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NT07-13 Cruise Report

July 3 (Naha) – July 7 (Naze), 2007
R/V Natsushima and *ROV Hyper-Dolphin*

Geomicrobiology of the Iheya North hydrothermal field

Iheya North: Dives #710-713

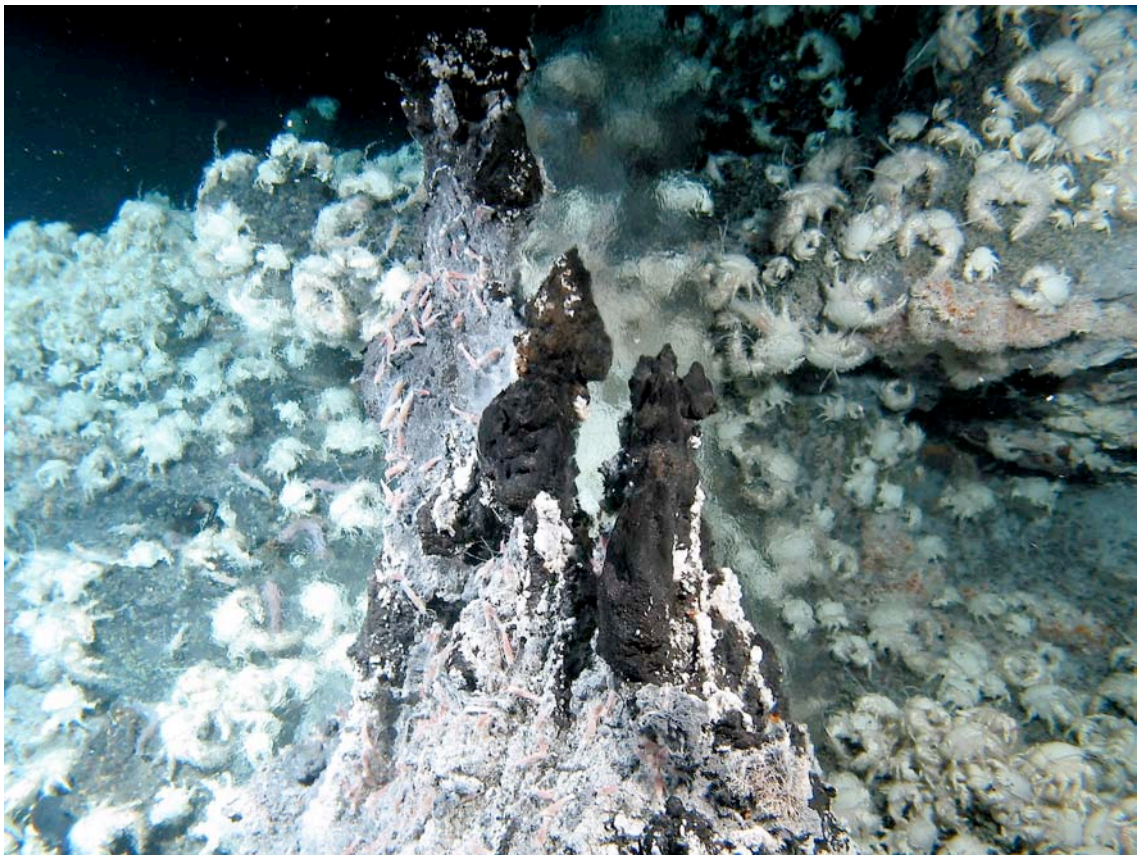

Photo: Chimney structure on Pseudo-SBC

**Japan Agency for Marine-Earth Science & Technology /
University of Tokyo / Tokyo Institute of Technology /
Kochi University / Kyoto University / University of Colorado**

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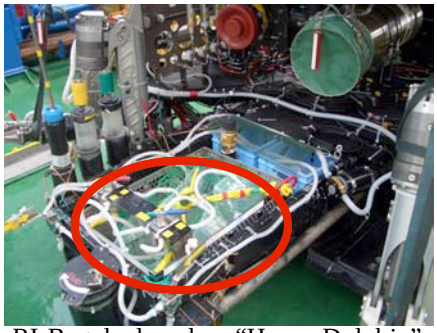
1.1 Expedition Overview

Satoshi Nakagawa

We performed NT07-13 cruise at the Iheya North hydrothermal field, from 3rd to 7th of July, 2007. In a continuing series of NT07-11, the cruise was done for geomicrobiological and biogeochemical studies in hydrothermal fields. The survey was conducted by means of ROV *Hyper Dolphin* and its mother vessel R/V *Natsushima*.

First, sampling and onboard analyses of various hydrothermal habitats, including vent fluids, fluids surrounding animal colonies, sediments, animals, and chimney structures, were successfully performed. One of our major foci was “mixing zones”, where discharged hydrothermal fluids and seawater mix. These area are quite important habitats for both hydrothermal macrofauna and microorganisms. Interestingly, in different mixing zones at hydrothermal fields in Mid-Okinawa Trough, different macrofauna, i.e. polychaete, galetheid, and mussels, are flourishing. Our previous studies indicated that different mixing zones support different active microbial communities. However, quite little is known about microbial activity in each of the mixing zone. In addition, its impacts on geochemical carbon/nitrogen/sulfur flow have never been estimated. What controls the zonation of hydrothermal vent macrofauna and microbes? Together with further geochemical analyses, we will perform wide arrays of microbial analyses including cultivation-, enzymatic-, DNA-, and RNA-approaches. The multidimensional comparisons of various mixing zones should allow us to link the zonation of macrofauna to the zonation of major chemolithoautotrophic microbial activity.

Second, we successfully performed in-situ tracer experiments by using the newly developed sampling system, called “RI-Bag”. The system is simple and inexpensive, which consists of pump, tubes, check valves, multidirectional valves, 6L vinyl bags, and ROV homer. During this cruise, we used only stable isotope (^{15}N). After the fluid-sampling, the RI-Bag was deployed and incubated on seafloor. After the incubation, the sampler was recovered, and fluids were prepared for shore-based studies such as scintillation, MAR-FISH, and geochemical analysis. This



RI-Bag deployed on “Hyper-Dolphin”.

approach should allow us to quantify the in-situ microbial activity and concomitant carbon/nitrogen flow.

1.2 ACKNOWLEDGEMENTS

We are grateful to all crew and captain Ishiwata of "*R/V Natsushima*" for their safe navigation and their skillful handling of the vessel. Great thanks are due to the commander Mr. Mitsufuji and "*ROV Hyper-Dolphin*" operation team for the sampling and observation of deep-sea hydrothermal fields in Mid-Okinawa Trough with safe and accurate operations. We also thank Mr. Okada (Nippon Marine Enterprise, Ltd) and Mr. Yoshida (JAMSTEC) for their heartfelt supports to our works. We thank all the JAMSTEC personnel who have strongly supported this cruise. Finally, to others who were directly or indirectly involved in helping make this cruise so successful, we extend our wholehearted thanks with all the best regards and wishes.

