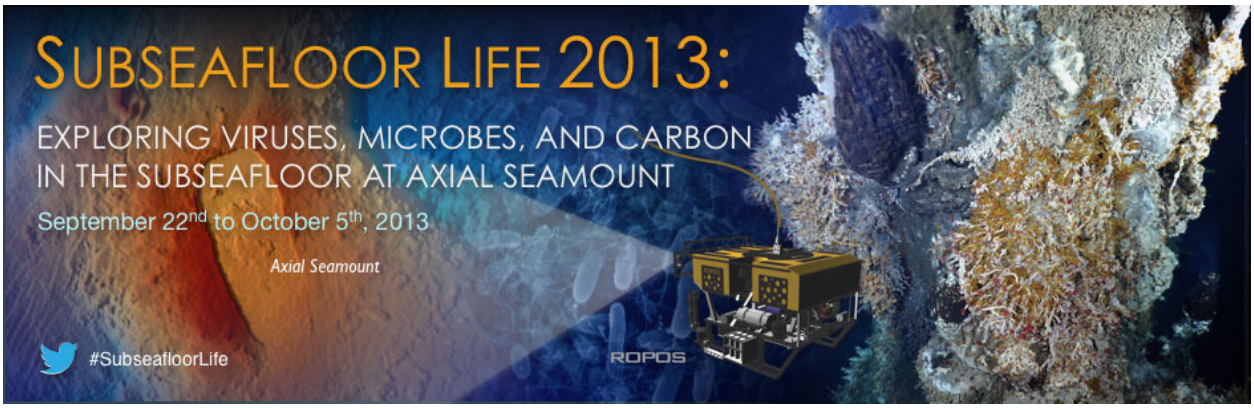
Subseafloor Life 2013 Cruise Report

Axial Seamount, Juan de Fuca Ridge



***R/V* Falkor FK010**

September 22, 2013-October 5, 2013

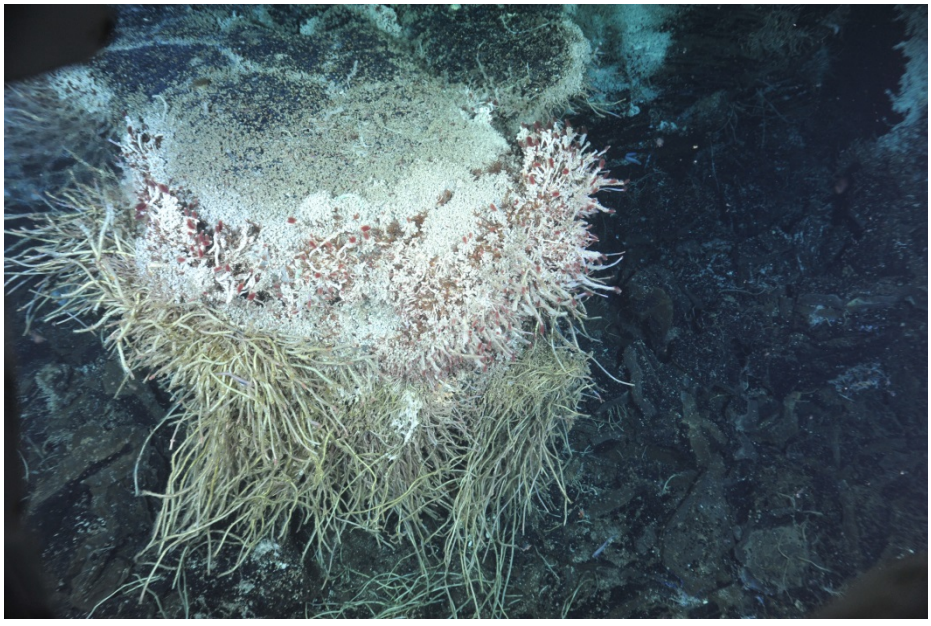
Victoria, British Columbia to Victoria, British Columbia

Chief Scientist: Julie Huber

R/V Falkor Captain: Bernd Buchner

ROPOS Expedition Leader: Keith Tamburri

Cruise report prepared by: Julie Huber



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1.0 Expedition Summary

Julie Huber, Chief Scientist

This expedition to Axial Seamount on *R/V* Falkor was based on a science proposal to SOI entitled "Functional Dynamics, Interactions and Biogeochemical Impact of Chemolithoautotrophic Subseafloor Microbial Ecosystems at Axial Seamount, a Mid-Ocean Ridge Cabled Observatory." The goal of the work was to investigate community membership and gene repertoire of active primary autotrophs that live in the rocky outer layer of Axial Seamount, evaluate the growth, metabolite production, and energy consumption rates for autotrophs, investigate abundance, production, structure, and gene repertoire of co-located viral communities, and determine chemical and isotope signatures of subseafloor communities using Remotely Operated Vehicle (ROV) ROPOS to achieve a deeper understanding of how the viral and microbial communities interact and alter the flow of carbon and nutrients in this subseafloor ecosystem. Funding for the science portion of this cruise was leveraged through a grant to Julie Huber and co-investigators Lisa Zeigler Allen, David Butterfield, Jim Holden, and Giora Proskurowski from the Marine Microbiology Initiative at the Gordon and Betty Moore Foundation. The weather we faced during this expedition was incredibly terrible, yet we still managed to achieve five successful ROV dives at Axial Seamount, as detailed in this report.

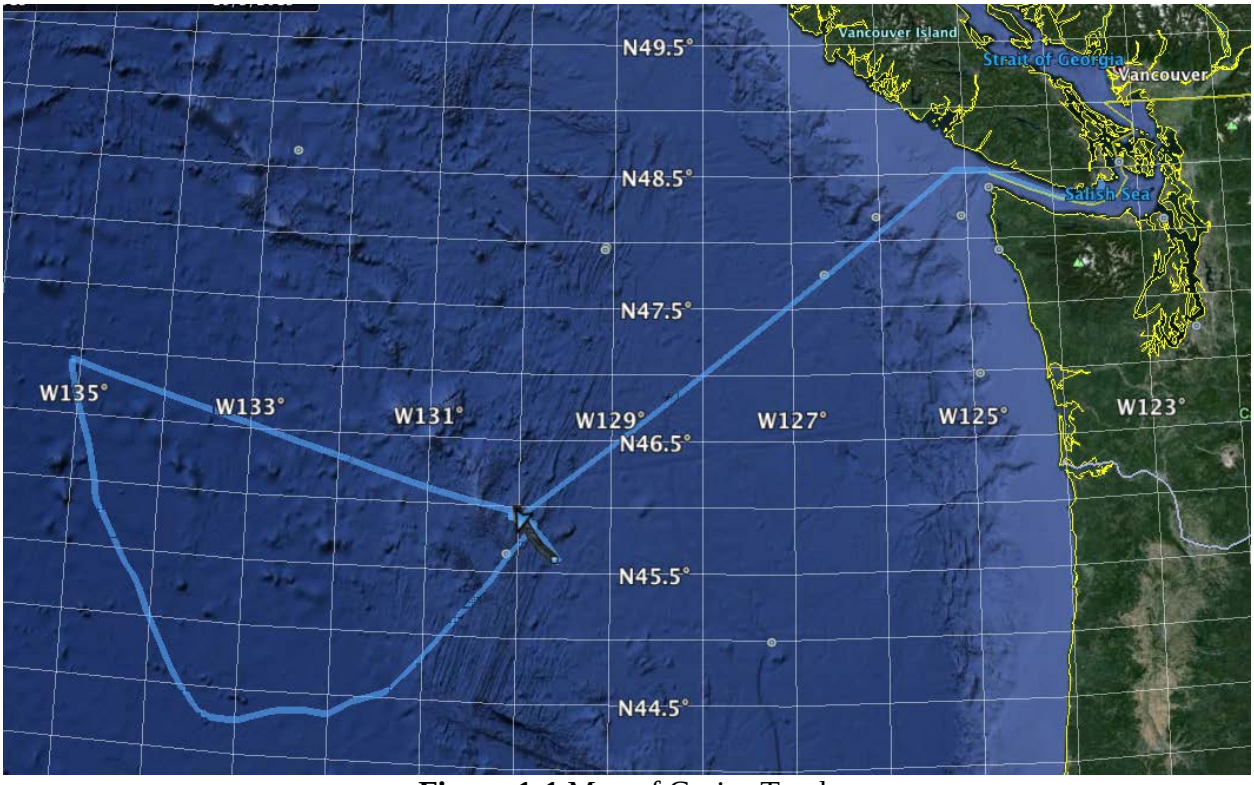

Figure 1-1 Map of Cruise Track

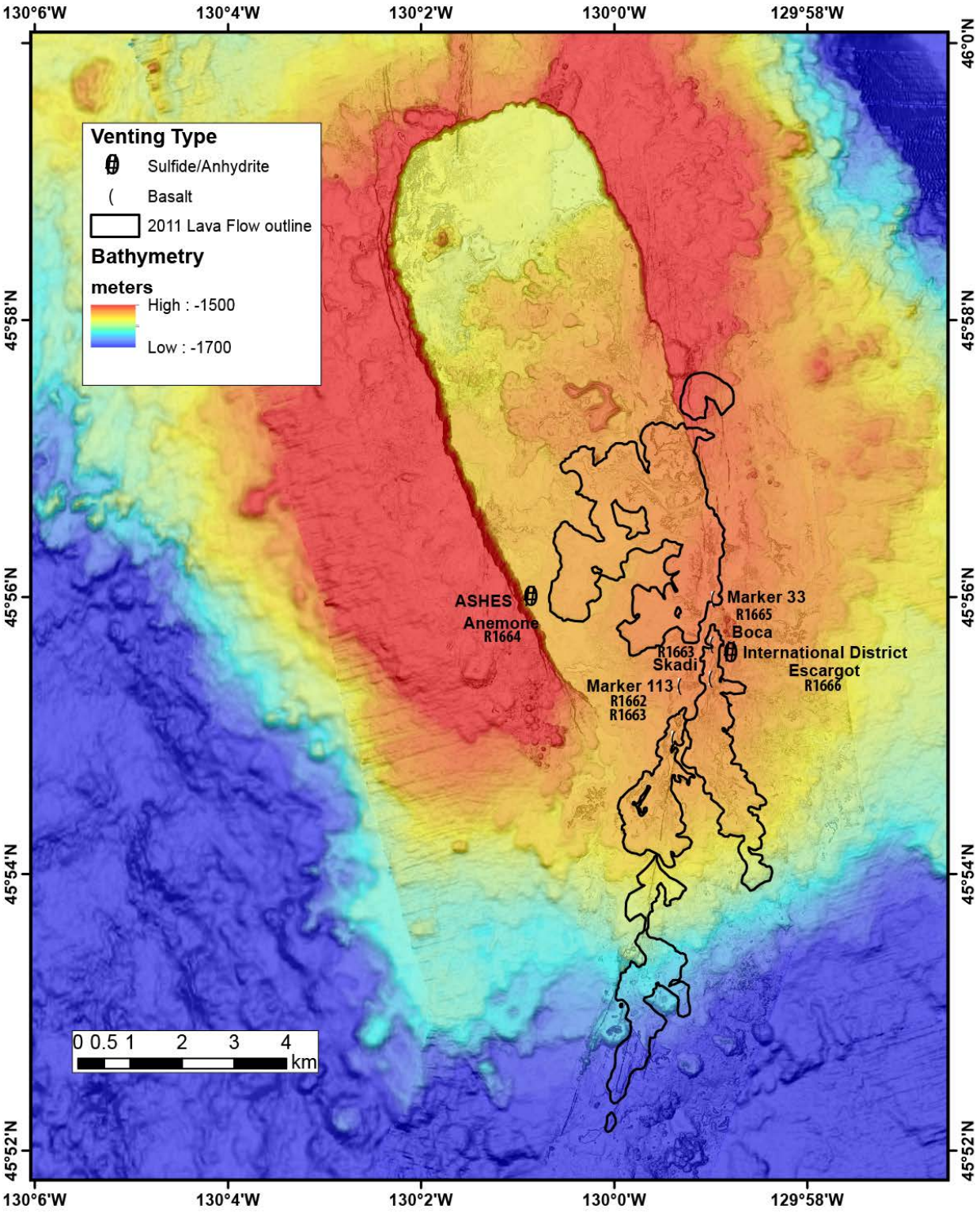

Figure 1-2 Map of Axial Seamount with ROPOS dive and vent locations marked

2.0 Science Cruise Participants

Name	Affiliation	**Expertise**
Lisa Zeigler Allen	JCVI	Viruses
Dave Butterfield	U. Washington	Vent fluid chemist
Caroline Fortunato	Marine Biological Lab	Microbiology
Jim Holden	U. Mass.	Microbiology
Julie Huber	Marine Biological Lab	Microbiology
Ben Larson	U. Washington	Vent fluid chemist
Giora Proskurowski	U. Washington	Vent fluid chemist
Kevin Roe	U. Washington	Vent fluid chemist
Lucy Stewart	U. Mass.	Microbiology

Figure 2-1 FK010 Science Party, ROPOS, and Crew

3.0 Operations Summary
Julie Huber, Chief Scientist (all times are local PST in this narrative)

Our science party arrived at the ship at 0900 Friday, September 20. With the help of the ship's crew, we had all of our gear aboard by 1700. We had to use our personal cabins for extra gear storage, as well as the helicopter pad for our crates.

On Saturday, September 21, we continued setting up our laboratory. On Saturday afternoon at 1600, we moved to anchorage in Victoria Harbor.

On Sunday, September 22 at approximately 1400, we did a test dive with ROPOS and the Large Volume Water Sampler (LVWS). This was ROPOS dive R1661. The LVWS successfully pumped over 60 liters of water in 10 minutes and was considered a success. Due to inclement weather at the dive site, we stayed on the mooring an extra 12 hours, and left for Axial Seamount at 0900 Monday, September 23.

We arrived on station Wednesday, September 25 at 0600. Conditions were still not appropriate for diving due to wind and swell. Instead, we used an acoustic hydrophone over the starboard boat deck to try to "talk" to some energy-harvesting "vent cap" devices that sit on top of a few active vents at Axial Seamount. These devices were installed by Dr. Dave Dyer of the Applied Physics Laboratory in August, and Dave asked us to check in on them if we had free time. The acoustic hydrophone was put over the side of the ship on a long cable then connected to a laptop on the ship, and a signal was sent to the seafloor. Drs. Dave Butterfield, Ben Larson, and the marine technicians on board spent about 12 hours sending signals and trying to talk to the 3 devices, with varying degrees of success.

On Thursday, September 26 at 1330, we were finally given the go ahead to dive. We achieved four dives over the next 36 hours. The detailed logs are appended in Section X in table format, with a description of each dive above them. Note that all times in the dive logs are UTC. Dives R1662, R1663, R1664, and R1665 were completed in this time period. ROPOS was recovered in 35 knot gusts early the morning of Saturday, September 28.

We were weathered out due to a nasty storm from early morning Saturday, September 28 through Monday, September 30. During this time, the ship headed southwest, then west, then north, to stay at a comfortable heading. We saw steady winds above 40 knots, with gusts in excess of 60 knots. A wind record is found in Appendix X. During this time, the science party continued processing samples from the previous dives, which was sometimes made difficult by water coming into the laboratory through the starboard launching bay and the movement of the ship. One glass bottle in the ovens in the dry lab being used for a microbial incubation experiment was broken.

During the storm, equipment was also lost and damaged on the fantail on September 29th, as reported by Keith Tamburri.

On Tuesday, October 1, the decision was made to return to the dive site to see if conditions were improved enough for a final dive or two. We steamed ~220 miles back to Axial, heading

southeast, and arrived Wednesday, October 2. We remained on weather hold until 0900 Thursday, October 3. Dive 1666 began at 0900, but was quickly aborted due to loss of telemetry. After 8 hours of fixing the ROV, at 1700, we launched again, as dive 1667. Dive 1667 was back on deck at 11pm, and we began steaming back to Victoria, BC.

Friday, October 5 was spent in transit. We arrived at Victoria, BC Ogden point at 0900 on Saturday, October 6.

Throughout the cruise, we had four “Science at Sea” seminars, given by Julie Huber, Giora Proskurowski, Jim Holden, and David Butterfield. In addition, marine technician Leighton Rolley gave a seminar about finding Terra Nova.

4.0 Discipline Summaries

4.1 Chemistry

David Butterfield, Giora Proskurowski, Ben Larson, and Kevin Roe

Due to the extremely bad weather during most of the cruise, we completed only two full HFPS dives. The focus was on a small number of diffuse vent sites to collect sufficient volume of fluids to do a wide range of microbiological experiments and to characterize fully the chemistry of the target sites. We sampled the high-priority vent sites Marker 113, Skadi, Marker 33 and Boca with HFPS. A total of 23 fluid samples were collected with HFPS, along with 10 IGT samples and 7 RNA filters.

Hydrothermal Fluid Particle Sampler Configuration (HFPS)

Because the configuration of HFPS changes slightly with time, we provide some details of how it was set up. Valve position 1 was the large volume bag (4-liter) used for microbiology experiments on board (Stable Isotope Probing, or SIP). Valve positions 2 through 9 were occupied by pistons. Pistons 2-5 were titanium with Teflon end caps. Pistons 6-9 were PVC. Even-numbered positions were filtered through Millipore http 0.4 micron polycarbonate membrane filters. Positions 10, 11, and 14-16 were 47mm diameter, 0.2micron pore size, flat membrane filters in McClane filter holders with preservative reservoirs filled with RNA-Later preservative. The preservative was passively added to the filter in-situ after the sample was taken. Positions 17-24 were collapsible Tedlar bag samples. The even-numbered bags were filtered through 0.4 micron membrane filters, with the exception that bag 18 was not filtered to provide more unfiltered water for microbiological experiments. Bag 17 was set up with an acid-cleaned Teflon sample bag for gold analysis and processed specifically for gold. Samplers with all Teflon and titanium parts were preferred for the hottest samples. The standard HFPS intake nozzle was used without any additional attachment/adapter.

Chemical Sensors on HFPS.

