	Aliquots	Preliminary Temp. (°C)	RNA Later
J2-815	J2-815-11	J2-815-11-R2	63-80	264.7	-
Event #	**Chemistry**	**Notes:**			
1179	W1-IGT5 W2-IGT6	Lat: 20 45.792484 S Long: 176 11.498248 W Heading: 339.85			
Description: Beehive structure. Little turrets formed, though mostly anhydrite mush. Whole sample will be homogenized.					



Lowering number	Site number	Sample name	Aliquots	Preliminary Temp. (°C)	RNA Later
J2-815	J2-815-14	J2-815-14-R1	0	287	-
Event #	**Chemistry**	**Notes:**			
1851	-	Lat: 20 45.952757 S Long: 176 11.579400 W Heading: 339.37			
Description: Lots of animals, worms and worms casings. Hard structure.					



Lowering number	Site number	Sample name	Aliquots	Preliminary Temp. (°C)	RNA Later
J2-815	J2-815-15	J2-815-15-R1	119,116	220	-
Event #	**Chemistry**	**Notes:**			
1930	-	Lat: 20 45.953329 S Long: 176 11.586914 W Heading: 333.63			
Description: Many animals, worms, on exterior of sulfide. Not a great sample.					



Lowering number	Site number	Sample name	Aliquots	Preliminary Temp. (°C)	RNA Later
J2-815	J2-815-16	J2-815-16-R1	97-106	-	-
Event #	**Chemistry**	**Notes:**			
1962	-	Lat: 20 45.954715 S Long: 176 11.594939 W Heading: 234.91			
Description: A flange sample, with a tough outer crust. Covered in animals.					

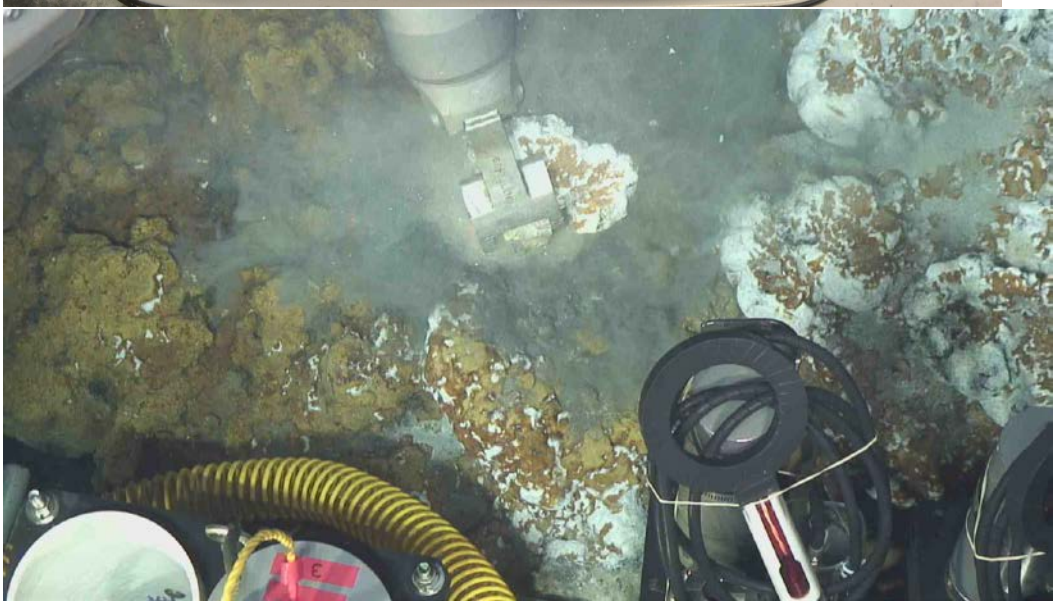
Lowering number	Site number	Sample name	Aliquots	Preliminary Temp. (°C)	RNA Later
J2-815	J2-815-ABE	J2-815-ABE-R1	108-115	-	-
Event #	**Chemistry**	**Notes:**			
-	-	ABE Vent Field			

Description: Pieces from the Jason basket, there is no temperature or event. The outer layer was soft and was homogenized for cultures.

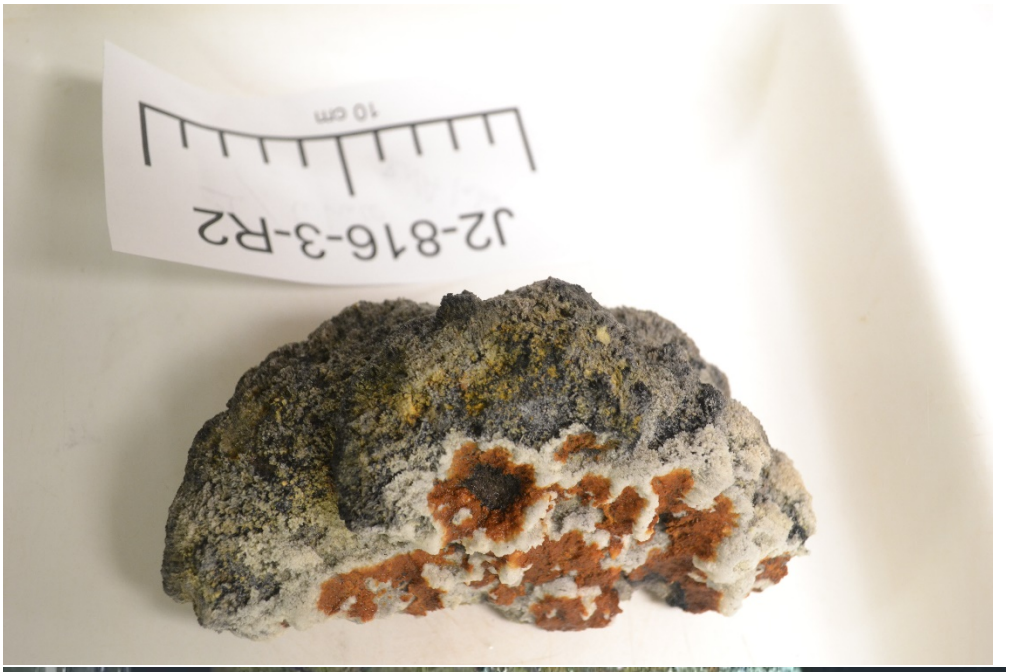
Lowering number	Site number	Sample name	Aliquots	Preliminary Temp. (°C)	RNA Later
J2-816	J2-816-3	J2-816-1-B1	202-205	-	-
Event #	**Chemistry**	**Notes:**			
2014		Lat: 22 10.771032 Long: 176 36.121758 Head: 110.72			
Description: Confirmed to contain manganese on board the ship.					

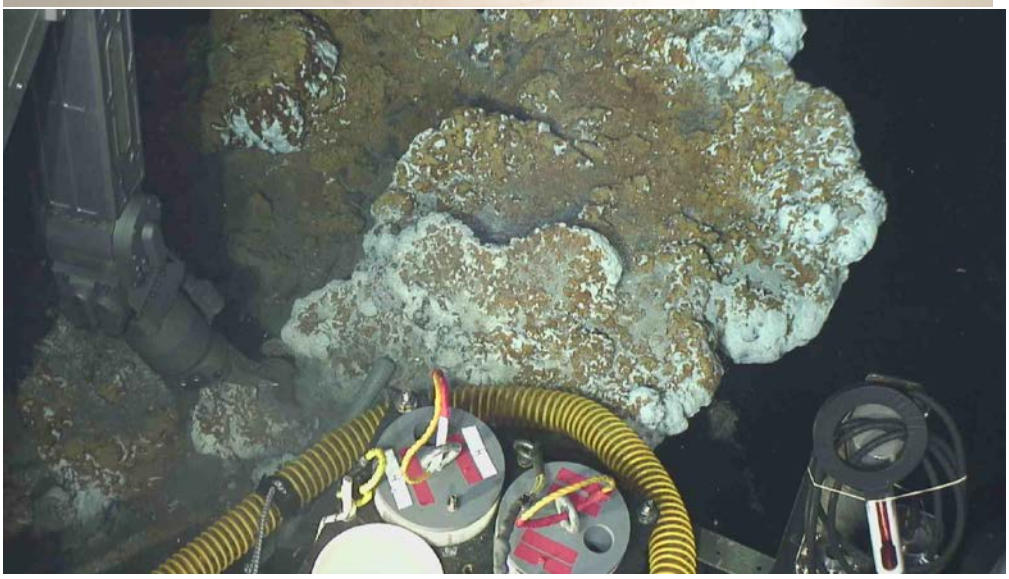
Lowering number	Site number	Sample name	Aliquots	Preliminary Temp. (°C)	RNA Later
J2-816	J2-816-3	J2-816-3-B1	185-201	-	-
Event #	**Chemistry**	**Notes:**			
2232		Lat: 22 10.811268 S Long: 176 36.071424 W Head: 88.14			
Description: Slurp from the toilet bowl area at Mariner.					

Lowering number	Site number	Sample name	Aliquots	Preliminary Temp. (°C)	RNA Later
J2-816	J2-816-3	J2-816-3-R1	125-160,206-208	139	-
Event #	**Chemistry**	**Notes:**			
2152	-	Lat: 20 10.810572 Long: 176 36.069006 Hdg: 305.00			
Description: The outer 2-3 mm were subsampled. Subsample #1 contained the white biofilm. Subsample #2 contained the orange biofilm. The remaining material was combined with 3-R1 for culturing (137-148). Culture aliquots 206-208 included samples from 3-R1, 3-R3, and water from 3-B1.					