2.1 NT07-13 Participants

2.1.1 Shipboard Scientists

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Research scientist

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Extremobiosphere Research Center

Japan Agency for Marine-Earth Science and Technology (JAMSTEC)

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Marine Technician
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Mr. Katsunori Yoshida
Staff
Safety and Environment Management Office
Japan Agency for Marine-Earth Science and Technology (JAMSTEC)

2.1.2 ROV Hyper-Dolphin Operation Team

Operation Manager	Kazuya MITSUFUJI
1st Submersible Staff	Mitsuhiro UEKI
2nd Submersible Staff	Atsumori MIURA
2nd Submersible Staff	Hideki SEZOKO
2ndSubmersible Staff	Keigo SUZUKI
2nd Submersible Staff	Tetsuya ISHIZUKA
3rd Submersible Staff	Katsushi CHIBA
3rd Submersible Staff	Atsushi TAKENOUCH

2.1.3 R/V NATSUSHIMA Crews

Captain	Masayoshi ISHIWATA
Chief Officer	Koji SAMESHIMA
2nd Officer	Tokuro KOBAYASHI
3rd Officer	Yuki FURUKAWA
Chief Engineer	Minoru TSUKADA
1st Engineer	Kouji FUNAE
2nd Engineer	Yoshinobu HIRATSUKA
3rd Engineer	Humihiko NATSUI
Chief Radio Operator	Fukuo SUDA
2nd Radio Operator	Yohei YAMAMOTO
Boat Swain	Mikio ISHIMORI
Able Seamen	Kozo YATOGO
Able Seamen	Kuniharu KADOGUCHI
Able Seamen	Kazushiro OHSAKO
Sailer	Naoki IWASAKI
Sailer	Yoshiaki MATSUO
Sailer	Tomohiro KIMURA
No.1 Oiler	Kiyoshi YAHATA
Oiler	Takeshi FUKUBARA
Oiler	Kazuo ABE
Oiler	Masanori SATOH
Oiler	Keiya TANIGUCHI

Chief Steward	Takeshi MIYAUCHI
Steward	Toshiharu KISHITA
Steward	Shigeta ARIYAMA
Steward	Futoshi HATAKEYAMA
Steward	Shinobu OYU

2.2 *R/V Natsushima & ROV Hyper Dolphin*

Ocean research vessel *Natsushima* was built to support the manned submersible *SHINKAI 2000* in 1980s. *R/V Natsushima* was reconstructed as a support vessel of *ROV Hyper Dolphin*.

2.2.1 General information about *R/V Natsushima*

Length: 67.4m	Bow thruster: 1	Width: 13.0m	Maximum speed: 12kt
Depth: 6.3m	Duration: 8400 mile	Max capacity: 55 persons	
Gross Tonnage: 1553t	Main prop: 2 axis, CPP		

Research equipment

(1) PDR

This can record a water depth at right below and make contour map together with navigation data.

Max depth: more than 3000m　　Record Range: 200～800m (changeable)

Frequency: 12kHz +/-5%　　Output: more than110dB (0dB ubar at 1m)

Directivity: conical beam pattern

Beam width: 15deg. +/-5 deg. (-3dB)

Pulse width: 1, 3, 10, 30msec

(2) XBT equipment

XBT profile a vertical water temperature by free-fall probe.

Maximum measurable depth :1830m, Measure range :-2 deg. - +35 deg.

(3) Navigation equipment

Position of the ship is measured by DGPS within about 3m error. ROV and transponder are measured by acoustic positioning system.

(4) Laboratory

There are laboratories at the back part of second deck. Each room has AC100V power supply and LAN. The video of HPD diving and deck-camera video are distributed to the laboratories and every cabin.

- Second laboratory: There are two desktop PCs (windows and Mac), equipment for video editing, color copy with printer, meeting desk and white board. Hi-vision video of HPD is distributed to this laboratory. You can copy from a digital β cam and S-VHS to S-VHS/VHS, Hi8 and DV.
- Third laboratory: There are two sinks, refrigerator (-80deg. low temperature refrigerator, Incubator, domestic refrigerator, ice maker, ice crasher) and reagent water system (ORGANO, Mili-QSPTOC). And sea water for experiment is supply to the sink.
- Dry laboratory: There are a work desk and a shelf for baggage. This room has 4 beds to be used as a private one in case that there are many researchers.

At the work deck, there is a rock-cutter room

- Rock-cutter room: There are a rock cutter and two grinders. And exclusive video player is set to describe rocks with playing video of ROV diving.

2.2.2 General information about *ROV Hyper-Dolphin*

Hyper Dolphin is 3000m ROV which was built by SSI (Canada) in 2001. The vehicle has two manipulators, a Hi-definition super harp TV camera, and a color CCD TV camera. In addition, digital photo camera, black and white TV camera for back side monitoring, altitude sensor, depth sensor (with temperature sensor), sonar for obstacle avoidance sonar.

Principal specification

Length : about 3.0m	Depth capability : Maximum 3000m
Breadth : about 2.0m	Payload weight : -100kg (in the air)
Height : about 2.3m	Speed in the water : 0-3kt
Weight in the air : about 3800kg	Manipulators : 2 sets

(1) Manipulator capability

Pivot : 7 pivoted , Working load : in the water 68kg (max outreach), Length of arm : 1.53m

Grip strength : 450kg, Hoisting power : max 250kg (vertical)

Hand opening width : right 77mm, left 195mm

(2) TV camera

High-definition TV camera : 1, Color CCD TV camera : 1, Black-and-white TV camera : 1

(3) Digital photo camera

Type : Seamax DPC7000 (DSSI)

(4) Obstacle avoidance sonars

Type : SIMRAD MS1000, Range : 10, 20, 25, 50, 100, 200m change

Detective distance : max 200m, Transmission frequency : 330kHz±1kHz

(5) Altitude sonar

Type : SIMRAD MS1007, Frequency : 200kHz, Measure range : -200m, Accuracy : -2m

(6) Depth sensor (with temperature sensor)

Type : made by Paroscientific, Inc, Range of measuring depth : -4000m

Range of measuring temperature : -2-40deg.

2.3 Ship Operation Log

Satoshi Okada

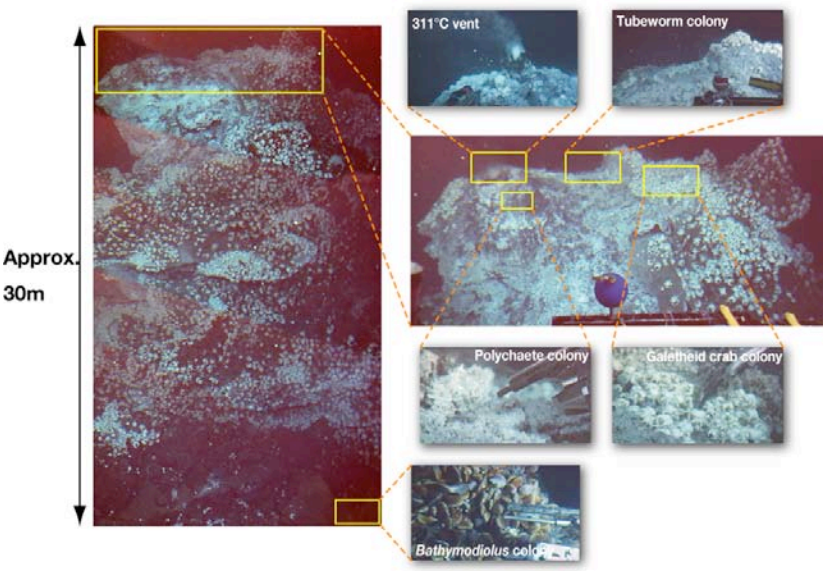
Shipboard Log & Ship Track(NT07-13 07/07/03 - 07/07/07)				Position/Weather/Wind/Sea condition (Noon)
Date	Time	Description	Remark	
03,Jul,07	9:00	embarkation science group		7/3 12:00
	10:00	departure from NAHA SHINKO		26-28.7N, 127-31.7E
	10:45～11:30	on board seminar	for safety NATSUSHIMA life	fine
				SSW-4(Moderate breeze)
				Sea smooth
04,Jul,07	3:00	ariived at research area		7/4 12:00
	6:28	released XBT		27-47.5N, 126-53.8E
	8:10	launched HPD		fine
	8:25	started HPD#710 dive		SW-6(Strong breeze)
	9:25	arrived at bottom	D=997m	Sea moderate
	9:32	leave the bottom	D=996m	
	10:02	surfaced HPD		
	10:14	recovered HPD		
	11:35	launched HPD		
	11:51	started HPD#711 dive		
	13:03	arrived at bottom	D=1054m	
	14:32	leave the bottom	D=998m	
	15:02	surfaced HPD		
	15:22	recovered HPD		
05,Jul,07	8:14	launched HPD		7/5 12:00
	8:29	started HPD#712 dive		27-47.4N, 126-54.0E
	9:22	arrived at bottom	D=1054m	fine
	15:46	leave the bottom	D=1043m	SW-5(Fresh breeze)
	16:15	surfaced HPD		Sea moderate
	16:29	recovered HPD		
06,Jul,07	8:09	launched HPD		7/6 12:00
	8:24	started HPD#712 dive		27-47.4N, 126-54.0E
	9:18	arrived at bottom	D=986m	cloudy
	14:41	leave the bottom	D=1113m	SW-6(Strong breeze)
	15:14	surfaced HPD		Sea moderate
	15:27	recovered HPD		
	15:30	left the research area for NAZE		
07,Jul,07	8:00	arrived at NAZE		
	9:00	left the ship and concluded NT07-13	NT07-13 scientists	