Positions 12 and 13 were dedicated to chemical sensors. A new Seabird (Bellvue, WA) 63 Optical Oxygen sensor was plumbed in series with a AMT (Rostock, Germany) deep-sea glass pH electrode. Both sensors responded consistently. The oxygen sensor on HFPS appeared to continue to work normally throughout the Falkor cruise. We did not fully evaluate the pH data from the AMT sensor during the cruises, but it appeared to respond normally throughout both cruises. We will perform a post-Falkor cruise calibration.

Isobaric Gas Tight (IGT) Samplers

On each dive ROPOS was equipped with two Isobaric Gas Tight (IGT) fluid samplers owned by Proskurowski. The IGTs are titanium samplers designed to capture deep-sea fluids and maintain bottom pressure throughout the dive to prevent degassing of the fluids and allow for the most accurate analyses of gas composition. The IGTs are controlled via an Inductively Couple Loop (ICL) modem connected to a RS-232 serial channel ported through the vehicles telemetry system. The communications with the sampler allows for temperature-guided alignment of sampler intake, typically resulting in high quality samples. Despite the weather, FK010 resulted in five successful ROPOS dives, and 10 high-quality IGT samples. While there is no doubt that

more samples would have been preferred, 10 quality IGT samples is not atypical for a 10-day expedition, and should be considered a success.

Sample Processing and Analysis

For HFPS samples, Kevin Roe analyzed hydrogen sulfide, dissolved silica and ammonia on board by spectrophotometry. Dave Butterfield analyzed pH and alkalinity. Giora Proskurowski and Ben Larson analyzed hydrogen and methane on Proskurowski's HP gas chromatograph. We processed nearly all of the HFPS samples for gas analysis. If a gas headspace was present, the entire gas volume was removed and combined into a gas sample bag, the volume of the gas was measured at room T and P, and the methane and hydrogen content of the gas was analyzed on the GC. Immediately after the gas removal (within 1 minute), a liquid sample was taken and the gas content of the liquid was also analyzed. The total sample volume of the liquid was determined by weight, by piston displacement, or by tally of all the sub-sample volumes. The measurements are combined to calculate the total methane and hydrogen content of the fluid.

Our shore-based analytical plan for HFPS will analyze major elements (Na, K, Mg, Ca, Cl, SO4) by ion chromatography, minor elements (Li, F, B, Sr, Rb, Fe, Mn) by Atomic Absorption, ICP-OES, ion-selective electrode, and other techniques, a suite of trace metals (Fe, Mn, Cu, Zn, Pb, Mo, Ni, Ag, Cd, Bi, U and others) by ICP-MS, S isotopes on H_2S and SO_4 by mass spectrometry in collaboration with ETH-Zurich, O and H isotopes of water at UW, stable C on DIC (Giora), Sr and Pb isotopes on selected samples. Sub-samples of unfiltered, low-temperature vent fluids were saved for cell counts. Sub-samples of a few samples were saved for virus counts. Nutrient samples (filtered and purged with nitrogen, but not acidified per discussion with Annie Bourbonnais) were frozen and will be sent to Annie Bourbonnais for both nutrient analysis and isotopes of N and O on nitrate. Replicate nutrient samples (filtered, acidified, and purged with nitrogen) were saved and frozen to be analyzed for nutrients (by either the PMEL nutrient lab or the UW nutrient lab). Samples for analysis of N isotopes on nitrite were preserved with NaOH solution and frozen, to be analyzed by Annie Bourbonnais. DOC will be analyzed on selected samples from each vent site.

For IGT samples, Proskurowski and Larson analyzed for methane and hydrogen, and Roe and Butterfield analyzed hydrogen sulfide, pH, and alkalinity. An IGT sample contains ~150mL of fluid, and after shipboard analyses were complete, aliquots were preserved for shorebased analyses of DIC, 13C-DIC, major ions, trace metals, and nutrients.

Table 4.1-1 HFPS Samples

Sample #	Lab #	HFS#	Target	Type	Start	Stop	Tmax	Tavg	Volume	pH
R1663-1	R1663-LVB1	1	Mkr 113/62	LVB	8:22:33	8:39:27	24.7	24.1	4002	
R1663-2	R1663-P5	5	Mkr 113/62	unfilt piston	8:40:49	8:43:50	24.3	24	701	6.17
R1663-3	R1663-PF8	8	Mkr 113/62	filtered piston	8:45:15	8:48:34	24.1	23.6	704	7.12
R1663-4	R1663-P9	9	Mkr 113/62	unfilt piston	8:49:21	8:53:27	23.9	23.5	702	6.19
R1664-5	R1663-RNA14	14	Mkr 113/62	RNA filt	8:59:41	9:18:35	24.7	24.4	3002	
R1663-6	R1663-RNA15	15	Mkr 113/62	RNA filt	9:19:20	9:36:41	24.7	24.5	3004	
R1663-7	R1663-B21	21	Mkr 113/62	unfilt bag	9:37:59	9:40:51	24.5	24.4	644	7.77
R1663-8	R1663-B23	23	Mkr 113/62	unfilt bag	9:41:39	9:44:32	24.8	24.5	643	6.17
data only	R1663-SENS13	13	Mkr 113/62	sensors		O2=0.163mL/L		pH=3.206V	at 24.6C	
R1663-9	R1663-PF2	2	Skadi-high T	filtl piston Ti	11:37:37	11:40:58	129.9	123.4	701	5.21
R1663-10	R1663-P3	3	Skadi-high T	unfilt piston Ti	11:41:41	11:44:47	125.5	119.8	699	5.16
R1663-12	R1663-B18	18	Skadi-diffuse	unfilt bag	12:40:13	12:43:17	34.8	34.5	642	6.23
R1663-13	R1663-B19	19	Skadi-diffuse	unfilt bag	12:45:01	12:48:16	35.6	35.3	642	
R1663-14	R1663-BF20	20	Skadi-diffuse	filt bag	12:50:28	12:54:43	35.2	34.9	626	6.21
R1663-15	R1663-RNA10	10	Skadi-diffuse	RNA filt	12:57:34	13:20:47	36	35.5	3001	
R1663-16	R1663-RNA11	11	Skadi-diffuse	RNA filt	13:21:47	13:42:30	35.5	35.2	3001	
R1663-17	R1663-BF22	22	Skadi-diffuse	filt bag	13:44:40	13:48:59	35.6	35.4	642	6.19
data only		13	Skadi-diffuse	sensors		O2=0.134 ml/L		ph=3.227V	at 35.5C	
R1665-1	R1665-LVB1	1	Mkr 33/166	LVB	04:16:21	04:33:15	29.1	27.6	4002	
R1665-2	R1665-PF2	2	Mkr 33/166	filtl piston Ti	04:34:25	04:37:26	29.1	28.3	701	5.50
R1665-3	R1665-P3	3	Mkr 33/166	unfilt piston Ti	04:38:29	04:41:31	28.4	27.8	701	5.52
R1665-4	R1665-RNA14	14	Mkr 33/166	RNA filt	04:48:03	05:05:03	27.6	26.1	3002	
R1665-5	R1665-B18	18	Mkr 33/166	unfilt bag	05:08:03	05:10:46	28.5	27.3	629	5.54
R1665-6	R1665-B19	19	Mkr 33/166	unfilt bag	05:11:52	05:14:40	28.2	26.7	628	
R1665-7	R1665-RNA15	15	Mkr 33/166	RNA filt	05:16:29	05:33:39	26.7	25.3	3001	
R1665-9	R1665-PF4	4	Mkr 33/166	filt piston	06:18:25	06:21:19	27.4	27	626	5.61
R1665-10	R1665-BF20	20	Boca	filt bag	7:43:04	7:46:02	6.8	6.7	642	6.88
R1665-12	R1665-BF22	22	Boca	filt bag	7:51:48	7:55:07	6.7	6.7	641	6.91
R1665-11	R1665-B21	21	Boca	unfilt bag	7:46:54	7:49:55	6.8	6.7	642	
R1665-13	R1665-B23	23	Boca	unfilt bag	7:55:54	7:58:51	6.7	6.7	641	
R1665-14	R1665-P5	5	Boca	unfilt PVC piston	8:00:57	8:04:13	6.7	6.6	703	6.90
R1665-15	R1665-RNA10	10	Boca	RNA filt	8:05:39	8:23:17	6.8	6.7		

Table 4.1-2 HFPS Sample Split Summary

Sample #	Target	H_2S/Si	pH/alk	Majors	Trace Metal	Nutrient N iso	NO_2 iso	Microbio	Sulfur isotopes	DOC	Virus counts
R1663-1	Mkr 113/62	10		10		30 din#166 + HCl					
R1663-2	Mkr 113/62	30	35	35	250	50 din#179	45 bno2#118	40	45	90	60
R1663-3	Mkr 113/62	30	35	35	510	45 din#140	45 bno2#155				
R1663-4	Mkr 113/62	35	35	35	240	45 din#146	45 bno2#114	40	45	90	
R1663-7	Mkr 113/62	15	10	20				610			
R1663-8	Mkr 113/62	35	35	35	125	45 din#189		260			
R1663-9	Skadi-high T	25	35	35	350	45 din#192	45 din#199		45	100	
R1663-10	Skadi-high T	35	35	35	360	45 din#156	45 bno2#164	40	45		
R1663-12	Skadi-diffuse	10	35	35		45					
R1663-13	Skadi-diffuse			12				585			
R1663-14	Skadi-diffuse	25	35	35	180	50 din#183			45	90	
R1663-17	Skadi-diffuse	35	35	35	230	45 din#172	40 bno2#195			90	
R1663-18	Skadi-diffuse										
R1665-1	Mkr 33/166	15		40							
R1665-2	Mkr 33/166	35	35	35	460	50 din#138	50 bno2#132	50 Nuts-UW	45		
R1665-3	Mkr 33/166	40	35	35	250	45 din#163	50 NutsUW	40			60
R1665-5	Mkr 33/166	30	35	35	230	45 din#171	45 bno2#109	40	45	90	60
R1665-6	Mkr 33/166	20			microcosm			606			
R1665-9	Mkr 33/166	35	35	35	420	45 din#185	45 Nuts-UW		45	90	
R1665-10	Boca	35	35	35	130	45 din#101	45 bno2#120		45		
R1665-12	Boca	50	35	35	250	45 din#167	45 bno2#156		45	100	
R1665-11	Boca										
R1665-13	Boca										
R1665-14	Boca	30	35	35	235	45 din#177	45 bno2#177	40	45 Nuts-UW	90	60

Table 4.1-3 IGT Samples

Number	Date (UTC)	Identifier	Full Sample Number	Description	Site	Latitude	Longitude	Depth
R1662-2	9/27/13 02:24	IGT3	R1662-2-IGT3	IGT3 at Marker 113, 23 C	Mrkr 113	N45° 55.3671′	W129° 59.2902′	1521.78m
R1662-3	9/27/13 02:39	IGT4	R1662-3-IGT4	IGT4 at Marker 113, 23.6 C	Mrkr 113	N45° 55.3650′	W129° 59.2891′	1521.96m
R1663-11	9/27/13 12:07	IGT1	R1663-11-IGT1	IGT1 at Skadi, Tmax ~ 120 C. Temperature varied considerably between 35C and 120C	Skadi	N45° 55.4096′	W129° 58.9858′	1520.99m
R1663-18	9/27/13 14:03	IGT2	R1663-18-IGT2	IGT2 Skadi, low T diffuse site. Tmax = 32.8 C, temperature steady 32.3-32.9 C.	Skadi	N45° 55.4069′	W129° 58.9867′	1522.07m
R1664-1	9/27/13 20:03	IGT4	R1664-1-IGT4	IGT4 at high temp Anemone, Tmax 213	Anemone	N45° 55.9939′	W130° 00.8187′	1542.1m
R1664-3	9/27/13 22:09	IGT3	R1664-3-IGT3	IGT3 at high temp Anemone, Tmax 234	Anemone	N45° 55.9921′	W130° 00.8213′	1542.77m
R1665-8	9/28/13 05:42	IGT2	R1665-8-IGT2	IGT2 at Marker 33, Tmax was 40.65	Mrkr 33	N45° 55.9946′	W129° 58.9340′	1515.85m
R1665-16	9/28/13 08:17	IGT1	R1665-16-IGT1	IGT1 at Boca, Tmax=7.0	Boca	N45° 55.6584′	W129° 58.9445′	1516.99m
R1667-2	10/4/13 04:21	IGT2	R1667-2-IGT2	Escargot LoT, Tmax 41.3°C	EscargotLo	N45° 55.5832′	W129° 58.7549′	1519.86m
R1667-3	10/4/13 04:39	IGT1	R1667-3-IGT1	Escargot HiT, Tmax 270°C	EscargotHi	N45° 55.5830′	W129° 58.7449′	1517.27m

Table 4.1-4 IGT Sample Shipboard Results

Number	Max T °C	Sample Avg T °C	TCD H_2 umol/kg	stdev TCD H_2 umol/kg	CH_4 umol/kg	stdev CH_4 umol/kg
R1662-2	24.6	21.1	< 1	n/a	17.7	0.8
R1662-3	23.8	23.3	< 1	n/a	18.3	0.4
R1663-11	122.8	85.2	62.1	1.7	15.6	0.7
R1663-18	33.2	32.6	< 1	n/a	4.8	0.4
R1664-1	212.7	207.7	272.5	20.1	156.3	8.3
R1664-3	232.4	227.8	351.2	14.7	200.1	6.4
R1665-8	40.7	39.4	1.3	0.8	30.1	0.9
R1665-16	7.8	6.8	< 1	n/a	0.8	0.04
R1667-2	40.3	36.2	< 1	n/a	12.0	0.34
R1667-3	270.2	270	433.6	21.2	146.6	17.61

4.2 Viral Ecology

Lisa Zeigler Allen

The primary objective was to collect and process ~160 L of diffuse flow fluids for microbial and viral abundance estimates, viral production assessment, nutrient concentrations, flow cytometric capture of populations and single organisms, and metagenomic analysis of prokaryotic and viral communities. A secondary goal was to collect and process ~160 L off-axis, non-vent bottom water, for abundance estimates, viral production assessment and metagenomic analysis of prokaryotic and viral communities.

The in-situ filtration sampling device that was used to collect and process large volumes of water (LVWS) was fitted to a sled appropriate for use with ROPOS. A temperature probe was mounted to the LVWS sample collection arm for real-time capture of diffuse flow fluid temperature variations. When needed, e.g., due to temperature drop due to entrainment of cold deep fluids, the pumps were paused and the arm readjusted to maintain temperature.

The majority of filtering activities take place at depth including pre-filtering through a 200 µm Nytex net, then serial filtration through 3.0 µm and two 0.2 µm membrane filters. The 0.2 µm filtered water containing viral particles is then collected in tedlar bags and brought to the surface for further processing. Using tangential flow filtration (TFF) the ~160 L of 0.2 µm filtered water is concentrated to ~250-500ml, glycerol added, and stored at -80 °C before transport on dry ice to JCVI for further analyses.

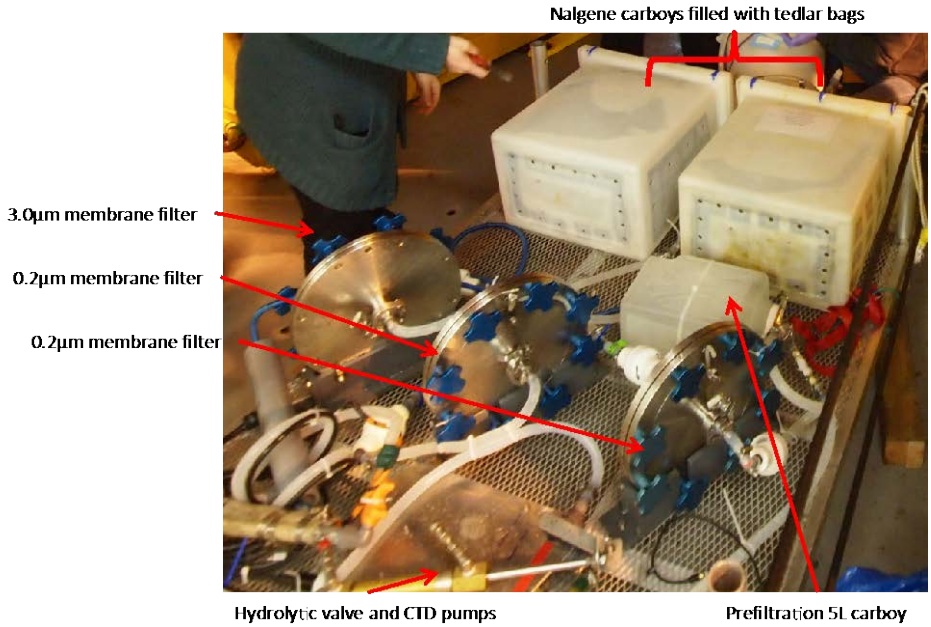

Figure 4.2-1 Large Volume Water Sampler on ROPOS

Dive Summary for LVWS Sampling

During the ROPOS dives equipped with the LVWS, samples were collected after filtering fluids for: ~2 hours (1662), 1.2hours (1664), and 50minutes (1667). In each case 140-160L of fluids were brought up from depth and further processed. The volume of water filtered across membrane filters varied due to total filtering time. Samples were taken to evaluate microbial and viral abundance within the unfiltered water, filtered and concentrated water. Membrane filters were preserved for community genomic (DNA) and transcriptomic (RNA) analyses. Additionally, shipboard assays were conducted to assess the rate of microbial mortality due to viral lysis. Briefly, samples were washed from the membrane filter using vent fluid that had been filtered using the tangential flow filtration unit with a 50kD cutoff size. The ultrafiltrate contains no viral particles, yet still maintains some of the fluid properties present at the time of sample.

Fluid Collection Details for LVWS

A. Prior to deployment deck activities:

1. Rinse collection apparatus with 1:100 bleach to Milli-q water solution follow with Milli-q water only.
2. Set up a 200µm nytex pre-filter in the 5L collection carboy, filled with Milli-q water.
3. **Use forcepts and gloves for ALL filter contact**.
4. Load the duplicate serial 293mm filter stand units (in series 3.0µm 'Y' to two 0.2µm).
5. Load tedlar bags into Nalgene box carboys and secure with tie-wraps.
6. Prime system using Milli-q and peristaltic pump.
7. Attach LVWS sled to ROPOS.
8. Check hydrolytic valve and CTD pumps.