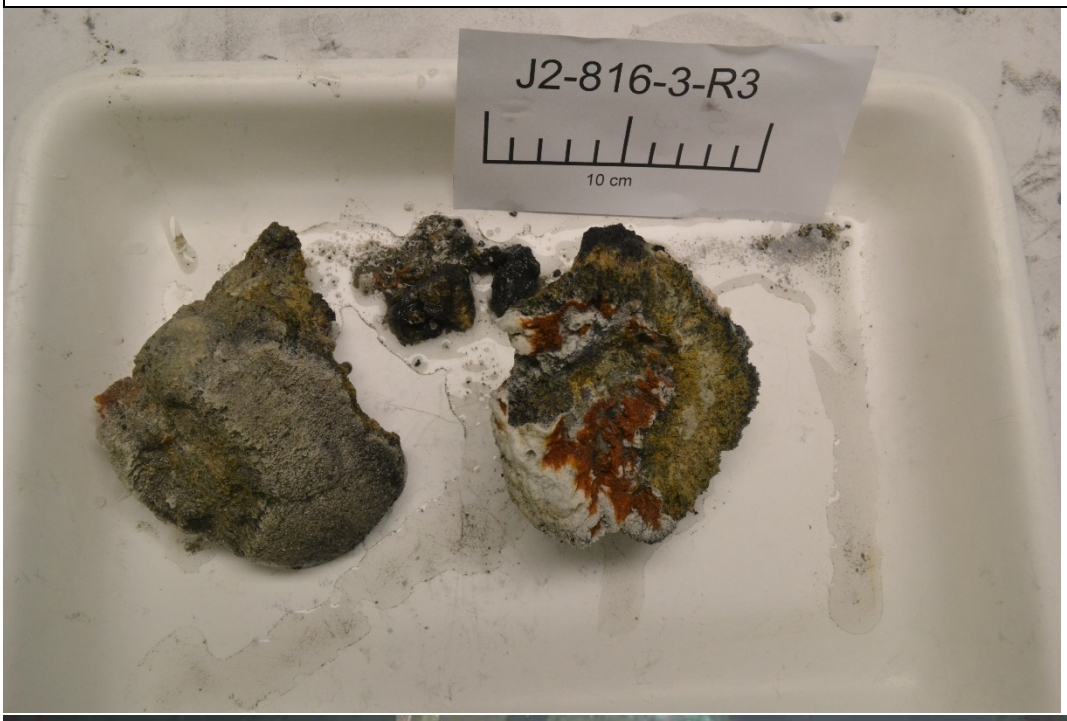


Lowering number	Site number	Sample name	Aliquots	Preliminary Temp. (°C)	RNA Later
J2-816	J2-816-3	J2-816-3-R2	258-261	139	Yes
Event #	**Chemistry**	**Notes:**			
2167	W1-IGT6, W2-IGT5	Lat: 22 10.909546 S Long: 176 36.069216 W Hdg: 288.34			
Description: RNA Later sample. Subsample 1 included the side most of the orange biofilm. Subsample 2, included the other side without orange biofilm, and mostly white.					

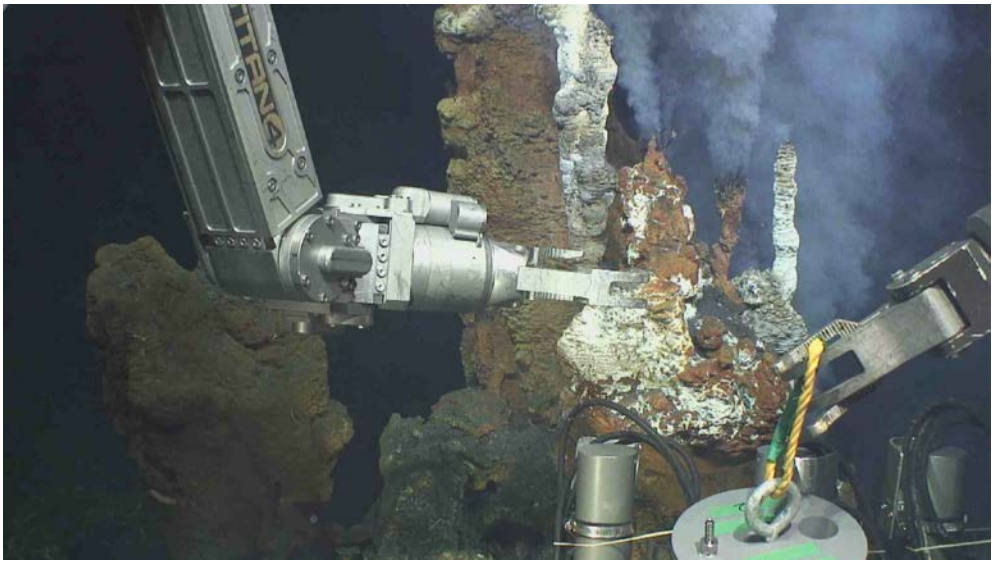




Lowering number	Site number	Sample name	Aliquots	Preliminary Temp. (°C)	RNA Later
J2-816	J2-816-3	J2-816-3-R3	161-184	139	-
Event #	**Chemistry**	**Notes:**			
2213		Lat: 22 10.809504 S Long: 176 36.071424 W Hdg: 178.78			
Description: The mostly white was subsample #1. The orange areas were subsample #2. For both areas the outer 2-3mm were subsampled.					

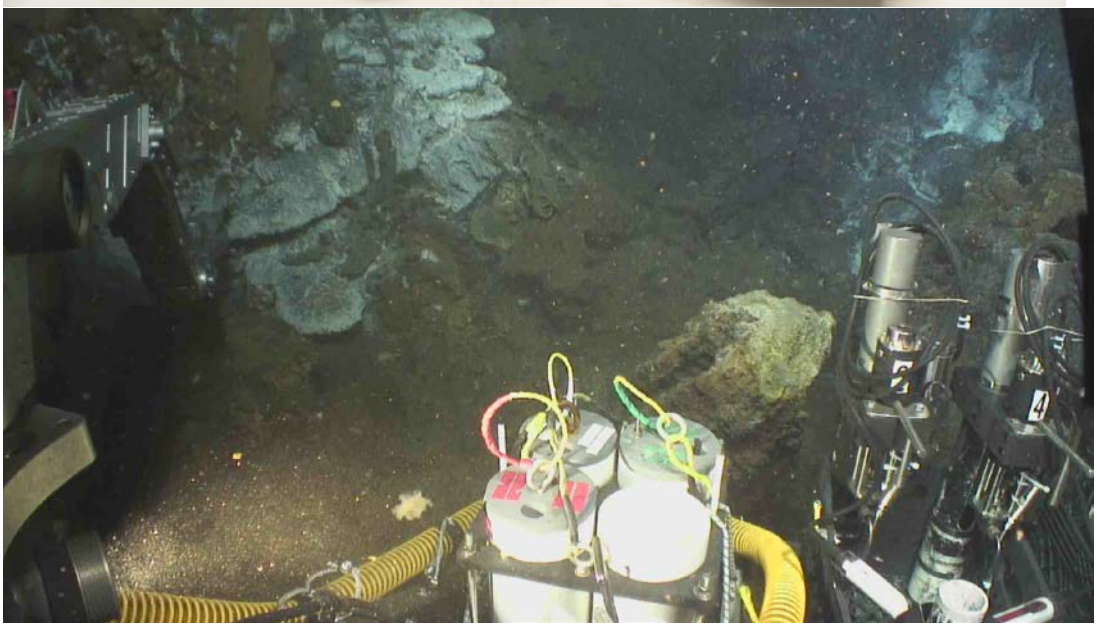


Lowering number	Site number	Sample name	Aliquots	Preliminary Temp. (°C)	RNA Later
J2-817	J2-817-2	J2-817-2-R1	245-257	319	-
Event #	**Chemistry**	**Notes:**			
2331	W1-IGT6, W2-IGT5	Lat: 22 10.808310 S Long: 176 36.074688 W Hdg: 317.33			
Description: Large defined structure with chalcopyrite lined conduit. The structure is very hard with orange rust. Around 2-3mm scrape of all soft white areas with black under layer and crust like paint. Subsample 2 included further scrapings					

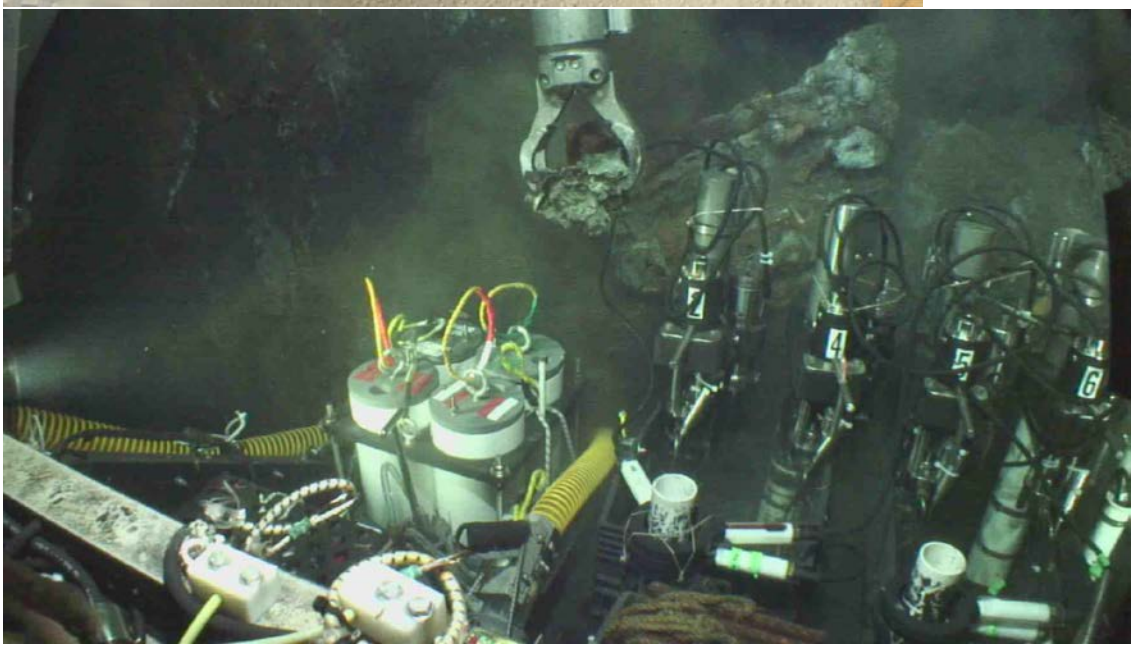


Lowering number	Site number	Sample name	Aliquots	Preliminary Temp. (°C)	RNA Later
J2-817	J2-817-3	J2-817-3-R1	231-244	-	-
Event #	**Chemistry**	**Notes:**			
2502	-	Lat: 22 10.803210 S Hdg: 180.47 Long: 176 36.095352 W W1-IGT6 and W2-IGT5 were taken from same mound with a max temperature of 319°C. However, the flange itself temperature was not recorded			

