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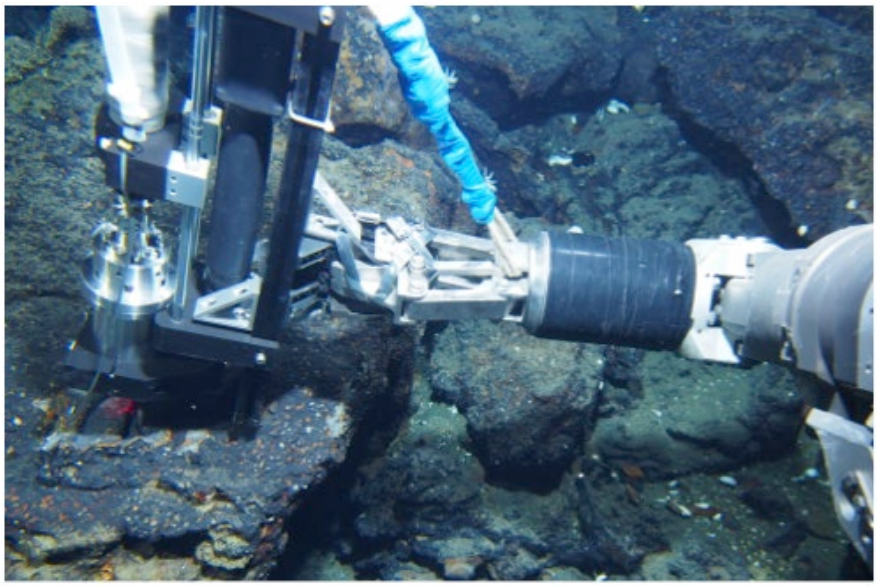
R/V Natsushima cruise report

NT14-21

11th December – 23rd December, 2014

In Situ Chemical Sensor and Microbiological Study of the Bayonnaise Knoll and Okinawa Trough



The University of Tokyo
Kyoto University, University of Shizuoka
Enoshima Aquarium, JAMSTEC

Preface

This report describes the dives of the ROV Hyper-Dolphin between 11st and 23th December 2014 at the Izu-Ogasawara arc and the Okinawa trough, performed during the NT14-21 cruise of the R/V Natsushima.

This cruise was conducted based on three separate proposals; #S14-22 'Application of spectroscopic chemical sensors to survey hydrothermal vent deposits', proposed by Blair Thornton of the University of Tokyo, #S14-13 'Host-symbiont recognition mechanisms in the chemosynthetic ecosystem: functional analysis of abundantly expressed lectin-like protein in host animal', proposed by Satoshi Nakagawa of Kyoto University, and #S14-35(2) 'Time-resolved in situ colonization experiments of basalt at seafloor to understand a deep biosphere ecosystem', proposed by Satoshi Mitsunobu of the University of Shizuoka.

A total of three dives were performed over three days. In situ multi-element analysis of hydrothermal deposits was performed at depths of over 1000m in the Iheya North field under proposal #S14-22. A large number of invertebrates were sampled from the Iheya North field to study host-symbiont recognition mechanisms specific to chemosynthetic ecosystems under proposal #S14-13. Incubation vessels, seawater and rock samples were recovered from the Bayonnasie knoll as part of a time-resolved in situ colonization experiment under proposal #S14-35(2).

The research party would like to thank the crew members of the R/V Natsushima lead by Captain Hiraoki Masujima, the members of the ROV HPD operation team lead by Tomoe Kondo, marine technician Mitsuteru Kuno, and Yuta Yamamuro together with the staff of JAMSTEC and Nippon Marine Enterprise for their dedicated efforts which contributed greatly to the success of this cruise.

December 2014
Blair Thornton (NT14-21 Chief Scientist)

Notice on use

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Contents

1. Cruise information

Cruise ID	NT14-21
Research vessel	R/V Natsushima
Title of the cruise	In situ chemical sensor and microbiological study of the Bayonnaise Knoll and Okinawa trough
Chief scientist	Blair Thornton (Institute of Industrial Science, The University of Tokyo)
Cruise period	11th December 2014 to 23rd December 2014 (Yokosuka Sumitomo ~ Naha)
Survey sites	Bayonnaise Knoll and Okinawa trough

Title of Proposals

Proposal 1	Application of spectroscopic chemical sensors to survey hydrothermal vent deposits, *Blair Thornton (Institute of Industrial Science, The University of Tokyo)*
Proposal 2	Host-symbiont recognition mechanisms in the chemosynthetic ecosystem: functional analysis of abundantly expressed lectin-like protein in host animal, *Satoshi Nakagawa (Graduate School of Agriculture, Kyoto University)*
Proposal 3	Time-resolved in situ colonization experiments of basalt at seafloor to understand a deep biosphere ecosystem, *Satoshi Mitsunobu (Institute for Environmental Sciences, University of Shizuoka)*

2. Cruise Log

2.1 Survey area and time schedule

A total of 3 out of a planned 8 dives were performed during this cruise. The first dive (HPD#1757) was performed at the Bayonnaise Knoll in the Izu-Ogasawara arc under Proposal 3. After a failed dive attempt (HPD#1758), the second dive (HPD#1759) was performed at the Iheya North Field in the Okinawa Trough under Proposal 1. The third and final dive (HPD#1760) was performed in the same location, under Proposal 2. The remaining dives were cancelled due to bad weather conditions. The ships track and ROV dive sites are shown in Figures 1 to 3. Table 1 shows the time schedule of the cruise.