B. Filtration during deployment

1. Temperature probe and sample collection wand is used to target diffuse flow fluids at ~20-50°C.
2. Valve is opened and pumps are turned on pulling fluids into filtration system.
3. The pumps average 3.5 L min^{-1}.
4. Measure and record total volume pumped on station data sheet.
5. When filtering is completed pumps are turned off and valve closed.

Sample Archiving Details for LVWS

A. Membrane filters for microbial and viral genomic and transcriptomic analysis

1. Remove 3.0µm and one 0.2µm filter from Millipore stand with forceps, and place on top of sterilized filter paper box (sterilize with alcohol wipes).
2. Fold the remaining of each filter in half with cells on inside. Continue to fold until the filter will fit into a seal-a-meal bag.
3. Add 10ml of DNA buffer (1x Tris-EDTA, 50mM EDTA, 50 mM EGTA) and 10ml of RNAlater.
4. Seal filters in separate sample bags. Label with date, dive, station and filter size.
5. Store at -80°C on R/V Falkor.

B. Membrane filter for viral production assay

1. Remove the second 0.2μm filter and aseptically cut using a sterile razor blade.
2. Place in 2L carboy
3. Add 1 L of ultrafiltrate (permeate) fluid (50kD filtered) from TFF.
4. Shake to remove microbes
5. Aliquot into 4 production assay vessels at equal volumes.
6. Take microscopy sample, 5ml fluid with 1% glutaraldehyde and DOC sample.
7. Incubate at 55°C for 48 hours.
8. Take microscopy sample and add glycerol, store at -80°C.

C. Viral particle concentration - 0.2μm filtered fluids contained in tedlar bags

1. Transfer water from bags on deck to clean 50L carboys using peristaltic pump.
2. Insert all lines (feed, retentate and permeate) into the carboy (this is referred to as recirculation mode). Ensure that the permeate valve is closed. Thread the feed line through the pump head and set the occlusion knob on the peristaltic pump to 5.
3. Ramp the pump speed up slowly until you reach 20. Once water starts to come out of the retentate line, turn the occlusion knob to 3 and move the permeate line to another acid-cleaned carboy.
4. Slowly ramp up the speed of the pump to 45. Open the permeate valve slowly until a steady "trickle" comes out of the permeate line. The water that comes out of the permeate line is ultra-filtered seawater and should be virus free. 50L of ultrafiltrate in a clean carboy. This will be used for cleaning purposes.
5. Concentrate the 0.2μm water to ~250-500ml.
6. Collect microscopy samples, 4x 15ml with 1% glutaraldehyde.
7. Collect 50ml of ultrafiltrate in 10% glycerol as sample processing control.
8. Add molecular biology grade glycerol to a 10% final concentration.
9. Freeze VC at -80°C.

D. Unfiltered (raw water) fluid from 5L prefiltration carboy

1. Aliquot 50ml into falcon tubes for viral production assay, 4x50ml and incubate at 55C for 48 hours. Take microscopy sample at T0 and T48 –2x5ml with 1% glutaraldehyde.
2. Take 2x50ml fluid and add 10%final volume glycerol for flow-cytometric analysis.
3. Take 2x5ml microscopy samples with 1% glutaraldehyde added.
4. Collect 4x50ml in acid washed nutrient analysis bottles.
5. Give aliquot to Dave Butterfield for silicate and other chemistry analyses.

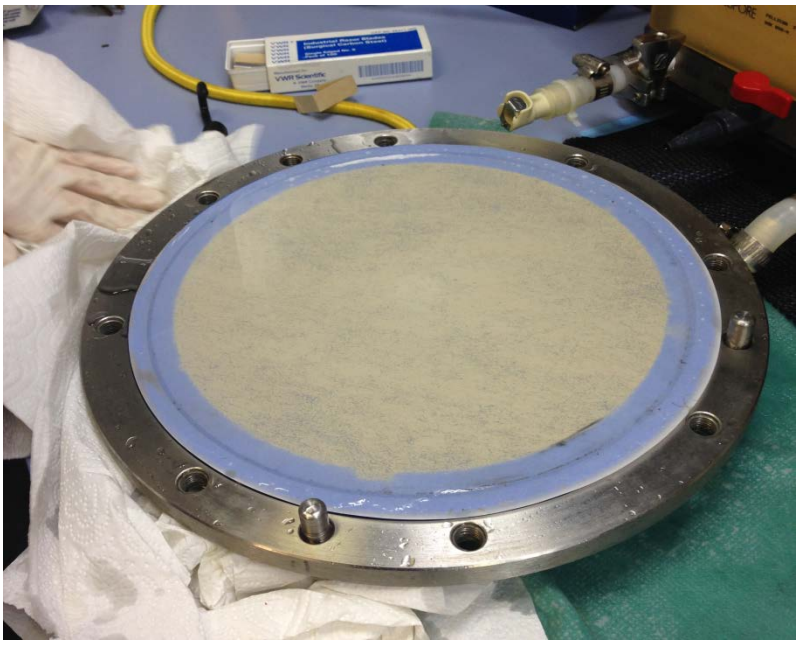

Figure 4.2-2 3.0 µm 293mm membrane filter from ROPOS Dive 1662 showing microbial biomass and marine snow captured during filtration of diffuse flow fluids.

Figure 4.2-3 Setting up viral production assay in wrapped sterile 150ml bottles. Each bottle was filled with 100ml of ultrafiltrate containing microbes washed from 0.2µm membrane.

Table 4.2-1 LVWS Samples

Sample ID	Site ID	Lat/Long	Date	Time (local) start	Time (local) end	Total Filter Time (H:M)	Sample Depth(m)	Column depth (m)	Temp° C (Avg)	Volume Filtered	Volume Collected
R1662 LVWS	Marker 113	45.922741 / -129.988104	9/26/2013	17:20	19:24	2:04	1520m	1520m	22	426L	160L
R1664 LVWS	Anemone	45.933251/-130.013790	9/27/2013	13:52	15:08	1:16	1542m	1542m	40	267L	160L
R1667 LVWS	Off-axis	45.926057/-129.970665	10/3/2013	18:40	20:10	0:50	1515m	1560m	2.5	178L	140L

Table 4.2-2 LVWS Sample Details

	Prefiltration carboy (Raw water)							Membrane Filters		
Sample ID	4XVP/ temp	2x flow cytometry	2x micro glut	Nutrients	Silicate (Dave)	Other (Dave)	Other (SIP)	3um DNA/RNA	0.2um DNA/ RNA	0.2um for VP
R1662 LVWS	Y, 55C	Y	Y	Y	Y	Y	Y	Y	Y	Y
R1664 LVWS	Y, 55C	Y	Y	Y	Y	Y	Y	Y	Y	Y
R1667 LVWS	Y, 55C	Y	Y	Y	Y	Y	Y	Y	Y	Y

Table 4.2-3 LVWS Sample Details

	Viral particle Concentrate		Viral Production Assay							
Sample ID	VC (glycerol)	(VC) Microscopy (Glut)	0.2um VP micro	Volume, Temp	Temp ©	VP T0	VP T48	DOC Ultrafiltrate	DOC VP T0	DOC VP T48
R1662 LVWS	3x150ml	4x15ml	2x5ml	4x100ml	55	2x5ml	Y	Y	Y	Y
R1664 LVWS	5x100ml	4x15ml	2x5ml	3x100ml	55	2x5ml	Y	Y	Y	Y
R1667 LVWS	13x50ml	4x15ml	2x5ml	4x50ml	55	2x5ml	Y	Y	Y	Y

4.3 Microbial Physiology
Jim Holden and Lucy Stewart

The goal of our research is to model the habitability of hydrothermal vent environments by thermophilic and hyperthermophilic anaerobic microorganisms and the biogeochemical impact of these organisms on the deep sea. On this cruise, we determined the concentrations of cultivatable methanogens, autotrophic sulfur reducers, autotrophic iron reducers, and anaerobic heterotrophs that grow at 55 and 80°C, and determined the potential growth restraints (e.g., N availability, H_2 concentration, trace metal or vitamins) on natural assemblages of methanogens at the same temperatures. Low-temperature (< 30°C) hydrothermal fluid samples were collected using the hydrothermal fluid sampler and processed from vents at Marker 113 (R1663-unfiltered bag 21), Skadi (R1663-unfiltered bag 19), Marker 33 (R1665-unfiltered bag 19), and Boca (R1665-unfiltered bag 21). From each bag (~640 ml per bag), 400 ml were used for methanogen microcosm incubations, 100 ml were used for three-tube most-probable-number estimates, 18 ml were preserved in 3-4% formaldehyde for total cell counts, and 5 ml and the remaining fluid were filtered onto a 0.2 μm pore size membrane filter and preserved with 4% paraformaldehyde for fluorescence in situ hybridization (FISH) counts of specific cell types. Overall, 324 Balch tubes were inoculated for viable cell estimates and 64 serum bottles were filled with diffuse vent fluid and incubated as microcosms. We also filtered and preserved 5 ml, 25 ml, 50 ml, and 100 ml of fluid for FISH analysis from each of the two large volume bags (R1663-01 and R1665-01) collected for SIP experiments by the Huber lab, and preserved 40 ml of fluid from each low-temperature unfiltered hydrothermal fluid sample collected for total cell counts. Final results cannot be determined at sea, but there are very good indications that we had growth of methanogens in our microcosm and MPN experiments at 55 and 80°C and in our heterotroph MPNs at the same temperatures.

4.4 Molecular Microbial Ecology
Julie Huber and Caroline Fortunato

Our main objective on this cruise was to conduct stable isotope probing experiments with diffuse vent fluids from Axial enriched with labeled DIC under various temperature to determine which microbes are actively fixing carbon at Axial. In addition, we collected diffuse fluids that were in situ filtered and preserved (with RNALater, in duplicate) to determine and quantify functional repertoire of total active microbial communities and compare to SIP experiment. The final goal was to preserve all diffuse fluid samples collected for single cell genomics analyses. We sampled fluids with two samplers, the HFPS and LVWS. We obtained samples from all 5 ROPOS dives.

Molecular Microbial Ecology Sampling with HFPS

RNA filters: Four filter holders charged with RNAlater and containing a 0.2 μm flat filter were loaded onto the HVFS. At each vent site, 3L was pumped through each filter and flooded with RNA later for immediate preservation. Duplicate (when possible) filters were taken at each vent site. Once on deck, filters were removed from their holders, folded into quarters and placed into sterile 50mL Falcon tubes with ~15mL of fresh RNA later. Tubes were kept at 4ºC for 24 hours and then moved to -80ºC for the remainder of the cruise.

SIP experiments: On each HFPS dive, 4L of vent fluid was collected using a large volume bag (LVB). This fluid was then used to fill six evacuated 500mL bottles. Water was pumped from the LVB into each bottle using a peristaltic pump and needle. To ensure dissolved gases did not escape as the bag volume decreased, a water filled carboy was used to pump water and keep pressure on the bag. Before filling, each bottle was spiked with either 13C or 12C labeled sodium bicarbonate to a final concentration of 10 μM after filling. Bottles were filled to 530ml and incubated at 30ºC, 55ºC, 80ºC, with two bottles at each temperature, a labeled experiment (13C) and an unlabeled control (12C). If there was enough water left in the bag, a seventh bottle was filled for a 13C labeled time point at 30ºC. After filling each bottle, 1 ml of 10% HCl was added to ensure a pH < 6.5. After the pH was corrected, 20ml (~900 μmoles) of 99.99% H2 gas was added to each bottle to have a concentration of 20uM H2 in solution. After the H2 was added, bottles were incubated for 36 hours (18 hours for the time point). Once the 36 hour incubation was complete, bottles were filtered through 0.22μm Sterivex filters, preserved with RNA later, and frozen at -80ºC. 5mL of the filtrate from each bottle was collected in evacuated serum vials for DIC analysis. To ensure the vent fluid collected was similar in composition to the rest of the fluid collected on the dive, 60mL was taken from the LVB for nutrient analysis in the Butterfield lab.

HFPS SIP experiments were completed at Marker 113 (FS903) and Marker 33 (FS904).

Single Cell Genomics: Water from the LVB was also used for single cell genomics. 1ml of water was added to a sterile cryovial with 100μl of filter-sterilized GlyTE. Vials were then inverted for mixing and incubated at room temperature for 5min before being frozen at -80ºC. Triplicate samples were taken. Single cell genomics samples were taken for Marker 113 (FS903) and Marker 33 (FS904)

Molecular Microbial Ecology Sampling with LVWS:

SIP experiments: On each LVWS dive, a 5L carboy was collected for SIP. For each experiment, water from the carboy was pumped into four evacuated 1L bottles. Prior to filling, each bottle was spiked with 13C or 12C labeled sodium bicarbonate to a final concentration of 10μM after filling. All bottles were filled to 1060 mL and incubated at 80ºC. Each experiment had two time points, 18 and 36 hours with two bottles for each time point, a labeled experiment (13C) and an unlabeled control (12C). After filling each bottle, 2.5 ml of 10% HCl was added to ensure a pH < 6.5. After the pH was adjusted, 60ml of 99.99% H2 was added to each bottle. At the end of each incubation (18 or 36 hours), bottles were filtered onto 0.22 μm Sterivex filters, preserved in RNA later, and frozen at -80ºC.

LVWS SIP experiments were completed at Marker 113 (LVWS1), Anemone (LVWS2), and a background sample outside the caldera (LVWS3).

Table 4.4-1 Samples for Molecular Microbial Ecology

Huber Sample #	Sample #	Sample Type	RNA/DNA	SIP	SCG	FISH	Vent	Date	Start Time	End Time	Tmax	Tavg	Volume Filtered (mL)
FS902	R1663-15	RNA	x				Skadi	9/27/2013	12:57	13:20	36	35.5	3001
FS902	R1663-16	RNA	x				Skadi	9/27/2013	13:21	13:42	35.5	35.2	3001
FS903	R1663-5	RNA	x				Marker 113	9/27/2013	8:59	9:18	24.7	24.4	3002
FS903	R1663-6	RNA	x				Marker 113	9/27/2013	9:19	9:36	24.7	24.5	3004
FS903	R1663-1	LVB		x	x	x	Marker 113	9/27/2013	8:22	8:39	24.7	24.1	4002
FS904	R1665-1	LVB		x	x	x	Marker 33	9/28/2013	4:16	4:33	29.1	27.6	4002
FS904	R1665-4	RNA	x				Marker 33	9/28/2013	4:48	5:05	27.6	26.1	3002
FS904	R1665-7	RNA	x				Marker 33	9/28/2013	5:16	5:33	26.7	25.3	3001
FS905	R1665-15	RNA	x				Boca	9/28/2013	8:05	8:23	6.8	6.7	n/a
LVWS1	R1662-1	Carboy	x				Marker 113	9/26/2013	17:20	19:24		22	160L
LVWS2	R1664-2	Carboy	x				Anemone	9/27/2013	13:52	15:08		40	160L
LVWS3	R1667-1	Carboy	x				Background Seawater	10/3/2013	18:40	20:10		2.5	140L

Table 4.4-2 SIP Experimental Details from HFPS

Date	Huber Sample #	Sample #	Bottle Label	Vent	Start Time	End Time	Volume (mL)	DIC for Giora (5 mL)
9/27/2013 to 9/28/2013	FS903	R1663-1	FS903-30-12C	Marker 113	12:34	22:50	530	x
9/27/2013 to 9/28/2013	FS903	R1663-1	FS903-30-13C	Marker 113	12:34	22:50	530	x
9/27/2013 to 9/28/2013	FS903	R1663-1	FS903-55-12C	Marker 113	12:34	23:10	530	x
9/27/2013 to 9/28/2013	FS903	R1663-1	FS903-55-13C	Marker 113	12:34	23:10	530	x
9/27/2013 to 9/28/2013	FS903	R1663-1	FS903-80-12C	Marker 113	12:34	23:40	530	x
9/27/2013 to 9/28/2013	FS903	R1663-1	FS903-80-13C	Marker 113	12:34	23:40	530	x
9/27/2013 to 9/28/2013	FS903	R1663-1	FS903-30-13C TP1	Marker 113	12:34	5:45	530	x
9/28/2013 to 9/29/2013	FS904	R1665-1	FS904-30-12C	Marker 33	5:33	16:45	530	x
9/28/2013 to 9/29/2013	FS904	R1665-1	FS904-30-13C	Marker 33	5:33	16:45	530	x
9/28/2013 to 9/29/2013	FS904	R1665-1	FS904-55-12C	Marker 33	5:33	17:05	530	x
9/28/2013 to 9/29/2013	FS904	R1665-1	FS904-55-13C	Marker 33	5:33	17:05	530	x
9/28/2013 to 9/29/2013	FS904	R1665-1	FS904-80-12C	Marker 33	5:33	17:25	530	x
9/28/2013 to 9/29/2013	FS904	R1665-1	FS904-80-13C	Marker 33	5:33	17:25	530	x
9/28/2013 to 9/29/2013	FS904	R1665-1	FS904-30-13C TP1	Marker 33	5:33	23:10	530	x

Table 4.4-3 SIP Experimental Details from LVWS

Date	Huber Sample #	Sample #	Bottle Label	Vent	Start Time	End Time	Volume (mL)
9/27/2013 to 9/28/13	LVWS1	R1662-1	LVWS1-80-12C-TP1	Marker 113	0:20	20:32	1060
9/27/2013 to 9/28/13	LVWS1	R1662-1	LVWS1-80-13C-TP1	Marker 113	0:20	20:32	1060
9/27/2013 to 9/28/13	LVWS1	R1662-1	LVWS1-80-12C-TP2	Marker 113	0:20	13:07	1060
9/27/2013 to 9/28/13	LVWS1	R1662-1	LVWS1-80-13C-TP2	Marker 113	0:20	13:07	1060
9/28/2013 to 9/29/13	LVWS2	R1664-2	LVWS2-80-12C-TP1	Marker 33	21:15	15:15	1060
9/27/2013 to 9/28/13	LVWS2	R1664-2	LVWS2-80-13C-TP1	Marker 33	21:15	15:15	1060
9/27/2013 to 9/28/13	LVWS2	R1664-2	LVWS2-80-12C-TP2	Marker 33	21:15	n/a	1060
9/27/2013 to 9/28/13	LVWS2	R1664-2	LVWS2-80-13C-TP2	Marker 33	21:15	11:00	1060
10/4/2013 to 10/5/13	LVWS3	R1667-1	LVWS3-30-13C-TP1	Background	1:50	19:30	530
10/4/2013 to 10/5/13	LVWS3	R1667-1	LVWS3-30-12C-TP2	Background	1:50	11:00	530
10/4/2013 to 10/5/13	LVWS3	R1667-1	LVWS3-30-13C-TP2	Background	1:50	11:00	530
10/4/2013 to 10/5/13	LVWS3	R1667-1	LVWS3-55-12C-TP2	Background	1:50	12:00	530
10/4/2013 to 10/5/13	LVWS3	R1667-1	LVWS3-55-13C-TP2	Background	1:50	12:00	530
10/4/2013 to 10/5/13	LVWS3	R1667-1	LVWS3-80-12C-TP2	Background	1:50	8:00	530
10/4/2013 to 10/5/13	LVWS3	R1667-1	LVWS3-80-13C-TP2	Background	1:50	8:00	530

5.0 Dive Summaries

5.1 R1662 Dive Summary

ROPOS Configuration: Large Volume Water Sampler, 2 IGTs (#3 and #4), and ROPOS temperature probe

Dive Target: Marker 113, 1526m 45.922741 -129.988104

Summary: ROPOS landed at Marker 113 (Marker 62). We looked around for a good temperature, using the ROPOS temperature probe. We then filled the LVWS for ~2 hrs, integrated with another ROPOS temperature probe. We then fired 2 IGTs (#3 and #4) and recovered the vehicle.