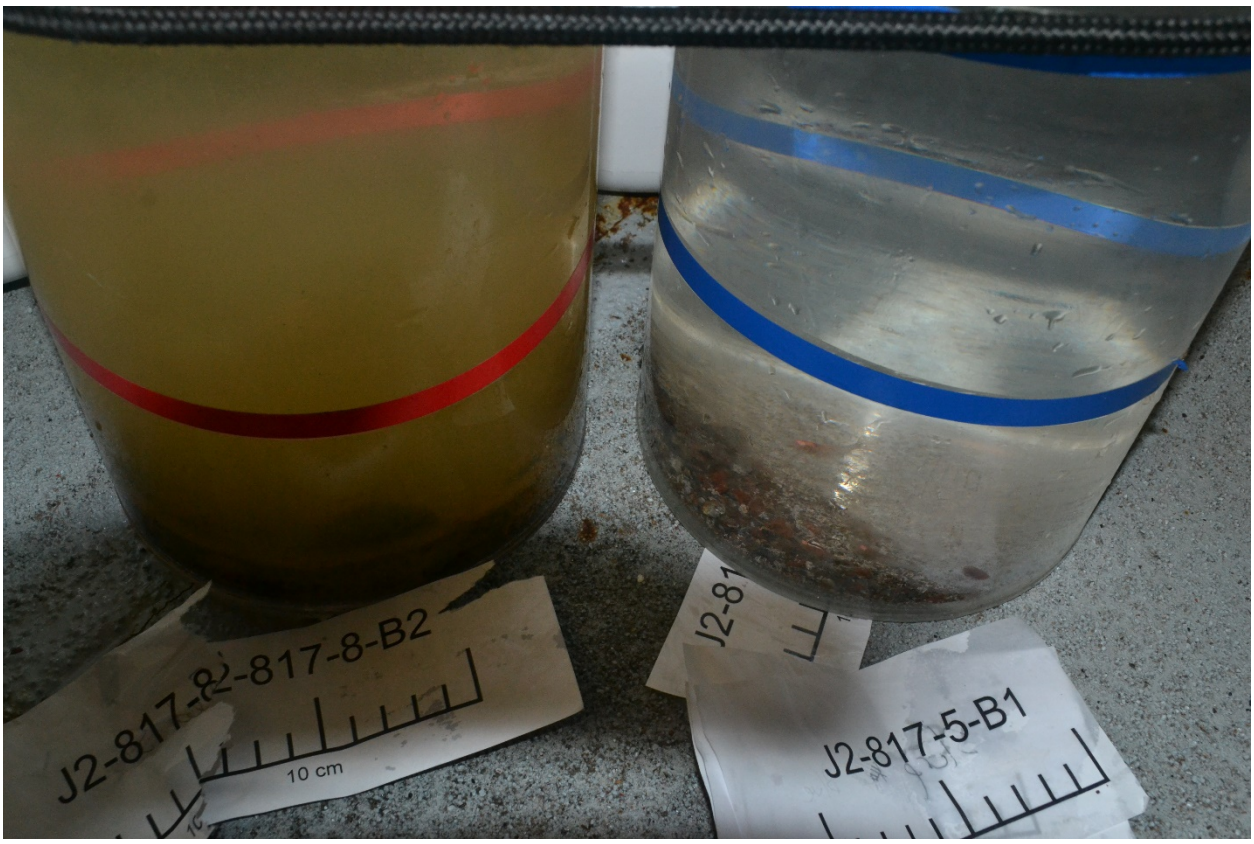
Description: Two pieces of flange were collected with a white biofilm. The outer 4mm were scraped off. Noted as a primo sample. Subsample #2 was a second pass through to collect more biomass for culture tubes.



Lowering number	Site number	Sample name	Aliquots	Preliminary Temp. (°C)	RNA Later
J2-817	J2-817-4	J2-817-4-R1	340	-	-
Event #	**Chemistry**	**Notes:**			
2554	-	Lat: 22 10.832052 S Long: 176 36.120654 Hdg: 198.15			
Description: Complex open conduit with thick chalcopyrite lining. Active open conduit with thick lining to Guy for mineralogy. Inactive open conduit to Anna-Louise for outreach.					



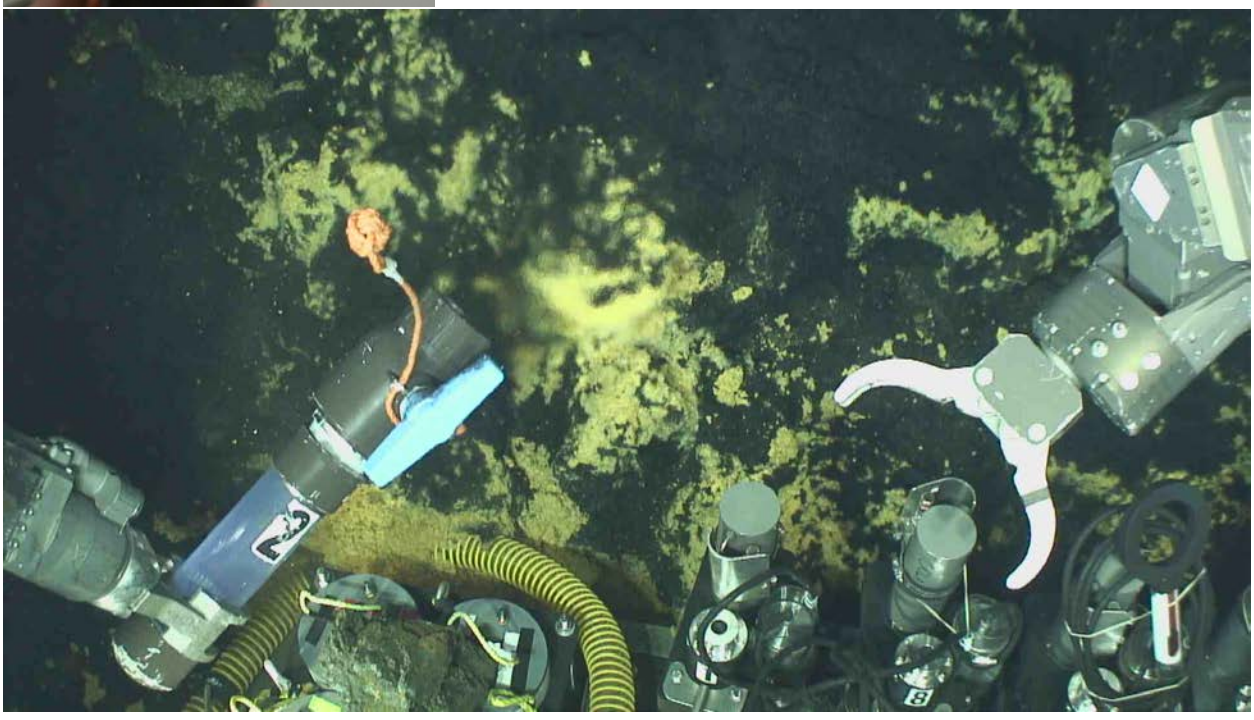
Lowering number	Site number	Sample name	Aliquots	Preliminary Temp. (°C)	RNA Later
J2-817	J2-817-5	J2-817-5-B1	341-349 360-367	9	-
Event #	**Chemistry**	**Notes:** Lat: 22 10.832052 S Long: 176 36.120654 W Head: 178.24			
2665					
Description:					



