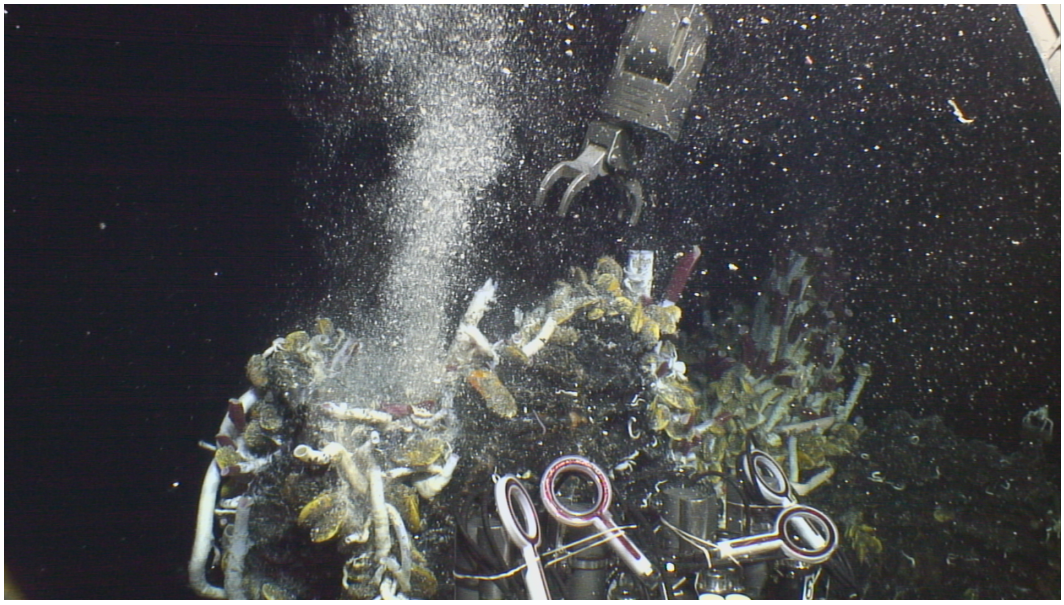
An Integrated Study of Energy Metabolism, Carbon Fixation, and Colonization Mechanisms in Chemosynthetic Microbial Communities at Deep-Sea Vents



AT26-10

R/V Atlantis and *ROV Jason II*

December 27, 2013 – January 26, 2014

Puntarenas, Costa Rica – Panama City, Panama

Stefan M. Sievert, Chief Scientist

Research funded by National Science Foundation

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Cruise Report AT26-10

1. Cruise Objectives

Deep-sea hydrothermal vents, first discovered in 1977, are exemplary ecosystems where microbial chemosynthesis rather than photosynthesis is the primary source of organic carbon. Significant gaps remain in our understanding of the underlying microbiology and biogeochemistry of these fascinating ecosystems. Missing are the identification of specific microorganisms mediating critical reactions in various geothermal systems, metabolic pathways used by the microbes, rates of the catalyzed reactions, amounts of organic carbon being produced, and the larger role of these ecosystems in global biogeochemical cycles. To fill these gaps, we are carrying out an interdisciplinary, international hypothesis-driven research program to understand microbial processes and their quantitative importance at deep-sea vents. Specifically, we will address the following objectives:

- Determine key relationships between the taxonomic, genetic and functional diversity, as well as the mechanisms of energy and carbon transfer, in deep-sea hydrothermal vent microbial communities.
- Identify the predominant metabolic pathways and thus the main energy sources driving chemoautotrophic production in high and low temperature diffuse flow vents.
- Determine energy conservation efficiency and rates of aerobic and anaerobic chemosynthetic primary productivity in high and low temperature diffuse flow vents.
- Determine gene expression patterns in diffuse-flow vent microbial communities during attachment to substrates and the development of biofilms.

This is an interdisciplinary project funded by NSF Dimensions of Biodiversity Grant '**Dimensions: Collaborative Research: An Integrated Study of Energy Metabolism, Carbon Fixation, and Colonization Mechanisms in Chemosynthetic Microbial Communities at Deep- Sea Vents**' involving PIs from four US institutions (WHOI – Stefan Sievert [lead], Jeffrey Seewald, Craig Taylor; Rutgers – Costantino Vetriani; Carnegie Institution of Washington – Dionysis Foustoukos; Bigelow Laboratory – Ramunas Stepanauskas), and collaborators in Germany (Dr. Musat [now at the Helmholtz Centre for Environmental Research, Leipzig], Dr. Schweder [Greifswald University]), France (Dr. Le Bris [UPMC, Banyuls-sur-Mer]), and China (Dr. Wang [Jiao Tong University, Shanghai]). We additionally have collaborations with Dr. Solveig Bühring at Marum, Bremen, Germany (lipid biomarker analyses), and Dr. Leonid Germanovich, who joined the cruise and measured the fluid flow rate of the studied vents.

2. Summary of Activities

The research expedition with *R/V Atlantis* and *ROV Jason II* was funded by NSF Dimensions of Biodiversity Grant '**Dimensions: Collaborative Research: An Integrated Study of Energy Metabolism, Carbon Fixation, and Colonization Mechanisms in Chemosynthetic Microbial Communities at Deep- Sea Vents**'. *R/V Atlantis* left Puntarenas, Costa Rica on December 29, 2013 and arrived in Panama City, Panama on January 27, 2014. The field site was located at the deep-sea hydrothermal vent field at 9° 50' N on the East Pacific Rise. We collected

samples from a number of sites, but our main activities took place at a diffuse-flow vent site named 'Crab Spa'. The dive program was got cut short by 3 days due to a medical emergency of a crewmember. An extensive research program was carried out that included the following:

a) A total of 65 water samples were taken with isobaric gastight samplers (IGT). Twenty-two of these were used to determine the chemistry of diffuse-flow and black-smoker fluids while the remainder were used for microbial incubation experiments (see below). Dissolved H_2, CH_4, CO, O_2, $\sum H_2S$, $\sum NH_3$, H_2O_2, and pH (25°C) were also measured at sea. Upon return to the laboratory at WHOI, the abundance of major cations and anions were determined by ion chromatography. While at sea samples were filtered and archived (frozen) for future measurement of the abundance and isotopic composition of dissolved nitrogen species in both high temperature and diffuse fluids.
b) IGTs were also used to perform incubations under simulated *in situ* conditions on board the ship. Originally designed for chemical sampling from hydrothermal fluids, these samplers are able to maintain fluids from the deep sea at *in-situ* pressure. The samplers were modified for use as a high pressure experimental chamber that would allow the external control of the chemical environment inhabited by microbial populations collected with low temperature vent fluid samples.
c) IGT's were also used to feed a high-pressure flow through system developed by Co-PI Dr. Dionysis Foustoukos at Carnegie.
d) Filtration of hydrothermal vent fluids with a Large Volume Pump (LVP) to collect biomass for subsequent 'omic' analyses (metagenomic, metatranscriptomic, metaproteomic) and lipid analyses. In total, we had 12 LVP deployments, of which 4 were at 'Crab Spa'.
e) Deployment and recovery of microbial colonizers to study microbial biofilm formation and colonization. This is work done by Co-PI Dr. Costa Vetriani at Rutgers.
f) Collection of chimney samples for microbial community analyses and cultivation. Samples were taken by Co-PI Dr. Costa Vetrinani and were also provided to international collaborator Fengping Wang from Shanghai, China.
g) In situ chemistry measurements (pH, temperature, sulfide, iron) were performed by international collaborator Dr. Nadine Le Bris from Banyuls-sur-Mer, France.
h) Tube worms and mussels were collected to perform analyses of the their endosymbionts (work by international collaborator Dr. Thomas Schweder, Greifswald, Germany).
i) Dr. Leonid Germanovich from Georgia Tech joined the cruise to perform flow-rate measurements of the various vents sites visited during the cruise.
j) We had an extensive outreach component, which involved two science writers, one for the WHOI Dive & Discover website (David Levin) and a science writer from Scholastic Magazine (Jennifer Barone). We further had a live feed to the New Bedford Ocean Explorium, which featured an exhibition of deep-sea hydrothermal vents as part of an NSF grant provided to Stace Beaulieu at WHOI (NSF 1202977). Sievert also visited schools (5-6 grade) before and after the cruise to talk about the research.

Table 1 Summary of Dive Program

Dive #	Date [GMT]	Max depth [m]	Hours in Water	Hours on Bottom	Deployments	
					Elevator	LVP
J2-758	2014/01/02 - 2014/01/07	2512	103.9	99.9	4	2
J2-759	2014/01/08 - 2014/01/12	2519	90.1	86.3	7	4
J2-760	2014/01/13 - 2014/01/15	2521	54	50.25	3	2
J2-761	2014/01/16 - 2014/01/18	2520	70.1	66.9	4	3
J2-762	2014/01/19 - 2014/01/19	2512	5.7	2.6	-	1
Total			322.75	306	18	12

Table 2 Main vent sites visited during cruise

	Longitude (W)	Latitude (N)	Depth
L vent	104 16.74	9 46.25	2528
P vent	104 17.47	9 50.28	2506
Mk L-O (sandwiches)	104 17.46	9 50.27	2507
Bio 9	104 17.30	9 50.30	2503
Crab Spa (Tica)	104 17.48	9 50.39	2503
Riftia colony (Tica)	104 17.49	9 50.39	2511
Teddy Bear	104 17.51	9 50.50	2514
M vent	104 17.53	9 50.97	2500
Flea vent	104 17.60	9 50.81	2519
Bio vent	104 17.61	9 50.95	2499
Perseverance	104 17.59	9 50.95	2507

Abbreviations of instruments mentioned in report

IGT – Isobaric gas-tight sampler
ISO – In situ optode
LTS – Long-term sensor
LVP – Large volume pump
MJ – Major sampler
SIP – Small in situ pump
SPOT – in situ chemical sensor

3. Science Participants

Name	Affiliation	Status
Stefan Sievert, Chief Scientist	WHOI	Scientist
Jeff Seewald	WHOI	Scientist
Craig Taylor	WHOI	Scientist
Jesse McNichol	WHOI	Grad Student
François Thomas	WHOI	Post-Doc
Kerry McCullough	WHOI	Undergrad Student
Sean Sylva	WHOI	Technician
Xi Wei	UFZ Leipzig, Germany	Grad Student
Miriam Sollich	MARUM, Bremen, Germany	Grad Student
Costantino Vetriani	Rutgers	Scientist
Donato Giovanelli	Rutgers	Post-Doc
Ashley Grosche	Rutgers	Grad Student
Dionysis Foustoukos	Carnegie	Scientist
Ileana Perez Rodriguez	Carnegie	Post-Doc
Matthew Rawls	Carnegie	Undergrad Student
Nadine le Bris	UPMC, Banyuls, France	Scientist
Erwan Peru	UPMC, Banyuls, France	Technician
Horst Felbeck	SIO	Scientist
Ruby Ponnudurai	Greifswald, Germany	Post-Doc
Xiao Xiang	Jiao Tong University, Shanghai, China	Scientist
Leonid Germanovich	University of Georgia	Scientist
David Levin	Boston	Science Writer
Jennifer Barone	Scholastic Magazine	Science Writer

4. Jason Navigation

Jason navigation on this cruise was derived from a combination of accurate absolute navigation and precise relative navigation. The Sonardyne Ranger ultra short baseline system (USBL) embedded on Atlantis acoustically tracks Jason determining a range, bearing and depth from the ship. These positions are then converted into absolute positions by tying them to the ships GPS derived position. Relative navigation of Jason is derived from bottom tracking using a DVL (doppler velocity log). When Jason reaches the seafloor and obtains bottom lock with the DVL an absolute position is determined by taking an average of many USBL fixes. Jason's relative position is then reset to this absolute position. Jason's movement relative to the seafloor is then tracked very precisely using the DVL. Occasionally the reset process is repeated when DVL bottom lock is lost or drifts. Medea, elevators and the LVP were tracked using the USBL system. The Atlantis USBL system was calibrated on the previous cruise with good results. The quality of navigation on the cruise was very good. The spread of USBL fixes was approximately 10-15 meters which is much better than the 1% of water depth specification of the system. DVL navigation was also of good quality. The relatively flat hard seafloor at 9º N allowed for consistent doppler bottom lock. Known vent sites were easily found and relocated on subsequent lowerings. Elevators and the LVP were accurately tracked and easily found on the seafloor.