Table 5.1-1 R1662 IRLS Log

Date (UTC)	Observation	Latitude	Longitude	Depth (m)	Heading
9/26/2013 22:31	Sub in the water and lemons going on	45.922897	-129.988413	43.88	129.3
9/26/2013 22:47	last lemon	45.92273	-129.988283	130.74	128.9
9/26/2013 22:50	winch control handed to console	45.922763	-129.988257	194.96	130.38
9/26/2013 22:56	Test framegrab.	45.922782	-129.988195	308.26	128.92
9/26/2013 22:59	framegrab test	45.922797	-129.988192	365.8	130.11
9/26/2013 23:00	test framegrab	45.922807	-129.98825	379.96	127.21
9/26/2013 23:12	framegrab test	45.922792	-129.988212	661.43	129.07
9/26/2013 23:19	Julie test entry	45.92282	-129.988085	852.67	197.91
9/26/2013 23:45	bottom in sight	45.922823	-129.988198	1510.47	139.65
9/26/2013 23:47	we landed at Marker 113	45.922818	-129.988198	1519.9	128.61
9/26/2013 23:47	tubeworms, limpets, Marker 62 (physical marker) in site	45.922815	-129.988173	1520.12	101.49
9/26/2013 23:48	Lots of shimmery flow	45.922778	-129.988183	1521.99	93.98
9/26/2013 23:49	Positioning to take some temperatures	45.922798	-129.988157	1521.57	93.92
9/26/2013 23:50	Going to use ROPOS temperature probe to poke around	45.922803	-129.988188	1521.62	93.9
9/26/2013 23:52	only seeing 5 degrees so far	45.922802	-129.988167	1521.82	93.7

Table 5.1-1 R1662 IRLS Log Continued

Date (UTC)	Observation	Latitude	Longitude	Depth (m)	Heading
9/26/2013 23:53	10 to 11 degrees, need to reposition	45.922802	-129.988167	1522.05	93.68
9/26/2013 23:54	moved temp probe down, at 14 deg C	45.922802	-129.988167	1521.25	93.54
9/26/2013 23:55	Taking temp at Mkr 113	45.922803	-129.988172	1521.61	93.48
9/26/2013 23:56	still poking around for temperature	45.922803	-129.988167	1521.73	93.31
9/26/2013 23:58	repositioning vehicle	45.922813	-129.988177	1521.37	87.07
9/27/2013 0:02	moving around to find best position	45.922777	-129.98818	1520.68	69.93
9/27/2013 0:03	going to take another temp measurement in this clump of worms	45.922772	-129.988163	1522.03	69.85
9/27/2013 0:03	taking another temperature 15-23 deg	45.922775	-129.988165	1521.83	69.83
9/27/2013 0:05	Tmax 24.8 deg C. Moving to the right a bit now.	45.92278	-129.988162	1522.06	69.72
9/27/2013 0:06	repositioning again	45.922782	-129.98816	1521.71	69.34
9/27/2013 0:08	Temperature measurement again. 22 deg C	45.922787	-129.988175	1521.82	69.06
9/27/2013 0:09	Tmax is around 25 so far here	45.922787	-129.988175	1522.04	69.07
9/27/2013 0:09	we can see an MTR here last year. Can see its line. Tmax 26 now	45.922788	-129.988172	1521.89	69.07
9/27/2013 0:10	This is MTR 4127. Stable temp at 26.5. Want to try one more spot.	45.922787	-129.988182	1521.71	69.07
9/27/2013 0:11	Temp measurement with ROPOS probe again	45.922788	-129.988182	1522	68.8
9/27/2013 0:14	Repositioning for better sampling position	45.922767	-129.98817	1521.31	57.23
9/27/2013 0:17	Moving sampling nozzle for LVSW into sampling position.	45.922782	-129.988172	1521.88	61.61
9/27/2013 0:20	LVWS. Begin pumping. T = 21.5-22.3 C hdg 062 depth 1522.6 m. **R1662-1**	45.922788	-129.988172	1522.09	61.69
9/27/2013 0:22	LVWS. Temperature dropped to 15 C and is now climbing to 22 C.	45.922787	-129.988168	1521.97	61.74

Table 5.1-1 R1662 IRLS Log Continued

Date (UTC)	Observation	Latitude	Longitude	Depth (m)	Heading
9/27/2013 0:26	LVWS. T = 20.2 C	45.922783	-129.988165	1521.9	61.83
9/27/2013 0:29	LVWS. T around 18 C	45.922778	-129.988167	1521.96	61.9
9/27/2013 0:37	LVWS. Temperature dropped briefly to 15 C, but recovered to 24 C after slight repositioning of intake.	45.922785	-129.988158	1522.21	62.09
9/27/2013 1:39	LVWS. Starboard arm has lost power, but is holding the sampler in place properly. Temperature has remained fairly constant at ~24 C.	45.922783	-129.988158	1521.8	62.2
9/27/2013 1:47	Repositioning to continue the sample	45.922778	-129.988167	1521.84	62.21
9/27/2013 1:49	Pump back on	45.922778	-129.988162	1521.62	62.22
9/27/2013 1:49	Temperature back up to 24 deg	45.922785	-129.988192	1521.86	62.22
9/27/2013 2:17	still sampling	45.922775	-129.988158	1521.87	62.17
9/27/2013 2:18	25.3 degrees	45.922777	-129.988157	1521.91	62.16
9/27/2013 2:22	scale worm on sampler	45.922798	-129.988175	1521.94	62.17
9/27/2013 2:23	tmax was 25.7, average 24 at end	45.922783	-129.988183	1521.84	62.17
9/27/2013 2:24	stowing LVWS intake, getting ready to take 2 IGT samples	45.922797	-129.988177	1521.78	62.16
9/27/2013 2:24	Positioning for IGT3 sample at same location at LVWS. Bottle #3.	45.92279	-129.988188	1521.91	62.17
9/27/2013 2:25	IGT3 out of holster	45.922785	-129.98817	1521.78	62.16
9/27/2013 2:26	IGT. T = 23 C, starting sampling **R1662-2**	45.922778	-129.988165	1521.71	62.16
9/27/2013 2:33	IGT. T dropped slightly to 19 C	45.922782	-129.988167	1521.99	62.17
9/27/2013 2:35	IGT. Sample finished. T was 18 C during sampling.	45.922778	-129.98815	1521.63	62.17
9/27/2013 2:39	IGT. Preparing to collect another IGT sample from the same site as IGT3 and LVWS.	45.92275	-129.988152	1521.96	62.16
9/27/2013 2:41	IGT. This is IGT4. **R1662-3**	45.922773	-129.988167	1521.87	62.14

Table 5.1-1 R1662 IRLS Log Continued

Date (UTC)	Observation	Latitude	Longitude	Depth (m)	Heading
9/27/2013 2:43	IGT4 T = 23.6 C, beginning sampling.	45.922733	-129.988247	1521.74	62.16
9/27/2013 2:45	Temperature is remaining stable during sampling.	45.922775	-129.988162	1521.97	62.17
9/27/2013 2:50	Sampling complete at Marker 113. Finished on the bottom. Ascending from the bottom.	45.922783	-129.988173	1521.54	62.16
9/27/2013 3:26	600m	45.922968	-129.988458	598.78	129.43
9/27/2013 3:51	Floats coming off	45.922905	-129.988347	60.66	172.39
9/27/2013 3:54	Vehicle visible from surface	45.923022	-129.98832	43.66	79.69
9/27/2013 4:09	Vehicle on deck.	45.923155	-129.98832	4.02	101.62
9/27/2013 4:10	End of Dive R1662	45.923155	-129.98832	3.62	1.2

Table 5.1-2 Sample Summary for R1662

Number	Date (UTC)	Identifier	Description	Latitude	Longitude	Depth
R1662-1	9/27/2013 0:20	LVWS	LVWS at Marker 113	N45° 55.3688′	W129° 59.2911′	1520.47m
R1662-2	9/27/2013 2:24	IGT3	IGT3 at Marker 113	N45° 55.3671′	W129° 59.2902′	1521.78m
R1662-3	9/27/2013 2:39	IGT4	IGT4 at Marker 113	N45° 55.365′	W129° 59.2891′	1521.96m

Sample Photos from ROPOS Dive 1662

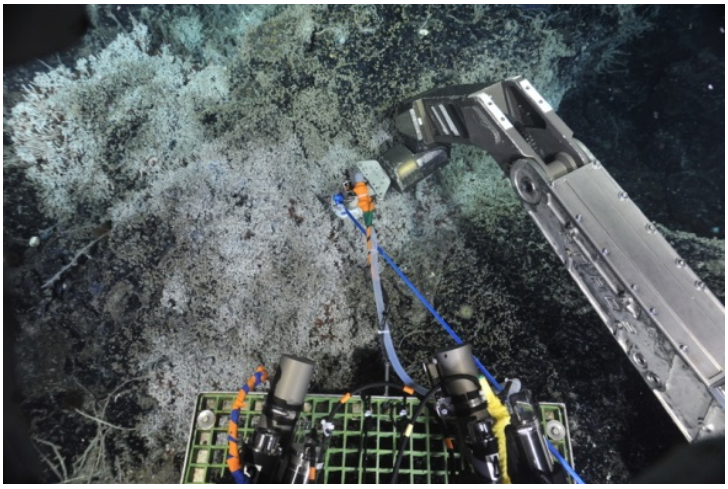

Figure 5.1-1 Sample R1662-1, Marker 113

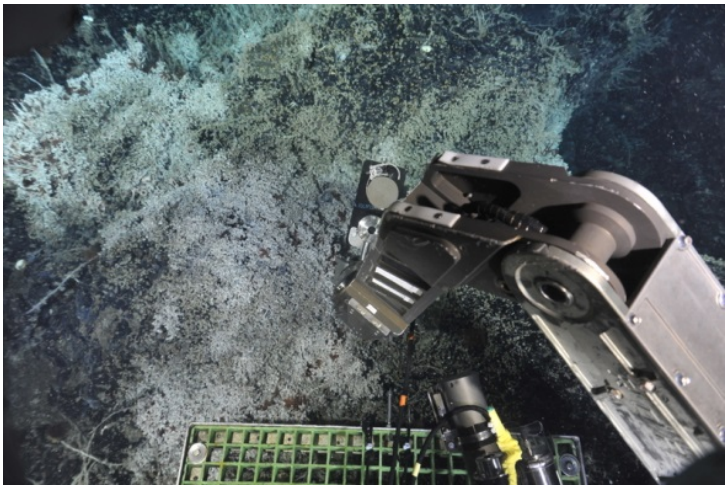

Figure 5.1-2 Sample R1662-2, Marker 113

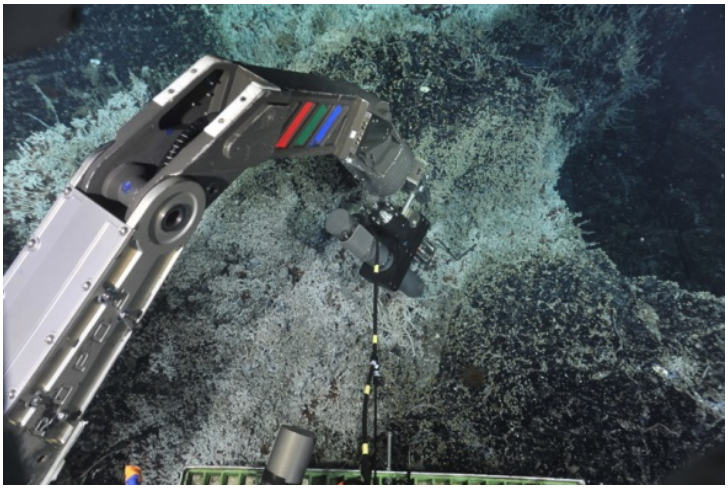

Figure 5.1-3 Sample R1662-3, Marker 113

5.2 R1663 Dive Summary

ROPOS Configuration: Hydrothermal Fluid and Particle Sampler, 2 IGTs (#1 and #2), and ROPOS temperature probe

Dive Target: Marker 113, 1526m 45.922741 -129.988104

Summary: ROPOS landed at Marker 113 (Marker 62). We looked around for a good temperature, using the ROPOS temperature probe. We then filled half of the bottles/filters/bags at Marker 113 We then transited ~500 m to Skadi. We found high temperatures (~130-215 °C) on the old sulfide mound and sampled there, then also sampled in a small tube worm bush near the large mound for lower temperature samples (~35 °C). IGTs (#3 and #4) were fired at both places within Skadi (high and low temperature). We then recovered the vehicle.

Table 5.2-1 R1663 IRLS Log

Date (UTC)	Observation	Latitude	Longitude	Depth(m)	Heading
9/27/2013 6:36	ROPOS off deck.	45.923097	-129.987938	0.52	106.11
9/27/2013 6:39	ROPOS is wet	45.923097	-129.987938	1.28	101.98
9/27/2013 6:43	ROPOS clear to dive	45.923097	-129.987938	3.58	99.98
9/27/2013 6:45	Starting to put lemons on	45.922652	-129.987953	36.56	91.07
9/27/2013 6:50	Recording oxygen on HFPS on the way down, which we can later compare to the vehicle CTD	45.92308	-129.988398	60.11	95.43
9/27/2013 6:51	Vehicle CTD is now on	45.923648	-129.988582	61.22	92.6
9/27/2013 6:53	Clear to dive	45.924418	-129.983608	72.03	94.54
9/27/2013 6:54	Heading down to 1520m	45.924418	-129.983608	78.67	94.49
9/27/2013 7:00	Passing 200m	45.923307	-129.988427	197.77	94.5
9/27/2013 7:14	Passing 500m	45.923202	-129.987882	502	94.77
9/27/2013 7:40	Passing 1000m	45.922965	-129.988132	1072.54	95.03
9/27/2013 7:58	Passing 1500m	45.922758	-129.988398	1504.48	97.96
9/27/2013 7:59	Bottom in sight- big collapsed lava pillar	45.922742	-129.988442	1517.93	100.3
9/27/2013 8:00	About 20 meters away from Marker 113	45.922722	-129.98843	1518.99	96.79
9/27/2013 8:00	Lava drainback feature with Alvin weight in the middle	45.922718	-129.988343	1520.13	91.57

Table 5.2-1 R1663 IRLS Log Continued

Date (UTC)	Observation	Latitude	Longitude	Depth(m)	Heading
9/27/2013 8:01	Marker 113 in view	45.922752	-129.988288	1519.69	83.37
9/27/2013 8:03	Getting into position for sampling at Marker 113 (where Marker 62 is)	45.922758	-129.988222	1519.42	54.62
9/27/2013 8:07	Getting in to same position as last dive at Marker 113	45.922793	-129.988197	1520.34	50.94
9/27/2013 8:17	HFPS. T = 24.8 C running O2 sensor	45.922787	-129.988213	1520.33	62.98
9/27/2013 8:18	HFPS. O2 = 0.163 ml/l, pH voltage = 3.206, T = 24.6 C	45.922787	-129.98821	1520.25	62.97
9/27/2013 8:21	HFPS. Bottle #1 LVB starting. **R1663-1**	45.922798	-129.988212	1520.29	62.93
9/27/2013 8:37	HFPS: Tmax = 24.7 C, Tave = 24.1 C, T2 = 15 C, vol = 4002 **R1663-1**	45.922778	-129.988242	1520.29	63.31
9/27/2013 8:40	HFPS: unfiltered piston #5. Tmax = 24.3 C, Tave = 24.0 C, T2 = 15 C, vol = 700 ml **R1663-2**	45.922782	-129.988212	1520.28	63.35
9/27/2013 8:45	HFPS: filtered piston 8. Tmax = 24.1 C, Tave = 23.6 C, T2 = 14.3 C, vol. 700 ml **R1663-3**	45.922788	-129.988207	1520.06	63.46
9/27/2013 8:49	HFPS: unfiltered piston#9. Tmax = 23.9 C, Tave = 23.5 C, T2 = 14.3 C, Vol = 702 ml **R1663-4**	45.922803	-129.988208	1520.46	63.53
9/27/2013 8:54	HFPS: RNA filter #14. Start pumping. **R1663-5**	45.9228	-129.988208	1520.38	63.62
9/27/2013 9:18	HFPS. RNA filter #14. Tmax = 24.7 C, Tave = 24.4 C, T2 = 15.2 C, Vol = 3002 ml **R1663-5**	45.9228	-129.988128	1520.39	64.12
9/27/2013 9:19	HFPS: Begin filtering RNA filter #15 at Marker 113. **R1663-6**	45.922807	-129.988172	1520.64	64.12
9/27/2013 9:33	HFPS: RNA filter #15. Tmax = 24.7 C, Tave = 24.5 C, T2 = 15.3 C, Vol = 3004 ml **R1663-6**	45.9228	-129.988195	1520.72	64.11
9/27/2013 9:37	HFPS: unfiltered bag #21. Tmax = 24.5 C, Tave = 24.4 C, T2 = 15.1 C, Vol = 644 ml **R1663-7**	45.922782	-129.988208	1520.78	64.11