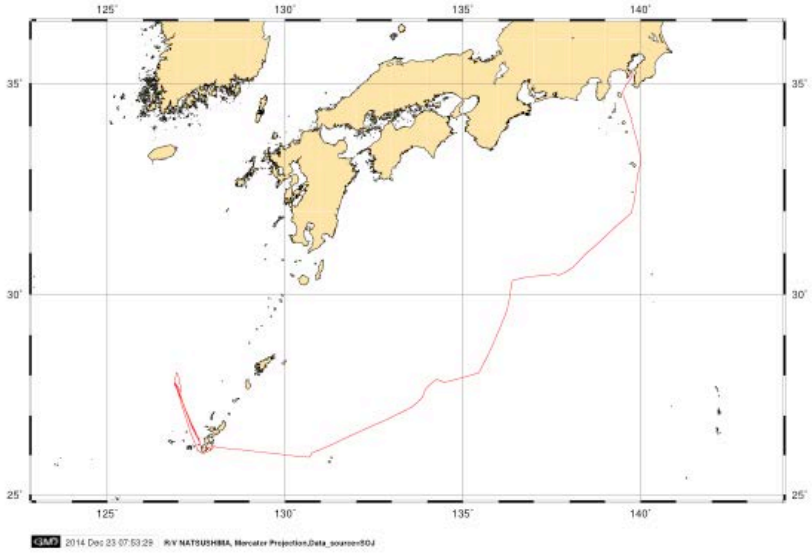

Figure 1 Ship track during NT14-21

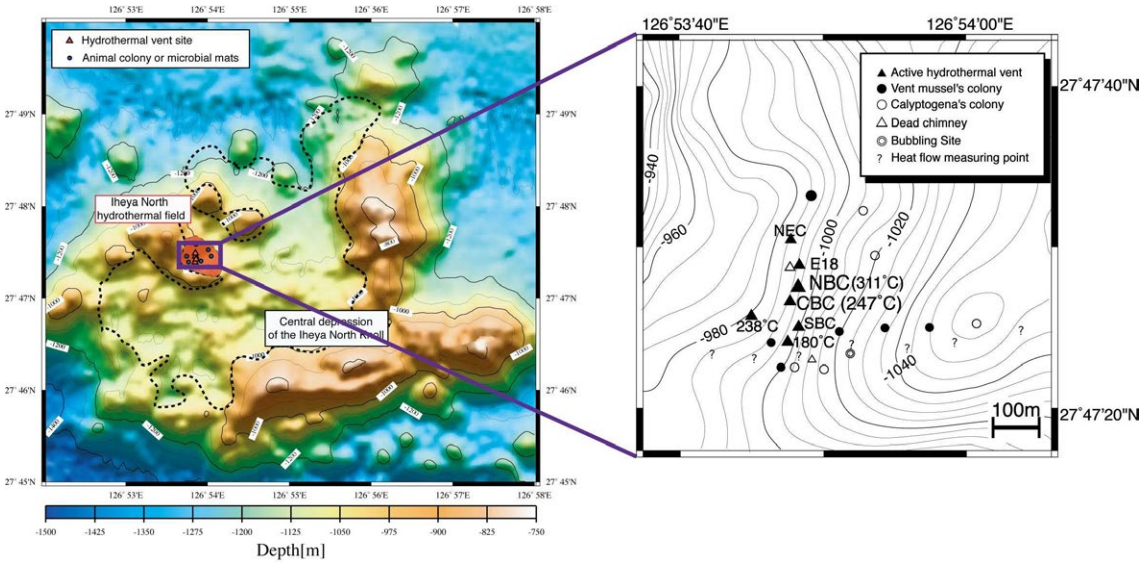

Figure 2 Iheya North Field track during NT14-21

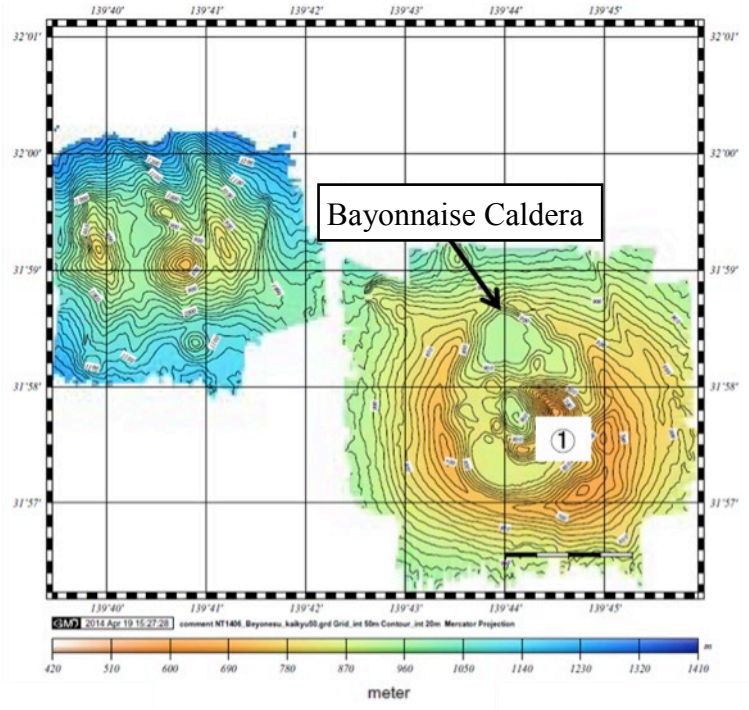

Figure 3 Bayonnaise Caldera

Date	Local Time	Description
11-Dec-14		**Sail out, proceeding to research area**
	09:00	Scientists onboard.
	09:30	Scientists meeting.
	10:00	Departure from Sumitomo heavy industry, Ltd.
	10:30-11:00	Briefing about ship's life and safety.
12-Dec-14		**HPD DIVE#1757(Myojin North knoll)**
	09:00	Arrived at research area (Myojin North knoll)
	09:00	Released XBT. (31-57.8447N, 139-44.7664E)
	09:47	Hoisted up HPD.
	10:40	HPD landed (D=883m)
	13:25	HPD left the bottom (D=778m)
	14:07	Recovered HPD and then proceeding to Free Fall site.
13-Dec-14 to 14-Dec-14		**Proceeding to Free Fall site**
15-Dec-14		**Free Fall Umbilical Cable.**
	08:00	Hoisted up Free Fall towing gear.
	10:01	Stopped winch at run out 3,500m.
	12:03	Wind up 1st Free Fall.
	12:04	Started 2nd Free Fall.
	13:49	Stopped winch at run out 3,500m.
	14:09	Wind up 2nd Free Fall.
	15:58	Recovered the gear and then proceeding to Yonabara bay.
16-Dec-14 to 18-Dec-14		**Avoided rough sea at Yonabara bay** Proceeding to research area (Iheya North knoll)
19-Dec-14		**HPD DIVE#1758 (Iheya north knoll: Operations aborted)**
	06:36	Released XBT. (27-47.7072N, 126-53.7260E)
	07:46	Hoisted up HPD.
	08:10	Started Dive.
	08:54	HPD unable to dive due to currents
	09:10	Recovered HPD.
	09:48	Hoisted up HPD.
	10:05	Started Dive.
	12:40	HPD unable to dive due to currents
	12:55	Recovered HPD.
	13:33	Hoisted up HPD.
	13:47	Operations aborted due to damaged umbilical
20-Dec-14		**Avoided rough sea at off Naha.** Proceeding to research area (Iheya North knoll)
21-Dec-14		**HPD DIVE#1759(Iheya north knoll)**
	06:00	Arrived at research area.
	07:28	Hoisted up HPD.
	08:35	HPD landed (D=999m)
	17:08	HPD left the bottom (D=995m)
	18:00	Recovered HPD.
22-Dec-14		**HPD DIVE#1760(Iheya north knoll)**
	10:22	Hoisted up HPD.
	11:39	HPD landed (D=994m)
	14:12	HPD left the bottom (D=1034m)
	15:23	Recovered HPD.
23-Dec-14		**Disembarked at Naha-shinkou**
	09:00	Disembarked at Naha-shinkou.