3. Introduction of Iheya North hydrothermal field

Satoshi Nakagawa

The Iheya North hydrothermal field is one of the most extensively studied hydrothermal fields around the world in aspects of microbiology and geochemistry. Its specific features include (1) extremely high concentrations of CO_2 and CH_4 in vent fluids, (2) phase-separation- and –segregation-controlled vent fluid chemistry, and (3) existence of active, potentially abundant subvent biosphere.

The geomicrobiological survey in the Iheya North hydrothermal field focuses on the “mixing zones”, where discharged hydrothermal fluids and seawater mix. The mixing zones are quite important habitats for both hydrothermal vent macrofauna and microorganisms. Interestingly, in different mixing zones, different kinds of macrofauna, i.e. polychaete, galetheid, and mussels, are colonizing. Although little is known about what the segregation means, it potentially reflects the physicochemical differences of mixing zones. Additionally, the segregation potentially reflects the differences of microbial community structure and/or microbial activity in each mixing zone, since the hydrothermal macrofauna strongly depend on the symbiotic and/or free-living microorganisms for their energy and carbon sources. It has been generally regarded that primary microbial energy-yielding reaction in mixing zones is the oxidation of reduced sulfur compounds provided from hydrothermal fluids. However, our preliminary studies demonstrated that microorganisms dominating mixing zones were capable of oxidizing not only sulfur-compounds but also molecular hydrogen. In addition, hydrothermal fluids contain high concentrations of methane and ammonium, which could also be energy sources for some



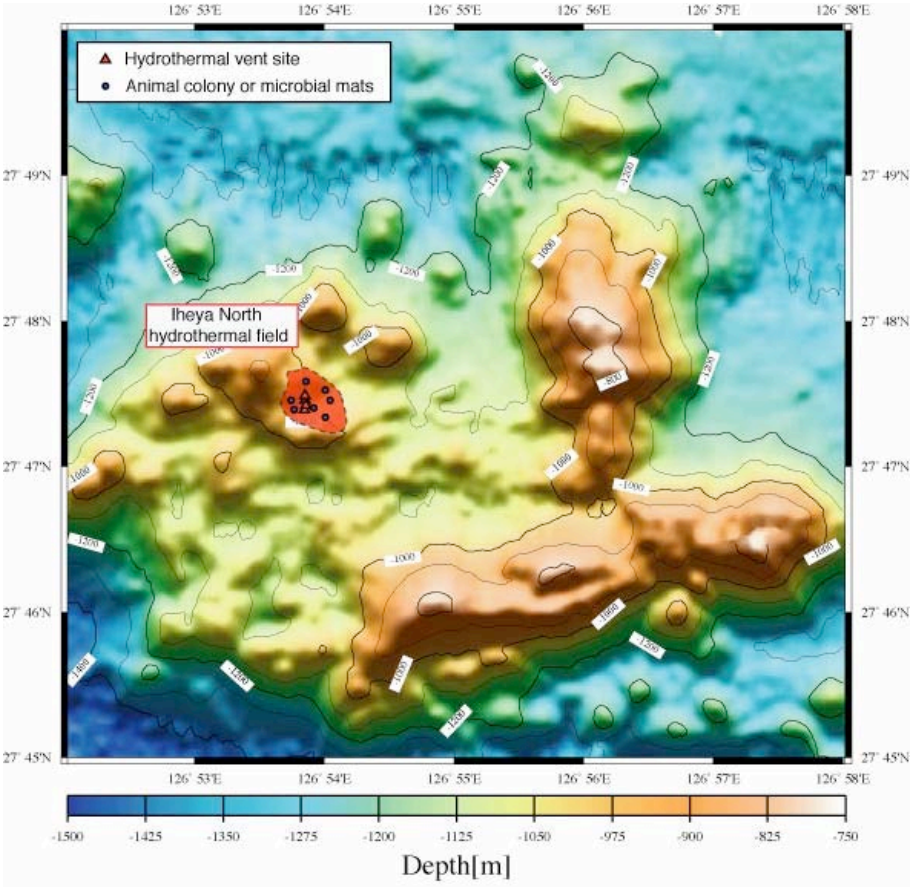
Mixing zones at the NBC in the Iheya North hydrothermal field. From Nakagawa et al. (2007).

microbes. Our primary objectives are i) to clarify the differences of microbial community structures and physicochemical parameters in each of the mixing zones, ii) to evaluate the primary microbial energy metabolism, especially energy-yielding reaction, in each of the mixing zones, and iii) to assess the effects of the microbial activity on geochemical carbon-, sulfur-, nitrogen-, and hydrogen-flows.

On the basis of our previous microbiological studies using samples obtained during NT02-06, members of the *Proteobacteria*, especially epsilon- and gamma-*Proteobacteria*, commonly represent the numerously most abundant microbial populations in a variety of mixing zones. The ratio of the free-living epsilon-*Proteobacteria* to total cell numbers was found to decrease with increasing distance between vent emission and habitats studied. Although members of the epsilon-*Proteobacteria* had no cultured representatives until recently, our cultivation-dependent studies on the epsilon-*Proteobacteria* members for the first time revealed that this group of bacteria had an extensive metabolic repertoire, including hydrogen- and sulfur-compounds-oxidation, coupled with the reduction of oxygen, nitrate (denitrification and ammonification), and sulfur compounds. Our subsequent genetic and enzymatic characterizations of epsilon-Proteobacteria partially revealed their energy and carbon metabolic pathways. In addition, we recently published genome sequences of two epsilon-*Proteobacteria* strains isolated from the Iheya North field. Genome sequences and comparative genomic analyses revealed that the complete gene structures that were responsible for the various energy metabolisms. However, little is still determined about what energy-yielding pathway exactly do they utilize in their habitats, and how do they interact with other micro- and macro-organisms.

During NT05-03 and YK06-09 cruises performed in 2005 and 2006, we focused on the several different macrofauna colonies. We investigated microbial community structure by combined use of culture-dependent and –independent microbiological methods. Together with the microbiological surveys, biogeochemical characterizations were performed. Cultivation-dependent analyses revealed that numerously abundant culturable populations drastically varied in different mixing zones. However, cultivation-independent analyses did not support the microbial segregation. Since we could not collect enough numbers of samples, we could not clarify the difference in the microbial activity and the physicochemical parameters in each of the macrofaunal habitats.