5. Summaries of research groups

A. WHOI research team: Dr. Jeff Seewald, Dr. Stefan Sievert, Dr. Craig Taylor, Ms. Kerry McCulloch, Mr. Jesse McNichol, Mr. Sean Sylva, Dr. François Thomas plus Xi Wei and Miriam Sollich

Isobaric Gas Tight Samplers

A total of 65 water samples were taken with isobaric gastight samplers (IGT). Twenty-two of these were used to determine the chemistry of diffuse-flow and black-smoker fluids while the remainder were used for microbial incubation experiments (see below). Dissolved H_2, CH_4, CO, O_2, $\sum H_2S$, $\sum NH_3$, H_2O_2, and pH (25°C) were also measured at sea. Upon return to the laboratory at WHOI, the abundance of major cations and anions were determined by ion chromatography. While at sea samples were filtered and archived (frozen) for future measurement of the abundance and isotopic composition of dissolved nitrogen species in both high temperature and diffuse fluids. IGTs were also used to perform incubations under simulated *in situ* conditions on board the ship. Originally designed for chemical sampling from hydrothermal fluids, these samplers are able to maintain fluids from the deep sea at *in-situ* pressure. The samplers were modified for use as a high pressure experimental chamber that would allow the external control of the chemical environment inhabited by microbial populations collected with low temperature vent fluid samples. By withdrawing fluid samples at selected time intervals, we were able to monitor both the growth of microbes and changes in *in situ* fluid chemistry under conditions that very closely mimic the natural environment. This was the first time these samplers were used for measuring microbial activities. The incubations were performed under variety conditions by adding different electron donors and acceptors. Further, incubations were carried out at different temperatures. Onboard the ship, a full suite

of chemical measurements (H_2, H_2S, O_2, NH_3 and CH_4) were carried out to monitor microbial activity in the incubations. Post-cruise, nitrate was also measured, which together with the shipboard measurements, gave a precise accounting for the majority of substrates responsible for microbial growth in this system. Microbial growth was monitored on board the ship by acridine orange direct counts. Samples were also preserved during the cruise for subsequent analysis using a combination of CARD-FISH and NanoSIMS to identify microbes and to determine the amount of inorganic carbon being fixed by the cells (collaboration with Dr. Musat). Finally, subsamples were taken for single genome analyses (work by Co-PI Dr. Ramunas Stepanauskas at Bigelow), and biomass was preserved from each sample for DNA extraction to monitor the effect of the experimental conditions on the microbial community composition.

Large Volume Pump

Filtration of hydrothermal vent fluids with a Large Volume Pump (LVP) to collect biomass for subsequent 'omic' analyses (metagenomic, metatranscriptomic, metaproteomic) and lipid analyses. In total, we had 12 LVP deployments, of which 4 were at 'Crab Spa' (Table 3).

Table 3 Summary of LVP Deployments

Deploy Number	Site of Sampling	Filtration Start Date, Time (GMT)	Filtration Time (hrs)	Release of Pump Date, Time (GMT)
1	Crab Spa with animals	2014/01/03, 8:00	12	2014/01/03, 20:09
2	Crab Spa, animals removed	2014/01/04, 22:00	16	2014/01/05, 15:07
3	Crab Spa	2014/01/09, 6:00	8	2014/01/09, 5:02
4	Crab Spa	2014/01/10, 6:00	8	2014/01/10, 14:31
5	Teddy Bear	2014/01/10, 9:00	8	2014/01/11, 17:17
6	Hole to Hell	2014/01/12, 5:00	8	2014/01/12, 13:05
7	Teddy Bear	---	0	2014/01/14, 12:00
8	Teddy Bear	2014/01/14, 8:00	6	2014/01/15, 00:10
9	L-Vent	2014/01/16, 7:00	8	2014/01/16, 16:39
10	Riftia colony	2014/01/17, 9:00	8	2014/01/17, 17:25
11	Riftia colony	2014/01/18, 7:00	8	2014/01/18, 5:09
12	Riftia colony	---	0	2014/01/19, 05:31

Oxygen measurements

In situ oxygen measurements were carried out with an Aanderaa Oxygen Optode 4330F (ISO). Data were streamed real time via Jason-II communication.

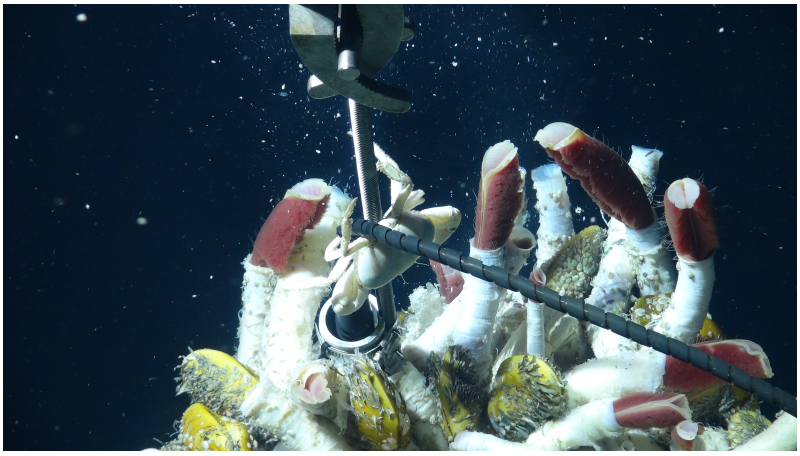


Fig. 1 Oxygen sensor at Crab Spa.

B. CIW research team: Dr. Ileana Perez-Rodriguez, Mr. Matt Rawls, and Dr. Dionysis I. Foustoukos

1. Shipboard continuous culturing experiments:

The CIW team was responsible for the shipboard continuous culturing incubations of vent fluids collected from Crab Spa and Tica hot springs during the AT26-10 expedition at 9°N EPR by utilizing our high-pressure bioreactor (Fig. 2). This was accomplished through a collaborative effort with Jeff Seewald and Sean Sylva

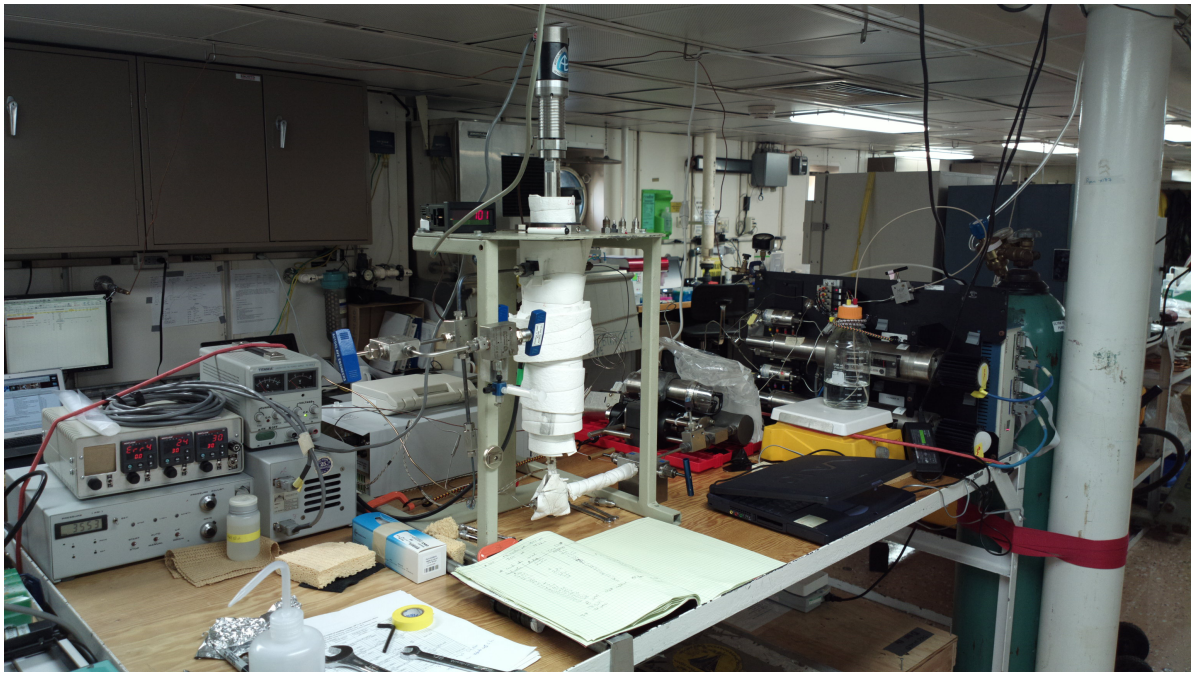


Figure 2. The CIW high-pressure bioreactor deployed onboard the R/V Atlantis. Continous culturing experiments of microbial communities from Crab Spa and Tica were conducted at in-situ pressure (250 bar) and temperature (30-50°C) conditions by integrating vent fluid sampling techniques (Isobaric-Gas-Tight sampler, WHOI) with our experimental facility.

(WHOI), who deployed isobaric gas-tight samplers (IGTs) to collect hydrothermal vent fluids at the diffuse flow sites. Experiments were designed to study the cycling to N through the metabolic processes of denitrification and dissimilatory nitrate reduction to ammonia (DNRA) under *in-situ* deep-sea vent temperature and pressure conditions.

We studied the evolution of nitrate reducing microorganisms at mesophilic (30^{o}C) and thermophilic (50^{o}C) conditions at pressures ranging from 5 to 250 bar. Vent fluids (16 IGTs) were delivered in the bioreactor and homogeneously mixed with aqueous media solution enriched in dissolved nitrate, hydrogen and ^{13}C labeled bicarbonate to facilitate the growth of nitrate reducing microorganisms (Fig. 3). The two distinct sets of experiments were lasted for 356 and 100 hours. In short, experimental results constrained the function and metabolic rates of the denitrifying microbial communities in the Crab Spa fluids, while DNRA metabolic pathways were identified for the populations residing in the moderate temperature vent fluids (60^{o}C) of the Alvinella colony at Tica.

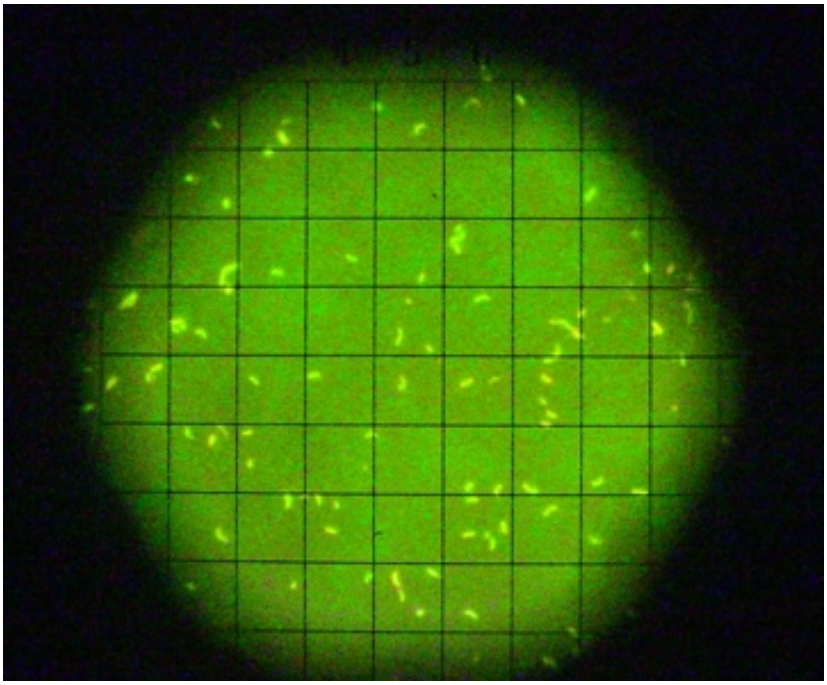


Figure 3. Epi-fluorescence microscopy of acridine orange stained microorganisms collected from the Crab Spa diffuse flow site and cultured at *in-situ* pressure conditions (250 bar). Photo courtesy of Matt Rawls.