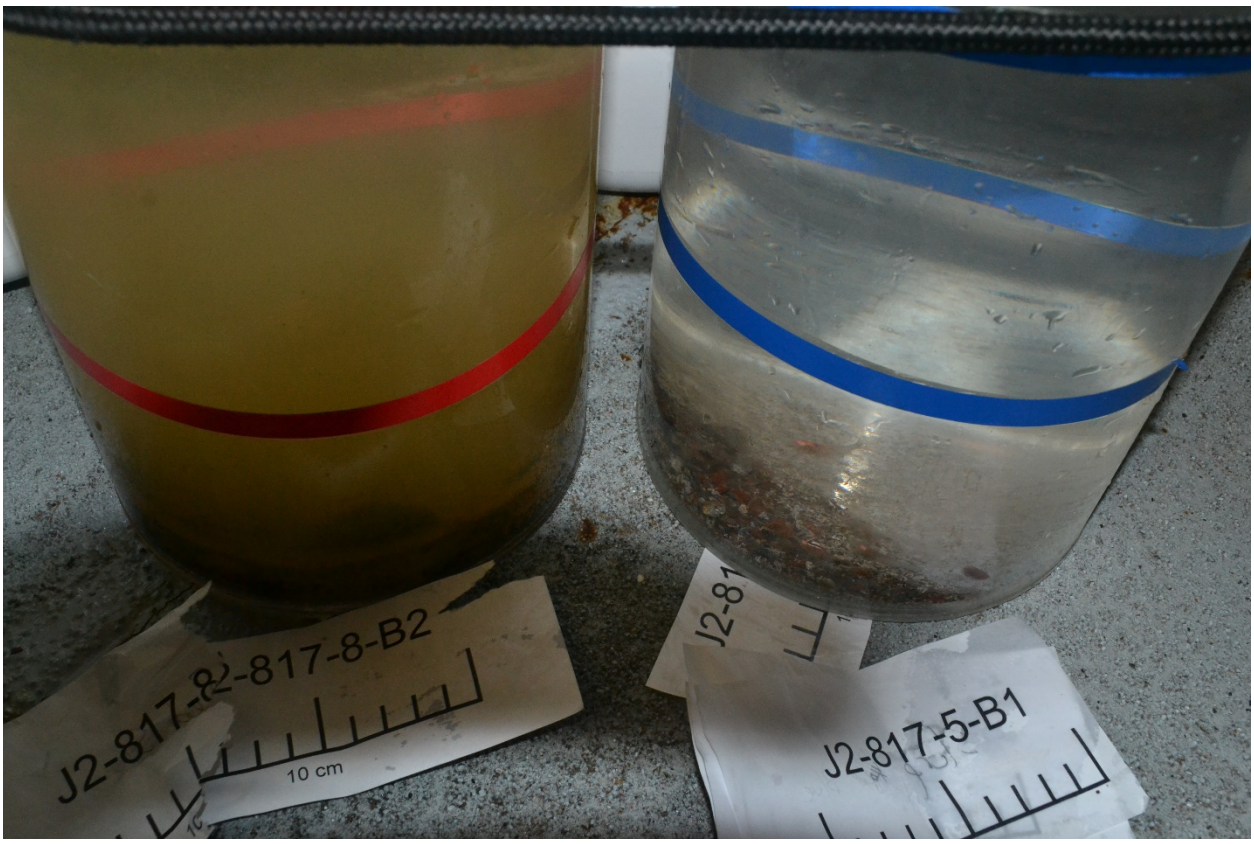
Lowering number	Site number	Sample name	Aliquots	Preliminary Temp. (°C)	RNA Later
J2-817	J2-817-6	J2-817-6-R1	209-230	232	-
Event #	**Chemistry**	**Notes:**			
2736	-	Lat: 22 10.839822 S Long: 176 36.120204 W Hdg: 272.82			
Description: Chimney structure, long spike about 20cm in length. Several smaller wall pieces with white areas. Removed 3-5mm of outer wall biofilm for subsample 1. Small pieces were added for subsample 2 and homogenized.					

Lowering number	Site number	Sample name	Aliquots	Preliminary Temp. (°C)	RNA Later
J2-817	J2-817-7	J2-817-7-R1	280-291	363	-
Event #	**Chemistry**	**Notes:**			
2879	W1-IGT1 W2-IGT8	Lat: 22 10.814874 S Long: 176 36.121524 W Hdg: 157.33			
Description: Not a nice sample, but very hot sample. Lots of anhydrite on inside and rock was soft. No real apparent biofilm. Subsample #2 includes small pieces, then homogenized for serum tubes.					

Lowering number	Site number	Sample name	Aliquots	Preliminary Temp. (°C)	RNA Later
J2-817	J2-817-8	J2-817-8-B1	310-317	38.19	-
Event #	**Chemistry**	**Notes:**			
2976	-	Lat: 22 12.848940 S Long: 176 36.482706 W Head: 185.87			
Description: Scoop sample of manganese iron mat.					

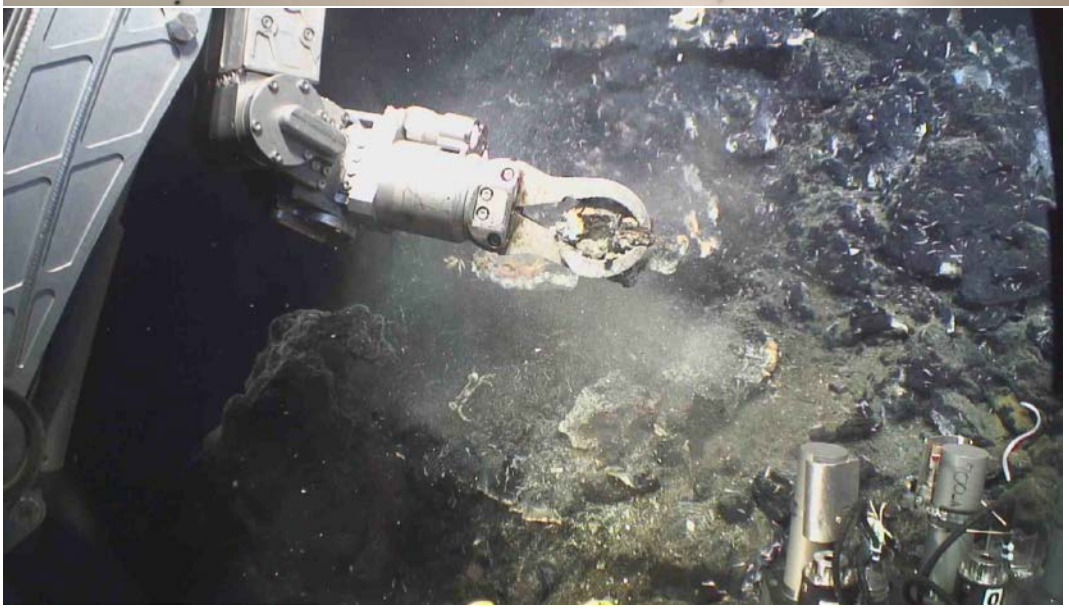


Lowering number	Site number	Sample name	Aliquots	Preliminary Temp. (°C)	RNA Later
J2-817	J2-817-8	J2-817-8-B2	350-359	38.19	-
Event #	**Chemistry**	**Notes:**			
2990		Lat: 22 12.841266 S Long: 176 36.480246 Head: 186.28			
Description: Slurps.					

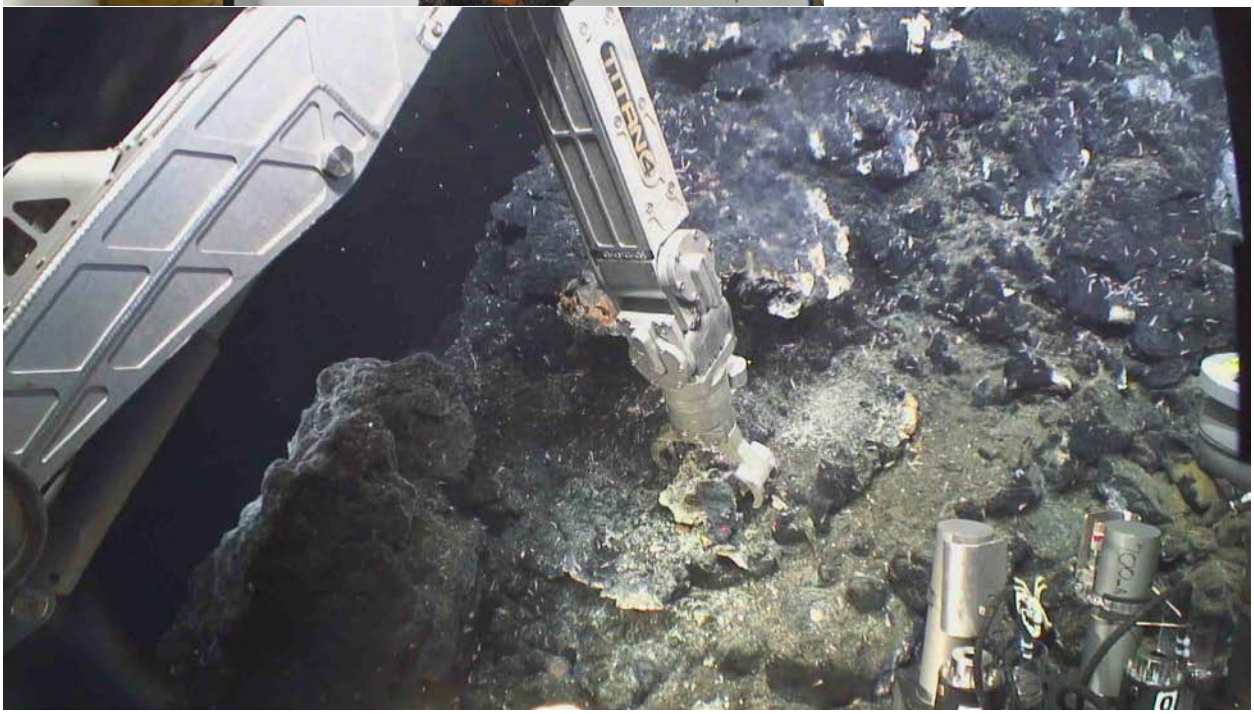


Lowering number	Site number	Sample name	Aliquots	Preliminary Temp. (°C)	RNA Later
J2-817	J2-817-10	J2-817-10-R1	262-275	116	-
Event #	**Chemistry**	**Notes:**			
3188	W1-IGT3 W2-IGT7	Lat: 22 12.941904 S Long: 176 36.516726 Hdg: 162.23			
Description: Rock sample contained mostly manganese (confirmed by color metric assay). Shrimp and polychaete worms on rock when collected. Some anhydrite was also observed.					