Table 1 Time schedule of NT14-21

2.2 Research party

Name	Affiliation
Blair Thornton*	The University of Tokyo
Toshihiko Ohki	The University of Tokyo
Takumi Sato	The University of Tokyo
Tomoko Takahashi	The University of Tokyo
Satoshi Nakagawa	Kyoto University
Tomoo Watsuji	Japan Agency for Marine-Earth Science and Technology
Kaori Motoki	Japan Agency for Marine-Earth Science and Technology
Suguru Nemoto	Enoshima Aquarium
So Fujiyoshi	Kyoto University
Satoshi Mitsunobu	University of Shizuoka
Hiroko Makita	Japan Agency for Marine-Earth Science and Technology
Mitsuteru Kuno	Nippon Marine Enterprise

Table 2 Research Party (*chief scientist)

3. Instrumentation and methods

3.1 Objectives

The main objectives of the cruise were as follows:

Proposal 1: Application of spectroscopic chemical sensors to survey hydrothermal vent deposits

Our group is investigating the application of laser-induced plasmas as a method to perform in situ, multi-element chemical analysis of the composition of liquids and solid deposits on the seafloor. During this cruise, we deployed an in situ sensor called the ChemiCam, which was developed under the 'Program for the development of fundamental tools for the utilization of marine resources' of the Japanese Ministry of Education. ChemiCam uses a technique known as laser-induced breakdown spectroscopy (LIBS) to perform atomic emission spectroscopy on site at depths of up to 3000m. During NT13-23, we deployed ChemiCam in Iheya North Field and performed on site chemical analysis of both liquids and the exposed surface of hydrothermal deposits blocking the C0013E artificial vent orifice [1] and well resolved spectra were obtained. The main goal of this cruise is to improve the operational efficiency of ChemiCam and test a number of operational features that have been implemented. First, while the measurements of liquids can be considered routine, the measurement of solids is complicated since the focal point of the laser has to be precisely aligned with the surface of the solid. In order to make this task more manageable, a guide laser has been integrated with ChemiCam, together with a laser-focusing system that measures the intensity of light reflected from targets surface and uses this signal to control a linear stage. In addition, one of the major disadvantages of LIBS is that it can only measurement of exposed surfaces. In order to overcome this issue, we developed a deep-sea grinder to remove the weathered surface of the deposits, so that measurements can be made of freshly exposed surfaces that are more representative of whole rock composition. The main objective was to use these tools to enable efficient, 'sub-surface' measurements of hydrothermal deposits using ChemiCam.

Proposal 2: Host-symbiont recognition mechanisms in the chemosynthetic ecosystem: functional analysis of abundantly expressed lectin-like protein in host animal

One of our major research foci is "symbiosis in deep-sea vents". Deep-sea vents are light-independent, highly productive ecosystems fueled primarily by chemolithoautotrophic microorganisms. Most of the invertebrates in deep-sea vent areas thrive in the ever-changing physical and chemical gradients through their relationship with chemolithoautotrophic symbionts. Each generation of deep-sea vent invertebrates inhabiting near active vents, e.g. shrimps, squat crabs and gastropods, acquire their specific endo- or epi-symbiotic bacteria from the environment. However, little is known about the molecular mechanism through which host-microbe recognize with each other. Recently, glycoconjugates have been recognized as common legislators of host-microbial interactions including both symbiosis and pathogenicity. For example, the attachment of *Helicobacter pylori*, a pathogenic species of *Epsilonproteobacteria*, to

[1] Thornton et al. 'Development of a Deep-Sea Laser Induced Breakdown Spectrometer for In Situ Multi-element Chemical Analysis', Deep-sea Research I 95 (2015) 20-36

fucosylated or sialylated glycans produced by various gastric epithelial lineages and their progenitors skews the destiny of colonization toward pathogenicity. Our previous work indicated deep-sea vent specific chemolithoautotrophs have the ability to form unique N-linked glycans. These support to the hypothesis that the capacity to synthesize diverse glycan structures may have arisen in part from the need of both host and symbionts to both evade pathogenic relationships and to coevolve symbiotic relationships with non-pathogenic resident microorganisms. During this cruise, fluids, animals, and chimney structures were successfully collected, and we prepared samples for multi-omics analyses including glycomic analysis. For comparison, we will isolate and characterize free-living microorganisms from hydrothermal samples, i.e. fluids and chimney structures.

Proposal 3: Time-resolved in situ colonization experiments of basalt at seafloor to understand a deep biosphere ecosystem

The main objective of our research project is to understand the litho-biosphere ecosystem beneath the sea-floor that is supported by oxidation of ferrous iron (Fe(II)) in ocean crust basalt. In order to achieve this, our group is performing a "time-resolved in situ colonization experiment with fresh basalt". During this cruise, (i) we recovered incubation vessels from the Bayonnaise knoll that were installed during the NT14-06 cruise, and (ii) collected seawater and rock samples from the points where the incubation vessels were installed.

3.2 Instruments

During this cruise, several different payloads were mounted on Hyper-Dolphin (HPD). These can be grouped into sensors for real-time measurement, tools to manipulate the environment, sampling tools and on-site experimental apparatus. During the dives the readings of the some of the sensors were monitored in the ROV control room by members of the research party, and several sensors (DO, turbidity) used were operated as stand-alone units.

3.2.1 ChemiCam

ChemiCam, see Figure 4, is a chemical sensor developed to perform in situ, multi-element analysis of the composition of liquid and solids at depths of up to 3000m. The focusing probe is attached to the ROV manipulator on a single axis linear stage. The probe is moved into the vicinity of the measurement target using the ROV manipulator, after which the laser is focused onto the target using the laser-based focusing system. Both systems are controlled using a PC via a single RS232 communication line on the ROV and the measured data can be monitored in real-time. ChemiCam uses a form of atomic emission spectroscopy, called LIBS, which observes the optical emissions of plasmas generated by focusing a high power pulse laser into the bulk liquid, or onto immersed solid targets. Two types of LIBS device were deployed during this cruise. ChemiCam D is a 3000m depth rated device that uses a direct optic that focuses the laser to generate plasmas directly in bulk seawater to measure its chemical composition. ChemiCam F is a 3000m depth rated device, principally designed to measure the composition of solids. The latter system uses a fiber optic

probe that is attached to the ROV manipulator (shown in Figure 4) and can target specific regions of the seafloor for analysis. Both devices perform measurements at a sampling rate of 1Hz and the results of the obtain spectra can be observed in real-time in the ROV control room. The ChemiCam systems are the 2nd generation of ocean going LIBS devices, following the prototype I-SEA system that was deployed during NT12-07. The most significant difference between the ChemiCam and I-SEA systems is the use of a long duration ns-pulse laser, which has been demonstrated to present significant advantages in the quality of spectral emissions that can be observed underwater and at high pressure. During this cruise, a number of improvements have been made to improve the operational efficiency of ChemiCam, including a laser-based focusing system, an improved laser for target ablation and an integrated CT sensor for seawater composition measurement. The red-laser used to focus ChemiCam can be seen in the photo in Figure 5, which was taken during #1759 of this cruise.