During this cruise, we collected additional samples from each of the mixing zones. Together with the geochemical analysis, we will perform the measurement of microbial activity by the combined use of cultivation-, enzymatic-, DNA-, and RNA-approaches. In addition, in-situ tracer experiments and in-situ filtration represent our new approaches. The multidimensional comparisons of mixing zones will provide new insights into survival strategies of microorganisms, interactions between microorganisms and macrofauna, and effects of microbial activities on the geochemical energy flux.



Map 1. Iheya North hydrothermal field

4. Preliminary Results

4.1 Microbiology

S. Nakagawa

During the NT07-13, we collected various hydrothermal samples including hydrothermal plumes, vent fluids, chimney structures, and vent animals from Iheya North hydrothermal field. Samples were characterized by geochemical analysis (described below) and using multiple sensors. Immediately after recovery, all the samples were prepared for multidisciplinary shore-based study (described below).

For molecular analyses, which target microbial DNA, RNA, enzymes, and etc, cells in fluid samples were harvested on 0.2 μm pore size filters, and then immediately stored at –80ºC. For the (MAR-)FISH analysis, cells were fixed, gathered, and frozen.

The chimney samples were basically subsampled into two parts, i.e. exterior surface and inside structure, and then anaerobically slurried for cultivation or stored at -80 ˚C.

Overall, all samplings for microbiology have been successfully performed onboard during this cruise.

4.2 Biogeochemistry

S. Kawagucci, A. Makabe, T. Narita

During the NT07-13, we collected various hydrothermal samples from Iheya North hydrothermal field using WHATS, Bag, and Niskin samplers. Following geochemical parameters have been determined onboard ship to avoid chemical alterations during sample storage.

4-2-1. pH and alkalinity

The pH and Alkalinity were determined for unfiltered water samples. A pH meter with a combined glass electrode (Radiometer, PHC2401-8) was used. Measurements were done within an hour after sample recovery from the WHATS bottle. Calibration was conducted daily using JSCS buffer solutions (pH=6.865 and 4.010).

Alkalinity was determined by titration with hydrochloric acid. For calculation of the endpoint, Gran plot is employed using the pH/ion meter(PHM240). Calibration factor was checked by analysis of IAPSO standard seawater (which alkalinity must be 2.325mM). Analytical precision is estimated as within 3%.

4-2-2. Colorimetric method

For determination of SiO_2 and NH_4, water samples filtered by 0.2μm were used. Using a colorimeter (Hach, DR2010), concentrations of dissolved silica (SiO_2) and ammonium ion (NH_4) were analyzed following classical methods; molybdenum blue method (λ=812nm) for SiO_2 and indo-phenol method (λ=640nm) for NH_4. Analytical precision is usually estimated as within 3% for seawater analysis. However, sometimes the precision is somewhat worse for the case of hydrothermal fluids, because of wide range of concentrations (SiO_2) and of interference by specific species (NH_4).

4-2-3. Result of onboard analysis

Dive No.	Sampler		Depth m	pH	Alk.	SiO2 ug/L	NH4 uM
#711	B1	control	1054	7.24	2.58	121	<1
(7/4/2007)	B2	Galetheid colony	979	6.56	2.58	133	<1
	N1	plume?	1030	7.28	2.69	112	<1
#712	B1	control	1054	6.48	2.50	112	<1
(7/5/2007)	B2	Bathymodiolus	1000	6.40	2.42	118	<1
	B3	1m above NBC vent	979	5.14	2.32	1213	14
	B4	just above polychaetes	980	5.14	2.55	163	3
	N1	Alt=10m	1045	7.32	2.37	107	3
	W1	control	1054	7.31	2.46	110	<1
#713	W1	NBC top vent	980	4.98	2.57	12557	175
(7/6/2007)	B1	polychaetes	980	6.21	2.32	157	1
	B2	Galetheid colony	980	6.56	2.38	170	<1
	W3	Pseudo SBC	1006	4.99	2.18	13073	161
	M直上		1079				<1
	MU		1079				3
	MM		1079				4
	ML		1079				4

4.3 Geophysics

A. Masaki

1. Introduction

During the NT07-11 and NT07-13 cruise, intense heat flow measurements were made in the Iheya North and Minami-Ensei sites in the middle Okinawa Trough. The objectives in each field are described in the following:

*Hydrothermal regime in the Iheya North Hydrothermal field

A complex of big active chimneys was located in the Iheya North Hydrothermal Field in the middle Okinawa Trough. To infer the hydrothermal regime in this field a detailed heat flow measurements has been under way. First measurement was made during the NT02-06 cruise in 2002, to take an East-West transect across the hydrothermal area. It showed a very high heat flow (>10 W/m2) within the active area, and generally uniform heat flow (1-4 W/m2) in the surrounding area. During this cruise further investigation, including the area we have never observed before.

*Hydrothermal regime in the Minami-Ensei field

There are too many isolated chimneys located in the Minami-Ensei. And this is the first time to try to measure heat flow value around there.

2. Instrument

We prepared four heat flow probes used by Hyper Dolphin.

Stand-Alone Heat Flow meter (SAHF) is designed to measure heat flow by manned submersibles or ROVs. Five thermistors situated within the probe at 11 cm intervals. Since SAHF takes measurements as “OFF LINE” system, heat flow can be measured while observer is conducting something else at that position or else at that position or elsewhere. We prepared four SAHFs, designated as SAHF#6, SAHF#7, SAHF#8, and SAHF#9 are equipped with LED, which flashes during operation.

While Hyper-Dolphin(HD) is descending or ascending, SAHF is set in a case beside a sample basket prepared by HD operational team. After HD lands on the seafloor, SAHF is grabbed by HD’s left manipulator and takes the reference temperature for 5 minutes. SAHF is then put vertically into sediment and measure temperature gradient for at 15

minutes. Thermal conductivity is necessary to obtain a heat flow value, which is not available on current SAHF. We simply assumed a constant value of w W/m/K for all SAHF data.

Fig1. shows the photograph and graphical description of SAHF. The following is description of SAHF.

Description:

Material	Alloy of titanium
Weight	4.0 kg in air, 2.6 in seawater
Length of pressure case	294 mm
Diameter of pressure case	85 mm
Length of probe	600 mm
Diameter of probe	13.8 mm (filled by silicon oil inside)
Number of thermistors	5
Intervals of thermistors	110 mm
Accuracy	0.01 ℃
Resolution	0.001 ℃
External Interface	RS232C (9600bps, 8bit, Non-parity, 2 stop-bit)