Lowering number	Site number	Sample name	Aliquots	Preliminary Temp. (°C)	RNA Later
J2-817	J2-817-10	J2-817-10-R2	299-308	116	-
Event #	**Chemistry**	**Notes:**			
3247	W1-IGT3 W2-IGT7	Lat: 22 12.942024 Long: 176 36.516612 Hdg: 160.3			
Description: Flange sample with lots of manganese. Many small broken fragments.					



Lowering number	Site number	Sample name	Aliquots	Preliminary Temp. (°C)	RNA Later
J2-817	J2-817-10	J2-817-10-R3	296-298	116	Yes
Event #	**Chemistry**	**Notes:**			
3254	W1-IGT3 W2-IGT7	Lat: 22 12.942030 Long: 176 36.516606 Hdg: 160.23			
Description: RNA Later sample. No presoak, removed outer layer+- into cryovials with RNA later and leave at 4°C overnight. Lots of pyrite and manganese. Flange sample, mostly scraping red/orange layer. The white layer is very hard.					



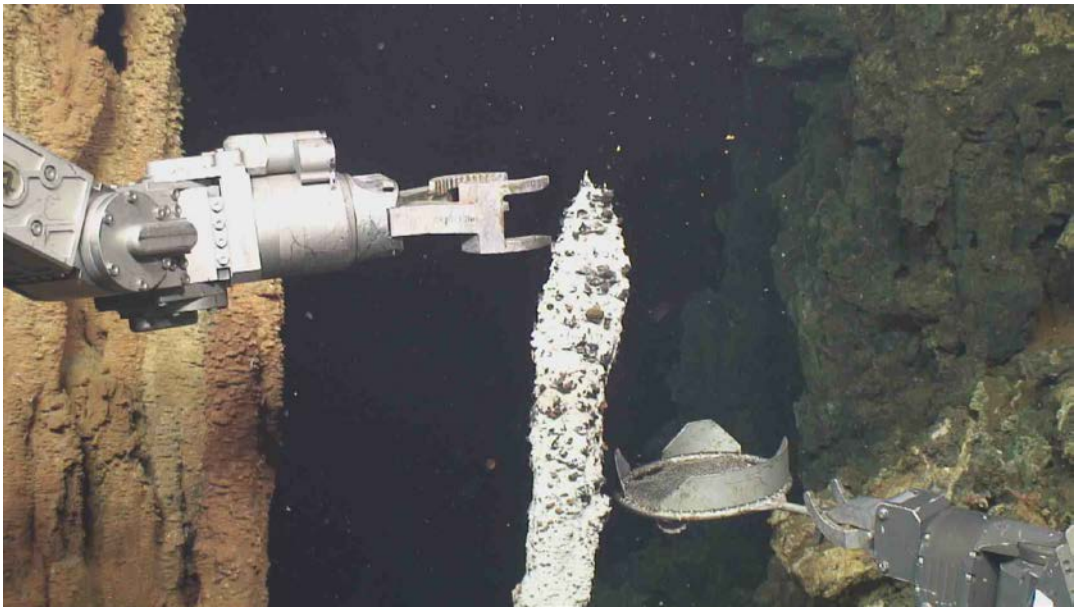
Lowering number	Site number	Sample name	Aliquots	Preliminary Temp. (°C)	RNA Later
J2-817	J2-817-10	J2-817-10-R4	336-339	116	-
Event #	**Chemistry**	**Notes:**			
3275	W1-IGT3 W2-IGT7	Lat: 22 12.942108 S Long: 176 36.516660 W Hdg: 159.15			
Description: Large flange with bivalves on the black side. The black manganese layer was scraped for culturing. The other side was covered with 'pyrite' crystals. The lip, red area, was also sampled.					

Lowering number	Site number	Sample name	Aliquots	Preliminary Temp. (°C)	RNA Later
J2-817	J2-817-11	J2-817-11-R1	318-335	361	-
Event #	**Chemistry**	**Notes:**			
3408	W1-IGT5B W2-IGT6B	Lat: 22 10.814592 S Long: 176 36.117486 W Hdg: 25.85			
Description: Many chunks of rock, removing outer 4mm. Chalcopyrite.					

Lowering number	Site number	Sample name	Aliquots	Preliminary Temp. (°C)	RNA Later
J2-818	J2-818-1	J2-818-1-R1	368-372	-	-
Event #	**Chemistry**	**Notes:**			
3710	-	Lat: 22 10.838010 S Long: 176 36.128250 W Hdg: 301.49			
Description: Small chunks of sulfide chimneys, used for culturing.					

Lowering number	Site number	Sample name	Aliquots	Preliminary Temp. (°C)	RNA Later
J2-818	J2-818-2	J2-818-2-R1	412-429	-	-
Event #	**Chemistry**	**Notes:**			
3760	-	Lat: 22 10.840038 S Long: 176 36.131166 W Hdg: 359.27			

Description: Very hard structure, but softer than 818-3-R1 and 4-R1. Removed the outer 2-3mm of the white biofilm. The sample had a strong sulfide smell.



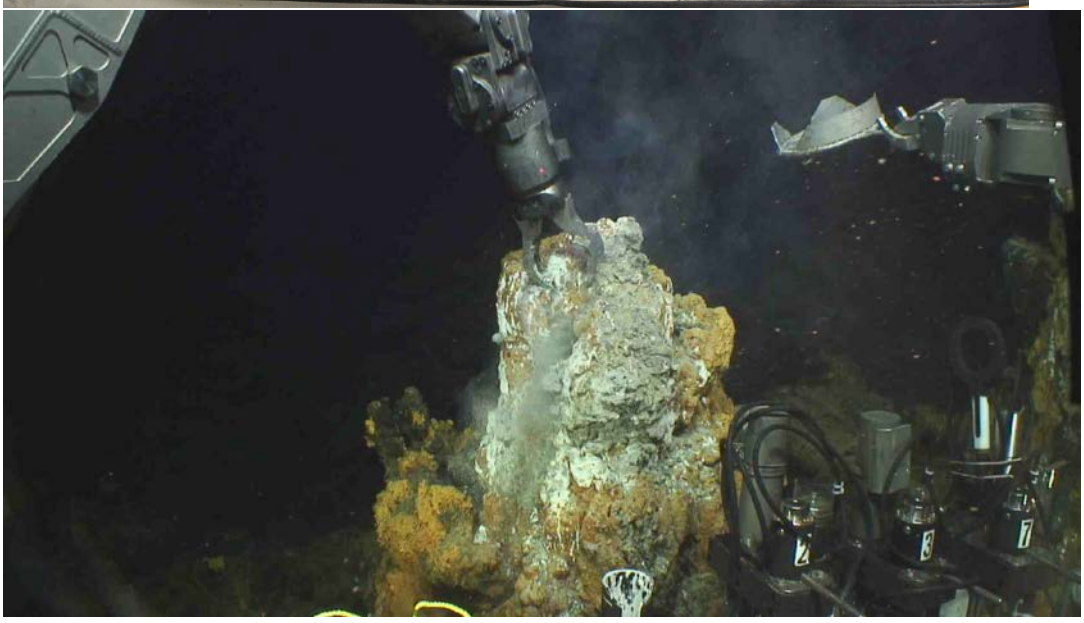
Lowering number	Site number	Sample name	Aliquots	Preliminary Temp. (°C)	RNA Later
J2-818	J2-818-3	J2-818-3-R1	394-403	300	-
Event #	**Chemistry**	**Notes:**			
3850	W1-IGT4 W2-IGT5	Lat: 22 10.844436 S Long: 176 36.122484 W Hdg: 88.68			
Description: Very hard structure, not easy to remove biofilm. White layer was scraped off for molecular. The remaining pieces were homogenized for culturing.					

Lowering number	Site number	Sample name	Aliquots	Preliminary Temp. (°C)	RNA Later
J2-818	J2-818-4	J2-818-4-R1	376-393	111	-
Event #	**Chemistry**	**Notes:**			
3938	-	Lat: 22 10.841742 S Long: 176 36.117990 W Hdg: 74.89			
Description: White biofilm. Some chalcopyrite-lined conduits.					

Lowering number	Site number	Sample name	Aliquots	Preliminary Temp. (°C)	RNA Later
J2-818	J2-818-4	J2-818-4-R2	519-533	307	Yes
Event #	**Chemistry**	**Notes:**			
4157	W1-IGT2 W2-IGT3	Lat: 22 10.837008 S Long: 176 36.114978 W Hdg: 107.16			
Description: Sulfide chimney broke apart during collection. The sample was almost to large for the biobox. The upper part of the chimney, with a white biofilm, was scraped for RNAlater vials. The lower section, orange was not kept.					

Lowering number	Site number	Sample name	Aliquots	Preliminary Temp. (°C)	RNA Later
J2-818	J2-818-6	J2-818-6-R1	466-474	82.8	Yes
Event #	**Chemistry**	**Notes:**			
4281	-	Lat: 22 10.805610 S Long: 176 36.068394 W Hdg: 233.01			
Description: RNAlater sample, the outer layer was soft and scraped off into cyrovials. The white and orange layers were kept together. Sample taken near Toilet Bowl.					