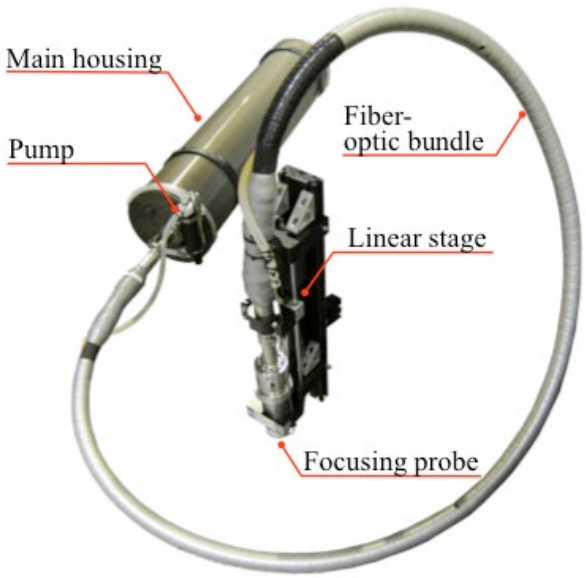

Figure 4 ChemiCam F

Physical and electrical information		
Length (main housing) [m]	1.3	
Diameter [m]	0.3	
Maximum depth [m]	3000	
Operational mode	Mode 1	Mode 2
Measurement target	Dissolved ions	Mineral deposits
Weight in air [kg]	140	160
Weight in water [kg]	25	40
Power consumption [W]	130	140
Peripherals	CT sensor	Pump Linear stage
Power supply [VAC]	100	
Communication	RS232 or Ethernet	
Optical characteristics		
Focusing method	Direct	Optical head via 4 m fiber
Focusing optic	10 × Objective lens	5 × Cassegrain
Laser type	Q-switched DPSSL Nd:YAG	
Pulse energy (at target) [mJ]	30	20
Pulse duration [ns]	150 to 250	150 to 250
Laser wavelength [nm]	1064	
Spectrometer type	Czerny-Turner	
Inlet slit dimensions [mm]	0.5 × 8	
Spectral range [nm]	400 to 800	295 to 550
Spectral resolution [nm]	1.6	0.8
Detector type	ICCD (Gen. III)	
Number of pixels	1024 × 256 (i.e. 1024 ch)	

Table 3 Specification of ChemiCam

Figure 5 Guide laser (red spot) used to align and focus ChemiCam's main pulsed laser, which generates a plasma from the target once a second.

3.3 Sampling and manipulation tools

3.3.1 Grinder

A deep-sea grinder was deployed during HPD#1759 to remove the weathered surface of hydrothermal deposits and expose a fresh, 'sub-surface' layer, which can then be measured by ChemiCam. The main hydraulic unit of the ROV supplies hydraulic pressure, and the flow can be controlled from the control room of the ROV. The grinder is operated using the ROV manipulator. The specifications of the grinder are as shown in Table 4.

Hydraulic pressure	13.7MPa (2,000psi)
Flow rate	20L/min
Maximum rotation	2,400rpm
Torque	11Nm
Weight	9kg (in air) 7kg (in water)
Size	350 x 151 x 108mm

Table 4 Specifications of the grinder

Figure 6 Grinder used to remove the weathered surface of deposits during HPD#1759

3.3.2 Slurp gun

A suction sampler that consists of a tube connected to a pump was used to collect deep-sea vent animals into a cylindrical sample chamber (maximum two cylinders per dive). For in-situ fixation (so called DEATH mode), a small sampling chamber (3.5 L in volume) was used instead of the cylindrical chamber. After animal collection at the seafloor, individuals in the sampling box were immediately immersed in a RNA stabilization reagent on site (RNAlater, QIAGEN, JAPAN) colored in yellow of phenol red. The system consisted of the sampling box, a flexible 6-L plastic bag (Sekisui Chemical, Japan) containing 5 L of the fixation solution, and a silicon tube (φ9 mm, TOGAWA, Japan) with a valve used to connect the bottom part of the sampling box and the plastic bag. The high-density fixation solution in the flexible bag was poured into the sampling box by opening the valve of the flexible bag located above the sampling box.

3.3.3 Niskin bottle

A water sampler was used to collect water samples at ROV landing points.

3.3.4 Payload for in situ colonization experiment

Figure 7 shows the payload for the in situ colonization experiment.

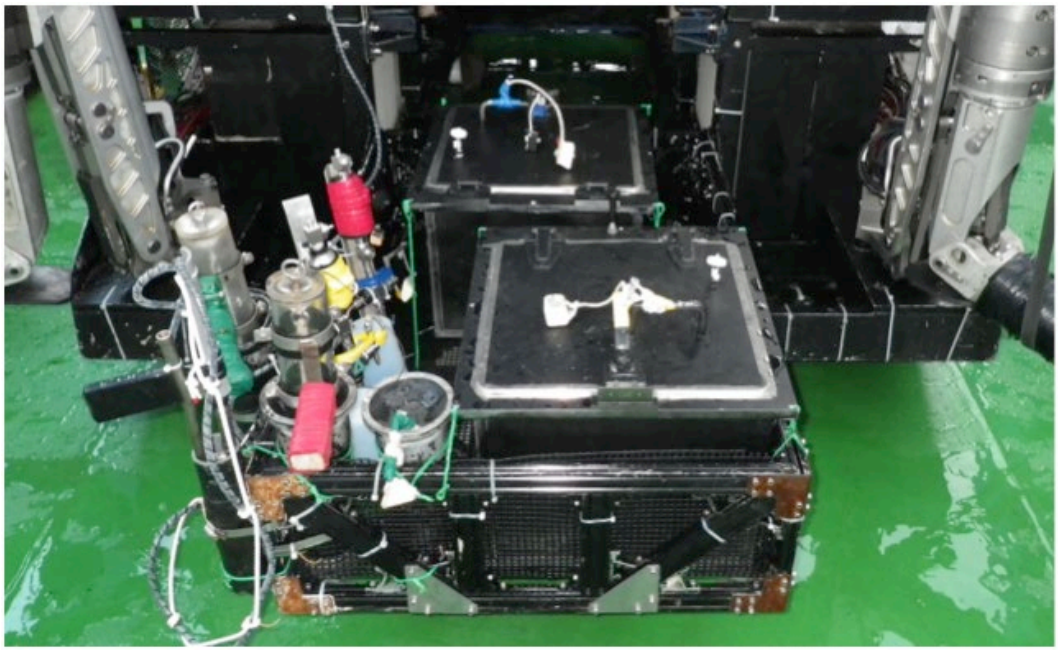

Figure 7 On-line thermometer, Niskin water sampler (x2), Sampling box for in situ incubator (x2), DO meter, M-type sediment sampler (x2), Vacuum water sampler (x2)

3.4 Sample preparation

3.4.1 Host-symbiont recognition studies

During the NT14-21, we collected various hydrothermal samples including chimney structures, fluids, and hydrothermal vent animals from the Iheya North hydrothermal field. Immediately after recovery, all samples were prepared for the multidisciplinary shore-based microbiological study (described below).