3. Operation

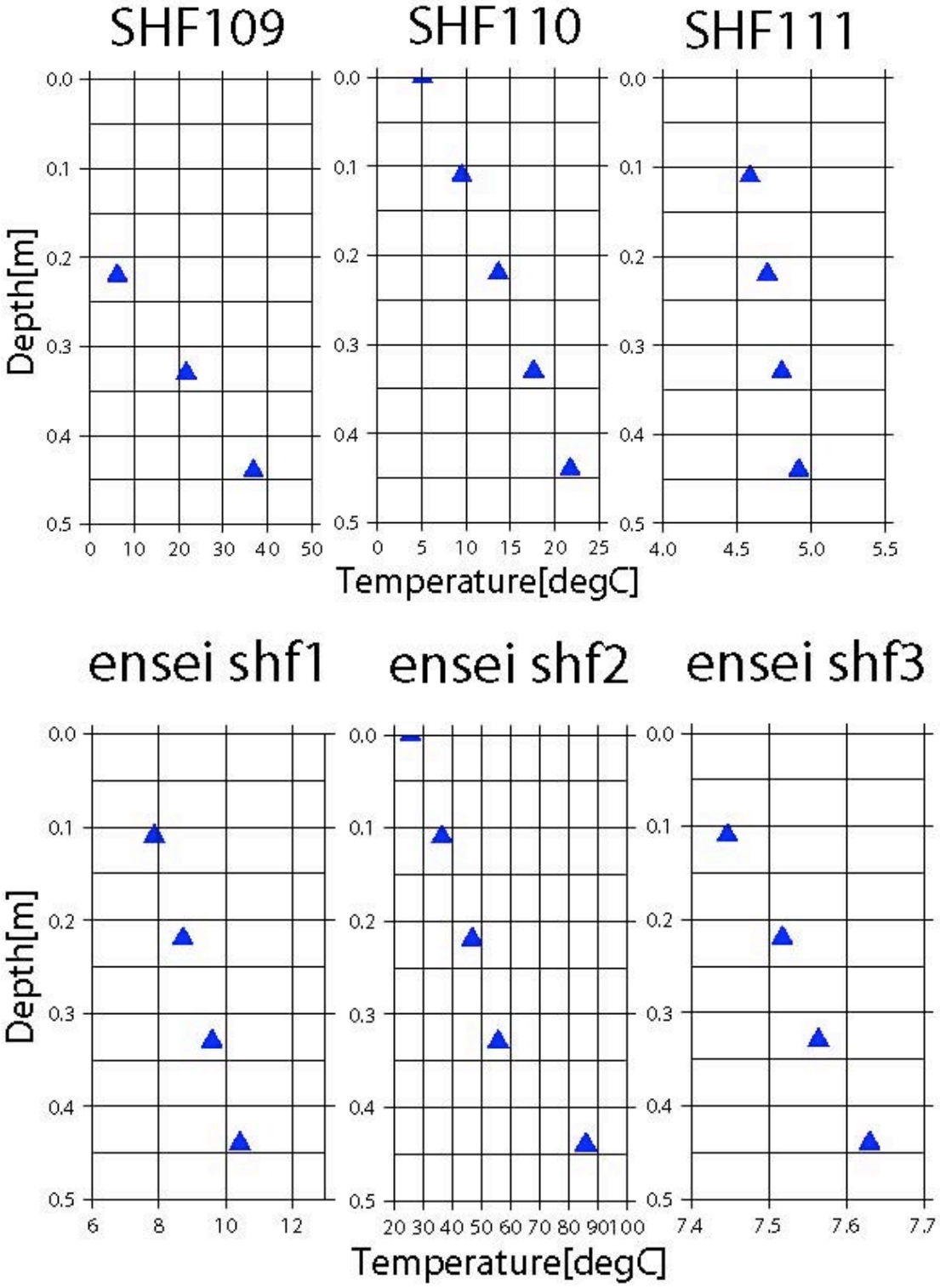
SAHF measurements were made during 4 dives (696, 699, 703, 704, 712, 713) in the Iheya North and Minami-Ensei site. Details of temperature data are shown in Fig2.