Table 5.2-1 R1663 IRLS Log Continued

Date (UTC)	Observation	Latitude	Longitude	Depth(m)	Heading
9/27/2013 9:41	HFPS: unfiltered bag #23. Tmax = 24.8 C, Tave = 24.5 C, T2 = 15.8 C, Vol = 643 ml **R1663-8**	45.922817	-129.988218	1520.66	64.1
9/27/2013 9:46	HFPS: O2 reading. T = 24.7C, O2 = 0.131 ml/l, pH voltage = 3.203 V	45.922798	-129.98821	1520.53	64.11
9/27/2013 9:56	HFPS wand has been stowed on basket. Used port manipulator to look for MTR deployed last year. Could not find it.	45.922778	-129.988198	1520.64	64.17
9/27/2013 10:12	Found MTR from last year at edge of precipice 3 m from this year's sampling site.	45.922773	-129.988243	1520.7	102.82
9/27/2013 10:14	Begin transit to Skadi.	45.922868	-129.988173	1515.61	84.02
9/27/2013 10:25	Transiting over large area of collapse zone. Basalt is black with minimal sediment in depressions.	45.923148	-129.98635	1518.93	83.34
9/27/2013 10:32	Now transiting over laminated sheet flow.	45.9233	-129.985178	1519.21	84.48
9/27/2013 10:34	Reached contact zone between 2011 eruption and old sheet flow.	45.923343	-129.984982	1520.47	85.22
9/27/2013 10:48	Arrived at Skadi venting area	45.923467	-129.983188	1520.45	32.73
9/27/2013 10:49	Looking at small old sulfide mound with extensive diffuse venting.	45.923467	-129.98315	1520.15	51.04
9/27/2013 10:57	Taking first temperature measurement using ROPOS T probe. Tmax = 30.5 C in tubeworm bush	45.923437	-129.983148	1520.89	28.56
9/27/2013 11:02	New location 1 m away. Tmax = 32.2 C	45.923457	-129.983142	1520.9	28.44
9/27/2013 11:05	Second T measurement. Tmax = 32.9 C	45.923458	-129.983153	1521.05	66.05
9/27/2013 11:07	Moving to old sulfide mound for T measurements.	45.923487	-129.983157	1519.57	127.96
9/27/2013 11:10	Briefly found temperature as high as 130 C.	45.923552	-129.983073	1520.98	222.43

Table 5.2-1 R1663 IRLS Log Continued

Date (UTC)	Observation	Latitude	Longitude	Depth(m)	Heading
9/27/2013 11:11	Next T was 21 C	45.923512	-129.983122	1521.1	222.62
9/27/2013 11:13	Moved into bunch of palmworms. Tmax = 217.8 C	45.92353	-129.983113	1521.04	222.59
9/27/2013 11:18	HFPS: filtered piston #2. Tmax = 129.9 C, Tave = 123.4 C, Vol = 700 ml, T2 = 51 C. **R1663-9**	45.923498	-129.983102	1520.87	223.03
9/27/2013 11:41	HFPS: unfiltered piston #3. Tmax = 125.5 C, Tave = 119.8 C, Vol = 699 ml, T2 = 51 C **R1663-10**	45.923503	-129.983142	1521.12	222.28
9/27/2013 12:07	IGT1: Begin sampling. Tmax ~ 120 C. Temperature during sampling varied considerably between 35 C and 120 C. **R1663-11**	45.923493	-129.983097	1520.99	222.54
9/27/2013 12:10	Finished sampling. Tmax = 122 C but fluctuated down to 45 C.	45.923508	-129.983082	1521.15	222.52
9/27/2013 12:14	Moving from Skadi mound to recover MTR from Marker 113 that was in claw and set down on seafloor for sampling. Will stow on IGT1 bungee cord.	45.92351	-129.983107	1521.2	222.71
9/27/2013 12:17	Skadi venting is on the top edge of a collapse zone. Extensive pockets of diffuse venting in the area hosting tubeworm bushes.	45.923478	-129.983187	1521.06	166.5
9/27/2013 12:31	Move into position for low T vent fluid sampling.	45.923442	-129.98312	1521.63	69.54
9/27/2013 12:38	HFPS: unfiltered bag #18. Tmax = 34.8 C, Tave = 34.5 C, T2 = 18.1 C, vol = 642 ml **R1663-12**	45.923442	-129.983108	1521.6	69.2
9/27/2013 12:44	HFPS: unfiltered bag #19. Tmax = 35.6 C, Tave = 35.3 C, T2 = 16.7 C, vol = 642 ml **R1663-13**	45.923435	-129.983132	1521.45	69.09
9/27/2013 12:49	HFPS: filtered bag #20. Tmax = 35.2 C, Tave = 34.9 C, T2 = 15.0 C, Vol = 626 ml **R1663-14**	45.92344	-129.983123	1521.74	69.12
9/27/2013 12:55	HFPS: RNA filter #10. Begin filtering. **R1663-15**	45.923452	-129.983127	1521.45	69.32
9/27/2013 13:14	HFPS: RNA filter #10 finished. Tmax = 36.0 C, Tave = 35.5 C, T2 = 15 C, vol = 3001 ml **R1663-15**	45.923452	-129.983127	1521.4	69.24

Table 5.2-1 R1663 IRLS Log Continued

Date (UTC)	Observation	Latitude	Longitude	Depth(m)	Heading
9/27/2013 13:21	HFPS: RNA filter #11. Begin filtering. **R1663-16**	45.92344	-129.983092	1521.59	69.35
9/27/2013 13:23	HFPS: RNA filter #11 finished filtering. Tmax = 35.5 C, Tave = 35.2 C, T2 = 14.8 C, vol = 3001 ml **R1663-16**	45.923448	-129.983117	1521.71	69.36
9/27/2013 13:43	HFPS: filter bag #22. Tmax = 35.6 C, Tave = 35.4 C, T2 = 15 C, vol =642 ml **R1663-17**	45.923447	-129.983128	1521.86	69.38
9/27/2013 13:50	HFPS: O2 reading = 0.134 ml/l, pH voltage = 3.227 V	45.92344	-129.983122	1521.56	69.36
9/27/2013 14:01	Stowing HFPS sample wand. Preparing to collect IGT sample from same location.	45.923435	-129.983117	1521.53	69.36
9/27/2013 14:03	IGT2: Collecting sample at low T diffuse site. Tmax = 32.8 C, temperature steady 32.3-32.9 C. **R1663-18**	45.923448	-129.983112	1522.07	69.34
9/27/2013 14:12	Tmax = 33.0 C, finished sampling, and stowing IGT.	45.923438	-129.983113	1521.94	69.31
9/27/2013 14:16	Stowing IGT onto basket.	45.923447	-129.983118	1521.78	69.35
9/27/2013 14:17	Leaving bottom, beginning ascent to the surface.	45.923443	-129.983143	1521.17	69.03
9/27/2013 14:40	Ascent	45.923218	-129.982822	986.39	129.44
9/27/2013 15:19	Floats coming off	45.92325	-129.983825	68.28	129.65
9/27/2013 15:28	ROPOS coming up	45.922248	-129.982992	18.92	131.25
9/27/2013 15:32	ROPOS on surface	45.922223	-129.982995	3.13	125.1
9/27/2013 15:35	ROPOS on deck. End of dive.	45.922223	-129.982995	3.19	131.38

Table 5.2-2 Sample Summary for ROPOS Dive 1663

Number	Time	Identifier	Description	Latitude	Longitude	Depth
R1663-1	9/27/2013 8:21	HFPS 1	HFPS. Bottle #1 LVB. Tmax = 24.7 C, Tave = 24.1 C, T2 = 15 C, vol = 4002	N45° 55.3679′	W129° 59.2927′	1520.29m
R1663-2	9/27/2013 8:40	HFPS UP5	HFPS: unfiltered piston #5. Tmax = 24.3 C, Tave = 24.0 C, T2 = 15 C, vol = 700 ml	N45° 55.3669′	W129° 59.2927′	1520.28m
R1663-3	9/27/2013 8:45	HFPS FP8	HFPS: filtered piston 8. Tmax = 24.1 C, Tave = 23.6 C, T2 = 14.3 C, vol. 700 ml	N45° 55.3673′	W129° 59.2924′	1520.06m
R1663-4	9/27/2013 8:49	HFPS UP9	HFPS: unfiltered piston#9. Tmax = 23.9 C, Tave = 23.5 C, T2 = 14.3 C, Vol = 702 ml	N45° 55.3682′	W129° 59.2925′	1520.46m
R1663-5	9/27/2013 8:54	HFPS RNA 14	HFPS: RNA filter #14. Tmax = 24.7 C, Tave = 24.4 C, T2 = 15.2 C, Vol = 3002 ml	N45° 55.368′	W129° 59.2925′	1520.38m
R1663-6	9/27/2013 9:19	HFPS RNA 15	HFPS: RNA filter #15 at Marker 113. Tmax = 24.7 C, Tave = 24.5 C, T2 = 15.3 C, Vol = 3004 ml	N45° 55.3684′	W129° 59.2903′	1520.64m
R1663-7	9/27/2013 9:37	HFPS UB21	HFPS: unfiltered bag #21. Tmax = 24.5 C, Tave = 24.4 C, T2 = 15.1 C, Vol = 644 ml	N45° 55.3669′	W129° 59.2925′	1520.78m
R1663-8	9/27/2013 9:41	HFPS UB23	HFPS: unfiltered bag #23. Tmax = 24.8 C, Tave = 24.5 C, T2 = 15.8 C, Vol = 643 ml	N45° 55.369′	W129° 59.2931′	1520.66m
R1663-9	9/27/2013 11:18	HFPS FP2	HFPS: filtered piston #2. Tmax = 129.9 C, Tave = 123.4 C, Vol = 700 ml, T2 = 51 C.	N45° 55.4099′	W129° 58.9861′	1520.87m
R1663-10	9/27/2013 11:41	HFPS UP3	HFPS: unfiltered piston #3. Tmax = 125.5 C, Tave = 119.8 C, Vol = 699 ml, T2 = 51 C	N45° 55.4102′	W129° 58.9885′	1521.12m
R1663-11	9/27/2013 12:07	IGT1	IGT 1: Begin sampling. Tmax ~ 120 C. Temperature during sampling varied considerably between 35 C and 120 C.	N45° 55.4096′	W129° 58.9858′	1520.99m
R1663-12	9/27/2013 12:38	HFS UB18	HFPS: unfiltered bag #18. Tmax = 34.8 C, Tave = 34.5 C, T2 = 18.1 C, vol = 642 ml	N45° 55.4065′	W129° 58.9865′	1521.6m
R1663-13	9/27/2013 12:44	HFPS UB19	HFPS: unfiltered bag #19. Tmax = 35.6 C, Tave = 35.3 C, T2 = 16.7 C, vol = 642 ml	N45° 55.4061′	W129° 58.9879′	1521.45m

Table 5.2-2 Sample Summary for R1663

Number	Time	Identifier	Description	Latitude	Longitude	Depth
R1663-14	9/27/2013 12:49	HFPS FB20	HFPS: filtered bag #20. Tmax = 35.2 C, Tave = 34.9 C, T2 = 15.0 C, Vol = 626 ml	N45° 55.4064′	W129° 58.9874′	1521.74m
R1663-15	9/27/2013 12:55	HFPS RNA 10	HFPS: RNA filter #10 Tmax = 36.0 C, Tave = 35.5 C, T2 = 15 C, vol = 3001 ml	N45° 55.4071′	W129° 58.9876′	1521.45m
R1663-16	9/27/2013 13:21	HFPS RNA 11	HFPS: RNA filter #11. Tmax = 35.5 C, Tave = 35.2 C, T2 = 14.8 C, vol = 3001 ml	N45° 55.4064′	W129° 58.9855′	1521.59m
R1663-17	9/27/2013 13:43	HFPS FB22	HFPS: filter bag #22. Tmax = 35.6 C, Tave = 35.4 C, T2 = 15 C, vol =642 ml	N45° 55.4068′	W129° 58.9877′	1521.86m
R1663-18	9/27/2013 14:03	IGT2	IGT2: Collecting sample at low T diffuse site. Tmax = 32.8 C, temperature steady 32.3-32.9 C.	N45° 55.4069′	W129° 58.9867′	1522.07m

Sample Photos From Dive R1663

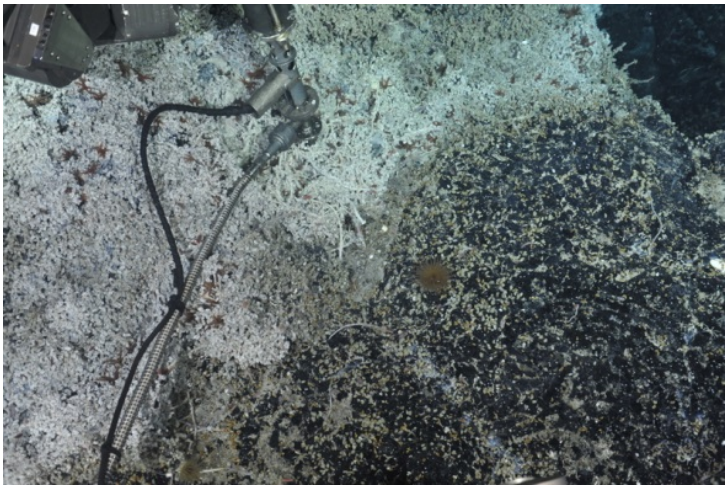

Figure 5.2-1 Sample R1663-1, Marker 113

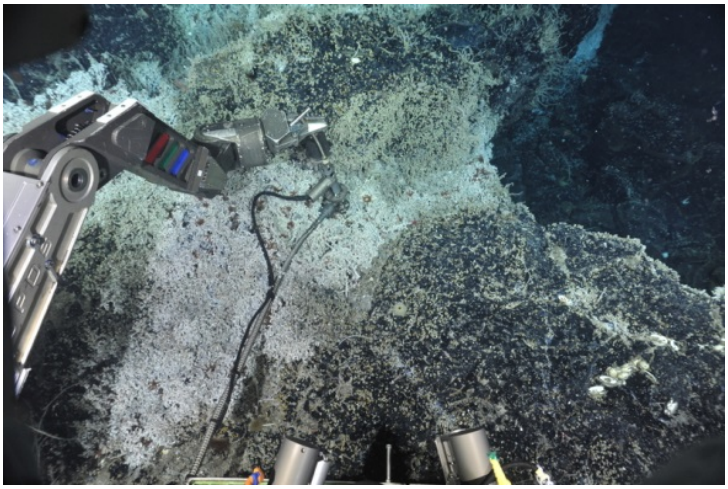

Figure 5.2-2 Sample R1663-5, Marker 113

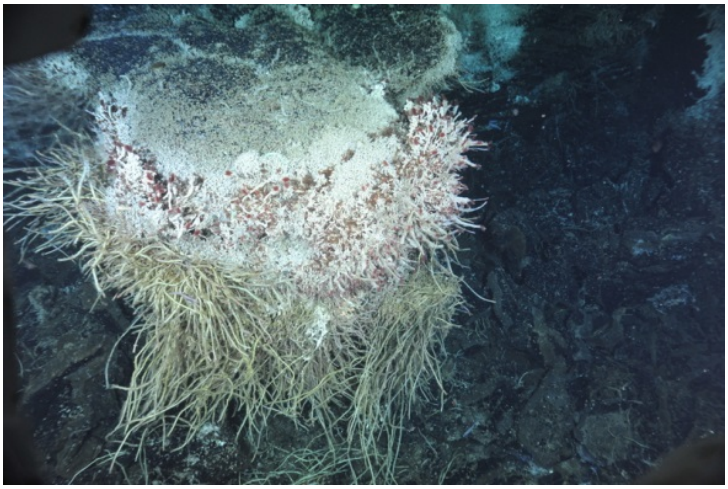

Figure 5.2-3 Marker 113 site

Sample Photos From Dive R1663 Continued

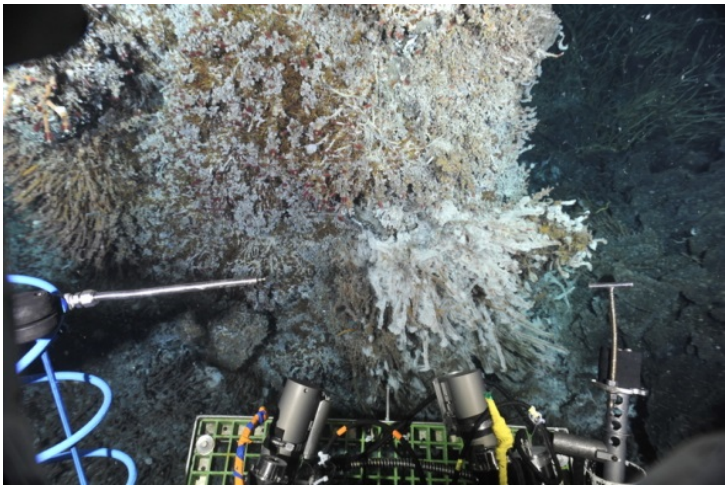

Figure 5.2-4 R1663, Skadi High Temperature

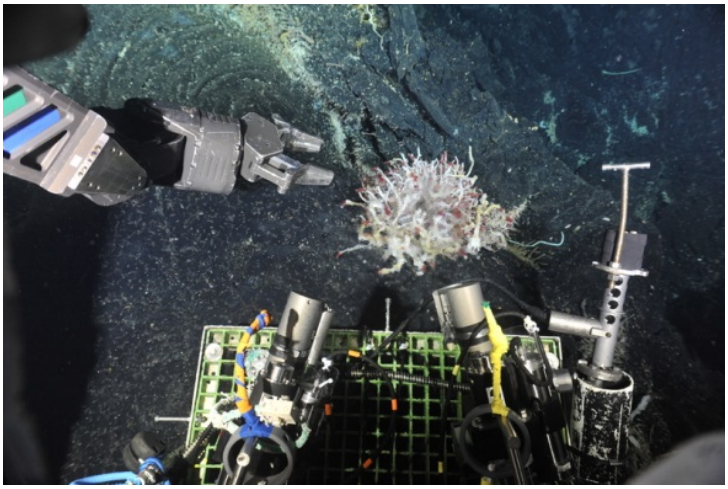

Figure 5.2-5 R1663, Skadi Low Temperature

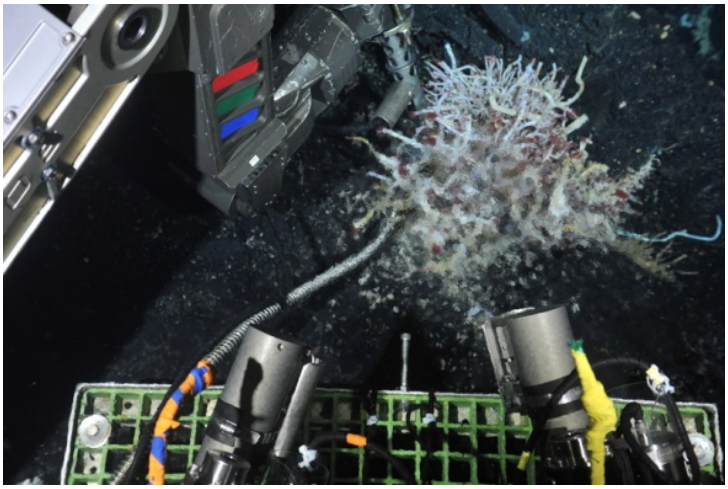

Figure 5.2-6 R1663-15, Skadi Low Temperature

5.3 R1664 Dive Summary

ROPOS Configuration: Large Volume Water Sampler, 2 IGTs (#3 and #4), and ROPOS temperature probe

Dive Target: Anemone Vent 1543m 45.933251 130.013790

Summary: ROPOS landed near Anemone Vent in the ASHES vent field. We looked around for a good temperature, using the ROPOS temperature probe. We then filled the LVWS for ~1.75 hrs, integrated with another ROPOS temperature probe. We then fired 2 IGTs (#3 and #4) and recovered the vehicle.