Fluids were kept under a N_2 atmosphere at 4 °C for cultivation. For culture-independent analysis, cells in fluids were collected by filtration, and then stored at -80 °C. Similarly, chimney structures were sub-sampled into two parts, i.e. exterior surface and inside parts, and then anaerobically slurried for cultivation or stored at -80 °C for culture-independent analysis.

Hydrothermal vent animals, e.g. Shinkaia shrimps and Bathymodiolus mussels, were dissected into the symbiont-housing tissue and other body parts. The samples were fixed, anaerobically slurried, or frozen for shore based study. Overall, all samplings for microbiology were successfully performed onboard during this cruise.

3.4.2 In situ colonization experiment

For chemical analysis, collected sediments were N2 purged and stored in a refrigerator during cruise and transported to our laboratory. Water samples for chemical analysis were acidified with HCl and stored in room temperature during cruise. For the analysis of microbial communities in seawater, the other water samples were filtrated with 0.2 mm membrane filters, and then the filters were stored at -80^{o}C freezer for 16S rRNA-DNA analysis.

Solid samples in the recovered vessels were stored at -80^{o}C for following microbial and chemical analyses. The analyses would be performed by 16S rRNA-DNA technique for the microbial community analyses and synchrotron microscope technique for the chemical analyses.

4. ROV operation

4.1 HPD #1757

Site: Bayonnaise Knoll
Landing point: 31-57.436 N, 139-44.560 E, 833m

During dive #1757, incubation vessels were successfully recovered at hydrothermal and non-hydrothermal areas in Bayonnaise Knoll. During the recovery, temperature and DO were measured by in situ sensors at the installation points. At the installation points, both water and sediment samples were also collected.

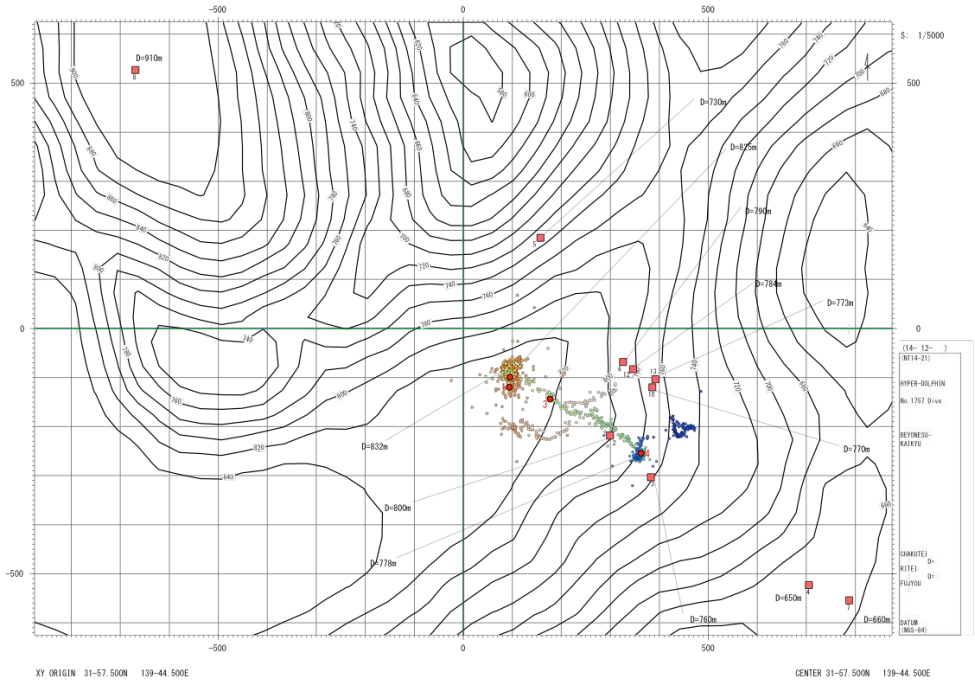

Figure 8 ROV track during HPD#1757

Time	Depth(m)	Remark
10:40	833	Reach the seafloor
10:41	833	Niskin seawater sampling
11:09	834	DO measurement
11:26	834	Collection of in situ incubator (set in NT14-06-4)
11:30	835	Temperature measurement
11:39	835	Vacuum seawater sampling
11:50	834	M-style sediment sampling
12:18	776	Niskin bottle seawater sampling
12:28	778	Temperature measurement
12:31	780	DO measurement
12:49	780	Vacuum (red) seawater sampling
12:56	780	Collection of in situ incubator (set in NT14-06-1)
13:13	780	M-style (green) sediment sampling
13:25	778	End of dive

Table 5 HPD#1757 Dive summary

4.2 HPD #1758

Dive aborted before arriving a seafloor due to damaged ROV umbilical.

4.3 HPD #1759

Site: NBC (North Big Chimney), Izena North Knoll
Landing point: 27-47.433 N, 126-53.794 E, 999m

The main objectives of this dive were LIBS measurements using ChemiCam F and sample of Shinkaia crosnieri. The ROV manipulator was used to hold ChemiCam F`s focusing probe near sample test pieces and also near the seafloor. The laser focusing system was used to guide the probe, and ChemiCam's linear stage was used to focus the main pulsed laser on the surface of the targets. The grinder was also deployed to remove weathered surfaces of natural deposits, and LIBS measurements were made of freshly exposed sub-surface layers at several locations around the base and near the top of the NBC mound. Samples were also obtained for laboratory analysis to verify the results of the in situ measurements.

We successfully collected hydrothermal samples, i.e. vent animals, fluids, and chimney

structures. Individuals of *Shinkaia crosnieri* were collected at NBC using the slurp gun (into two different canisters). Fluids were also collected at the seawater/vent fluids mixing area of NBC using Niskin bottles. A chimney structure on the NBC mound was collected using manipulators. Samples were prepared immediately for shore-based microbiological and biogeochemical analyses.