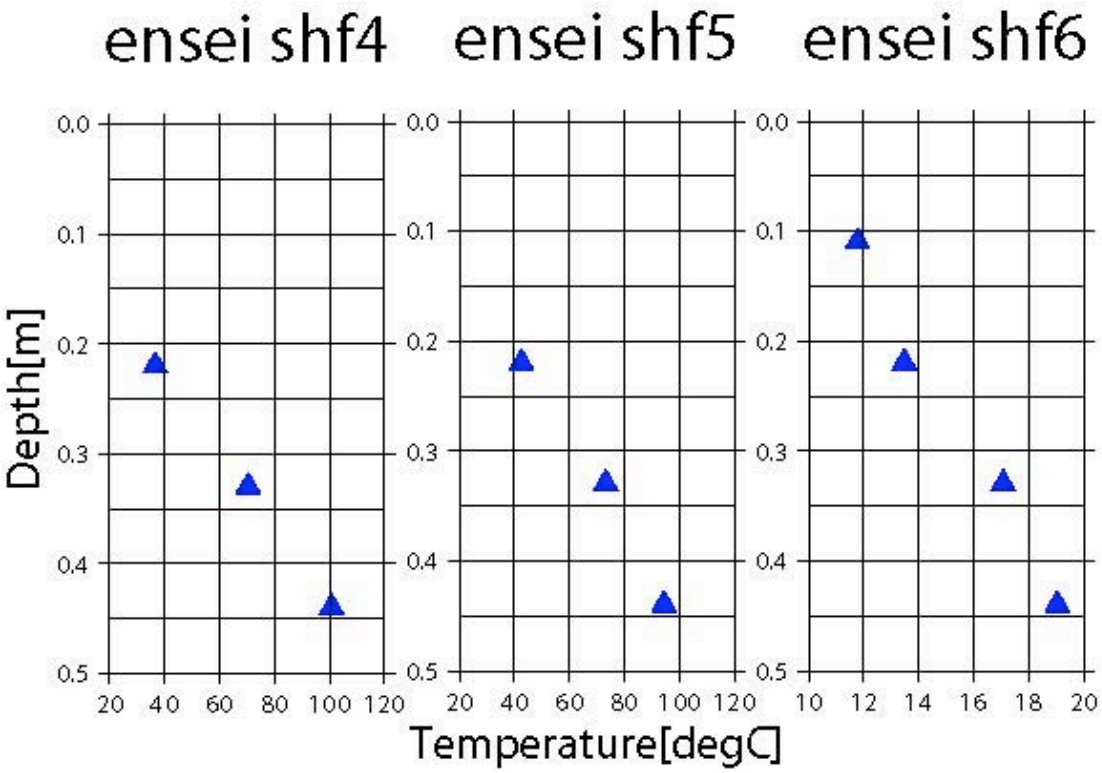
Fig2. Operation of SAHF

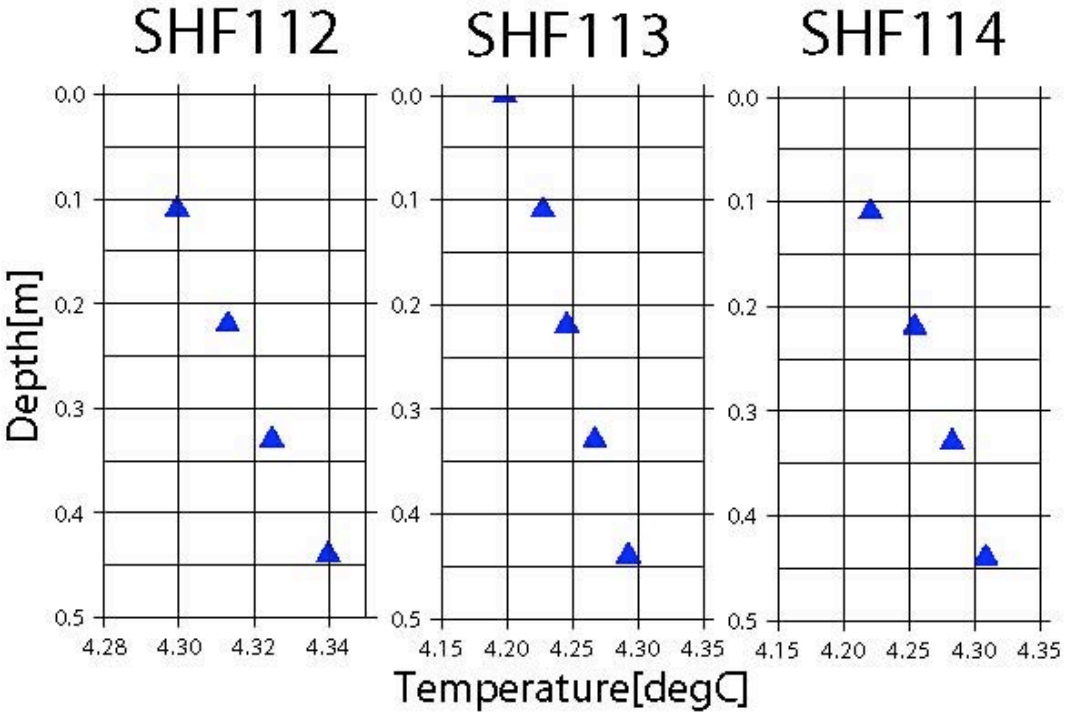
	dive	date	bottom temp	penetrate	pull	latitude	lontitude
shf109	696	2007.6.19	9:43:44	9:49:55	10:11:07	27-47.397	126-53.863
shf110			10:56:04	11:03:15	11:18:56	27-47.351	126-53.655
shf111			13:48:56	13:55:00	14:10:54	27-47.666	126-53.507
minamiensei							
ensei_shf1	699	2007.6.21	16:01:13	16:07:12	16:22:31	28-23.286	127-38.378
ensei_shf2	703	2007.6.24	12:50:45	12:57:42	13:12:47	28-23.446	127-38.392
ensei_shf3			15:12:03	15:18:49	15:34:37	28-23.551	127-38.492
ensei_shf4	704	2007.6.25	11:39:27	11:53:58	12:11:00	concentrated obserbation	nearby shf2
ensei_shf5				12:16:44	12:34:27	concentrated obserbation	nearby shf2
ensei_shf6				12:38:06	13:10:21	concentrated obserbation	nearby shf2
shf112	712	2007.7.5	14:56:15	15:04:04	15:19:33	27-47.683	126-53.895
shf113			15:24:24	15:28:38	15:44:23	27-47.677	126-53.972
shf114	713	2007.7.6	13:03:44	13:09:46	13:25:53	27-47.392	126-54.546
shf115			13:37:06	13:44:15	14:00:35		
shf116			14:14:09	14:20:10	14:36:00	27-47.394	126-54.750

4. Results

Fig3. All data




ensei shf4
ensei shf5
ensei shf6
Depth[m]
0.0
0.1
0.2
0.3
0.4
0.5
20 40 60 80 100 120
20 40 60 80 100 120
10 12 14 16 18 20
Temperature[degC]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SHF113
SHF114
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0.3
0.4
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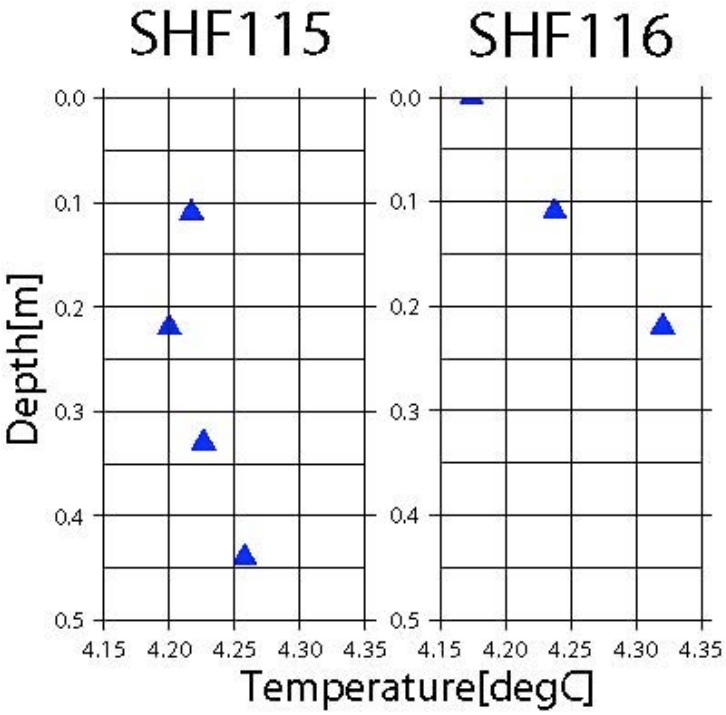
Temperature[degC]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SHF116
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0.5
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4.20
4.25
4.30
4.35
Temperature[degC]

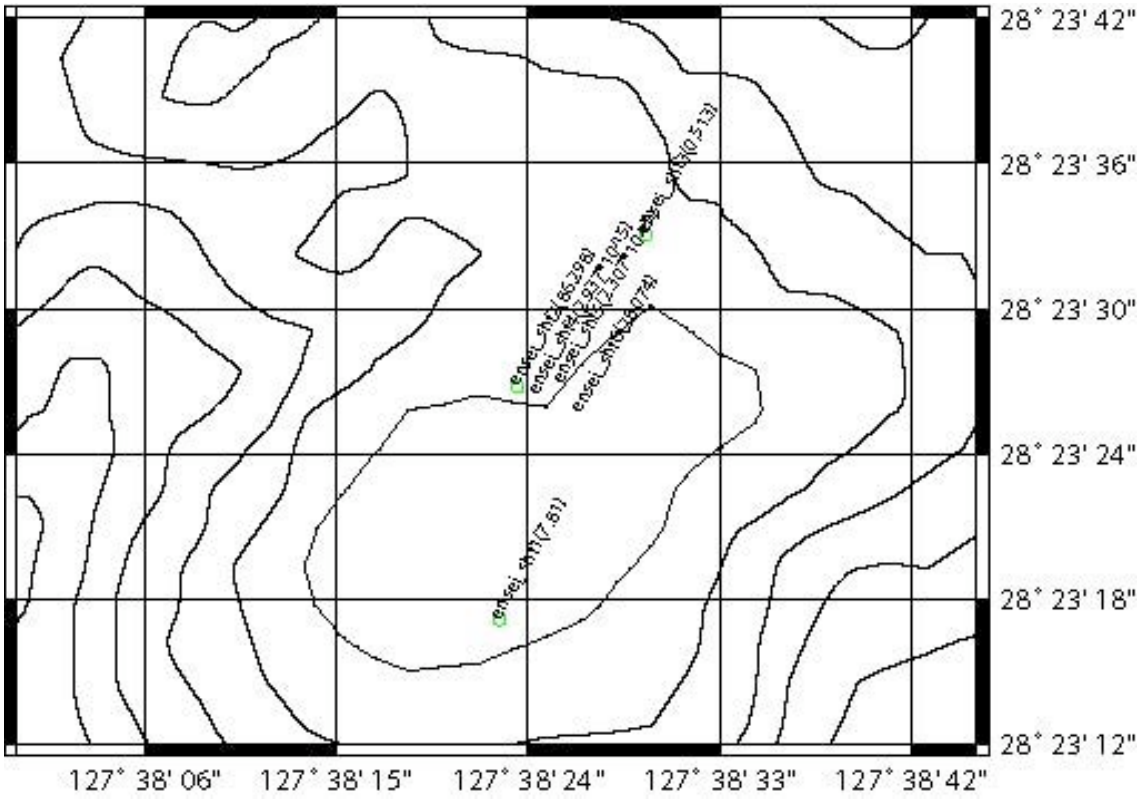


Fig5. Mapping the observation sites in Minami-Ensei (Thermal Gradient K/m)