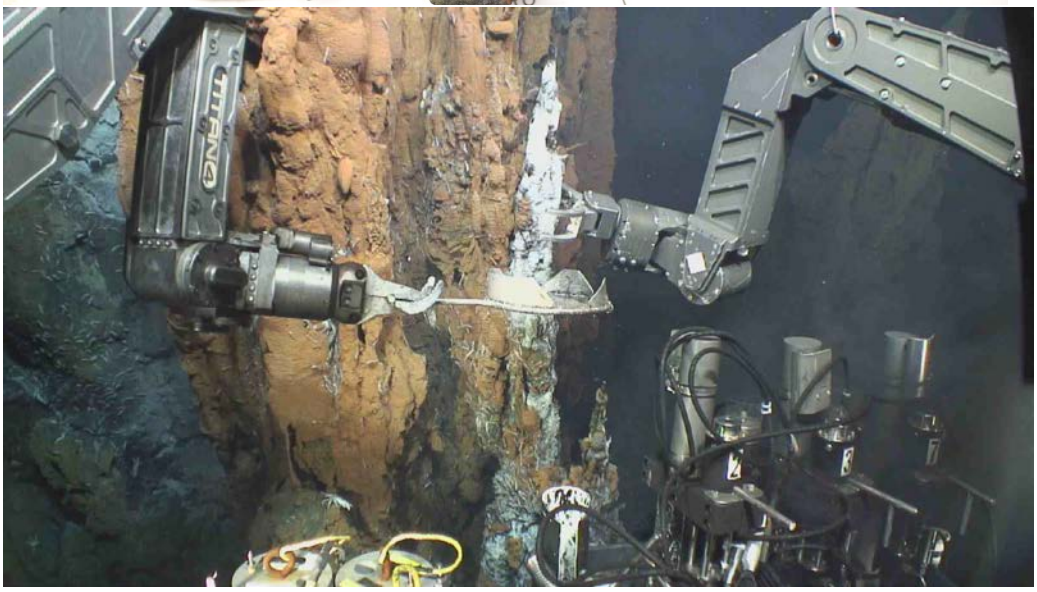
Lowering number	Site number	Sample name	Aliquots	Preliminary Temp. (°C)	RNA Later
J2-818	J2-818-6	J2-818-6-R2	430-439 441-447	82.8	-
Event #	**Chemistry**	**Notes:**			
4311	-	Lat: 22 10.805820 S Long: 176 36.068268 Hdg: 237.64			
Description: Removed 5mm of the soft outer layer. The white and orange areas were combined in this sample. A second subsample was used and homogenized for cultures.					



Lowering number	Site number	Sample name	Aliquots	Preliminary Temp. (°C)	RNA Later
J2-818	J2-818-8	J2-818-8-R1	475-489 494, 495	345	-
Event #	**Chemistry**	**Notes:**			
4429	W1-IGT7 W2 (Major)	Lat: 22 10.806366 Long: 176 36.090282 Hdg: 29.9			
Description: Nice sample, removed the outer 2-3mm. The soft outer layer is more porous, great thermophiles. Chalcopyrite conduit. Subsample 2 was deeper into the outer layer and used for culturing. The small pieces went to Guy and another piece with conduit was saved for Amy G.					

Lowering number	Site number	Sample name	Aliquots	Preliminary Temp. (°C)	RNA Later
J2-818	J2-819-9	J2-818-9-R1	-	340	-
Event #	**Chemistry**	**Notes:**			
4514	-	Lat: 21 59.453946 S Long: 176 34.143546 Hdg: 236.31			
Description: Complex open conduit with chalcopyrite lining.					

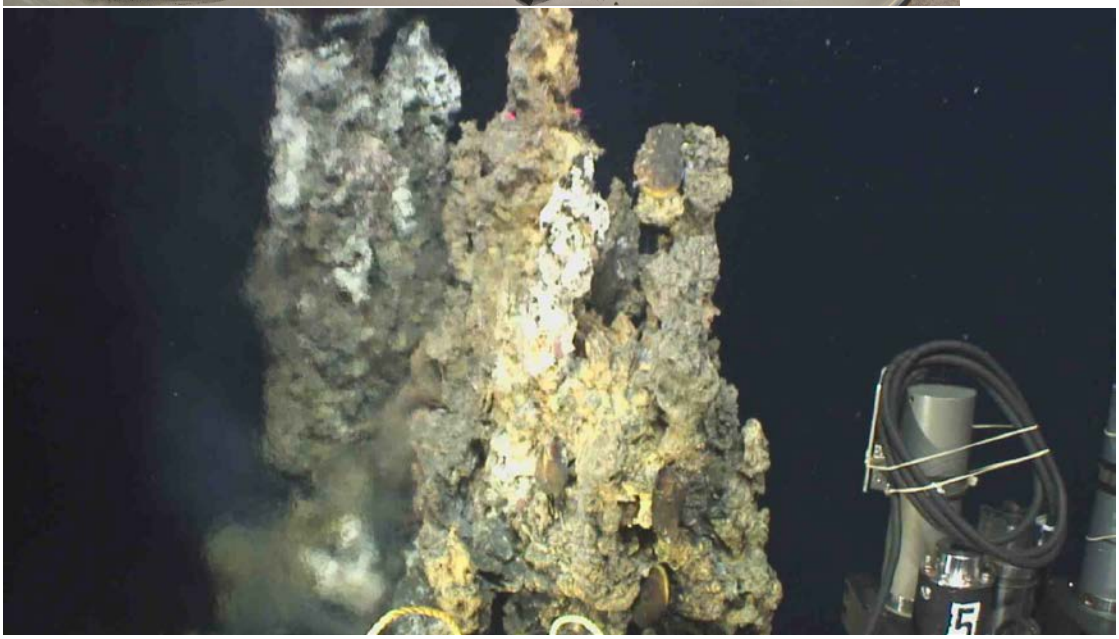
Lowering number	Site number	Sample name	Aliquots	Preliminary Temp. (°C)	RNA Later
J2-818	J2-818-10	J2-818-10-R1	448-465 493, 496, 497, 518	-	-
Event #	**Chemistry**	**Notes:**			
4533	-	Lat: 22 10.820502 S Long: 176 36.100164 Hdg: 302.11			
Description: The outer layer is thick bioflim that peeled away easily. The beehive-like sample has a strong sulfide odor. Enough was sampled for molecular work, then the remaining was homogenized for culturing. Traces of chalcopyrite in the inner sulfide.					



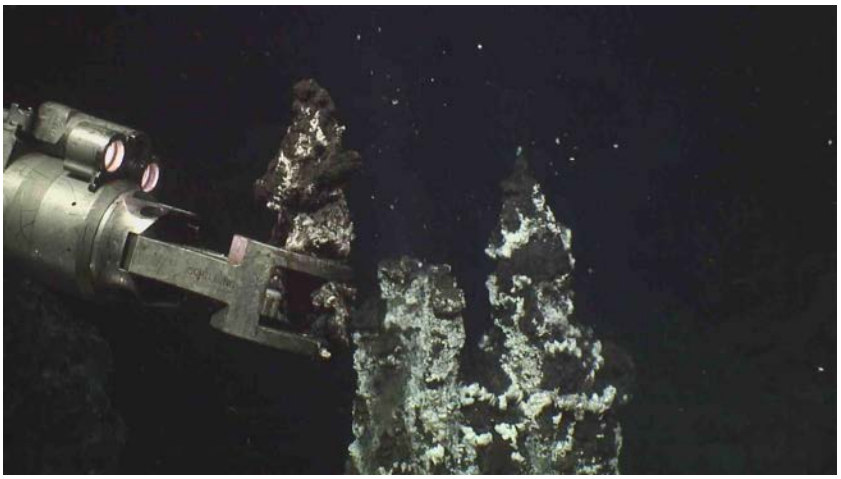
Lowering number	Site number	Sample name	Aliquots	Preliminary Temp. (°C)	RNA Later
J2-818	J2-818-11	J2-818-11-R1	498-517	-	-
Event #	**Chemistry**	**Notes:**			
4593	-	Lat: 22 10.811880 S Long: 176 36.066546 W Hdg: 32.21			

Description: Very soft extinct sulfides. Subsample #1 contains the black exterior. The interior, orange, was homogenized for subsample #2.

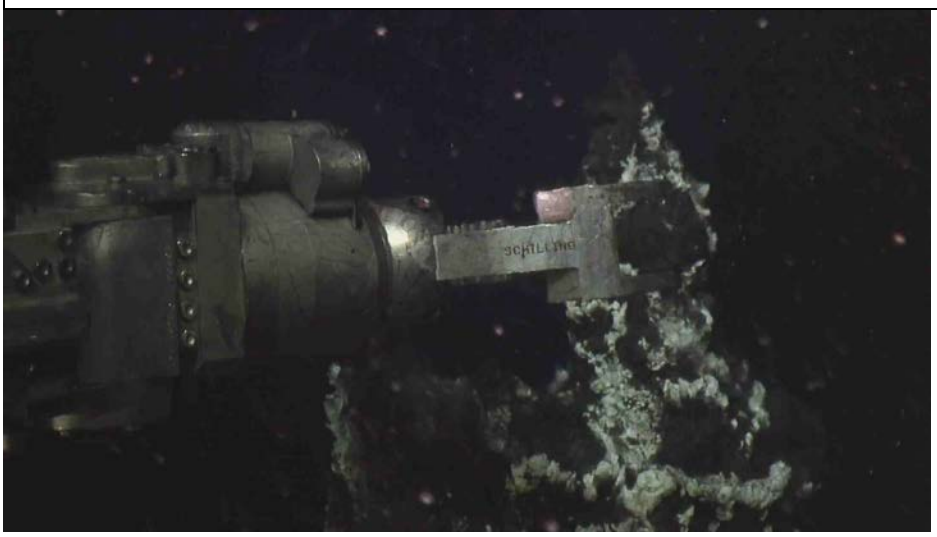
Lowering number	Site number	Sample name	Aliquots	Preliminary Temp. (°C)	RNA Later
J2-819	J2-819-1	J2-819-1-R1	555-564 579-582	168	-
Event #	**Chemistry**	**Notes:**			
4707	-	Lat: 21 59.453946 S Long: 176 34.143546 W Hdg: 236.31			
Description: Not a great sample. The whole sulfide was homogenized for molecular and culturing.					