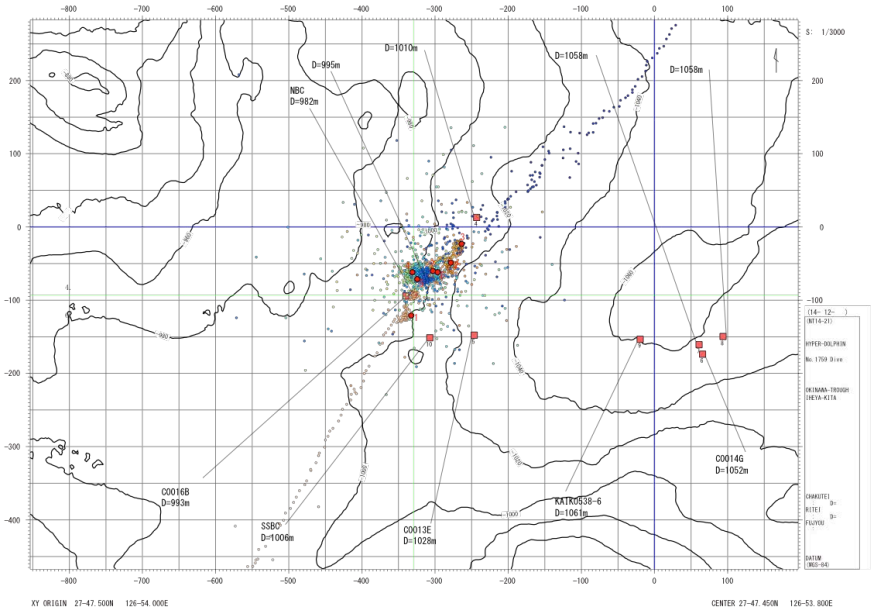

Figure 9 ROV track during HPD#1759

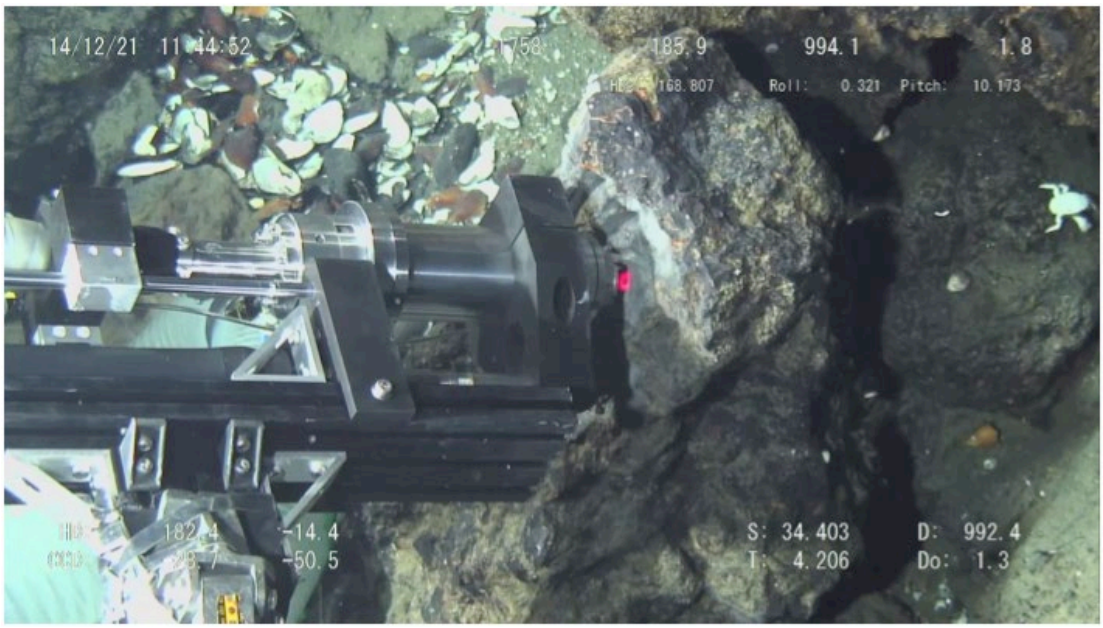

Figure 10 LIBS measurement of a near vertical cliff face after removing its surface layer using the grinder

Time	Depth(m)	Remark
7:32		Dive start
8:35	999	Reach the seafloor
9:23	1016	LIBS measurements of a test piece using ChemiCam F
9:48	1017	Measurements and sampling of rocks around the base of NBC mound
15:06	984	Sampling of Shinkaia crosnieri (cubic canister)
15:12	986	Sampling of Shinkaia crosnieri (cylindrical canister)
15:26	986	Niskin bottle seawater sampling
15:45	986	Measurement and sampling of rocks near the top of NBC mound
17:04	991	Sampling of an active chimney Niskin bottle seawater sampling
17:08	995	End of dive

Table 6 HPD#1759 Dive summary

4.4 HPD # 1760

Site: NBC (North Big Chimney), Izena North Knoll
Landing point: 27-47.433 N, 126-53.794 E, 999m

Vent animals, fluids, and chimney structures were collected at the base of CBC (Central Big Chimney). During the dive, the in-situ fixation of Shinkaia was performed. Bathymodiolus mussels and chimney structures were also collected using manipulators. Fluids were collected at the seawater/vent fluids mixing area using Niskin bottles. Samples were prepared immediately for shore-based microbiological and biogeochemical analyses.

ChemiCam D was used to continuously make spectroscopic measurements of seawater composition during this dive.

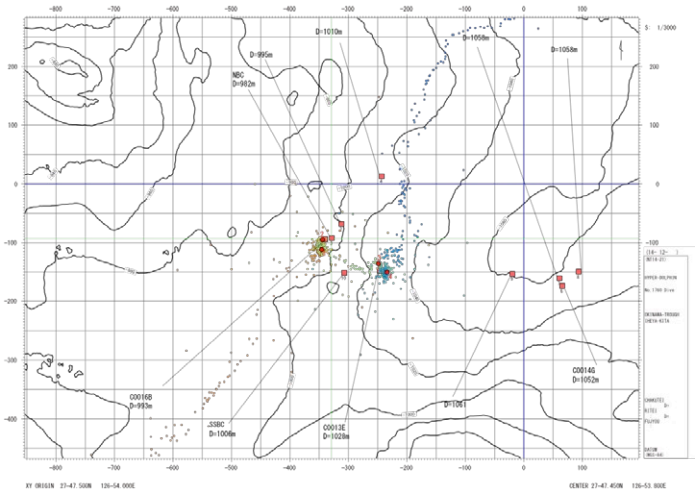

Figure 11 ROV track during HPD#1760

Time	Depth(m)	Remark
11:39	994	Reach the seafloor
11:57	985	Chimney sampling
12:10	987	Rock with Bathymodiolus japonicus sampling Niskin bottle seawater sampling
12:32	983	Sampling of Shinkaia crosnieri (cubic canister)
12:36	984	Sampling of Shinkaia crosnieri (death canister)
12:40	984	Niskin bottle seawater sampling
12:53	983	Chimney sampling
13:05	1022	H1178-1 marker check
13:06	1022	C0013E check
13:25	1031	Grinder used on C0013E vent deposits
14:12	1034	End of dive