Table 5.3-1 R1664 IRLS Log

Date (UTC)	Observation	Latitude	Longitude	Depth (m)	Heading
9/27/2013 18:31	ROPOS off deck	45.933583	-130.015537	1.52	162.02
9/27/2013 18:33	ROPOS is wet	45.933583	-130.015537	3.13	166.89
9/27/2013 18:36	Adding lemons	45.933583	-130.015537	13.49	164.42
9/27/2013 18:41	At 50m	45.933615	-130.014158	47.67	163.28
9/27/2013 18:48	Done with floats	45.933635	-130.014383	75.56	166.33
9/27/2013 18:49	Heading down	45.933635	-130.014383	89.8	164.2
9/27/2013 19:36	We are having trouble with navigation	45.933238	-130.01353	1306.64	135.19
9/27/2013 19:42	Still working out problem with navigation	45.933242	-130.013157	1501.05	135.43
9/27/2013 19:45	Bottom in site	45.936438	-130.017287	1534.86	266.55
9/27/2013 19:45	On bottom at ASHES, to the east of target	45.936437	-130.01739	1539.45	245.7
9/27/2013 19:46	Can see camera at Mushrooms	45.936447	-130.017503	1537.68	255.13
9/27/2013 19:47	Big rat tail fish	45.936438	-130.017588	1540.17	273.11
9/27/2013 19:47	Inferno vent in field of view	45.933583	-130.01351	1540.8	274.63
9/27/2013 19:49	Heading 221 toward Hell and Anemone	45.933568	-130.013537	1540.87	220.47
9/27/2013 19:50	We think we are looking at Hell	45.933452	-130.013707	1541.61	221.17
9/27/2013 19:51	We are at Anemone	45.933342	-130.013612	1540.68	158.74

Table 5.3-1 R1664 IRLS Log Continued

Date (UTC)	Observation	Latitude	Longitude	Depth (m)	Heading
9/27/2013 19:52	Positioning to begin sampling at Anemone, Marker 129	45.933267	-130.013592	1540.2	201.34
9/27/2013 19:54	Getting out ROPOS temperature probe	45.933242	-130.013623	1542.2	199.97
9/27/2013 19:55	placing temperature probe in little chimlet; at Anemone just below the Marker, heading 200	45.933243	-130.013625	1542.08	200.35
9/27/2013 19:57	got temperature up to 60	45.933243	-130.013625	1542.05	200.59
9/27/2013 19:58	Temperature measurement of 220 C!	45.933243	-130.013627	1542.06	200.65
9/27/2013 20:00	Going to take an IGT sample with IGT #4 at this 220 fluid	45.933243	-130.013622	1541.93	200.71
9/27/2013 20:03	IGT#4 at this 220 degree fluid at ASHES **R1664-1**	45.933232	-130.013645	1542.1	200.79
9/27/2013 20:05	Positioning IGT #4 **R1664-1**	45.93323	-130.013722	1542.04	201.13
9/27/2013 20:09	Starting sample at 20:13	45.93321	-130.013767	1541.92	201.23
9/27/2013 20:11	Max temp was 213	45.933215	-130.013792	1542.13	201.22
9/27/2013 20:12	Sample complete, stowing sampler.	45.933213	-130.013792	1541.97	201.23
9/27/2013 20:17	Setting up to use ROPOS temp probe to find cooler water for Lisa	45.933228	-130.013827	1542.56	201.52
9/27/2013 20:19	37 degrees here	45.933218	-130.013818	1542.35	201.52
9/27/2013 20:20	still probing around for temperature	45.933218	-130.01382	1542.11	201.53
9/27/2013 20:22	putting it right near the MTR that was deployed there	45.933228	-130.013802	1542.49	201.56
9/27/2013 20:22	over 180 degrees there, not good for LVWS	45.933228	-130.013802	1542.17	201.53
9/27/2013 20:25	Going to probe for temp with Lisa's intake now	45.933227	-130.013783	1542.44	201.5
9/27/2013 20:25	Sampling for viruses at Anemone	45.933227	-130.013783	1542.25	201.47
9/27/2013 20:27	Probing for temperature with Lisa's probe to see what we can get	45.933227	-130.013783	1542.24	201.41
9/27/2013 20:29	We are going to take the LVWS here	45.933227	-130.013783	1542.15	201.41

Table 5.3-1 R1664 IRLS Log Continued

Date (UTC)	Observation	Latitude	Longitude	Depth (m)	Heading
9/27/2013 20:30	Scratch that, still looking around for a place without so much junk	45.933227	-130.013783	1542.23	201.4
9/27/2013 20:36	Now heading about 30 on the other side of Anemone	45.933218	-130.013832	1542.15	29.35
9/27/2013 20:38	got 19 degrees there, trying again	45.933222	-130.013833	1542.25	31.07
9/27/2013 20:39	sometimes when we push down with the sampler we see bacterial floc released from beneath the seafloor	45.933235	-130.013803	1542.42	31.1
9/27/2013 20:40	Tmax around 24.5	45.933237	-130.013805	1541.9	31.07
9/27/2013 20:41	coming back around to other side of Anemone	45.933245	-130.013808	1541.25	45.91
9/27/2013 20:43	Heading 300, temperature is coming up. More bacterial bloomy stuff coming from beneath	45.933242	-130.013768	1542.04	300.6
9/27/2013 20:46	tmax around 20 or so	45.933232	-130.013765	1542.15	300.06
9/27/2013 20:48	Moving around again back to where we started, heading 230 or so	45.933237	-130.013767	1541.41	242.29
9/27/2013 20:50	Tmax 18 there	45.933232	-130.01373	1542.26	231.9
9/27/2013 20:51	Starting sampler	45.933222	-130.013717	1542.5	231.98
9/27/2013 20:53	Tmax was 24, then down to about 18 when we started pumping **R1664-2**	45.933212	-130.013713	1542.25	232.05
9/27/2013 20:56	still sampling	45.93322	-130.013717	1542.68	232.24
9/27/2013 20:56	stop pump, valve closed	45.933222	-130.013715	1542.52	232.26
9/27/2013 20:58	we are at 28 deg C right now	45.933213	-130.013703	1542.47	232.3
9/27/2013 20:58	turning pump back on at 36 deg C **R1664-2**	45.933223	-130.013717	1542.44	232.36
9/27/2013 20:59	Holding pretty steady around 35 degrees	45.933223	-130.01371	1542.39	232.42
9/27/2013 21:13	Tmax 38.5 so far here.	45.933212	-130.013737	1542.57	232.88

Table 5.3-1 R1664 IRLS Log Continued

Date (UTC)	Observation	Latitude	Longitude	Depth (m)	Heading
9/27/2013 21:20	Tmax 45.4	45.933223	-130.013713	1542.57	232.88
9/27/2013 22:08	Sample is done!	45.933198	-130.013692	1542.39	232.6
9/27/2013 22:09	sampling IGT #3 at Anemone	45.933202	-130.013688	1542.77	232.59
9/27/2013 22:12	Getting the sampler out	45.933182	-130.013693	1542.66	233.68
9/27/2013 22:13	Checking the temperature with IGT #3 **R1664-3**	45.93319	-130.013697	1542.69	228.67
9/27/2013 22:22	Still checking for temperature	45.933188	-130.013708	1542.62	228.84
9/27/2013 22:29	still messing around looking for temperatures around 220. Stuck around 90.	45.93319	-130.013693	1542.54	228.56
9/27/2013 22:30	Found it. Taking sample now at 234	45.933188	-130.013702	1542.53	228.59
9/27/2013 22:31	Tmax is at 225 **R1664-3**	45.933187	-130.013697	1542.65	228.59
9/27/2013 22:32	New tmax around 230	45.933207	-130.013727	1542.77	228.58
9/27/2013 22:34	Tmax 232.4. Sample Done.	45.933183	-130.013717	1542.98	228.6
9/27/2013 22:37	Securing samplers.	45.933178	-130.01372	1542.65	228.65
9/27/2013 22:38	Picked up MTR that was in Anemone vent right near where we sampled 232 degree fluid.	45.933187	-130.013717	1542.52	228.66
9/27/2013 22:39	MTR is in starboard arm nestled in the platform.	45.933192	-130.013728	1542.58	228.66
9/27/2013 22:40	ROPOS leaving the bottom	45.933192	-130.013728	1542.81	228.66
9/27/2013 23:42	Lemons coming off	45.934868	-130.012652	85.35	79.17
9/28/2013 0:04	ROPOS on surface	45.939807	-130.012453	4.29	80.45
9/28/2013 0:05	on surface	45.939807	-130.012453	4.5	82.04
9/28/2013 0:10	ROPOS out of the water	45.939807	-130.012453	2.66	105.88
9/28/2013 0:12	ROPOS on deck.	45.939807	-130.012453	2.47	153.74

Table 5.3-2 Sample Summary for R1664

Number	Time	Identifier	Description	Latitude	Longitude	Depth
R1664-1	9/27/2013 20:03	IGT #4	IGT#4 at high temp Anemone	N45° 55.9939′	W130° 0.8187′	1542.1m
R1664-2	9/27/2013 20:25	LVWS	LVWS at Anemone	N45° 55.9936′	W130° 0.827′	1542.25m
R1664-3	9/27/2013 22:09	IGT #3	IGT #3 at low temp Anemone	N45° 55.9921′	W130° 0.8213′	1542.77m

Sample Photos From Dive R1664

Figure 5.3-1 R1664 Anemone Site

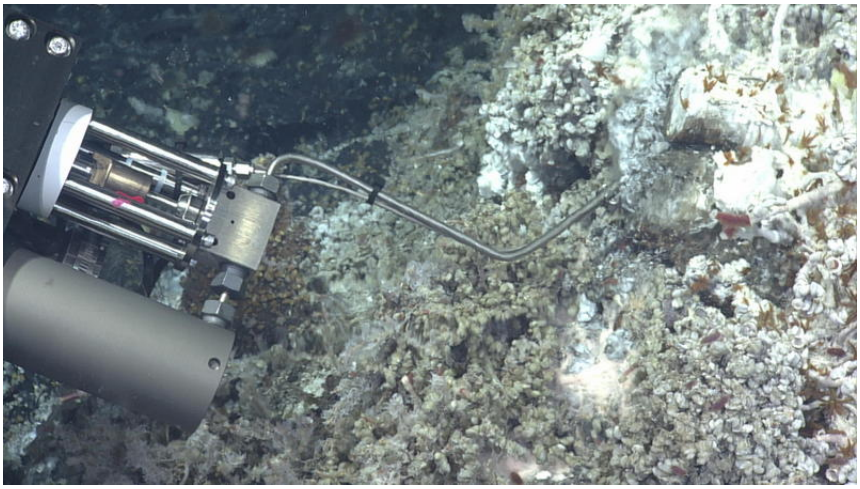

Figure 5.3-2 Sample R1664-1 at high temperature Anemone

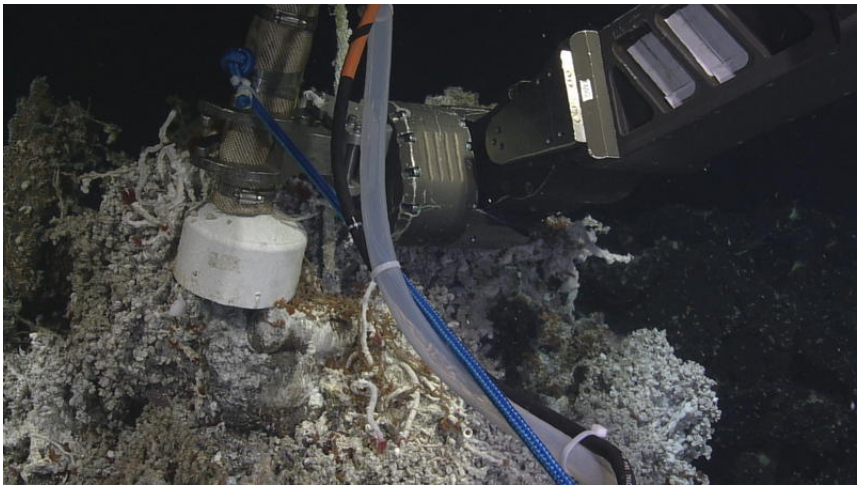

Figure 5.3-3 Sample R1664-2 at low tenmperature Anemone

5.4 R1665 Dive Summary

ROPOS Configuration: Hydrothermal Fluid and Particle Sampler, 2 IGTs (#1 and #2), and ROPOS temperature probe

Dive Target: Marker 33 1527m 45.933200 -129.982268

Summary: ROPOS landed at Marker 33 (Marker 166). We looked around for a good temperature, using the ROPOS temperature probe. We then filled half of the bottles/filters/bags at Marker 33 and fired one IGT. We then transited ~600 m south to Boca. We sampled low temperature flow there. The wind then rapidly began building and we very quickly filled the sampler and fired another IGT. We had a very quick and windy vehicle recovery.

Table 5.4-1 R1665 IRLS Log

Date (UTC)	Observation	Latitude	Longitude	Depth (m)	Heading
9/28/2013 2:17	Getting ready for launch	45.932977	-129.9831	1.59	86.64
9/28/2013 2:25	Beginning launch- ROPOS off deck	45.932977	-129.9831	1.68	137.56
9/28/2013 2:27	ROPOS is wet	45.932977	-129.9831	1.6	134.86
9/28/2013 2:30	ROPOS is clear to dive	45.932977	-129.9831	3.21	108.83
9/28/2013 2:37	Lemons going on	45.934782	-129.97862	60.44	114.43
9/28/2013 2:43	Logging oxygen and pH profile of the water column during descent	45.934768	-129.978262	68.59	13.76
9/28/2013 2:45	Lemons complete, ROPOS heading down	45.934102	-129.977363	64.47	129.9
9/28/2013 2:48	ROPOS heading down to Marker 33	45.93395	-129.977532	128.5	45.21
9/28/2013 3:38	bottom on the sonar now, 50m alt	45.933278	-129.982537	1483.24	164.82
9/28/2013 3:40	bottom in site	45.933347	-129.982437	1513.69	129.95
9/28/2013 3:42	We can see Marker 166, but really are at Marker 33	45.933258	-129.982295	1515.31	152.84
9/28/2013 3:43	Lots of lobate flow, this is lava from 2011. Red and white staining on rocks still.	45.933248	-129.982268	1515.96	154.98
9/28/2013 3:45	Nice area of shimmery flow	45.933238	-129.982257	1516.38	227.2
9/28/2013 3:45	Looking for a good place to set down and sample	45.933243	-129.982198	1515.5	222.58

Table 5.4-1 R1665 IRLS Log Continued

Date (UTC)	Observation	Latitude	Longitude	Depth (m)	Heading
9/28/2013 3:48	Taking some temperatures with the ROPOS temp probe first	45.933248	-129.982242	1516.31	210.67
9/28/2013 3:49	Tmax 37	45.933248	-129.982225	1516.36	211.34
9/28/2013 3:50	stowing ROPOS probe and getting out HFPS	45.93322	-129.982247	1516.23	211.29
9/28/2013 3:50	Sampling in a small clump of tube worms at Marker 33 where ROPOS probe got 37.7 deg C	45.933217	-129.982238	1516.35	211.23
9/28/2013 3:52	Turning on flush pump	45.933242	-129.982225	1516.56	211.19
9/28/2013 3:54	seeing around 30 with HFPS	45.933238	-129.982232	1516.2	211.22
9/28/2013 3:58	still working on finding that 37 degrees again	45.933232	-129.982233	1516.57	211.05
9/28/2013 3:58	intake of probe is mostly inserted between? in? some rocks	45.933238	-129.982238	1516.36	211.04
9/28/2013 4:00	only 8 degrees, trying again	45.933238	-129.982232	1516.53	211.02
9/28/2013 4:03	sample has not yet been started	45.93323	-129.98224	1516.39	210.99
9/28/2013 4:05	Tmax 29.8 so far, trying one more spot	45.933235	-129.982205	1515.97	210.96
9/28/2013 4:10	Still seeking temperature measurements	45.933243	-129.9822	1516.63	210.91
9/28/2013 4:15	Tmax 26 here so far	45.93323	-129.982223	1516.45	210.85
9/28/2013 4:15	ACTUALLY STARTING Large Volume Bag #1 **R1665-1**	45.933222	-129.982213	1516.1	210.84
9/28/2013 4:24	Flow is weak between cracks. Reducing flush rate raised temperature, but not to the level measured by ROPOS temperature probe. Palm worms, tubeworms, and limpets dominate.	45.933238	-129.982248	1516.48	210.77
9/28/2013 4:26	Tmax so far 28 deg	45.933237	-129.982255	1516.38	210.76
9/28/2013 4:33	Sample complete. Tmax 29,1 Tavg 27.6, volume 4002 mL, T2=14 **R1665-1**	45.933212	-129.98223	1516.27	210.7
9/28/2013 4:34	Filtered piston #2 at Marker 33, same spot as previous sample. **R1665-2**	45.933222	-129.98223	1516.47	210.71