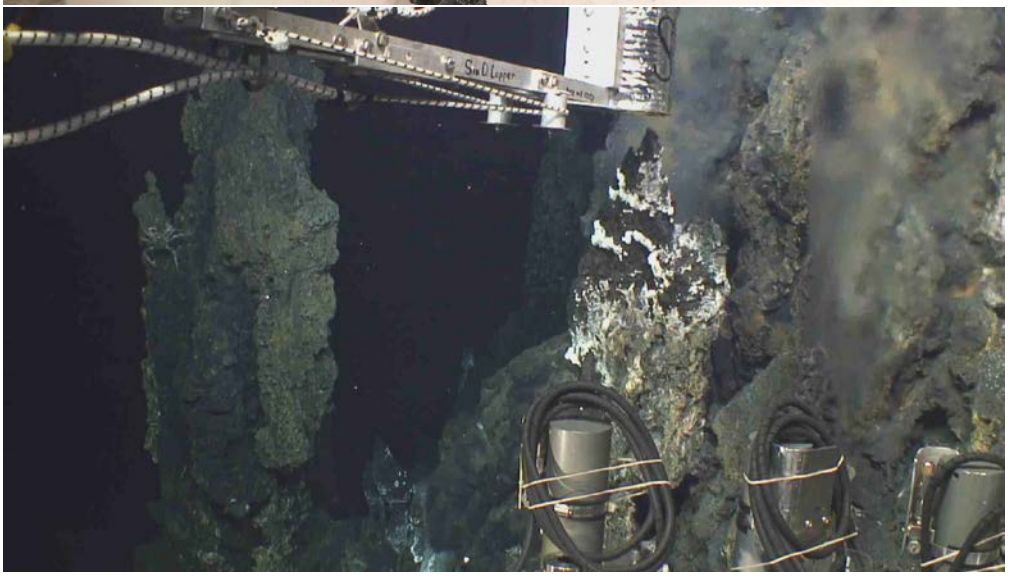
Lowering number	Site number	Sample name	Aliquots	Preliminary Temp. (°C)	RNA Later
J2-819	J2-819-2	J2-819-2-R1	583-600 623-640	260	-
Event #	**Chemistry**	**Notes:**			
4833	W1-IGT8 W2-IGT6	Lat: 21 59.413638 S Long: 176 34.139694 W Hdg: 261.13			
Description: Primo sample. The exterior has a nice crust, the interior was scraped out. Subsample #1 inside of chimney. Subsample #2 is exterior crust.					



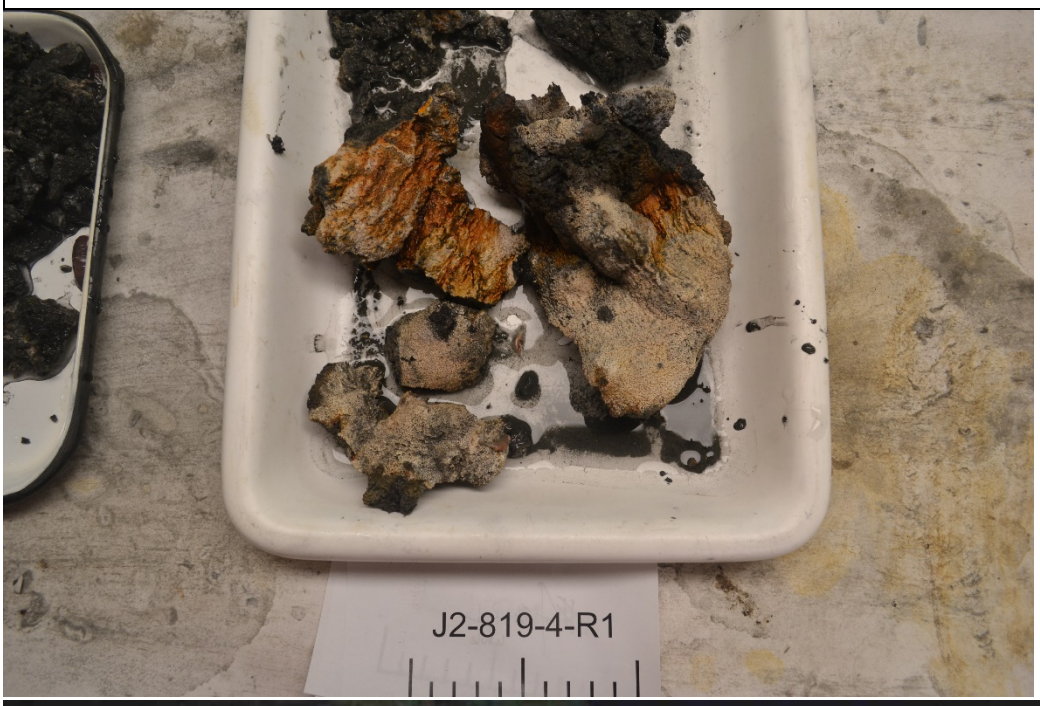
Lowering number	Site number	Sample name	Aliquots	Preliminary Temp. (°C)	RNA Later
J2-819	J2-819-2	J2-819-2-R2	601-622	260	Yes
Event #	**Chemistry**	**Notes:**			
4896	W1-IGT8 W2-IGT6	Lat: 21 59.413566 S Long: 176 34.140030 W Hdg: 260.17			
Description: RNAlater sample. White biofilm aliquoted into three cyrovials. The remaining sample was homogenized.					

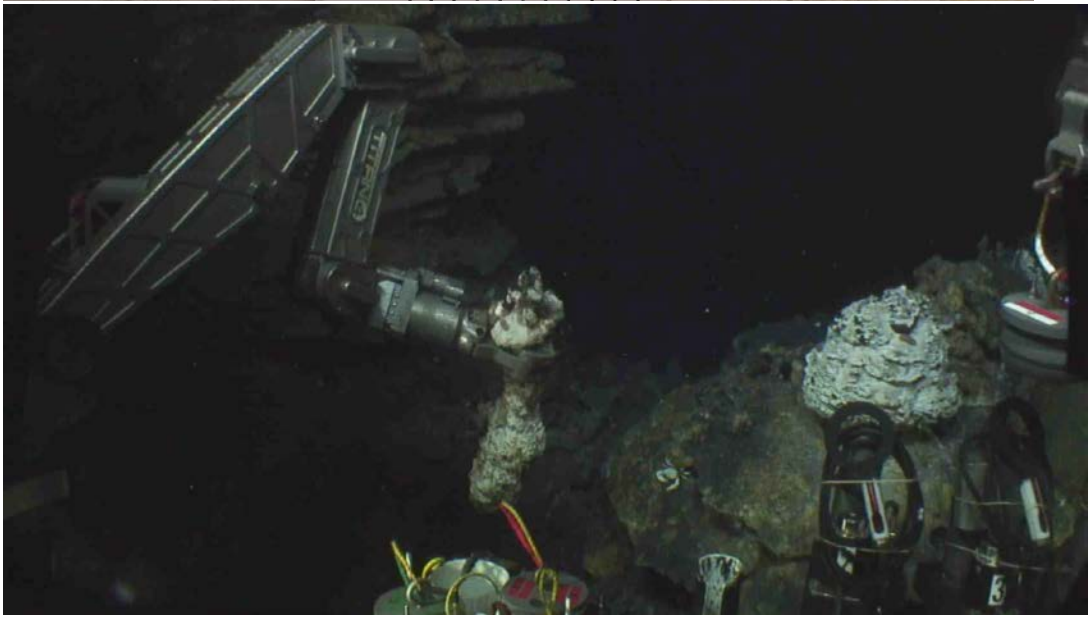


Lowering number	Site number	Sample name	Aliquots	Preliminary Temp. (°C)	RNA Later
J2-819	J2-819-3	J2-819-3-R1	565-578	289	-
Event #	**Chemistry**	**Notes:**			
5081	W1-IGT4 W2-IGT5	Lat: 21 59.409612 S Long: 176 34.145718 W Hdg: 165.18			
Description: Nice sample. White biofilm on outer layer. The whole sample was homogenized for molecular and culturing.					