Table 7 HPD#1760 Dive summary

5. Sample list

Name	Dive No.	Sea depth (m)	Sampler	Amount	Locality
Sediment samples					
HPD1757_M01(green)	1757	834	M-type corer	500 ml	Non-hydrothermal area
HPD1757_M02(red)	1757	778	M-type corer	500 ml	Hydrothermal area
Water samples					
HPD1757-V01	1757	834	Vacuum sampler	500 ml	Non-hydrothermal area
HPD1757-V02	1757	778	Vacuum sampler	500 ml	Hydrothermal area
HPD1757-N01	1757	833	Niskin sampler	2.6 L	Non-hydrothermal area
HPD1757-N02	1757	776	Niskin sampler	2.6 L	Hydrothermal area
Biological samples					
Colonization vessel 1	1757	834	-		Non-hydrothermal area
Colonization vessel 2	1757	778	-		Hydrothermal area

Table 8 Samples obtained from the Bayonnaise Knoll

Sample code	Description	Site	Date	Latitude		Longitude		Depth	Total	Distribution
HPD#1759_ R04	Chimney	NBC	12/21/2014	27	47.468N	126	53.816E	991	200g	200g (Kyoto)
HPD#1759-B01	*Shinkaia crosnieri*	NBC	12/21/2014	27	47.449N	126	53.800E	991	381	367 (Kyoto & JAMSTEC), 14 (Enosui)
HPD#1759-B02	*Shinkaicaris leurokolos*	NBC	12/21/2014	27	47.449N	126	53.800E	991	15	15 (JAMSTEC)
HPD#1759-B03	*Bathymodiolus japonicus*	NBC	12/21/2014	27	47.449N	126	53.800E	991	1	1 (Kyoto)
HPD#1759-B04	*provannasp. Aglabraff.*	NBC	12/21/2014	27	47.449N	126	53.800E	991	30	30 (Enosui)
HPD#1759-B05	*Lepetdrilus*sp.	NBC	12/21/2014	27	47.449N	126	53.800E	991	30	30 (Enosui)
HPD#1759-B06	polychaeta gen.et sp.	NBC	12/21/2014	27	47.449N	126	53.800E	991	20	20 (Enosui)
HPD#1759-B07	*Neoverruca* sp.	NBC	12/21/2014	27	47.449N	126	53.800E	991	1	1 (Enosui)
HPD#1759-B08	*Alvinocaris longirostris*	NBC	12/21/2014	27	47.449N	126	53.800E	991	1	1 (Enosui)
HPD#1759-B09	*Alvinocaris dissimilis?*	NBC	12/21/2014	27	47.449N	126	53.800E	991	1	1 (Enosui)
HPD#1759-W01	Fluid	NBC	12/21/2014	27	47.468N	126	53.816E	991	1700ml	1500ml (Kyoto), 200ml (Tokyo)
HPD#1759-W02	Fluid	NBC	12/21/2014	27	47.468N	126	53.816E	991	1700ml	1500ml (Kyoto), 200ml (Tokyo)
HPD#1760_ R02	Chimney	NBC-CBC	12/22/2014	27	47.449N	126	53.791E	983	400g	400g (Kyoto)
HPD#1760-B01	*Bathymodiolus platifrons*	NBC-CBC	12/22/2014	27	47.427N	126	53.849E	983	50	20 (Kyoto), 30 (Enosui)
HPD#1760-B03	Alvinocarididae gen.et al sp.	NBC-CBC	12/22/2014	27	47.427N	126	53.849E	983	10	10 (Enosui)
HPD#1760-B04	*Batyacmasea* sp.	NBC-CBC	12/22/2014	27	47.427N	126	53.849E	983	1	1 (Enosui)
HPD#1760-B05	*Shinkaia crosnieri*	NBC-CBC	12/22/2014	27	47.427N	126	53.849E	983	131	110 (Kyoto & JAMSTEC), 21 (Enosui)
HPD#1760-W01	Fluid	NBC-CBC	12/22/2014	27	47.449N	126	53.791E	987	1700ml	1500ml (Kyoto), 200ml (Tokyo)
HPD#1760-W02	Fluid	NBC-CBC	12/22/2014	27	47.449N	126	53.791E	983	1700ml	1500ml (Kyoto), 200ml (Tokyo)

Table 9 Samples obtained from the Iheya North Field

6. Preliminary results

6.1 In-situ multi-element chemical analysis

Successful measurements of solid test pieces and natural deposits with freshly exposed surfaces were made at various locations during HPD#1759 using ChemiCam F. An example of spectra is shown in Figure 12.

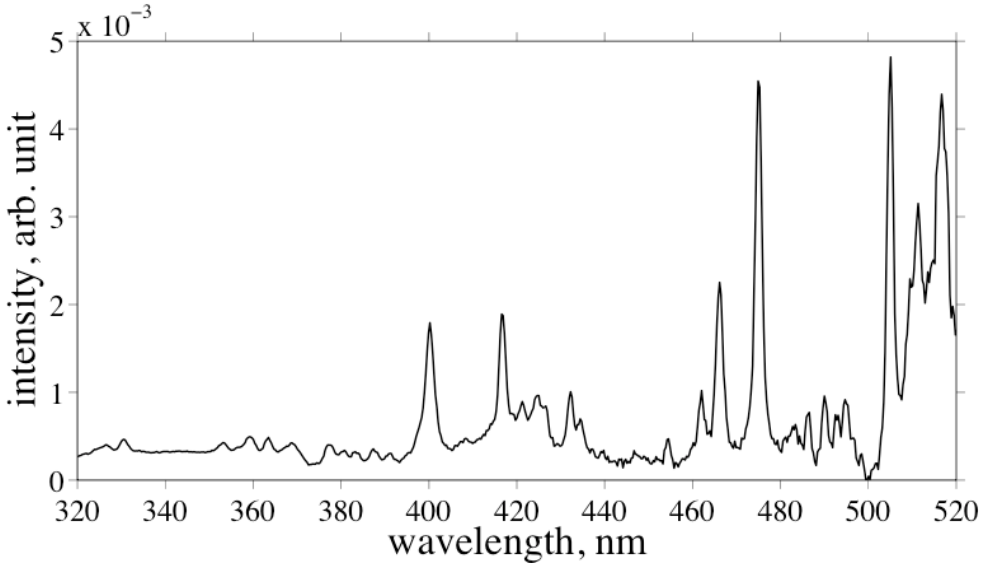

Figure 12 ChemiCam F LIBS measurement of a test piece at over 1000m depth

ChemiCam D was successfully operated to measure the chemical composition of seawater during HPD#1760. Figure 13 shows an example of a spectrum measured from seawater during the cruise at a depth of 980m.