During the course of the experiments we monitored the growth of deep-sea microbial communities by measuring the concentrations of dissolved aqueous species directly involved in nitrate based metabolism, such as NO_3, NH_4, H_2 and H_2S. We also monitored cell densities by utilizing an epi-fluorescence microscope (Sievert, WHOI). Dissolved gas and NH_4^+ concentrations were attained by gas and ion chromatography (Seewald - Sylva, WHOI). Subsamples were also collected for a number of offshore analysis to determine: i) the $^{15}N/^{14}N$ isotope composition of NO_3^- ,/NH_4^+ and constrain kinetic isotope effects associated with denitrification/DNRA (Perez-Rodriguez, CIW), ii) to study the rates of autotrophic carbon fixation by NanoSIMS (Dr, Musat, UFZ), iii) to perform single cell genomics on the microbial

populations grown in the bioreactor (Dr. Ramunas, Bigelow) and (iv) to isolate and characterize novel microoganisms from the communities cultured in our experiments (Dr. Perez-Rodriguez, CIW and Dr. Vetriani, Rutgers).

2. Other research activities:

We conducted a series of spectrophotometric analysis of diffuse and high-T focused vent fluids to identify the possible presence of metastable oxidants such as dissolved H_2O_2. Results revealed that H_2O_2 concentrations ranged from ~4 to 26 mM in vent fluids sampled from Crab Spa, M-vent, Bio9, Flea Vent, Tica and L-vent. Negative control measurements of ~ 2mM H_2O_2 were attained from the high temperature (~360^{o}C) fluids collected at P vent.

C. Rutgers research team: Dr. Costantino Vetriani, Dr. Donato Giovanelli, Ms. Ashley Grosche - List of samples collected

- Biofilm from colonizers. A total of 22 microbial colonizers were deployed during cruise AT 26-10, and 13 were recovered. Biofilm from each recovered colonizer were stored for laboratory work.
- Six fluid samples were collected with the Majors, and 11 with the LVP. Fluids collected with the majors were filtered on Supor 200 filters and stored at -80C. One quarter of each LVP filter was stored in RNA later.
- Eight chimney samples were collected, shared with our collaborators and stored for DNA and culture work.

Molecular analyses

Preservation

- Biofilm preserved in RNA later @ -80ºC
- Biofilm preserved for electron microscopy @ +4ºC
- Biofilm preserved for FISH analyses @ -20ºC
- Chimney preserved in RNA later @ -80ºC
- Fluids (1.5/2 L each site) filtered on 0.2 μm in RNA later @ -80ºC

Cultures

- Biofilm for cultures on board cultures
- Chimney for cultures on board cultures
- Chimney preserved under N_2 atmosphere @ +4ºC
- Tubeworms' tubes and other substrate on board cultures
- Tubes and other substrate preserved under N_2 atmosphere @ +4ºC

Fluids

- Use aliquots to inoculate media.
- Filter 1.5/2 L on suppor 0.2 μm filter and store in RNA later (overnight @ +4ºC, then @ -80ºC).

- Collect 300 ml for sites/deployment for hydrocarbon analyses. Run 5 ml methanol in the SPE, run 5 ml ddH_2O, run 100 ml fluids, run air to dry SPE. Store @ -20ºC.

Biofilms

- Collect small aliquots for inoculation (glass slides and basalt fragments).
- Include basalt fragments in Epoxy resins.
- A portion of the biofilm needs to be fixed for electron microscopy.
- Another portion should be preserved for FISH analysis (Formaldeyde, PBS wash, PBS ethanol preservation @ -20ºC).
- Preserve the rest of the biofilm in RNA later (overnight @ +4ºC, then @ -80ºC).

Sulfide Chimney

- Use small aliquots for inoculations.
- Preserve 3 to 5 full 50 ml falcon @ -20ºC.
- Preserve part of the chimney under N_2 atmosphere @ +4ºC for future inoculation in the lab.
- Store the rest of the sample in RNA later (overnight @ +4ºC, then @ -80ºC).

Solid substrates

- Scrape of potential biofilm from hard substrates (shells, tubes, basalts, etc...) and use to inoculate media.
- Preserve a portion of the substrate under N_2 atmosphere @ +4ºC for future inoculation in the lab.

D. Dr. Nadine Le Bris, Mr. Erwan Peru, LECOB CNRS, Univ.-Paris 6, Marie Station – Oceanological Observatory, Banyuls, France

In line with the objectives of the NSF DoB project, our aim for this cruise was to collect in situ environmental data to assess differences in energy availability and chemical constraints, in different habitats of free living chemosynthetic microbes and symbiotic organisms. The *R/V Atlantis* AT26-10 cruise in Jan 2014 offered the opportunity to complete the time series started in 2007, one year after the volcanic eruption at 9°50'N to monitor the reinstallation of chemosynthetic communities along the ridge segment and investigate recolonization dynamics in links with the evolution of habitat properties.

During this cruise, we implemented in situ electrochemical sensors (voltametry and potentiometry) (SPOT). The electrochemical methods implemented were potentiometry (pH and Ag_2S for sulfide), and cyclic voltammetry (0.8 mm silver and 0.1 mm Hg-amalgam).

The hand-held probe combining temperature, pH, sulfide and multianalyte voltammetry was operated using the manipulator arm of *ROV Jason-II* for short-term measurements. Series of scans were performed to assess local heterogenity of habitat and quantify chemical ranges. During the 4 Jason dives, 147 short-term measurement scans (about 3 minutes each) were acquired over 9 vent sites (Crab Spa, Tica, Bio9, P-Vent, M-vent, Teddy Bear, Flea-Vent, Hole to Hell) (Table 4).

Figure 5: Electrochemical sensors are installed on the ROV for in situ chemical measurements in combination to microbial colonization experiments (C.Vetriani, Rutgers Univ.)

In addition, 4 autonomous deployments (5 to 7-day long) were performed allowing combined measurements of temperature, pH, sulfide variations (Table 4). 4 characteristic locations in the Crab Spa/Tica areas were chosen: top of Crab Spa after fauna removal (Figure 5), adjacent Riftia, mussel aggregation, and small Alvinella patch at Tica. The objectives were to characterize habitats not only in terms of ranges and spatial gradients but also include their temporal features at daily to weekly scale.

Table 4: Short-term measurement scans

		Date	Start	End	Site
J2-758	JDV1A1	02/01/2014	22:25:23	22:29:46	Crab spa
J2-758	JDV1A2	02/01/2014	22:30:08	22:33:44	Crab spa
J2-758	JDV1A3	02/01/2014	22:34:32	22:37:54	Crab spa
J2-758	JDV1A4	02/01/2014	22:38:53	22:41:52	Crab spa
J2-758	JDV1A5	02/01/2014	22:44:00	22:53:16	Crab spa
J2-758	JDV1A6	02/01/2014	22:54:44	23:00:00	Crab spa
J2-758	JDV1A7	03/01/2014	07:47:40	07:51:14	P-vent
J2-758	JDV1A8	03/01/2014	07:52:17	07:56:20	P-vent
J2-758	JDV1A9	03/01/2014	07:57:34	08:01:37	P-vent
J2-758	JDV1A10	03/01/2014	08:03:36	08:06:38	P-vent
J2-758	JDV1A11	03/01/2014	08:07:38	08:10:37	P-vent
J2-758	JDV1A12	03/01/2014	08:11:24	08:14:15	P-vent
J2-758	JDV1A13	03/01/2014		13:25:11	P-vent
J2-758	JDV1B1	04/01/2014	00:02:42		Crab spa
J2-758	JDV1B2	04/01/2014	00:04:08	00:07:23	Crab spa
J2-758	JDV1B3	04/01/2014	00:07:50	00:10:55	Crab spa
J2-758	JDV1B4	04/01/2014	00:11:26	00:14:13	Crab spa
J2-758	JDV1B5	04/01/2014	00:15:07	00:17:52	Crab spa
J2-758	JDV1B6	04/01/2014	00:18:26	00:21:48	Crab spa
J2-758	JDV1B7	04/01/2014	00:22:26	00:25:15	Crab spa
J2-758	JDV1B8	04/01/2014		05:22:24	Crab spa
J2-758	JDV1B9	04/01/2014		05:26:45	Crab spa
J2-758	JDV1B10	04/01/2014		05:28:03	Crab spa
J2-758	JDV1B11	04/01/2014		05:32:05	Crab spa
J2-758	JDV1B12	04/01/2014		05:41:27	Crab spa

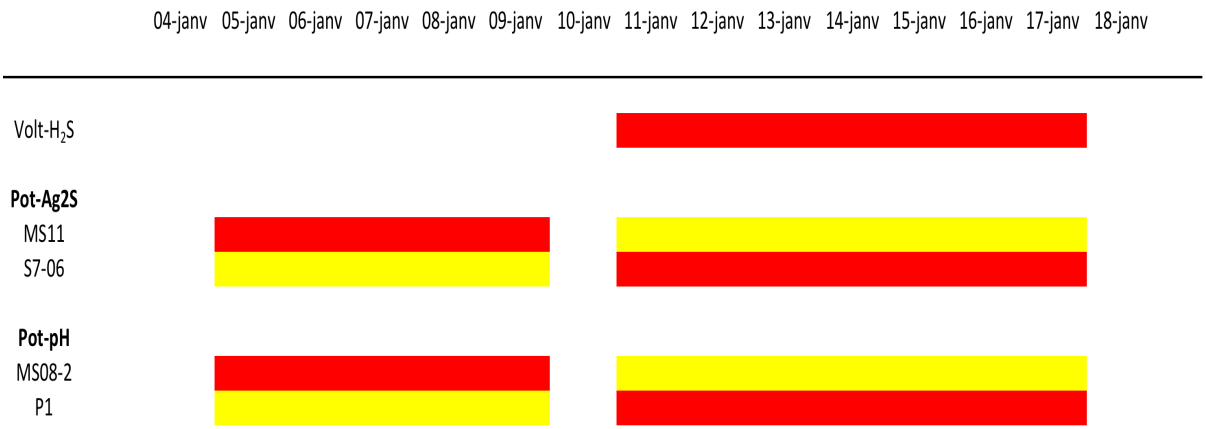
J2-758	JDV1B13	04/01/2014		05:48:45	Crab spa
J2-758	JDV1C1	05/01/2014	00:46:04	00:48:16	P-vent
J2-758	JDV1C2	05/01/2014	00:48:47	00:50:30	P-vent
J2-758	JDV1C3	05/01/2014	00:50:58	00:53:11	P-vent
J2-758	JDV1C4	05/01/2014	01:58:39		P-vent
J2-758	JDV1C5	05/01/2014	02:00:02		P-vent
J2-758	JDV1C6	05/01/2014	02:05:20	02:08:36	P-vent
J2-758	JDV1C7	05/01/2014	03:24:07	03:26:09	P-vent
J2-758	JDV1C8	05/01/2014	03:28:57	03:29:22	P-vent
J2-758	JDV1C9	05/01/2014	03:31:03	03:34:01	P-vent
J2-758	JDV1C10	05/01/2014	03:35:23	03:37:37	P-vent
J2-758	JDV1C11	05/01/2014	03:38:37	03:41:57	P-vent
J2-758	JDV1C12	05/01/2014	09:26:09		Bio9:alvinella structure
J2-758	JDV1C13	05/01/2014	09:28:49		Bio9:alvinella structure
J2-758	JDV1C14	05/01/2014		09:29:41	Bio9:alvinella structure
J2-758	JDV1C15	05/01/2014	09:30:07		Bio9:alvinella structure
J2-758	JDV1C16	05/01/2014	09:30:58		Bio9:alvinella structure
J2-758	JDV1C17	05/01/2014		09:33:29	Bio9:alvinella structure
J2-758	JDV1C18	05/01/2014	10:11:14		Bio9
J2-758	JDV1C19	05/01/2014	10:11:34		Bio9
J2-758	JDV1C20	05/01/2014		10:14:04	Bio9
J2-758	JDV1C21	05/01/2014	12:31:02	12:35:11	Tica
J2-758	JDV1C22	05/01/2014	12:35:43	12:39:15	Tica
J2-758	JDV1C23	05/01/2014	20:23:59		P-vent
J2-758	JDV1C24	05/01/2014	20:28:49	20:32:19	à côté de P-vent?
J2-758	JDV1C25	05/01/2014	20:33:41	20:35:15	à côté de P-vent?
J2-758	JDV1C26	05/01/2014	20:35:18	20:40:37	à côté de P-vent?
J2-758	JDV1C27	05/01/2014	21:23:02	21:26:59	à côté de P-vent?
J2-758	JDV1C28	06/01/2014	01:23:15		Crab spa
J2-759	JDV2A1	09/01/2014		01:01:41	Crab spa
J2-759	JDV2A2	09/01/2014		01:25:39	Crab spa
J2-759	JDV2A3	09/01/2014		01:28:24	Crab spa
J2-759	JDV2A4	09/01/2014	01:29:35	01:31:50	Crab spa
J2-759	JDV2A5	09/01/2014	07:50:05		Crab spa ou TICA?
J2-759	JDV2A6	09/01/2014	07:50:49		Crab spa ou TICA?
J2-759	JDV2A7	09/01/2014	07:53:34		Crab spa ou TICA?
J2-759	JDV2A8	09/01/2014	07:56:10	07:59:17	Crab spa ou TICA?
J2-759	JDV2A9	09/01/2014	08:17:39		Crab spa ou TICA?
J2-759	JDV2A10	09/01/2014	08:18:27		Crab spa ou TICA?
J2-759	JDV2A11	09/01/2014	08:21:44	08:24:42	Crab spa ou TICA?
J2-759	JDV2A12	09/01/2014	09:13:43		Crab spa ou TICA?
J2-759	JDV2A13	09/01/2014	09:14:26	09:18:37	Crab spa ou TICA?
J2-759	JDV2A14	09/01/2014	09:46:57		Crab spa ou TICA?
J2-759	JDV2A15	09/01/2014	09:47:47	09:50:02	Hole in basalt?
J2-759	JDV2A16	09/01/2014	10:08:36		Hole in basalt?
J2-759	JDV2A17	09/01/2014	10:08:54	10:11:30	Hole in basalt?
J2-759	JDV2A18	09/01/2014	11:47:04	11:48:30	Hole to hell?
J2-759	JDV2B1	10/01/2014	09:08:03		Hole to hell?
J2-759	JDV2B2	10/01/2014	09:11:42		Hole to hell?
J2-759	JDV2B3	10/01/2014	09:11:50	09:15:04	Hole to hell?
J2-759	JDV2B4	10/01/2014	09:30:13	09:33:06	Hole to hell?
J2-759	JDV2B5	10/01/2014	10:26:55		Hole in Basalt
J2-759	JDV2B6	10/01/2014	10:28:03	10:29:05	Hole in Basalt
J2-759	JDV2B7	10/01/2014	10:40:05	10:43:08	Hole in Basalt