Table 5.4-1 R1665 IRLS Log Continued

Date (UTC)	Observation	Latitude	Longitude	Depth (m)	Heading
9/28/2013 4:37	Ending sample **R1665-2**. Tmax 29.1, Tavg=28.3, vol 701 ml	45.933243	-129.982225	1516.36	210.69
9/28/2013 4:38	Unfiltered piston #3 at same spot, Marker 33 **R1665-3**	45.933227	-129.982237	1516.29	210.69
9/28/2013 4:41	Ending sample. Tmax 28.4, Tavg=27.8, Volume=701, T2=14 **R1665-3**	45.933247	-129.982233	1516.38	210.68
9/28/2013 4:42	Taking a scan with the oxygen sensor here	45.933252	-129.982258	1516.1	210.69
9/28/2013 4:44	Tmax of 29.7 in this crack so far	45.933232	-129.982228	1516.65	210.69
9/28/2013 4:47	Oxygen is about 0.3340, temperature fluctuating so not super stable	45.93323	-129.982225	1516.36	210.68
9/28/2013 4:47	stopping the sensors	45.933235	-129.982213	1516.44	210.69
9/28/2013 4:47	RNA Filter #14 at same spot, Marker 33 **R1665-4**	45.933237	-129.982212	1516.3	210.69
9/28/2013 4:48	Our heading for all of these samples is 211	45.933233	-129.982223	1516.37	210.7
9/28/2013 5:05	End of sample **R1665-4** Tmax=27.6, Tavg=26.1, volume=3002 ml	45.933228	-129.982242	1516.25	210.67
9/28/2013 5:06	Unfiltered bag #15 at same spot **R1665-5**	45.933232	-129.982223	1516.5	210.67
9/28/2013 5:10	Ending that sample. Tmax 28.5, Tavg=27.3, volume = 629ml, T2 = 13.9 **R1665-5**	45.933257	-129.982235	1516.28	210.68
9/28/2013 5:11	Same as the rest! Unfiltered Bag #19 **R1665-6**	45.933257	-129.982253	1516.4	210.68
9/28/2013 5:13	We just saw 4 knots of wind.	45.933247	-129.982237	1516.2	210.69
9/28/2013 5:14	Sample done. Tmax 282, Tavg 26.7, vol 628ml, tavg=12.8 **R1665-6**	45.933252	-129.982238	1516.29	210.69
9/28/2013 5:15	RNA Filter #13 at Marker 33 **R1665-7**	45.933252	-129.982238	1516.39	210.68
9/28/2013 5:34	Ending the sample **R1665-7**. Tmax 26.7, Tavg = 25.3, Volume = 3001, T2 = 12.4	45.93321	-129.982218	1516.03	210.75
9/28/2013 5:35	Doing another sensor scan in this spot	45.933233	-129.982238	1516	210.75
9/28/2013 5:38	oxygen was 0.345 at a temp of 25 C	45.933243	-129.982218	1516.3	210.74

Table 5.4-1 R1665 IRLS Log Continued

Date (UTC)	Observation	Latitude	Longitude	Depth (m)	Heading
9/28/2013 5:40	Stowing the HFPS intake to get out an IGT	45.933245	-129.98224	1516.04	210.72
9/28/2013 5:41	Picking up IGT#2	45.933243	-129.982235	1516.05	210.72
9/28/2013 5:42	Taking IGT #2 in the same spot as above	45.933243	-129.982233	1515.85	210.72
9/28/2013 5:46	Working on getting temperature	45.933235	-129.982233	1515.92	210.72
9/28/2013 5:47	Temperature is 29- saw 36.	45.933228	-129.982232	1516.07	210.73
9/28/2013 5:48	Starting IGT #2- 36.8 degrees. **R1665-8**	45.933225	-129.982243	1515.94	210.72
9/28/2013 5:49	Tmax so far is 40.3	45.933232	-129.982235	1515.99	210.74
9/28/2013 5:50	Sample ending	45.933233	-129.982237	1515.81	210.74
9/28/2013 5:50	Tmax was 40.65 **R1665-8**	45.933237	-129.982232	1516.18	210.74
9/28/2013 5:53	Going to do another sensor measurement with HFPS	45.933243	-129.9822	1516.08	210.73
9/28/2013 5:57	Putting HFPS probe back where Giora just took his sample	45.933212	-129.982257	1515.72	210.73
9/28/2013 5:59	Keep getting worms stuck on intake	45.933197	-129.982192	1516.04	210.44
9/28/2013 6:03	Scanning again	45.933215	-129.982217	1515.96	210.4
9/28/2013 6:04	Not getting a good temperature for scanning	45.933227	-129.982222	1516.15	210.42
9/28/2013 6:07	Going to sit at a different crack to try the sensors again	45.933242	-129.982242	1515.87	210.43
9/28/2013 6:13	stable at 28 so am going to scan	45.933217	-129.982198	1516.01	206.61
9/28/2013 6:16	oxygen was 0.276 at 27 degrees	45.933227	-129.982248	1515.89	206.61
9/28/2013 6:18	Taking a filtered piston #4 at a slightly different Marker 33 location. **R1665-9**	45.933233	-129.982223	1516	206.6
9/28/2013 6:22	sample complete **R1665-9**. Tmax 27.4, Tavg 27, volume 626ml, Tavg 14.5	45.933227	-129.982235	1516.06	206.6
9/28/2013 6:23	Stowing HFPS and heading south 600m to Boca	45.933213	-129.982247	1516.04	206.33

Table 5.4-1 R1665 IRLS Log Continued

Date (UTC)	Observation	Latitude	Longitude	Depth (m)	Heading
9/28/2013 6:23	Making our way to Boca	45.933223	-129.982252	1515.94	206.3
9/28/2013 6:28	lots of fresh lobate lava here	45.932733	-129.98242	1513.14	189.11
9/28/2013 6:29	Lava pillars and broken lava pools	45.932623	-129.98247	1514.65	188.2
9/28/2013 6:39	Old rubbly lava area here	45.931348	-129.982713	1516.54	178.12
9/28/2013 6:48	pillow lava section	45.930287	-129.982832	1518.71	178.01
9/28/2013 6:50	large rattail fish	45.930047	-129.982847	1517.82	177.84
9/28/2013 7:01	Nice singular pillar in the middle of large collapse	45.929062	-129.982945	1515.85	178.23
9/28/2013 7:05	ropos size collapse feature	45.928562	-129.982863	1515.96	169.66
9/28/2013 7:08	increased number of skylights as we enter the boca region of ex-snowblowers	45.928212	-129.982795	1515.42	169.47
9/28/2013 7:14	mrkr 170 at Boca	45.927698	-129.982448	1515.87	154.12
9/28/2013 7:17	MTR 52 located next to Mrkr 170, skylight with milky effluent right at marker, but from 2012 Nemo cruise report the Boca skylight sampled is not the one right here	45.927678	-129.982422	1516.95	154.8
9/28/2013 7:19	preparing to take temp probe of skylight near Mrkr 170	45.927672	-129.982417	1517.14	116.45
9/28/2013 7:27	taking temp probe measurements up to 7.5°C so far	45.927627	-129.982488	1516.96	114.75
9/28/2013 7:33	mini Boca skylight just above the larger skylight. Temp 6.8. This is where we are planning on sampling	45.927662	-129.982453	1517.13	120.39
9/28/2013 7:37	preparing to HFS sample mini-Boca, the small skylight adjacent to Boca at the top of the pillow mound associated with this feature	45.927627	-129.98244	1517.24	18.82
9/28/2013 7:41	mini-Boca steady 6.7Â°C fluid temp with HFPS	45.927638	-129.982393	1516.81	18.52

Table 5.4-1 R1665 IRLS Log Continued

Date (UTC)	Observation	Latitude	Longitude	Depth (m)	Heading
9/28/2013 7:42	HFPS-20 filtered bag 20 maxT=6.8 avgT=6.7 vol pumped=642 **R1665-10**	45.927613	-129.982387	1516.85	18.54
9/28/2013 7:46	HFPS-21 unfiltered bag 21 maxT=6.8 avgT=6.7 volume pumped=642 T2=4.6 **R1665-11**	45.927642	-129.982407	1516.96	18.53
9/28/2013 7:50	HFPS-22 filtered bag 22 maxT=6.7 avgT=6.7 volume pumped=641 T2=4.6 **R1665-12**	45.927647	-129.982407	1516.97	18.62
9/28/2013 7:56	HFPS-23 unfiltered bag maxT=6.7 avgT=6.7 volume pumped=642 T2=4.6 **R1665-13**	45.927637	-129.982388	1516.97	18.6
9/28/2013 7:59	HFPS-5 unfiltered piston 5 maxT=6.7 avgT=6.6 volume pumped=703 T2=4.2 **R1665-14**	45.927648	-129.982392	1516.98	18.72
9/28/2013 8:05	HFPS-10 RNA filter 10 maxT=6.8 avgT=6.7 volume pumped=3003 T2=4.6 **R1665-15**	45.927657	-129.982387	1516.85	18.62
9/28/2013 8:17	maxT=7.0 IG1 **R1665-16**	45.92764	-129.982408	1516.99	18.77
9/28/2013 8:19	IGT and HFPS in action before the storm... wind coming up to 20+ with gusts to 25	45.927645	-129.982413	1516.88	18.73
9/28/2013 8:29	ROPOS off bottom a couple of minutes ago. Dive ended quickly as winds increased from 10 knots to 25 knots in one hour.	45.927633	-129.982452	1440.36	185.54
9/28/2013 8:33	coming up at 31 m/min	45.927597	-129.982437	1322.85	30.25
9/28/2013 9:23	lemons coming off	45.930163	-129.980192	63	37.27
9/28/2013 9:30	last of the lemons	45.931303	-129.979535	17.56	35.89
9/28/2013 9:31	ROPOS at surface	45.931303	-129.979535	3.75	127.94
9/28/2013 9:37	sub on deck and secure	45.931303	-129.979535	3.25	84.17

Table 5.4-2 Sample Summary for R1665

Number	Time	Identifier	Method	Description	Latitude	Longitude	Depth
R1665-1	9/28/2013 3:50	HFPS LVB #1	HFPS	LVB#1 Tmax 29,1 Tavg 27.6, volume 4002 mL, T2=14	N45° 55.993′	W129° 58.9343′	1516.35m
R1665-2	9/28/2013 4:34	HFPS Filtered Piston 2	HFPS	HFPS-2 filtered piston Tmax 29.1, Tavg=28.3, volume 701 ml	N45° 55.9933′	W129° 58.9338′	1516.47m
R1665-3	9/28/2013 4:38	Unfiltered Piston #3	HFPS	HFPS-3 unfiltered piston Tmax 28.4, Tavg=27.8, Volume=701, T2=14	N45° 55.9936′	W129° 58.9342′	1516.29m
R1665-4	9/28/2013 4:47	RNA Filter #14	HFPS	HFPS-14 RNA Filter Tmax=27.6, Tavg=26.1, volume=3002 ml	N45° 55.9942′	W129° 58.9327′	1516.3m
R1665-5	9/28/2013 5:06	HFPS Unfiltered Bag #15	HFPS	HFPS-15 Unfiltered Bag Tmax 28.5, Tavg=27.3, volume = 629ml, T2 = 13.9	N45° 55.9939′	W129° 58.9334′	1516.5m
R1665-6	9/28/2013 5:11	HFPS Unfiltered Bag #19	HFPS	HFPS-19 Unfiltered BagTmax 282, Tavg 26.7, vol 628ml, tavg=12.8	N45° 55.9954′	W129° 58.9352′	1516.4m
R1665-7	9/28/2013 5:15	RNA Filter #13	HFPS	HFPS-13 RNA FilterTmax 26.7, Tavg = 25.3, Volume = 3001, T2 = 12.4	N45° 55.9951′	W129° 58.9343′	1516.39m
R1665-8	9/28/2013 5:42	IGT #2	IGT	IGT2-Tmax was 40.65	N45° 55.9946′	W129° 58.934′	1515.85m
R1665-9	9/28/2013 6:18	HFPS Filtered Piston #4	HFPS	HFPS-4 Filtered Piston Tmax 27.4, Tavg 27, volume 626ml, Tavg 14.5	N45° 55.994′	W129° 58.9334′	1516m
R1665-10	9/28/2013 7:42	HFS20 filtered bag #20	HFPS	HFPS-20 filtered bag 20 maxT=6.8 avgT=6.7 vol pumped=642	N45° 55.6568′	W129° 58.9432′	1516.85m
R1665-11	9/28/2013 7:46	HFPS-21 unfiltered bag 21	HFPS	HFPS-21 unfiltered bag 21 maxT=6.8 avgT=6.7 volume pumped=642 T2=4.6	N45° 55.6585′	W129° 58.9444′	1516.96m
R1665-12	9/28/2013 7:50	HFPS-22 filtered bag 22	HFPS	HFPS-22 filtered bag 22 maxT=6.7 avgT=6.7 volume pumped=641 T2=4.6	N45° 55.6588′	W129° 58.9444′	1516.97m
R1665-13	9/28/2013 7:56	HFPS-23 unfiltered bag	HFPS	HFPS-23 unfiltered bag maxT=6.7 avgT=6.7 volume pumped=642 T2=4.6	N45° 55.6582′	W129° 58.9433′	1516.97m

Table 5.4-2 Sample Summary for R1665 Continued

Number	Time	Identifier	Method	Description	Latitude	Longitude	Depth
R1665-14	9/28/2013 7:59	HFPS-5 unfiltered piston 5	HFPS	HFPS-5 unfiltered piston 5 maxT=6.7 avgT=6.6 volume pumped=703 T2=4.2	N45° 55.6589′	W129° 58.9435′	1516.98m
R1665-15	9/28/2013 8:05	HFPS-10 RNA Filter 10	HFPS	HFPS-10 RNA filter 10 maxT=6.8 avgT=6.7 volume pumped=3003 T2=4.6	N45° 55.6594′	W129° 58.9432′	1516.85m
R1665-16	9/28/2013 8:17	IGT#1	IGT	IGT1 maxT=7.0	N45° 55.6584′	W129° 58.9445′	1516.99m