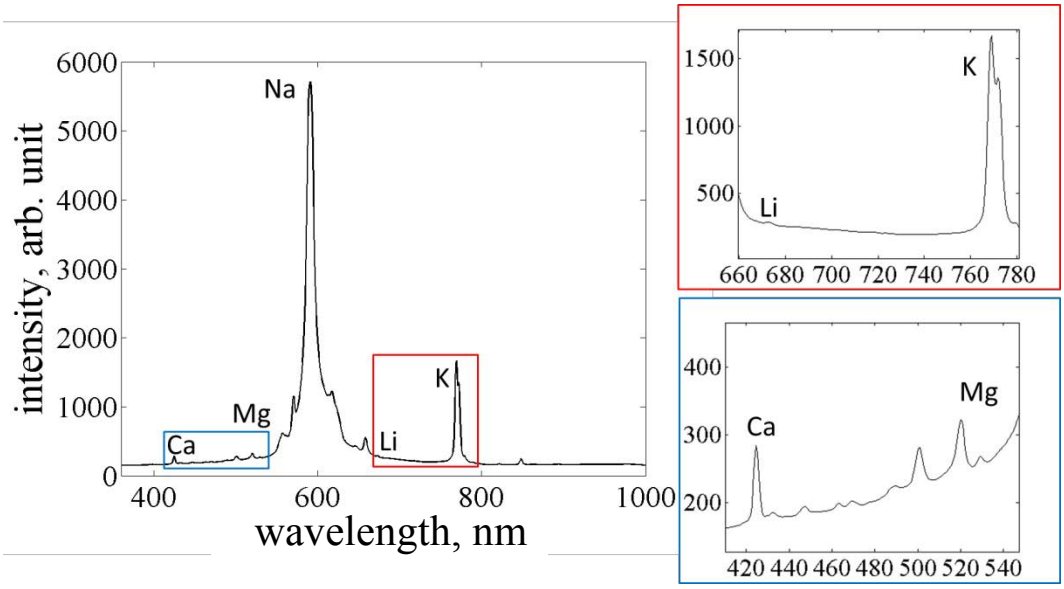

Figure 13 Spectral measurement of seawater made at 980m depth using ChemiCam D

7. Summary and future plans

The major objectives of the three proposals on this joint cruise were successfully achieved.

With regard to in situ chemical analysis, simultaneous multi-element analysis of both seawater and hydrothermal deposits were successfully achieved. Laboratory quality data was obtained from the two ChemiCam systems mounted on the ROV at depths of over 1000m. The improvements made to ChemiCam since its last deployment are clear, and ability to focus the main laser using a secondary guide laser and controllable linear stage improves the efficiency of operation of the device. Due to the limited dive time available, we were not able to perform a full survey of the NBC mound, however, we were able to successfully demonstrate measurements of freshly exposed 'sub-surface' deposits by combining LIBS measurements with a deep-sea grinder. Sampling was also performed for laboratory analysis in order to verify the results of the measurements made in situ during this cruise. Seawater measurements were also performed and spectral data has been obtained and will be analysed in our future work. With regards to the in situ device, future work will focus on improving operational efficiency by developing a new focusing stage taking into consideration our experience with the deployments of ChemiCam to date. With regards to measurement of liquids, we plan on extending the capabilities of the system to measure high-temperature (>100^{o}C) fluids on site.

With regard to host-symbiont recognition studies, the future studies can be separated into the following categories:

① Culture –independent molecular ecological surveys

Culture–independent molecular ecological methods allow us to characterize unculturable deep-sea vent bacteria. We will perform a variety of culture-independent analysis including those mentioned below.

Gene sequencing is essential for all phylogenetic analysis and identification of microorganisms. We will sequence specific genes (e.g. 16S rDNA, Methyl CoM reductase, dissimilatory sulfite reductase etc.) from each sample. Additionally, we will perform both meta-genomic and meta-transcriptomic analyses. Especially, Dr Watsuji and Ms Motoki will perform metatranscriptomic analysis using the in-situ fixed epibionts to characterize functionally active metabolic pathways in S. crosnieri epibionts. In addition, Dr. Nemoto will study the change in epibiont composition during rearing.

Glycan-profiling provides key insights into the molecular interactions between symbiont and host animals. We will purify glycans using enzymatic and chemical methods, and then analyze the structure using MALDI-TOFMS and 2D-HPLC. In addition, Ms. Fujiyoshi will investigate the glycan-binding protein of Shinkaia crosnieri.

② **Culture-dependent ecological surveys**

It is often noted that culturable microbes represent only 0.1-1 % of total microbes in environments. However, we have tried to cultivate previously uncultured organisms on the bases of data from culture-independent analyses from various hydrothermal vents, and has succeeded in cultivation of more than 10% of the members that were detected in culture-independent analyses in each habitat. Culturable populations of microbes will be evaluated by most probably number (MPN) method.

MPN analysis is a method to enumerate culturable populations of microbes. Hydrothermal samples were diluted in 10-fold steps into liquid media, which should support the growth and putative population of specific physiological types of microorganisms. The isolates obtained from the highest positive dilutions will be characterized since they are probably dominant in the habitat.

With regard to the in situ colonization experiments, potential substrates containing reduced iron (basalt, glassy basalt, pyrite, FeO) were loaded in colonization vessels. In our future work, we will examine the microbial community and chemical species of iron in the samples to reveal the microbial community supported by basaltic iron oxidation and the biogeochemical mechanisms. The microbial communities in samples will be analyzed by 16S rRNA-DNA technique focusing on archaea and bacteria domains. By comparing microbial communities in the substrates and seawater, we can identify potential microbial species as iron-oxidizer. The chemical species of iron (mainly oxidation states) would be determined by X-ray absorption fine structure (XAFS) with synchrotron based X-ray analysis. Particularly, the iron species on the target microbes are analyzed with nano-scale X-ray microscope, scanning transmission X-ray microscopy (STXM) technique. Combining results of microbial community and direct chemical speciation, the microbial community supported by basaltic iron oxidation and its biogeochemical mechanism would be investigated, cultivation-independently, which leads to understanding contribution of biological process in the basalt alteration in the ocean.

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