J2-759	JDV2B8	10/01/2014	19:41:02		Crab spa
J2-759	JDV2B9	10/01/2014	19:42:25	19:43:16	Crab spa ou TICA?
J2-759	JDV2B10	10/01/2014	19:43:37	19:47:01	Crab spa ou TICA?
J2-759	JDV2B11	10/01/2014	19:47:54	19:50:51	Crab spa ou TICA?
J2-759	JDV2B12	10/01/2014	19:51:54	19:53:22	Crab spa ou TICA?
J2-759	JDV2B13	10/01/2014	19:56:38	19:58:57	Crab spa ou TICA?
J2-759	JDV2C1	11/01/2014	02:20:12	02:24:38	North of Crab spa
J2-759	JDV2C2	11/01/2014	02:25:02	02:29:36	North of Crab spa
J2-759	JDV2C3	11/01/2014	02:57:30	03:00:38	Crab spa
J2-759	JDV2D1	12/01/2014	09:40:09	09:43:32	CV66 (near Tica)
J2-759	JDV2D2	12/01/2014	10:11:57	10:15:52	CV69
J2-759	JDV2D3	12/01/2014	10:17:07	10:20:30	CV69
J2-759	JDV2D4	12/01/2014	10:34:01		CV69
J2-759	JDV2D5	12/01/2014		10:39:50	CV69
J2-759	JDV2D6	12/01/2014	10:45:47	10:49:18	CV69
J2-759	JDV2D7	12/01/2014	10:52:51	10:53:27	CV69
J2-759	JDV2D8	12/01/2014	10:54:31		CV69
J2-759	JDV2D9	12/01/2014	10:54:41		CV69
J2-759	JDV2D10	12/01/2014	10:58:59	11:06:41	CV69
J2-759	JDV2D11	12/01/2014	11:52:02	11:54:13	Teddy Bear
J2-759	JDV2D12	12/01/2014	11:54:23	11:56:37	Teddy Bear
J2-759	JDV2D13	12/01/2014	11:57:17	11:59:32	Teddy Bear
J3-760	JDV3A1	13/01/2014	08:36:08	08:38:20	P-vent
J3-760	JDV3A2	13/01/2014	08:38:44	08:41:52	P-vent
J3-760	JDV3A3	13/01/2014	08:42:27	08:46:14	P-vent
J3-760	JDV3A4	13/01/2014	08:59:31	09:04:02	P-vent
J3-760	JDV3A5	13/01/2014	09:11:45	09:14:45	P-vent
J3-760	JDV3A6	13/01/2014	09:15:39	09:21:42	P-vent
J3-760	JDV3A7	13/01/2014	09:23:14	09:27:23	P-vent
J3-760	JDV3A8	13/01/2014	10:11:29	10:14:30	P-vent
J3-760	JDV3A9	13/01/2014	10:15:44	10:19:57	P-vent
J3-760	JDV3A10	13/01/2014	10:20:01	10:23:37	P-vent
J3-760	JDV3A11	13/01/2014	10:24:25	10:29:34	P-vent
J3-760	JDV3A12	13/01/2014	10:30:49	10:37:47	P-vent
J3-760	JDV3A13	13/01/2014	10:38:03	10:42:22	P-vent
J3-760	JDV3A14	13/01/2014	12:32:30	12:34:44	Crab spa
J3-760	JDV3B1	14/01/2014	08:37:06	08:41:34	Crab spa
J3-760	JDV3B2	14/01/2014	08:43:38	08:47:16	Crab spa
J3-760	JDV3B3	14/01/2014	08:48:11	08:52:42	Crab spa
J3-760	JDV3B4	14/01/2014	08:54:50	08:58:33	Crab spa
J3-760	JDV3B5	14/01/2014	08:59:30	09:02:50	Crab spa
J3-760	JDV3B6	14/01/2014	09:04:33	09:08:26	Crab spa
J4-761	JDV3B7	14/01/2014	09:12:15		Crab spa
J4-761	JDV4A1	17/01/2014	01:27:06		Crab spa
J4-761	JDV4A2	17/01/2014	01:32:42	01:34:08	Crab spa
J4-761	JDV4A3	17/01/2014	01:38:57		Crab spa
J4-761	JDV4A4	17/01/2014	01:39:41		Crab spa
J4-761	JDV4B1	17/01/2014	22:19:05	22:22:17	Crab spa
J4-761	JDV4B2	17/01/2014	22:23:11	22:25:43	Crab spa
J4-761	JDV4B3	17/01/2014	22:27:22		Crab spa
J4-761	JDV4B4	17/01/2014	22:27:35	22:30:09	Crab spa
J4-761	JDV4B5	17/01/2014	22:31:18	22:33:53	Crab spa
J4-761	JDV4B6	17/01/2014	22:35:04	22:38:01	Crab spa
J4-761	JDV4B7	18/01/2014	05:58		Teddy Bear

J4-761	JDV4B8	18/01/2014	06:04	Teddy Bear*
J4-761	JDV4B9	18/01/2014	06:08	Teddy Bear*
J4-761	JDV4B10	18/01/2014	06:11	Teddy Bear*
J4-761	JDV4B11	18/01/2014	07:08	Teddy Bear*
J4-761	JDV4B12	18/01/2014	07:11	Teddy Bear*
J4-761	JDV4B13	18/01/2014	07:17	Teddy Bear*
J4-761	JDV4B14	18/01/2014	09:19	Flea-vent
J4-761	JDV4B15	18/01/2014	09:23	Flea-vent
J4-761	JDV4B16	18/01/2014	09:30	Flea-vent
J4-761	JDV4B17	18/01/2014	09:58	Flea-vent*
J4-761	JDV4B18	18/01/2014	12:54	M-Vent
J4-761	JDV4B19	18/01/2014	13:01	M-Vent
J4-761	JDV4B20	18/01/2014	13:06	M-Vent
J4-761	JDV4B21	18/01/2014	13:10	M-Vent

*Location to be confirmed

Table 5: Deployment of autonomous sensors



E. Riftia and mussel group: Dr. Horst Felbeck and Dr. Ruby Ponnudurai

Our main objective for this cruise was to collect the deep-sea vent mussels *Bathymodiolus thermophilus* and the giant tube worms *Riftia pachyptila* samples. More specifically, we performed symbiont enrichment procedures from the gills of the mussels by exploiting the differential pelleting velocities of the symbiont and host cells. We intend to use these enrichments for the genome sequencing of symbiont DNA and for symbiont specific proteomic analysis to elucidate the mechanism of symbiosis in these mussels. Furthermore, we also samples tissues such as gills, mantle and foot from the mussels for more specific tissue based proteomics and metabolomics studies.

Our Riftia sampling was mainly focused on collecting trophosome, plume and vestimentum tissues for performing whole tissue proteomics approach and metabolic tissue profiling to detect specific metabolic differences between symbionts from light and from dark trophosome. Metabolic key enzymes of the bacterial *Riftia* symbionts will be localized by immunohistochemistry (IHC) in trophosome sections to detect

metabolically diverse symbiont subpopulations. As a prerequisite for these antibody-based detections which will be performed in Monika Bright's lab in Vienna, we sampled the trophosome and body wall tissues of several worms (male and female) based on their trophosome color. Furthermore, to address potential metabolic differences between the symbiont morphotypes viz. Rods and cocci in Riftia, we intend to subject these to cell sorting, according to size and shape, in Ramunas Stepanauskas' lab at Bigelow (Single Cell Genomics Center). To conserve the symbiont cells as intact as possible, we sampled the trophosome homogenate from several worms which was treated with a cryo preservative and frozen at -80˚C. Lastly, we also froze the trophosome, skin and whole worms without tubes for antimicrobial peptides project.

The following samples were obtained on this cruise for further analysis:

1) Trophosome, plume and vestimentum tissue samples of eight animals were frozen in cryovials in -80.
2) 12 worms were sampled for immunohistochemistry samples wherein the trophosome and body wall was fixed in paraformaldehyde and ethanol at 4C.
3) 5 worms were used for cryopreservation of trophosome homogenate for single cell sorting (at Bigelow Single cell genomics center)
4) 7 worms were used for preservation of whole troph and body wall to be sent to Monika Bright's lab in Univeristy of Vienna.
5) 3 worms were frozen without tube for antibody based studies.
6) 1 worm was sampled for symbiont enrichment using density gradient rate-zonal centrifugation.
7) 10 mussels were sampled for whole gill, mantle and foot tissue preservation in -80.
8) 10 mussels were sampled for symbiont enrichment using differential pelleting of gill homogenate.
9) 6 mussels were frozen in whole.

F. Flow Measurements – Dr. Leonid Germanovich

A total of 11 flow measurements at 7 locations were performed (Table 6). Different flow meters were used for diffuse-flow and focused flow.