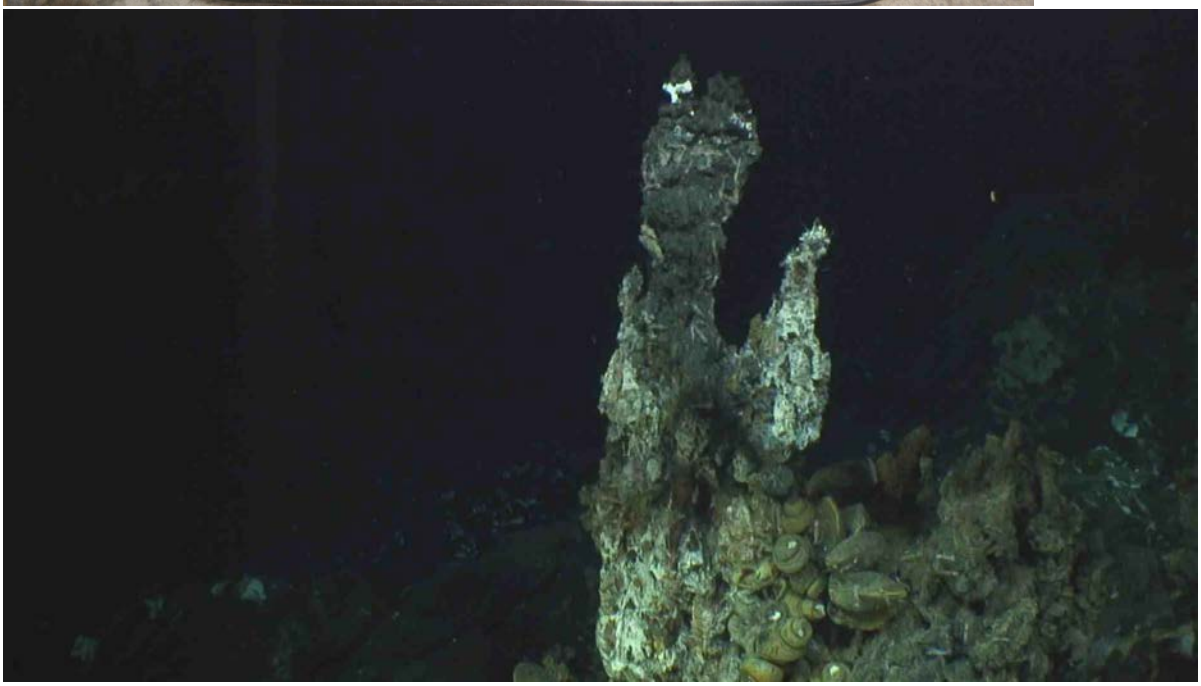


Lowering number	Site number	Sample name	Aliquots	Preliminary Temp. (°C)	RNA Later
J2-819	J2-819-4	J2-819-4-R1	598-714	268	-
Event #	**Chemistry**	**Notes:**			
5311	W1-IGT2	Lat: 21 59.464548 S Long: 34.152660 W Hdg: 143.23			
Description: Snails and polychaete worms present. Hard layers, bottom portion of sulfide collected. Removed the upper part of the outer crust (~5mm).					





Lowering number	Site number	Sample name	Aliquots	Preliminary Temp. (°C)	RNA Later
J2-819	J2-819-5	J2-819-5-R1	657-672 697	180	-
Event #	**Chemistry**	**Notes:**			
5709	-	Lat: 21 59.265630 S Long: 176 34.079760 W Hdg: 201.49			
Description: The chimney was soft and the outer 5mm was homogenized. The white and orange biofilms were mixed together for both culturing and molecular.					



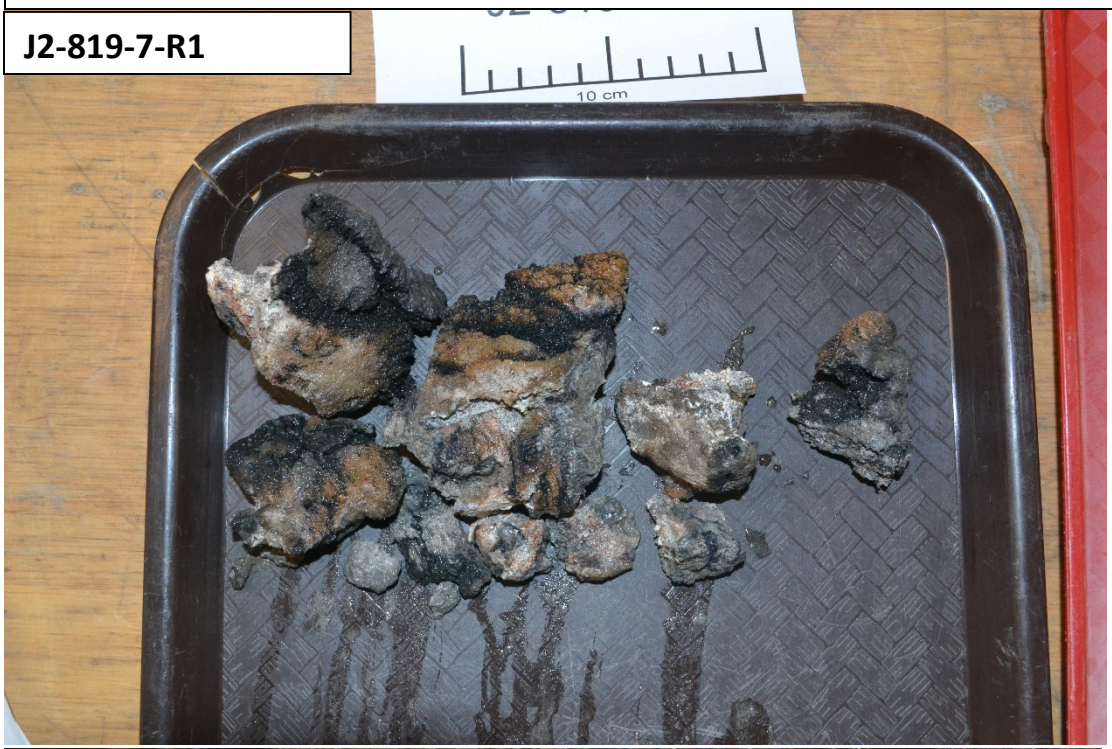
Lowering number	Site number	Sample name	Aliquots	Preliminary Temp. (°C)	RNA Later
J2-819	J2-819-6	J2-819-6-R1	641-656 696	246	-
Event #	**Chemistry**	**Notes:** Lat: 21 59.265630 S Long: 176 34.079760 W Hdg: 201.49			
5709	W1-IGT7 W2-IGT3				

Description: A soft beehive. The entire top of the chimney was homogenized for molecular and culturing.

Lowering number	Site number	Sample name	Aliquots	Preliminary Temp. (°C)	RNA Later
J2-819	J2-819-6	J2-819-6-R2	673-695	246	Yes
Event #	**Chemistry**	**Notes:**			
5767	W1-IGT7 W2-IGT3	Lat: 21 59.265984 S Long: 176 34.078542 W Hdg: 187.72			
Description: Nice outer crust with white biofilm.					

Lowering number	Site number	Sample name	Aliquots	Preliminary Temp. (°C)	RNA Later
J2-819	J2-819-7	J2-819-7-R1	748-768	232	Yes
Event #	**Chemistry**	**Notes:**			
5876	W1-IGT6B W2-IGT8B	Lat: 21 59.363243 S Long: 176 34.093994 W Hdg: 224.01			
Description: Flange sample in RNAlater. Orange biofilm sampled.					

J2-819-7-R1



Lowering number	Site number	Sample name	Aliquots	Preliminary Temp. (°C)	RNA Later
J2-819	J2-819-7	J2-819-7-R2	731-746 860	232	-
Event #	**Chemistry**	**Notes:**			
5882	W1-IGT6B W2-IGT8B	Lat: 21 59.363164 S Long: 176 34.093517 W Hdg: 223.99			
Description: The exterior part of the flange (5mm) was sampled for DNA. Lots of worm casings. More sample was added for culturing					

Lowering number	Site number	Sample name	Aliquots	Preliminary Temp. (°C)	RNA Later
J2-819	J2-819-8	J2-819-8-R1	842859	257	-
Event #	**Chemistry**	**Notes:**			
5961	W1-IGT5B W2-IGT4B	Lat: 21 59.364271 S Long: 176 34.110899 W Hdg: 356.34			
Description: Hard, chimney structure, has smell. The outer 1-2 mm for sample.					

Lowering number	Site number	Sample name	Aliquots	Preliminary Temp. (°C)	RNA Later
J2-819	J2-819-9	J2-819-9-R1	715-730 747	-	-
Event #	**Chemistry**	**Notes:**			
6036	-	Lat: 21 59.378843 S Long: 176 34.111913 W Hdg: 130.77			
Description: Small polychaete worms on exterior. Nice flange, 5mm - 1cm for molecular. Remove inside to the top of the flange for culturing.					

Lowering number	Site number	Sample name	Aliquots	Preliminary Temp. (°C)	RNA Later
J2-819	J2-819-10	J2-819-10-R1	804-819	-	-
Event #	**Chemistry**	**Notes:**			
6183	-	Lat: 21 59.425865 S Long: 176 34.152649 W Hdg: 116.64			
Description: Outer crust for sample. Strong sulfide smell.					

Lowering number	Site number	Sample name	Aliquots	Preliminary Temp. (°C)	RNA Later
J2-819	J2-819-11	J2-819-11-R1	769-778 779	300	-
Event #	**Chemistry**	**Notes:** Lat: 21.59428993 S Long: 176 34.155359 W Hdg: 237.26			
6280	W1-IGT7B W2-IGT2B				
Description: Small pieces of chimney with white biofilm. Whole sample homogenized.					

Lowering number	Site number	Sample name	Aliquots	Preliminary Temp. (°C)	RNA Later
J2-819	J2-819	J2-819-12-R1	820-841	130	Yes
Event #	**Chemistry**	**Notes:** IGT not fully in orifice			
6310	W1-IGT3B	Lat: 21 59.430389 S Long: 176 34.154330 W Hdg: 280.81			
Description: Visible anhydrite in the interior. A low biomass RNAlater sample. Scrape the outer few millimeters					

Lowering number	Site number	Sample name	Aliquots	Preliminary Temp. (°C)	RNA Later
J2-819	J2-819-12	J2-819-12-R2	780-787 790-797	130	-
Event #	**Chemistry**	**Notes:** IGT Not full in orifice Lat: 21 59.433203 S Long: 176 34.152799 W Hdg: 280.26			
6326	W1-IGT3B				
Description: Thin walls with visible anhydrite. Scraped the outer 1-2mm.