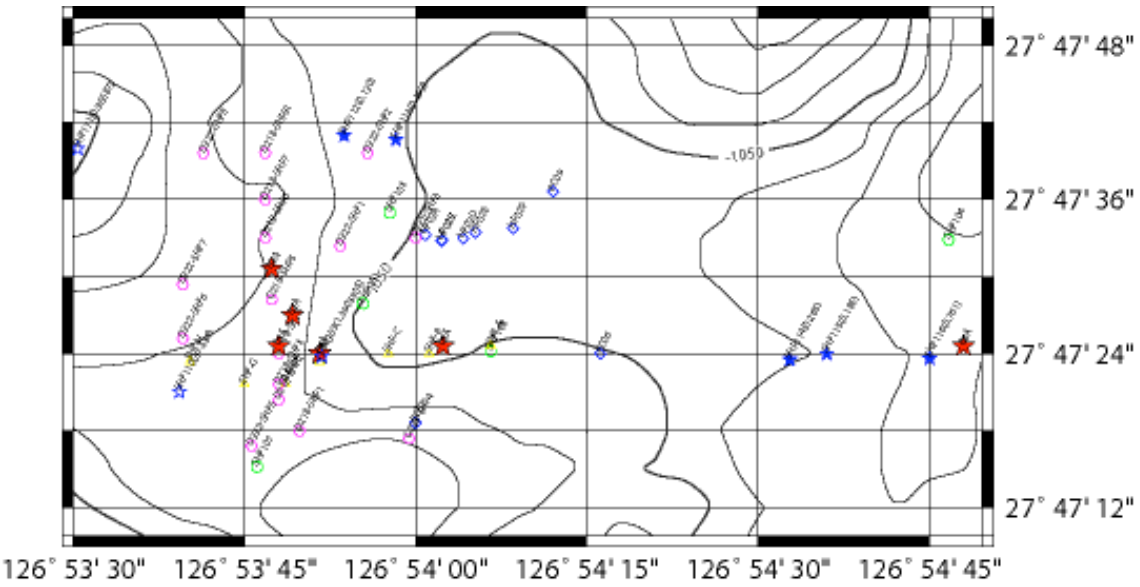


Fig6. The mapping of observation sites in Iheya North

5. Shore-based study
5.1 Microbiology

S. Nakagawa, T. Nunoura, K. Takai

Microbial ecology in deep-sea hydrothermal fields

We intend to investigate the microbial communities by the combined use of culture-dependent and culture-independent molecular ecological methods. The microbiological data will be coupled to geochemical and geophysical data.

• Culture-dependent ecological surveys

It is often noted that culturable microbes represent only 0.1-1% of total microbes in the environments, and thus culture–independent molecular ecological methods have become popular and indispensable in microbial ecology. However, it is nearly impossible to get direct into physiology and activities of microorganisms detected. Thus, cultivation is still an important and effective strategy in microbial ecology. Data from culture-independent molecular microbiological, geochemical and geophysical analyses provides the logical scheme to culture previously uncultured organisms. In fact, our group has been tried to cultivate previously uncultured organisms on the bases of data from culture-independent analyses from hydrothermal vents in Iheya North, Yonaguni Knoll IV, TOTO caldera, Lau Basin, Kermadec Arc, Suiyo Seamount, MAR (Lucky Strike, Rainbow, TAG, and Lost City) and CIR (Kairei and Edmond) hydrothermal systems, and has succeeded in cultivation of more than 10% of the members that were detected in culture-independent analyses in each habitat.

Using hydrothermal samples obtained through this cruise, we will try to culture previously abundantly detected Archaea and Bacteria; Methanogens, autotrophic sulfur reducers such as *Desulfurococcales*, *Aquificales, Deferribacterales* and *Epsilonproteobacteria*, autotrophic sulfur oxidizers such as *Aquificales, Alphaproteobacteria, Gammaproteobacteria* and *Epsilonproteobacteria*, nitrate or nitrite reducers such as *Aquificales, Deferribacterales,* and *Epsilonproteobacteria*, sulfate reducers such as *Archaeoglobales* and *Thermodesulfobacteriales* and *Deltaproteobacteria*, iron oxidizers and fermenters such as *Thermococcales* and *Thermotogales*. Culturable populations of these microbes will be evaluated by most probably number (MPN) method.

MPN analysis: This 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.

• Culture –independent molecular ecological surveys

Culture–independent molecular ecological methods allow us to catalogue microbial diversity and distribution. We will analyze the microbial diversity in hydrothermal samples by biomass evaluation, 16S rRNA gene clone analysis and quantitative PCR.

Evaluation of biomass: In order to evaluate the population and distribution of microbes, we will evaluate total microbial density by direct counting of DAPI or AO stained cells.

Quantitative PCR, a modification of two-step PCR, is a fluorescence assay used to quantify the target genes in samples. When used for 16S rDNA, we will study the population ratio between the domain Bacteria and Archaea using the specific probe for each domain. In addition, we also quantify the amount of functional genes by using this technique.

Gene sequencing is essential for all phylogenetic analysis and identification of microorganisms. We will construct clone libraries for target genes (e.g. 16S rDNA, Methyl CoM reductase, dissimilatory sulfite reductase etc.) from each sample and compare them.

Stable-Isotope-Probing (SIP) is a technique to detect microbes that are capable of utilizing specific substrates. For example, hydrogen oxidizing chemolithoautotrophs uptake CO_2 by hydrogen-oxidation if hydrogen is provided. The ^{13}C-labelled CO_2 will be incorporated into cellular molecules including DNA. The ^{13}C-incorporated DNA becomes heavier than pristine environmental DNA. These can be separated by density-gradient ultra-centrifugation, and then characterized by molecular analyses described above.

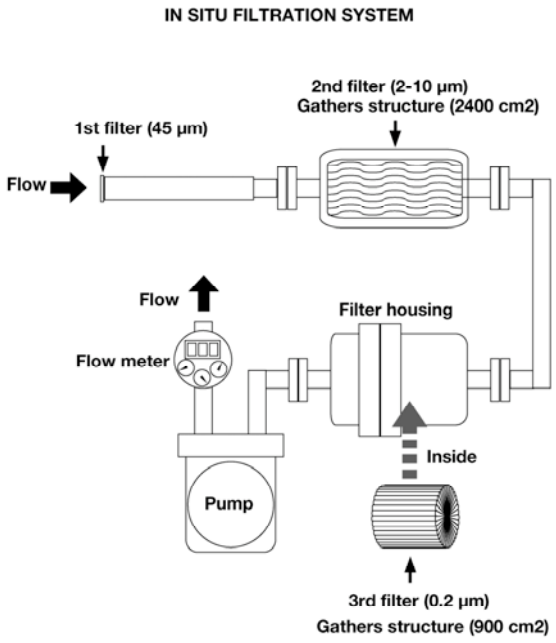
Environmental enzymology

M. Yamamoto

Environmental enzymology is a very brand-new technique to evaluate the activity of microbial community. The microbial community has a variety of metabolic potentials

such as hydrogen-oxidation, sulfur-oxidation, methane-oxidation, ammonia-oxidation, sulfur-reduction, sulfate-reduction, and nitrate-reduction. Environmental enzymology is a technique of measurement of key enzyme activity in the community under in situ conditions.