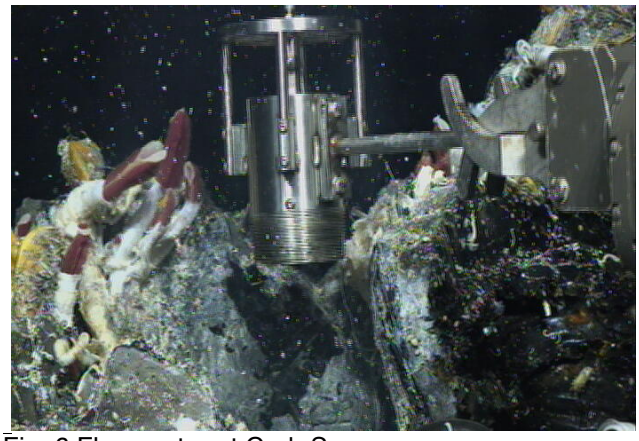


Fig. 6 Flow meter at Crab Spa

Table 6 Summary of flow measurements

Dive	Location	Deployment	Comment
J-758	Crab Spa	1	Prior to animal removal
J-758	Small chimney at Riftia colony near Crab Spa	2	
J-758	Mk L-O, diffuse-flow	3	Sandwich site, near P vent
J-758	Crab Spa	4	Prior to animal removal
J-758	Crab Spa	5	After animal removal
J-758	P vent, black smoker	6	
J-758	P vent, white smoker	7	
J-758	CV61, P vent	8	
J-759	M vent	10	
J-760	Bio9, black smoker	11	

6. Outreach

The Dive & Discover website of Expedition 15 was very successful and we were able to reach a broad audience to educate and inform them about our research and related activities (http://www.divediscover.whoi.edu/expedition15/index.html). A big part of the success of the website was the participation of science writer David Levin, as well as a dedicated staff at WHOI who, in addition to the funds provided by this grant, were supported by additional internal resources at WHOI. During the cruise, we had close to 93,000 visits to the website and the average visit duration was 18 minutes, substantially greater than 5.23 minutes reported by Google Analytics in February 2011 as the average amount of time that internet users spend at a website. Based on the statistics, Dive & Discover Expedition 15 ranks second only to Expedition 11, which had 5,000 more visits, but also was 12 days longer. We had followers from around the world, and the visitor statistics show that the site was heavily used by educators.

During the cruise, Sievert connected live to the science family night at the New Bedford Ocean Explorium, which featured an exhibition of deep-sea hydrothermal vents as part of an NSF grant provided to Stace Beaulieu at WHOI (NSF 1202977). Sievert also had Skype call during the cruise to the class of science teacher Carolyn Sheild at Clarke Middle School in Lexington, MA, and to a high school class at the Gymnasium Ramstein-Miesenbach, Germany.

In addition, we were lucky to have Jennifer Barone, a science writer from Scholastic Magazine, on board. She produced a number of short blog-like articles that were featured online during the cruise, and in the end a summary of the research was provided in an article that appeared in the May 5, 2014 issue of the ScienceWorld-Current Science, a magazine that is widely distributed at schools and is targeting children between grades 7 and 12. Jennifer Barone further wrote an article for Nautilus magazine on the origin of life that was inspired by her participation on the cruise (http://nautil.us/issue/17/big-bangs/in-search-of-lifes-smoking-gun). Sievert further visited a number of schools before and after the cruise to talk about the science and answer questions. One example was a presentation on April 17 at Clarke Middle School in Lexington, MA (http://clarkenewsletter.org), that also followed up on a Skype call Sievert had during the cruise with the class of science teacher Carolyn Sheild. We further had an article in the Feb 2014 issue of the Sea Technology Magazine reporting on our expedition and scientists Stefan Sievert and Jeff Seewald provided answers to questions.

7. Dive reports

Jason-2 Dive 758

2014/01/02 17:51 Jason in water
2014/01/02 19:54 Jason at bottom
2014/01/02 20:58 At Crab Spa, active community with Riftia and mussels

- Measure T in various spots at and around Crab Spa; make chemical measurements with SPOT and ISO; flow measurements

2014/01/03 0:35 Head to TICA

- Survey at TICA
- Find extensive Riftia colony with small chimney venting hot fluids and colonized with Alvinella ~ 5m away from base of Crab Spa
- Measure T, flow measurements , deploy colonizer CV60 on side of chimney colonized by Alvinella, measure T

2014/01/03 1:56 Move away during deployment of LVP-1
2014/01/03 3:14 Found LVP and move to Crab Spa
2014/01/03 3:31 At Crab Spa

- Take IGTs 4A (3:50), 8A (3:58), 3A (04:05), and 2A (4:11)
- Take SIP sample (4:20 – 5:44)
- Position LVP-1 wand into Crab Spa (6:11)

2014/01/03 6:28 Move to Pvent, Marker L-O
2014/01/03 7:01 At Pvent, Marker L-O

- Survey Marker L-O site and inspect Mullineaux's 'sandwiches'
    - Measure T and perform SPOT and ISO measurements on sandwiches in Riftia and mussel communities
    - Flow measurement

2014/01/03 9:28 At Pvent

- Measure T, deploy CV65 on top of Alvinella and CV61 near small 'hive'
- Take chimney sample (10:26), CH#1
- Measure T of black smoker (10:52)

2014/01/03 11:00 Moving to Trick-or-Treat vent (Mk 28)
2014/01/03 11:33 Arrived at Trick-or-Treat vent (Mk 28)

- Site is extinct, dead tubeworms and mussels

2014/01/03 11:48 Moving to Alvinella Pillar (arrive at 12:09)

- Not active

2014/01/03 12:13 At Mk14

- Not active

2014/01/03 12:18 Moving back North to look for other sites
2014/01/03 12:40 Back at Mk28
2014/01/03 13:14 At Pvent

- Sampling Riftia (RIF-1), measure T, SPOT measurements

2014/01/03 14:33 At Elevator-1

- Change IGTs, transfer SPOT, transfer tubeworms, transfer SIP, take Niskin, relaease elevator at 16:01, Jason moves off bottom

2014/01/03 18:35 Jason at bottom moving to Crab Spa to check on LVP-1 (arrive 18:40)

- Wand of LVP-1 had fallen off
- LVP-1 is being moved (18:44)
- LVP-1 wand picked up and secured (18:55)

2014/01/03 19:04 Moving to Tica to search for H-T

- Surveying area, no H-T found, except little chimney found earlier

2014/01/03 20:09 LVP-1 released
2014/01/03 21:26 Elevator-2 launched
2014/01/03 22:39 At Elevator-2
2014/01/03 23:44 Place elevator-2 at seafloor~10 m from Crab Spa
2014/01/04 00:00 At Crab Spa

- Perform SPOT, ISO, and flow measurements prior to sampling animals
- Start sampling Riftia and mussels (1:50), RIF-2, bring samples to elevator-2
- Removal of animals starts snow blower (2:55)
- Take IGT 7A (3:21), 5A (3:55), 1A (4:04), 6A (4:15)
- Move to elevator-2 to swap out IGTs
- Back to Crab Spa to measure T, SPOT, ISO at various spots
- Flow measurements, part of Crab Spa wall collapses

2014/01/04 08:59 Heading to Pvent (arrive 9:20)

- Surveying CV65 and 61
- Flow measurement of black smoker (9:45)
- Flow measurement of black smoker (11:00)

2014/01/04 11:24 Heading to Bio9

- Arrive at Mk27 (11:29), Riftia, crabs, serpulid worms, Hole to Hell?
- T and SPOT measurement
- Tall structures

2014/01/04 12:17 Leaving site

- Pass Mk2 of AT15-13 (12:21)
- Pass Mk20 of AT15-13 (12:27)

2014/01/04 At Pvent (12:31)

- Attempt to sample chimney failed

2014/01/04 12:44 Leaving for elevator-2 (arrive at 13:29, released at 14:02)
2014/01/04 17:48 LVP-2 launched
2014/01/04 18:10 At LVP-2, transfer to Crap Spa (arrive at 18:48)

- Positioning wand (19:52), T next to wand

2014/01/04 20:18 Leaving Crab Spa, go to Riftia colony close by

- Check on colonizer CV60, measure T

2014/01/04 20:59 Back at Crab Spa checking on LVP-2 wand, still in place
2014/01/04 21:41 Elevator-3 launch
2014/01/04 22:51 At Elevator-3 near Pvent, move elevator-3 to Mk L-O
2014/01/05 00:06-03:54 Work at Mk L-O sandwich site

- Measure T, SPOT on and around sandwiches
- Pick up old sandwiches and deploy new ones

2014/01/05 03:57 At Pvent
- Sample chimney CH#2 (4:48)
- IGT-UW1A (5:33), UW4A (5:44)
- Pvent white smoker flow measurement (6:20-7:43)

2014/01/05 7:55 At elevator-3
- Swapping IGTs, picking up SPOT

2014/01/05 08:54 At Alvinella structure near Bio9
- Measure T, SPOT, ISO, deploy CV64 on top of structure

2014/01/05 09:59 Found mussel cage near Bio9
- T, SPOT measurement

2014/01/05 11:50 Found mussel cage at Tica, near Mk30

2014/01/05 12:03-12:40 Working at Riftia clump at Tica
- Take T, SPOT, ISO measurements

2014/01/05 13:00 Inspect colonizer CV60, thick biofilm

2014/01/05 14:10 At Crab Spa
- Secure wand of LVP-2
- Take IGT 8B (14:32), IGT UW2A (14:45)
- Deploy colonizer CV62
- Release LVP-2 (15:07)

2014/01/05 17:10 At Elevator-3
- Swapping IGTs, trigger Niskin
- Released at 17:40

2014/01/05 20:09 At Mk L-O site w/ sandwiches

2014/01/05 20:15 At colonizer CV65 at Pvent
- SPOT measurements (20:28-20:40)

2014/01/05 20:50 At colonizer CV61 at Pvent
- Small chimney growing next to it
- Measure T, SPOT measurements (21:23-21:26)
- Flow meter measurements (21:41-21:46)

2014/01/05 21:52 Launch of Elevator-4

2014/01/05 23:33 At elevator-4

2014/01/06 00:25 Elevator-4 near Crab Spa, pick up long term sensor LTS-1

2014/01/06 00:35-01:28 Working at Crab Spa
- CV62 has rolled out of orifice, repositioning
- Position LTS-1 at Crap Spa
- T, SPOT, ISO measurements

2014/01/06 01:32 At elevator-4, picking up LTS-2

2014/01/06 01:35 – 02:05 Working at Riftia clump, CV60
- Deploying LTS-2 close to CV60
- Measuring T, ISO

2014/01/06 02:05 Heading north to Perseverance

2014/01/06 03:39 – 04:16 At BioVent
- Survey of area, one active, one inactive structure
- HOBO #148 in inactive structure

- Alvinella on active structures, large Riftias at base
- T measurement in Riftias and of hot fluids

2014/01/06 04:18 – 05:11 Working at Perseverance

- Huge mussel bed w/ Riftia clumps in large depression
- Very smoky, lots of stuff in water
- Deploy CV67 in mussel bed
- Measure T in various places

2014/01/06 05:59-06:19 Working at M vent

- Structure still inactive, but small flow on top
- T (21.7ºC) and ISO measurements (~26μM)

2014/01/06 06:27 At Robin's roost, inactive

2014/01/06 06:55 – 07:15 At Qvent

- No black smoker, but Alvinella colony
- Measure T (max 47ºC)

2014/01/06 07:15 – 07:29 looking for Riftia field, but cannot be found

2014/01/06 08:02 Mussel bed, inactive

2014/01/06 08:26 East Wall, inactive

2014/01/06 08:45 Mk CAV21 from AT15-25, inactive

2014/01/06 08:51 Come across new active site, south of East Wall, later named Teddy Bear

- Lots of stuff in water, white biofilm, microbial filaments on rocks, fluids emanating through cracks and holes
- Measure T (10.1ºC)

2014/01/06 09:23 Crack in basalt with diffuse flow, measure T (9.1ºC)

2014/01/06 10:01 Site w/ Riftia and mussels, measure T (21ºC)

2014/01/06 10:19 Mk P, mussels and Riftia

2014/01/06 10:26 At elevator-4 near Crab Spa, surveying area

2014/01/06 11:19 Working at Crab Spa

- Take IGT 3B (11:21), IGT 2B (11:35), IGT UW3A (11:58), IGT 4B (12:13)

2014/01/06 12:21 At elevator-4

- Swapping IGTs

2014/01/06 12:42 Back at Crab Spa

- Take IGT 1B (12:46)
- Pick up 'side wall' of Crab Spa', put in biobox of elevator-4