Sample Photos From Dive R1665

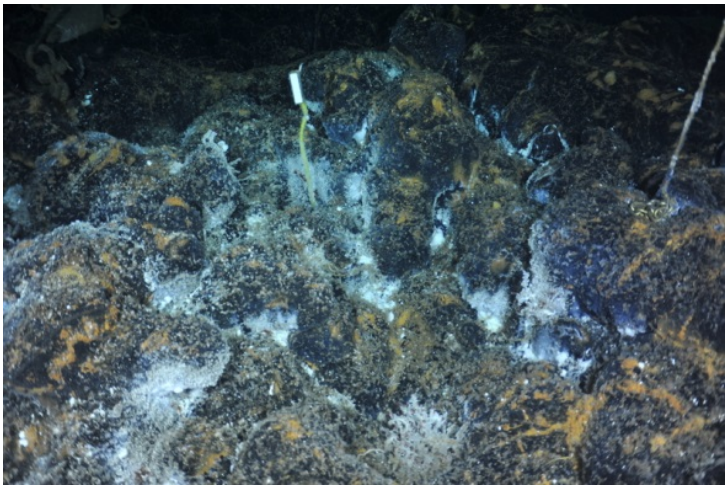

Figure 5.4-1 R1665 Marker 33 Area

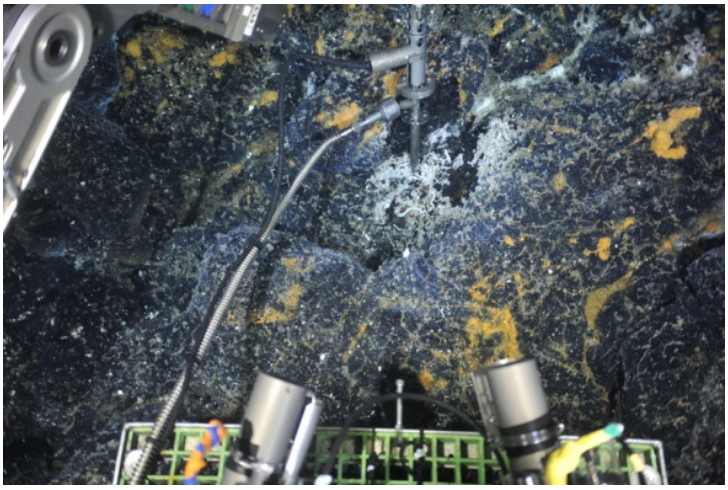

Figure 5.4-2 R1665 HFPS sampling at Marker 33

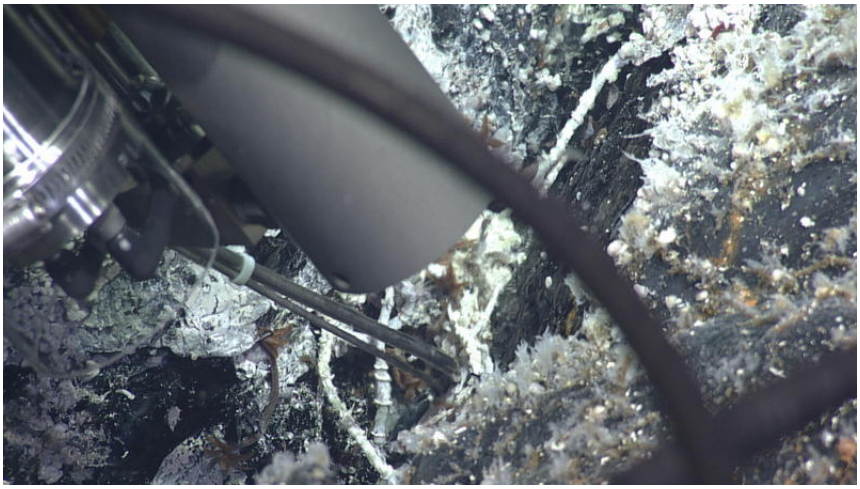

Figure 5.4-3 Sample R1665-5 at Marker 33

Sample Photos From Dive R1665 Continued

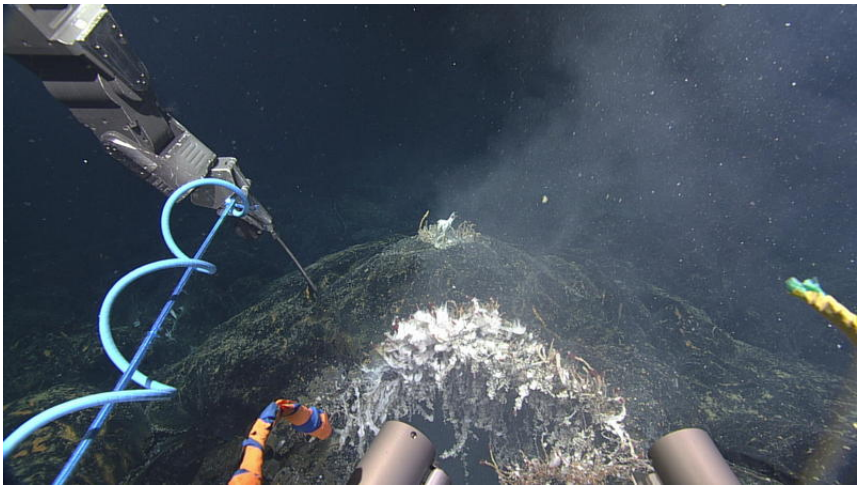

Figure 5.4-4 R1665 Boca Area

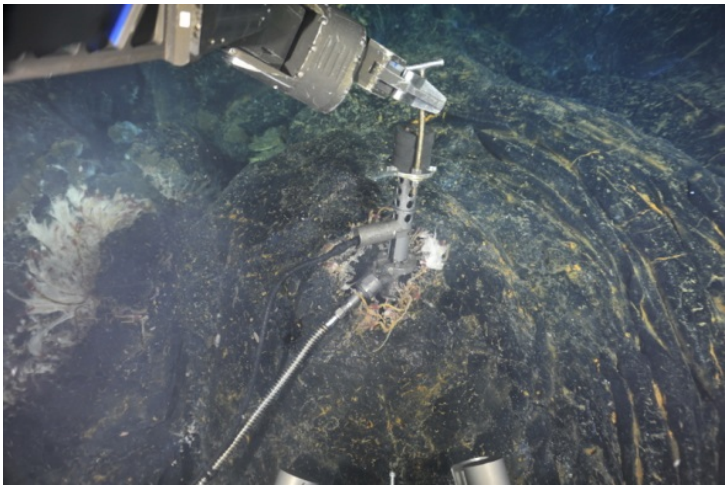

Figure 5.4-5 R1665-12 HFPS sampling at Boca

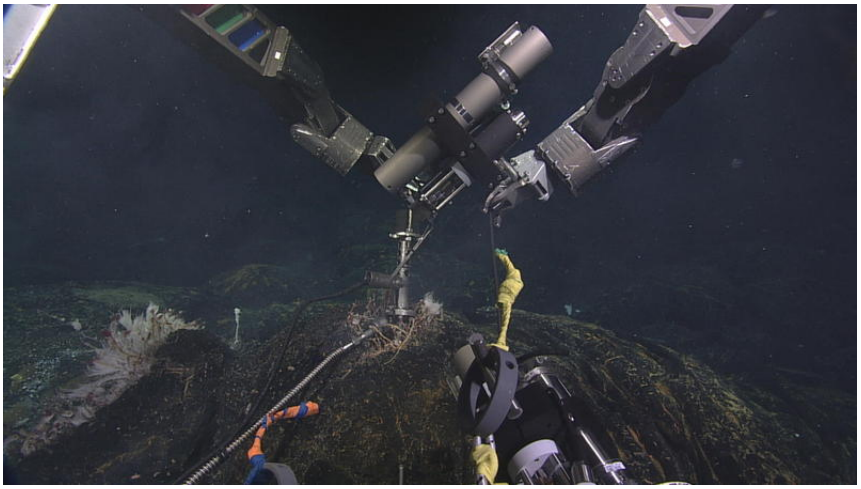

Figure 5.4-6 Sample R1665-15 IGT and HFPS sampling at Boca

5. 5 Dive Summary for ROPOS Dive R1667

ROPOS Configuration: LVWS, ROPOS temperature probe, and IGT #1 and #2

Dive Target: East of International district, 1534m, 45° 55.57 N, 129° 58.24 W

We landed on the bottom, then came up to about 1515 m and filled the sampler for 1.5 hours. We then transited 650m to Escargot in the International District. We fired the IGTs at Escargot, one in low temperature fluid, one in high temperature fluid. We then left the bottom.

Table 5.5-1 R1667 IRLS Log

Date (UTC)	Observation	Latitude	Longitude	Depth (m)	Heading
10/4/2013 0:19	ROV off deck	45.927263	-129.967653	0.43	7.47
10/4/2013 0:21	ROV in the water	45.927263	-129.967653	0.77	13.51
10/4/2013 0:26	Clear to dive, going down.	45.927263	-129.967653	3.16	359.73
10/4/2013 0:27	Stopping at 50	45.927263	-129.967653	41.98	357.81
10/4/2013 0:31	Lemons going on	45.927583	-129.968718	49.21	0.59
10/4/2013 0:40	Last lemon going on	45.92652	-129.968482	85.32	355.46
10/4/2013 0:41	ROPOS heading down	45.927128	-129.96875	87.3	354.71
10/4/2013 0:41	Heading down	45.927585	-129.968812	93.67	353.88
10/4/2013 1:04	Passing 675 m	45.925902	-129.970552	658.61	328.21
10/4/2013 1:38	20m of bottom, 1515m getting ready to start pumping	45.926057	-129.970622	1515.54	27.59
10/4/2013 1:40	Getting into position	45.926107	-129.970665	1515.31	15.88
10/4/2013 1:40	Pump started for LVWS **R1667-1**	45.926057	-129.970663	1515.27	17.1
10/4/2013 1:48	Happy Birthday J. 9 min. pumping	45.926068	-129.970463	1515.43	357.38
10/4/2013 2:30	50min. pumping. No tells yet.	45.926073	-129.970628	1515.13	352.22
10/4/2013 2:32	52min. tells on one carboy going	45.926217	-129.970593	1515.22	353.93
10/4/2013 3:02	LVWS pumping 1:20min so far. Happy Birthday J again.	45.92611	-129.970633	1515.26	352.47
10/4/2013 3:10	Close valve. Pumping stopped, 1:30min total pumping time. **R1667-1** complete	45.926087	-129.970712	1515.4	352.31

Table 5.5-1 R1667 IRLS Log Continued

Date (UTC)	Observation	Latitude	Longitude	Depth (m)	Heading
10/4/2013 3:13	We have the seafloor in view and are heading to Escargot	45.925982	-129.970537	1529.79	298.7
10/4/2013 3:20	Lots of rubbly lava with light sediment cover	45.926408	-129.972178	1533.48	310.01
10/4/2013 3:29	coming off the bottom to keep up with ship	45.927012	-129.974165	1519.6	270.56
10/4/2013 3:43	back on the bottom	45.926847	-129.976618	1526.82	268.93
10/4/2013 3:48	Still en route to Escargot	45.926638	-129.97753	1526.88	252.74
10/4/2013 3:51	The cable is in view	45.926607	-129.977907	1527.04	262.55
10/4/2013 3:57	We are on a ridge, Diva off in the distance	45.926442	-129.978827	1518.71	254.14
10/4/2013 3:58	We are at International District, Diva	45.926408	-129.97896	1519.59	218.62
10/4/2013 3:59	Marker 150 in view	45.926312	-129.978977	1518.17	312.26
10/4/2013 4:00	Escargot in view	45.926277	-129.979092	1516.77	323.58
10/4/2013 4:03	Getting into position to sample low temperature flow at Escargot. Lots of microbial mat, ciliate purple mat, etc.	45.926352	-129.979247	1517.48	72.46
10/4/2013 4:11	Sampling low temperature flow at Escargot	45.926372	-129.979188	1519.78	73.58
10/4/2013 4:12	Preparing to sample	45.92638	-129.979192	1519.89	73.51
10/4/2013 4:16	Saw tmax at 23 degrees so far. Lots of floc coming out	45.926393	-129.979205	1520.06	73.57
10/4/2013 4:18	TONS of floc coming out of the structure as we perturb it. Video Highlight.	45.926377	-129.979213	1519.72	73.7
10/4/2013 4:20	Still getting in position.	45.926388	-129.979243	1520.15	73.4
10/4/2013 4:21	Got up to 32.3 that time. Starting sample now. **R1667-2**	45.926387	-129.979248	1519.86	73.29
10/4/2013 4:21	Tmax so far is 41.	45.926383	-129.979258	1520.15	73.49
10/4/2013 4:24	Sample complete. Tmax 41.3 degrees.	45.926392	-129.979257	1519.9	73.35
10/4/2013 4:26	High temperature IGT at Escargot	45.926393	-129.979258	1519.8	69.13

Table 5.5-1 R1667 IRLS Log Continued

Date (UTC)	Observation	Latitude	Longitude	Depth (m)	Heading
10/4/2013 4:28	There is a HOBO probe where Giora wants to sample	45.926383	-129.979177	1516.92	313.35
10/4/2013 4:34	Breaking little chimney off to get better sampling orifice for us	45.926403	-129.979202	1517.29	239.49
10/4/2013 4:37	Saw Tmax of 165, should be able to get up to 270	45.926383	-129.979168	1517.47	239.36
10/4/2013 4:39	Starting the sample now, Tmax 268. **R1667-3**	45.926383	-129.979082	1517.27	239.39
10/4/2013 4:41	Tmax of 270 degrees	45.926408	-129.979152	1517.48	239.42
10/4/2013 4:42	We are done with that sample.	45.926403	-129.97918	1517.61	239.38
10/4/2013 4:45	Stowing IGTs for recovery	45.926367	-129.979138	1511.11	270.98
10/4/2013 4:46	Heading up	45.926342	-129.979052	1511.5	275.98
10/4/2013 5:53	At 35 m	45.926092	-129.978478	35.15	29.43
10/4/2013 5:55	ROPOS on surface	45.926093	-129.978498	4.42	32.91
10/4/2013 6:00	ROPOS on deck.	45.926093	-129.978498	4.36	46

Table 5.5-2 R1667 Sample Summary

Number	Date (UTC)	Identifier	Description	Latitude	Longitude	Depth
R1667-1	10/4/2013 3:46	LVWS	LVWS in background seawater	N45° 55.568′	W129° 58.2425′	1515.25m
R1667-2	10/4/2013 4:11	IGT #2	IGT #2 in Low Temperature Flow at Escargot, Tmax=41 C	N45° 55.5823′	W129° 58.7513′	1519.78m
R1667-3	10/4/2013 4:26	IGT #1	IGT #1 in High Temperature Flow at Escargot, Tmax=270 C	N45° 55.5814′	W129° 58.7518′	1519.19m

Sample Photos From Dive R1667

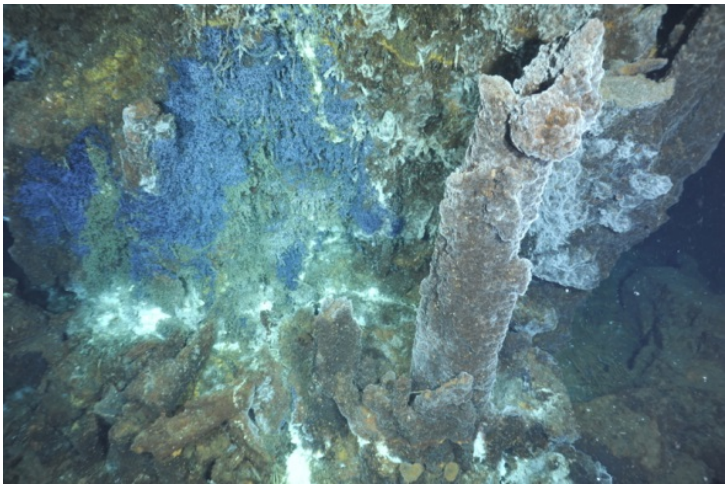

Figure 5.5-1 R1667 Escargot Base

Figure 5.5-2 R1667 Escargot Top

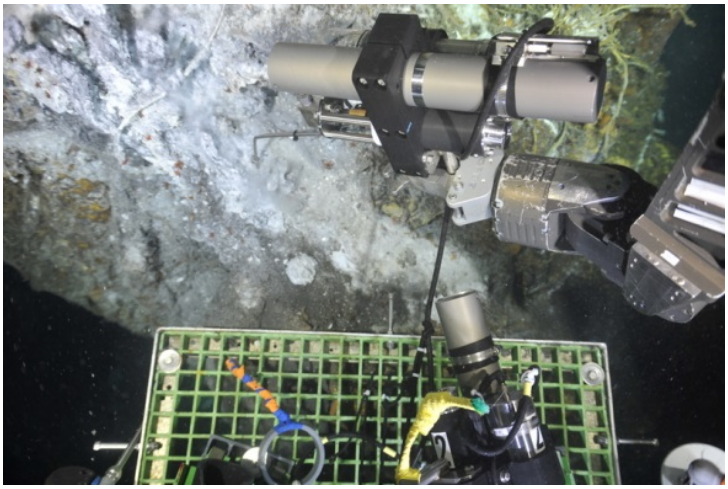

Figure 5.5-3 Sample R1667-3 IGT sampling at Escargot

6.0 Weather

Because we talked about it a lot.

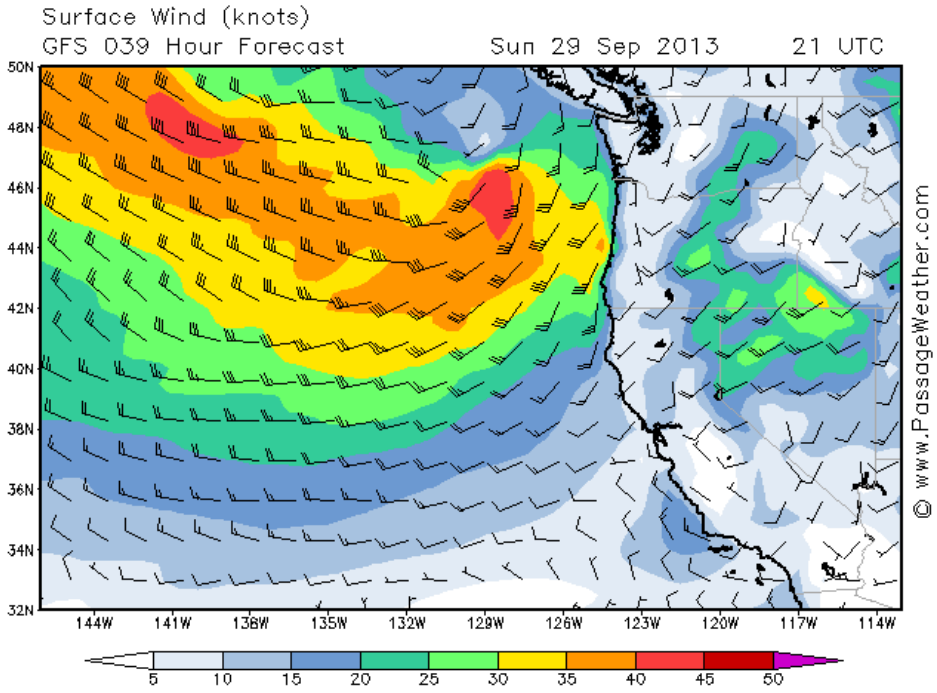

Figure 6-1 Weather forecast just before the storm

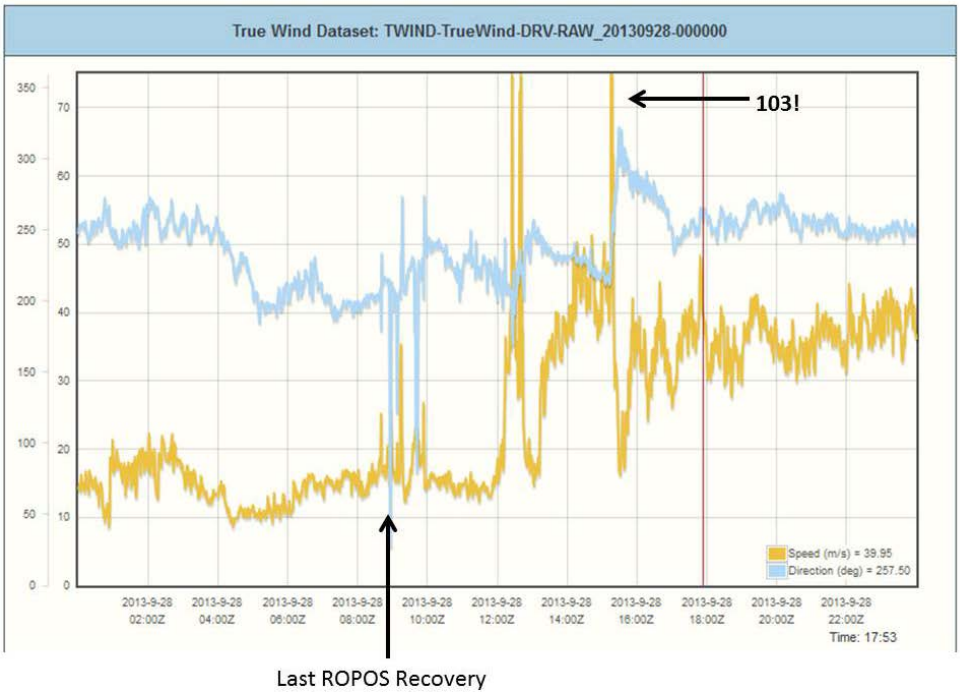

Figure 6-2 Wind speed and direction from the calm before the storm and then, the storm!