2014/01/06 13:19 – 14:02 At Riftia clump at Tica for Riftia and mussel collection (RIF-3)

- T and ISO measurement before collection
- Transfer animals to biobox on elevator-4

2014/01/06 14:55 Release elevator-4

2014/01/06 18:53 Pick up colonizer CV60

2014/01/06 19:36 Deploying new colonizer CV66, measure T

2014/01/06 20:06 – 22:04 Work at Bio9

- View of CV64
- Collect chimney CH#3 (21:15), measure T

- Slurp sample (0.2 μm), slurping white filaments around black smoker (21:34-21:44)
- IO in spot where filaments were (22:01)

2014/01/06 22:18 – 23:10 Working at Pvent

- Pick up CV65, measure T (22:31)
- Pick up CV61, measure T (22:39)
- Slurp Alvinella (22:50)

2014/01/06 23:43 Retrieve HOBO #145 at Trick-or-Treat
2014/01/06 23:50 Jason off bottom
2014/01/07 01:50 Jason on deck

Jason-2 Dive 759

2014/01/08 21:15 Jason in water
2014/01/08 22:52 Jason on bottom
2014/01/08 23:00 At LVP-3
2014/01/08 23:30 - 2014/01/09 01:01 Working at Crab Spa

- Deployment of LVP-3
- Take IGTs (UW)4A (23:47), 6A (23:56), 7A (00:03), (UW)1A (00:10)
- Position wand of LVP-3 (00:59), T near wand tip 23ºC

2014/01/09 01:17 – 01:41 Collect mussels at base of Crab Spa

- T, ISO and SPOT measurements

2014/01/09 01:42 Move to elevator-5
2014/01/09 02:00 At elevator-5

- Swap IGTs
- Transfer mussels
- Trigger Niskin

2014/01/09 02:49 Release elevator-5
2014/01/09 06:40 At Elevator-6 landing site
2014/01/09 07:10 Elevator-6 at Crab Spa
2014/01/09 07:23 Verify that LVP-3 wand is still in Crab Spa
2014/01/09 07:35 – 08:33 Working at Target 66, Riftia bush and mussel beds

- T, SPOT, ISO measurements

2014/01/09 08:36 At Mk P
2014/01/09 09:05 – 10:15 Work at site later named Teddy Bear

- Survey of area
- T, SPOT, ISO measurements
- Rock sample with bacterial filaments, RS#1 (09:28)

2014/01/09 11:10 Robin's Roost?
2014/01/09 11:27 – 12:33 Working at Mvent

- some surveying of surrounding, Robin's Roost w/ Alvinella?
- T, ISO, SPOT measurements on top of Mvent
- Take IGTs 3A (12:16), 8A (12:28)

2014/01/09 13:49 At elevator-6

- Swapping IGTs
- Transferring rock sample

2014/01/09 14:17 – 15:02 Working at Crab Spa

- Secure wand of LVP-3 (14:17)
- Take IGTs UW3A (14:35), 2A (14:48)
- Release LVP-3 (15:02)

2014/01/09 17:21 – 17:51 Sampling small Riftia in crack in Riftia clump near Crab Spa (RIF-4)

- Measure T, SPOT

2014/01/09 17:54 At elevator-6

- Transfer Riftia, IGTs, SPOT on elevator

2014/01/09 18:27 Release elevator-6
2014/01/09 22:09 At elevator-7 landing site
2014/01/09 22:46 Elevator-7 at Crab Spa
2014/01/09 23:00 Picking up LTS-2 and transfer to elevator-7
2014/01/09 23:13 At CV66 measuring T
2014/01/09 23:22 Picking up LTS-1 at Crab Spa and transfer to elevator-7
2014/01/09 23:40 – 23:59 Working at Crab Spa

- Take IGTs 5A (23:45), UW2A (23:55)

2014/01/10 00:02 At elevator-7 to swap IGTs
2014/01/10 00:17 Elevator-7 released
2014/01/10 03:21 At LVP-4 landing site
2014/01/10 03:59 – 05:39 Working at Crab Spa

- LVP-4 positioned at Crab Spa (04:09)
- Colonizer CV-62 fell off during LVP-4 wand positioning
- LVP-4 wand positioned in Crab Spa (04:38)
- Found CV 62 at base of Crab Spa (5:05) and repositioned (5:11)
- CV 62 fell again (05:18)
- CV 62 picked up again (05:21) and repositioned (05:26), measure T

2014/01/10 06:09 – 08:17 Working at Bio9

- Check on CV64
- Take chimney sample CH#4 (07:00), T after sampling 104ºC
- Take IGTs 1A (07:59), UW1B (08:12)

2014/01/10 08:27 at CV64 on top of structure near Hole to Hell
2014/01/10 09:21 Deploy colonizer CV71 at base of structure

- Measure T, ISO, SPOT in crack before deployment and T and SPOT afterwards

2014/01/10 10:36 Deploy CV63 in diffuse flow near Teddy Bear site

- Measure T, ISO, SPOT in crack before deployment and T and SPOT afterwards

2014/01/10 12:06 – 12:28 Working at Mvent

- Flow and T measurement (24.2 ºC)

2014/01/10 12:35 – 12:47 Working at Robin's Roost?, Alvinella colony

- Measure T

2014/01/10 12:53 Different vent site with Alvinella near Mvent, later named Flea Vent
- Measure T

2014/01/10 14:12 - Working at Crab Spa
- Secure LVP-4 wand
- Release LVP-4 (14:31)

2014/01/10 17:38 At Elevator-8 landing site

2014/01/10 18:32 Position elevator-8 near Crab Spa, swap IGTs

2014/01/10 18:58 – 19:32 Working at Crab Spa
- Take IGTs 2B (19:11), 3B (19:26)

2014/01/10 19:35 – 20:16 Working at Riftia colony near Crab Spa to collect Riftia
- SPOT measurements
- Riftia collection (RIF-5)

2014/01/10 20:25 At elevator-8
- Transfer Riftia, chimney, IGTs, SPOT
- Fire Niskin
- Release elevator-8 (21:21)

2014/01/11 00:46 At elevator-9 landing site

2014/01/11 01:35 Elevator-9 positioned near Crab Spa
- Pick up SPOT, LTS-3, LTS-4

2014/01/11 02:06 – 02:31 Working at Riftia colony near Crab Spa
- Deploy LTS-3 (red) in Riftia colony and LTS-4 (yellow) in mussels
- SPOT measurements

2014/01/11 02:33 – 03:03 Working at Crab Spa
- Take IGTs UW3B (02:40), 8B (02:51)
- SPOT measurements

2014/01/11 03:05 At elevator-9
- Transfer SPOT, IGTs, trigger Niskin
- Release of elevator-9 (03:43)

2014/01/11 03:47 – 04:05 Survey around base of Crab Spa, T measurements

2014/01/11 06:05 At LVP-5 landing site

2014/01/11 06:29 Position LVP-5 near Teddy Bear, survey area fro suitable site

2014/01/11 06:41 – 07:10 Working at diffuse flow site, measure T

2014/01/11 07:15 – 07:20 Working at different diffuse-flow site, measure T

2014/01/11 07:22 – 07:39 Working at different diffuse-flow site
- Measure T
- Deploy colonizer CV68 on top of crack
- ISO measurement

2014/01/11 07:41 – 07:44 Repositioning of LVP-5

2014/01/11 07:47 – 08:22 Working at LVP-5 deployment site
- Placing LVP-5 wand in hole
- Measure T, ISO
- Deploy colonizer CV70 on top of crack

2014/01/11 09:21 – 10:54 Working at Flea vent
- Measure T

- Take IGTs 6B (09:53), UW4B (10:30)
- Deploy colonizer CV70, measure T and ISO

2014/01/11 11:43 – 12:45 Working at Perseverance

- Check colonizer CV67
- Measure T and ISO

2014/01/11 16:34 Working at LVP-5 deployment site Teddy Bear

- Pick up rock samples with filaments, RS#2
- Secure LVP-5 wand
- Release LVP-5 (17:17)
- Reposition colonizer CV70

2014/01/11 19:02 At elevator-10 landing site, trigger Niskin

2014/01/11 19:49 Elevator-10 positioned near Crab Spa

2014/01/11 19:53 – 20:20 Working at Riftia colony near Crab Spa

- Measure T, collect Riftia (RIF-6)

2014/01/11 20:22 At elevator-10, transfer Riftias, basalt samples (Teddy Bear), IGTs

2014/01/11 21:08 – 21:45 Working at Crab Spa

- Take IGTs UW2B (21:16), 5B (21:30)
- Reposition colonizer CV62

2014/01/11 21:47 At elevator-10

- Transfer IGTs
- Release of elevator-10 (22:10)

2014/01/12 01:52 At elevator-11 landing site, pick up IGTs, SPOT, chimney bag

2014/01/12 02:21 At LVP-6 landing site, pick up LVP-6 and move to Hole-to-Hell

2014/01/12 03:03 LVP-6 positioned near Hole-to-Hell

2014/01/12 03:11 At CV64, bring colonizer in horizontal position, measure T

2014/01/12 03:30 – 04:14 Working at Hole-to-Hell

- Look for good spot to put wand for LVP-6
- Measure T
- Pick up LVP-6
- Place is very difficult to maneuver
- Putting in wand in outflow fails, during recovery hose came loose from wand, had to abandon LVP-6 deployment at Hole-to-Hell
- Placing LVP-6 away from venting for background deep-sea water sample

2014/01/12 04:47 - 06:20 Working at Bio9

- Getting chimney samples CH#5 (5:10) and #6 (5:34)
- Measuring T (363ºC)
- Deploy colonizer CV74 near where chimney was taken, measure T

2014/01/12 06:58 - Working at Crab Spa

- Take IGTs 7A (07:10), 4A (07:22)
- Take SIP (07:36 – 08:26)

2014/01/12 08:34 – 10:23 Working at CV66, Riftia colony

- Recovery of CV66 (08:54)
- Take IGTs 1B (09:02), UW1C (09:25)
- Deploy colonizer CV69 in Riftia, measure T, SPOT, ISO

2014/01/12 10:30 – 11:11Working at Crab Spa

- Measure SPOT, ISO at various locations within Crab Spa

2014/01/12 11:51 – 11:59 Working at Teddy Bear

- Measure SPOT around CV72

2014/01/12 12:49 – Trigger Niskin on Jason at LVP-6 near Bio9
2014/01/12 13:05 Release LVP-6
2014/01/12 13:11 – Jason leaves bottom, release of elevator-11
2014/01/12 14:10 – Jason on deck

Jason-2 Dive 760

2014/01/13 01:13 Jason in water
2014/01/13 02:48 Jason on bottom
2014/01/13 03:00 - 04:04 Working at Bio9, CV64

- Take major MJ no color #1 (03:03), blue #1 (03:32)
- Take IGT 2A at black smoker (04:00), T 367ºC

2014/01/13 04:25 At elevator-12

- Swap majors, transfer IGT2A, pick up ruler for flow meter

2014/01/13 05:25 - 06:18 Flow measurement at black smoker at Bio9, same as where IGT2A was taken
2014/01/13 06:45 – Working at Pvent, CV61

- Take major MJ red #1 (07:02) and MJ yellow #1 (07:07)

2014/01/13 07:19 – 10:48 Working at Mk L-O, sandwich site

- Repositioning sandwiches
- Measure T, SPOT

2014/01/13 11:10 At elevator-12, transfer majors, pick up IGT, move elevator to Crab Spa
2014/01/13 12:20 – 13:10 Working at Riftia colony near Crab Spa

- Sample Riftias and mussels (RIF-7)
- T and SPOT measurements
- Transfer animals to elevator

2014/01/13 13:15 - Working at Crab Spa

- Take IGTs 6A (13:22), 8A (13:37), UW3A (14:00)

2014/01/13 14:07 At elevator-12, transfer IGTs, SPOT, fire Niskin, switching flow meters
2014/01/13 14:30 Release elevator-12
2014/01/13 18:16 At LVP-7 landing site
2014/01/13 19:08 – 20:16 Working at Teddy Bear

- Deploy LVP-7
- Inspect CV72
- Inspect CV68, measure T
- Position wand of LVP-7 next to CV68 (19:50)

2014/01/13 20:21 – 21:02 Fly over of Teddy Bear area
2014/01/13 23:30 At elevator-13 landing site, transfer majors, SPOT, move to Perseverance

2014/01/14 00:09 – 01:33 Working at Perseverance

- Pick up colonizer CV67 and put in biobox on elevator
- Measure T in mussel patch on which CV67 was deployed
- Take major MJ red (00:34), and yellow (00:54)
- ISO, SPOT measurements
- Deploy colonizer CV73 on mussel patch (01:32)
- Swap majors, IGT at elevator

2014/01/14 02:15 – 03:07 Working at Biovent

- Take IGTs UW2A (02:27) after knocking off beehive structure, IGT drifted during deployment
- Deploy colonizer CV80 on Alvinella colony, measure T

2014/01/14 03:30 At elevator-13, picking up elevator

2014/01/14 04:35 – 05:51 Working at Flea vent, CV70

- Measure T around CV70
- Take major MJ no color #2 (04:46), MJ black #2 (04:59)
- SPOT, ISO around CV70
- Deploying colonizer CV75, measure T

2014/01/14 06:05 – 06:19 Working at Mvent

- Take chimney CH#7 with outflowing warm water (06:12)
- Measure T after taking chimney, 40.5ºC

2014/01/14 06:39 At elevator-13, picking it up

2014/01/14 08:29 – 09:40 Working at Crab Spa

- SPOT measurements along gradient within Crab Spa
- Measure at base of Crab Spa (09:22 – 09:38)

2014/01/14 09:40 – 10:29 Working at Riftia colony near Crab Spa

- Deploy colonizer CV76, measure T
- Repositioning of LTS-3

2014/01/14 10:31 At elevator-13

2014/01/14 11:00 – Working at Teddy Bear

- Secure wand of LVP-7
- SPOT measurements
- Release LVP-8 (12:00)

2014/01/14 13:57 At elevator-13, transfer chimney

2014/01/14 14:10 – 14:23 Collecting at Riftia colony close to Crab Spa (RIF-8)

2014/01/14 14:30 At elevator-13

- Transfer Riftia, SPOT, IGT, Major

2014/01/14 16:36 At LVP-8 landing site, moving to Teddy Bear

2014/01/14 17:22 – Working at Teddy Bear

- Deploy LVP-8
- Positioning wand (17:48)
- Pick up colonizer CV68
- Repositioning of LVP-8 wand (18:05)

2014/01/14 18:50 – Working at Crab Spa

- Take IGTs 7A (19:00), 3A (19:25), UW1A (19:35)

2014/01/14 19:46 At elevator-13

- Transfer IGTs
- Release elevator-13 (19:51)

2014/01/14 20:21 – 21:10 SIP sampling of ambient seawater, 20 m above seafloor
2014/01/15 00:04 – 00:21 Working at Teddy Bear

- Secure LVP-8 wand
- Deploying CV79 on crack where LVP wand was situated
- Releasing LVP-8 (00:21)

2014/01/15 02:20 – 03:00 Working at Bio9

- Retrieve CV64
- Retrieve mussel cage

2014/01/15 03:18 At elevator-14 landing site, transfer mussel cage, trigger Niskin, move to Crab Spa
2014/01/15 04:17 – Working at Crab Spa

- Take IGTs 4A (04:21), 1A (04:29), 2B (04:47)
- Retrieving colonizer CV62

2014/01/15 05:02 Release elevator-14
2014/01/15 05:03 Jason off bottom
2014/01/15 07:11 Jason on deck

Jason-2 Dive 761

2014/01/16 00:23 Jason in water
2014/01/16 02:43 Jason on bottom
2014/01/16 02:47 Jason at elevator-15, trigger Niskin, move to Lvent
2014/01/16 03:53 - 16:40 Working at Lvent

- HOBO #146 located, inactive structure
- Survey area for LVP positioning
- Get LVP-9 and move to Lvent
- T measurements
- Video of mystery creature (bacterial filaments?) (05:08), T around 96ºC
- Close up of old colonizer (05:23)
- Take IGTs UW2A (05:46), 8A (05:57)
- Take major MJ Black #1
- LVP-9 wand positioned (06:58)
- Take IGTs 6A (08:26), UW3A (09:08), transfer to elevator-15
- SPOT measurements near LVP-9 wand, (09:36 – 09:55)
- Recover old colonizer (10:06)
- Deploy colonizer CV83 (10:42), measure T
- Sample chimney CH#8 under flange (11:04), transferred to elevator-15
- Release elevator-15 (12:36)
- Collection of filamentous bacteria (mystery creature) with cloth bag (15:32)
- Take major MJ black #1 and MJ no color #1 at same spot (16:06 – 16:11)
- Deploy colonizer CV81 at same spot (16:17)

- Securing wand of LVP-9 (16:28)
- LVP-9 released (16:39)

2014/01/16 23:34 At Crab Spa
2014/01/17 00:21 At elevator-16 landing site, move to Crab Spa
2014/01/17 01:18 – 01:40 Looking for mussel cages near MkP at TICA

- Found one cage, SPOT measurement

2014/01/17 01:45 Transfer cage into biobox on elevator-16
2014/01/17 01:48 – 02:00 Working at Riftia colony near Crab Spa

- Collecting Riftias and mussels (RIF-9)

2014/01/17 02:03 At elevator-16

- Transfer animals, cloth bag with bacterial filaments, majors, SPOT
- Pick up IGTs

2014/01/17 02:49 Back at Riftia colony, CV60

- Take major MJ red #1 (02:57), MJ yellow #1 (03:03)

2014/01/17 03:09 Working at Crab Spa

- Overview of Crab Spa, mating octopuses (3:16)
- Take IGTs 7A (03:30), 6B (03:37), UW4A (04:01)

2014/01/17 04:16 Release elevator-16 with IGTs, majors, animals, cloth bag, mussel cage
2014/01/17 07:58 At LVP-10 landing site, move to Crab Spa
2014/01/17 08:34 – 09:03 Working at Riftia colony near Crab Spa

- Deploy LVP-10
- Position wand of LVP-10 in Riftia colony, measure T at tip suing data pencil (08:54)

2014/01/17 09:09 – 09:40 Working at Crap Spa

- ISO measurements at different positions
- Deploy colonizer CV78

2014/01/17 10:03 – 10:58 At Bio9, looking for missing mussel cage
2014/01/17 11:00 – Exploring south of Bio9

- Choo-choo, some mussels mostly dead (13:25)
- 14:38 – 14:48 Extensive vent, Riftia and mussels

2014/01/17 17:10 At LVP-10

- Secure LVP-10 wand
- Release LVP-10 (17:25)

2014/01/17 20:12 At elevator-17 landing site, trigger Niskin, move to Crab Spa
2014/01/17 21:50 – 23:34 Working at Riftia colony near Crab Spa

- Collecting Riftia, bring to elevator (RIF-10)
- SPOT at LTS site and in Riftia
- Survey for LVP deployment
- Picking up LTS-3 and 4 (red and yellow)
- Take IGTs UW1A (23:16), UW3B (23:26)

2014/01/17 23:38 At elevator-17

- Swap IGTs, transfer LTS

2014/01/18 00:00 – 00:17 Working at Crab Spa

- Take IGTs 8B (00:04), UW2B (00:13)

2014/01/18 00:20 At elevator-17

- Transfer IGTs
- Release elevator-17 (00:40)

2014/01/18 02:53 LVP-11 launched
2014/01/18 04:00 At LVP-11 landing site, move to Riftia colony near Crab Spa
2014/01/18 04:47 – 05:21 Working at Riftia colony near Crab Spa

- Deploy LVP-11
- Position wand of LVP-11 in crack and measure T at tip with data pencil (05:12)

2014/01/18 05:58 – 08:06 Working at Teddy Bear, CV72, CV79

- SPOT, ISO measurements at CV72
- Retrieve colonizer CV72 (06:36)
- T, SPOT, ISO measurements on CV79
- Retrieve CV79 (07:31)
- Take majors MJ black #2 (07:47) and MJ no color #2 (07:58) at location of CV79
- Retrieve CV63 (08:01)

2014/01/18 09:07 – 10:30 Working at Flea Vent, CV 70, 75

- T, SPOT, ISO measurements on top of colonizers
- Retrieving CV75 (09:45) and CV70 (10:19)

2014/01/18 10:38 – 11:00 Working at Mvent

- SPOT measurement
- Chimney sample CH#9 (10:56)

2014/01/18 12:32 – Working at Bio9, CV74

- T, SPOT, ISO around CV74
- Retrieve CV74 (13:22)
- SIP samples at black smoker (13:41 – 14:41)

2014/01/18 15:05 At LVP-11 site

- Secure wand
- Release LVP-11 (15:09)

2014/01/18 18:04 At elevator-18 landing site, pick up IGT, transfer majors, trigger Niskin
2014/01/18 19:07 – 19:43 Working at Crab Spa

- Take IGTs 6C (19:12), 3A (19:26)
- Collecting Rifia (RIF-11)

2014/01/18 20:11 – 20:40 At Bio9

- Take major MJ yellow #2 (20:27), MJ red #2 (20:34) at black smoker

2014/01/18 20:41 Jason off bottom, release of elevator-18
2014/01/18 23:14 Jason on deck

Jason-2 Dive 762

2014/01/19 01:25 Jason in water
2014/01/19 03:01 Jason on bottom

2014/01/19 03:07 Picking up LVP-12, moving to Riftia colony near Crab Spa
2014/01/19 03:39 – Working at Riftia colony near Crab Spa

- Survey to find T between 50-70ºC for LVP-12
- Positioning of LVP-12 wand
- Read T at tip w/ data pencil
- Dive aborted due to medical emergency of crew member (05:31)
- LVP-12 released (05:31)

2014/01/19 05:33 Jason off bottom
2014/01/19 07:11 Jason on deck

8. Appendix

A. Designation of Sample types, deployments, and measurements

Please follow these conventions for naming stations (locations) during Expediton AT26–10, for log–keeping in the Jason van, shipboard communication, and reports (text, figures, tables). Mixing different naming conventions, or leaving gaps or repeating sample/location IDs will lead to confusion and could contribute to sample/data loss or misinterpretation.
Please pay particular attention at start of your shift, when you are not sure about what IDs were used during previous shifts – ***check the log book(s) and other records to verify before assigning new IDs!*** Please check with Sievert and/or your shift leader with questions about these guidelines.

Sample collection types:

- IGT (Isobaric gas-tight sampler): IGT # (Jeff and Sean)
- MJ (Major sampler followed by color of sampler): MJ Color (Costa)
- Slurp sample: SS # (Costa and Donato)
    - Black and white chamber have small filter for microbial mats
    - Blue and yellow chamber have wider mesh for animals, like Alvinella
- Chimney sample: CHS # (Costa, Xiao, Leonid)
- Rock sample: RS # (Costa)
- Animals
    - Riftia sample: RIF # (Horst, Ruby)
    - Mussel sample: MUS # (Horst, Ruby)
    - Alvinella sample: ALV # (Costa)

Deployments

- LVP (Large Volume Pump): LVP # (Craig, Stefan)
- SIP (**S**mall **I**n-Situ-**P**ump): SIP # (Jeff, Sean, Stefan)
- Colonizer: Col # (Costa)
- Long-term sensor deployment: LTSD # (Nadine)

Measurements

- Flowmeter: (Leonid)
    - TFW-LT # (on Jason basket)
    - TFW-HT # (on elevator)
- In situ chemical sensor: SPOT # (Nadine)
- In situ Optode: ISO # (Craig, Stefan)
- Jason Temperature probe: J-TP #
- IGT Temperature probe: IGT-TP #

B. Tips for collecting tubeworms and mussels

If you just grab the middle and pull, they will be injured and be useless for most work. They do not have a blood clotting system and will bleed out and die from the smallest injury to their bodies. Try not to bend them when you put them into the biobox, injury is common when bent. That means, large worms are easier to collect but not very useful for most purposes.
The best Riftia are ones that barely fit into the biobox without folding them.

It is VERY important that the animals are not exposed to warm water during recovery. Even a relatively small leak will warm up the water in the biobox at the surface. If the water is warm, all animals will be dead, if it is cold most of them survive and could

actually be put in high pressure aquaria for extended periods of time.

Individual tubeworms: tap the top until they withdraw. Grab the top of the tube and pull.

Several tubeworms: They are usually connected at the bottom, i.e. if you grab one, you grab the whole bushel. One is injured, the rest are fine

Individual mussels: Difficult and requires extraordinary sensitivity without crushing them

Several to many mussels: Just grab one and a whole bunch will follow. They are usually connected

C. Collection of HD Video Clips during AT26-10

The HD video system is a useful tool, helping to capture high resolution clips that are useful for talks, as digitial supplements to papers, and for other purposes. However, having too much HD video in a single file can make the file difficult to use and require significant editing. In addition, it is best if each HD video clip presents a single operation/location/sample – this makes it easier to find the clip you want to insert into a talk or paper.

For these reasons, we are asking that HD **video clips during AT26-10 to be to ≤3 minutes in length.** To make this work, personnel responsible for HD video acquisition need to pay careful attention to the watch commander and clock. Please follow these rules:

• Don't start the HD video recorder until you know that the key operaiton of interest has begun or is about to begin. *If you are not sure, please ask!*

• Once the HD video recorder has been started, keep track of the elapsed time on the clock. As the clock passes 2 minutes, consider if it might be better to end the video, or perhaps to stop the initial clip and start another clip. *If you are not sure, please ask!*

• As video is being recorded (or soon after), update the video log to ***describe what is in the video*** being recorded. Please *use the sample/data/location ID conventions* posted for AT26-09 in refering to events recorded with HD video.

There may be times when HD videos longer than 3 minutes are desired. But ***there should be a very good reason for recording one long HD video rather than several shorter ones.*** Remember: you can record multiple short videos back to back, and this will be more useful in most cases, because the files will be shorter and easier to edit. It will also be easier to explain what is in each HD video file if each file comprises a single set of opertions.

D.

Procedure for SPOT probe measurements

Recommendations and preferred locations

- Do not expose the sensor tip to T>75 °C. If any doubt, check first w/high-T
- Never insert the probe in the mucus surrounding Alvinella tubes
- On mussel beds, take measurements close to siphons or in venting holes/cracks (often with white mats on shells)
- Before defaunating and area, take measurements at the highest and medium T°C and repeat it after fauna removal

Find an adequate area for a series of measurement scans

1. Identify an area (< $1m^2$) and ensure with Pilot that a good Jason position can be achieved for safe and stable manipulation of the probe
2. Rapidely map the temperature in the area, identify locations with highest T°C and intermediate ones.
3. Perform 3-min measurement scans successively on selected locations. For one area, typically, 5 locations would be fine
4. Check that the tip remains visible at least on one video. If not possible (masked by the manipulator arm, rock or animal), move to another point.

Performing a measurement scan

1. Ask Pilot to maintain the tip of the probe **motionless (c.a. +/- 2 cm) during 3 minutes for each scan.**
2. **Log the following events :** 1) SPOT probe in the manipulator arm , - **measurement scan start (add label)**, measurement scan stop, SPOT back in basket.
3. Record videos of the sensor tip continuously during scans. **Overlays should be always on.** Consider HD video recording.
4. **Close up videos of the tip during scan duration** (zoom on when the probe is in stable position and dezoom for a general view of the area while moving the probe.
5. Take video image captures during the meas.

ROV *Jason* Daily Report

Cruise Number: AT-26-10

Dive number: J2-758
Chief Scientist: Stefan Sievert

Report Date: 1/6/2014
Expedition Leader: Alberto Collasius Jr

Prepared By: Alberto Collasius Jr.

Vessel Location: Pacific
Position: 9 North
Weather: Pleasant/Deteriorating
Dive Times: GMT

Dive Activities/Future Activities: Gas Tight's, Temp probe, Optode sensor, Slurp, SPOT sensor and Flow Meter. Rock Collection and Tubeworm samples taken. Colonizers deployed and collected. 4 Elevators cycled during this dive with Gas tight's and other equipment listed above. 2 deployments of Large Volume Pump, that goes at its own 'elevator'.

Reason for Dive Termination: At Science request.

Completed Dive Summaries:

Dive No.	Dates	Max Depth	Hours Descending	Hours Ascending	Hours on Bottom	Hours in water	Time On Deck	Time on deck not available to science
J2-758	1/2-1/6 2014	2512	2.0	2.0	99.9	103.9		

Vehicle Status: Vehicle in very good working condition. All systems working properly.

Weather Forecast: Not Good!

Expedition Leader Comments: Great long dive. Planned well and executed well.

Chief Scientist Comments: This was my first experience with Jason and I am very impressed. The Jason team is excellent: Very helpful, supportive, and fun to work with. The dive went very well and we got a lot accomplished. I can't wait to have Jason in the water again.

Contact Numbers:

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ROV *Jason* Daily Report

Cruise Number: AT-26-10

Dive number: J2-759
Chief Scientist: Stefan Sievert

Report Date: 1/12/2014
Expedition Leader: Alberto Collasius Jr

Prepared By: Alberto Collasius Jr.

Vessel Location: Pacific
Position: 9 North
Weather: Pleasant
Dive Times: GMT

Dive Activities/Future Activities: Gas Tight's, Temp probe, Optode sensor, SPOT sensor and Flow Meter. Rock Collection and Tubeworm samples taken. Colonizers deployed and collected. Multiple elevators cycled during this dive with Gas tight's and other equipment listed above. Deployments of Large Volume Pump, that goes at its own 'elevator'. And a few Chimney collection's!

Reason for Dive Termination: At Science request.

Completed Dive Summaries:

Dive No.	Dates	Max Depth	Hours Descending	Hours Ascending	Hours on Bottom	Hours in water	Time On Deck	Time on deck not available to science
J2-758	1/2-1/6 2014	2512	2.0	2.0	99.9	103.9		
J2-759	1/8-1/12 2014	2519	1.6	2.1	86.3	90.1	43:30	43:30

Vehicle Status: Vehicle in very good working condition. All systems working properly. Temp probe grounded on the way to the surface. No impact on science. LSS grounded. No impact on Science. Niskin bottle on Stbd side was damaged. Small impact.

Weather Forecast: Fair.

Expedition Leader Comments: Another great long dive. Planned well and executed well. A few hiccups. One of the pump deployments did not go so well.

Chief Scientist Comments: Everything is going very well. The Jason team is great to work with. I have to especially praise the Jason team for fixing the tether in record time to get Jason in the water again. The dive was successful and we got very good samples. There is a very good team effort between science, Jason, and crew to cycle the elevators and the LVP to maximize bottom time of Jason and to increase productivity.

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ROV *Jason* Daily Report

Cruise Number: AT-26-10

Dive number: J2-760
Chief Scientist: Stefan Sievert

Report Date: 1/15/2014
Expedition Leader: Alberto Collasius Jr

Prepared By: Alberto Collasius Jr.

Vessel Location: Pacific
Position: 9 North
Weather: Deteriorating. 20+ knots and building.
Dive Times: GMT

Dive Activities/Future Activities: Gas Tight's , Temp probe, Major's , Optode sensor, SPOT sensor ,Rack recovery and Flow Meter. Rock Collection and Tubeworm samples taken. Colonizers deployed and collected. Multiple elevators cycled during this dive with Gas tight's and other equipment listed above. Deployments of Large Volume Pump, that goes as its own 'elevator'. And a few Chimney collection's!

Reason for Dive Termination: Weather deteriorating

Completed Dive Summaries:

Dive No.	Dates	Max Depth	Hours Descending	Hours Ascending	Hours on Bottom	Hours in water	Time On Deck	Time on deck not available to science
J2-758	1/2-1/6 2014	2512	2.0	2.0	99.9	103.9		
J2-759	1/8-1/12 2014	2519	1.6	2.1	86.3	90.1	43:30	43:30
J2-760	1/13-1/15	2521	1.6	2.1	50.25	54.0	9.9	0

Vehicle Status: Vehicle in very good working condition. All systems working properly. 300Khz Doppler grounded during dive. No impact on science.

Weather Forecast: Not good for the next 24 hours.

Expedition Leader Comments: Great dive. Had to terminate due to weather.

Chief Scientist Comments: Again, another great dive. With this dive we were able to fill in some gaps in the kind of samples received so far, like fluids w/ majors and extensive chemical monitoring. The Jason team is very accommodating and supports our science in the best way possible. Great work all around.

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ROV *Jason* Daily Report

Cruise Number: AT-26-10

Dive number: J2-761
Chief Scientist: Stefan Sievert

Report Date: 1/18/2014
Expedition Leader: Alberto Collasius Jr

Prepared By: Alberto Collasius Jr.

Vessel Location: Pacific
Position: 9 North
Weather: Deteriorating. 20+ knots and building.
Dive Times: GMT

Dive Activities/Future Activities: Gas Tight's , Temp probe, Major's , Optode sensor, SPOT sensor ,Rack recovery and Flow Meter. Rock Collection and Tubeworm samples taken. Colonizers deployed and collected. Multiple elevators cycled during this dive with Gas tight's and other equipment listed above. Deployments of Large Volume Pump, that goes as its own 'elevator'.

Reason for Dive Termination: Quick turn around

Completed Dive Summaries:

Dive No.	Dates	Max Depth	Hours Descending	Hours Ascending	Hours on Bottom	Hours in water	Time On Deck	Time on deck not available to science
J2-758	1/2-1/6 2014	2512	2.0	2.0	99.9	103.9		
J2-759	1/8-1/12 2014	2519	1.6	2.1	86.3	90.1	43:30	43:30
J2-760	1/13-1/15	2521	1.6	2.1	50.25	54.0	9.9	9.9
J2-761	1/16-1/18	2520	1.7	2.4	66.9	70.1	17.9	17.9

Vehicle Status: Vehicle in very good working condition. HPU for LARS acting up during recovery. Being troublshot as I write this. Power supply for LARS control failed during recovery. Being troubleshot as I write this.

Weather Forecast: Up around our limit for the next few days.

Expedition Leader Comments: Another great long dive. All vehicle systems working well as well as the Kraft we replaced during the last turn around. Replaced it because grippers were acting sticky. Had gone into this one and found a very corroded brass bushing that restricts movement of plunger. Will do the same to the one taken off.

Chief Scientist Comments: Another very successful dive. We continued our diverse sampling program and started to recover experiments as we are getting closer to the end of the cruise. Working with the Jason team continues to be both enjoyable and productive.

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ROV *Jason* Daily Report

Cruise Number: AT-26-10

Dive number: J2-762
Chief Scientist: Stefan Sievert

Report Date: 1/20/2014
Expedition Leader: Alberto Collasius Jr

Prepared By: Alberto Collasius Jr.

Vessel Location: Pacific
Position: 9 North
Weather: Deteriorating. 20+ knots and building.
Dive Times: GMT

Dive Activities/Future Activities: Dive was planned as most had been this cruise with all of the equipment listed in previous dive reports. We were on the bottom trying to get the first objective done (LVP install) when the call came to terminate the dive and get underway for Acapulco.

Reason for Dive Termination: Medical Emergency on board

Completed Dive Summaries:

Dive No.	Dates	Max Depth	Hours Descending	Hours Ascending	Hours on Bottom	Hours in water	Time On Deck	Time on deck not available to science
J2-758	1/2-1/6 2014	2512	2.0	2.0	99.9	103.9		
J2-759	1/8-1/12 2014	2519	1.6	2.1	86.3	90.1	43:30	43:30
J2-760	1/13-1/15	2521	1.6	2.1	50.25	54.0	9.9	9.9
J2-761	1/16-1/18	2520	1.7	2.4	66.9	70.1	17.9	17.9
J2-762	1/19	2512	1.4	1.5	2.6	5.7	-	-

Vehicle Status: Vehicle in very good working condition.

Weather Forecast: Up around our limit for the next few days.

Expedition Leader Comments: An unexpected end to a very productive cruise!

Chief Scientist Comments: I can only reiterate the comment of the expedition leader. It says it all. It has been a pleasure to work with the Jason team and I look forward to using Jason in the future.

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