There are many and various animals, such as polychaete, galetheid crab, tubeworm, and vent mussel, around deep-sea hydrothermal vents. Chemolithoautotroph is recognized to be ecologically significant as a primary producer in the environment. It is supposed that sulfur-compounds oxidation is the most dominant pathway for energy production in the environment. Whereas, it is thought that hydrogen, methane, ammonia and so on are also important energy sources. These presumptions are based on the experimental results of PCR analysis of 16S-rDNA and functional genes. To discuss the environmental energy flux, directly measurements of enzyme activity of the environmental samples, such as seawater, sediments, and animals, are very effective. In this cruse, we hope to collect environmental samples, which have high enzymatic activity. Especially, we try to collect concentrated bacterial cells from a large quantity of seawater. We are constructing “in situ filtration system”, which consists of an oil pressure pump, tandem connected filter membranes, and flow meter.



Ecological research for bacteriophages at deep-sea hydrothermal fields –a opening study for investigation of genetic elements in subsurface environments-

S. Ohno and H. Imachi

Our group has been studying hydrothermal field ecosystems. The most of our previous studies focused on prokaryotes ecology in the environments, but we have not paid attention to bacteriophages (phages) that is considered to be strongly affect to prokaryote communities. Phages are viruses infected to bacteria, which inject own

DNA and grow using host bacterial enzyme, finally lyse host bacteria and released themselves. In addition, it is well known that population of phages is 10 to 100-hold higher that that of prokaryotes in natural environments. Moreover, it is also known that phages are involved in aquatic food web for flux of dissolved organic matter was changed with or without phages. These previous findings indicate that phages are very important factor for ecosystems, even in the subsurface environments including deep-sea hydrothermal fields. However, despite of their importance, deep-sea phage ecology is little explored, and there are only two reports about phages in deep-sea hydrothermal filed, that only showed the abundance of virus-like particles. The objective of the cruise is to collect deep-sea water nearby hydrothermal vent for garnering basic know-how on phages thriving in the deep-sea hydrothermal fields. After getting back from the ship to laboratory, we will do the following experiments: (i) virus like particle abundance by direct counting using some nucleic staining reagents, (ii) taxonomical classification based on morphology by using TEM observation and (iii) isolation of phages using some representative prokaryotes in the hydrothermal filed as host.

Quantification of microbes by (MAR-)FISH

K. Yanagawa, and M. Sunamura

Fluorescence in situ hybridization (FISH) method enables to detect specific microbes at a single cell level under a fluorescent microscope. Using a specific oligonucleotide DNA probe libeled with fluorochrome, target microbial cells, which hybridized with the DNA probe, could be detected by the fluorescent signal of the labeled fluorochrome. A specific probe is designed based on 16S rRNA gene sequence of not only cultivable microbes but also uncultivated ones. Using this technique, microbial communities in natural environment have been elucidated and quantified, e.g. freshwater, activated sludge, arctic sediment, seawater, and hydrothermal plume.

Mixing zone between the discharged hydrothermal fluid and ambient seawater is suggested to be an important habitat for deep-sea vent microbes due to microbial physiological and ecological characters. Moreover, microbial community structures varied with the distance from the vent, indicating that the microbial community responded to a hydrothermal redox gradient. In this study, we will investigate chemolithoautotrophic microbes in the mixing zone at a single cell level, and search

for environmental triggers which affected microbial populations.

Most of microbes in hydrothermal field have been regarded as chemolithoautotrophic organisms that assimilate carbon dioxide as a sole carbon source, based on the physiological characters of cultured microbes isolated from the deep-sea hydrothermal environment. For uncultured phylogenetic groups, their autotrophy were presumed by the detection of carbon assimilation genes, e.g. RuBisCO, and by comparison with their closest neighbors from their phylogenetic position.

Microautoradiography (MAR) is an important tool for microbial ecology to detect utilization and uptake of chemicals at a single cell level. By combination of MAR and FISH, we can determine the trophic character of each phylogenetic group. In this study, we try to detect autotrophic microbial cells by microautoradiography(MAR) through in situ incubation with $^{14}CO_2$ and clarify the phylogenetic affiliation of the autotrophic microbial cells in the hydrothermal environment at a single cell level.

Symbiosis between microbes and deep-sea hydrothermal vent animals

T. Watsuji

Shinkaia crosnieri, family Galatheidae, inhabits hydrothermal vents and cold seeps on Okinawa Trough, which forms an abundant deep-sea life community including *Bathymodiolus* and *Alvinocaris*. In the total absence of photosynthesis, the food chain relies entirely on some bacteria extracting energy from the oxidation of reduced compounds present in these environments. Many species of deep-sea invertebrate are associated with such chemoautotrophic bacteria in a specialized tissue. *S. crosnieri* also has a unique structural feature of ventral vibrissae harboring epibiotic microflora. It is assumed that the association between *S. crosnieri* and this bacterial community is certainly symbiotic because the epibionts of ventral vibrissae couldn’t grow well in dead *S. crosnieri*. However, almost nothing is known about the microflora structure of the ventral vibrissae and interaction between *S. crosnieri* and these symbionts. Therefore, we planned to determine phylogenetic relationships of the epibiotic bacteria and the distribution of the bacteria by FISH, and to exam interaction between *S. crosnieri* and these epibionts.

In this cruise, individuals of *S. crosnieri* were collected from a North Big Chimney (NBC) of Iheya North in Okinawa Trough during the 711th dive by the ROV.

Immediately after retrieval, fresh ventral vibrissae were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS, pH 7.4), in preparation for *in situ* hybridization. After 12 hours, fixed ventral vibrissae were dehydrated in 50% ethanol and were stored at -20°C. Immediately after *S. crosnieri* collection, part of the specimens were frozen and stored at -20°C until DNA needed to investigate phylogenetic relationship is extracted. Moreover, part of living *S. crosnieri* was used for examining assimilation of inorganic stable carbon isotope through their epibionts. After culture in medium including ^{13}C-CH_4 or ^{13}C-$NaHCO_3$ for two days, individuals of living *S. crosnieri* were frozen and stored at -20°C. By analyzing these specimens, we will be able to determine whether the epibionts provide nutrient for *S. crosnieri*.

Iron-reducing microorganisms in deep-sea hydrothermal fields.

S. Kawaichi

Microbial Fe (III) reduction has an important role in the iron cycle of diverse environment. Previous studies have suggested that a diversity of hyperthermophilic microorganisms have the ability to reduce Fe (III). The fact that the ability of Fe (III) reduction is widespread among Bacteria and Archaea and is found in deeply branching thermophiles suggests that Fe (III) reduction could be an early form of microbial respiration and it have been conserved throughout microbial evolution. Although deep-sea hydrothermal environments are said to be iron-rich (e.g. chimney structures frequently covered with deposit of iron oxides), there were few reports on microbial Fe (III) reduction in deep-sea hydrothermal vents. One of the purposes of this cruise is to determine the quantity of Fe (III)-reducing microorganisms in deep-sea hydrothermal environment.

- **Microbial community in the chimney structure**

Culturable microorganisms are often estimated to represent less than 1% of total microorganisms in the environment. Thus culture-independent molecular biological methods have become very popular in microbial ecological study. Culture-independent methods provide important information on uncultured microbes. However, culture-independent methods hardly reveal the actual physiology and activities of microorganisms. Therefore, culture-dependent analyses are still important and effective techniques in microbial ecology.

Using the samples obtained in this cruise, we will try to culture and quantify the microorganisms inhabit the chimney structure. Culturable population of these microbes will be evaluated by most probably number (MPN) method.

MPN method: This is a cultivation method to enumerate populations of microorganisms. Slurry of chimney 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 microorganisms in the highest dilutions with positive growth are possibly dominant species in the cultured condition.

Evaluation of biomass: In order to evaluate the population and distribution of microbial components, we will evaluate the total microbial density by direct counting of DAPI stained cells.

16S rDNA clone library analyses: We will construct clone libraries for 16S rRNA gene with each subsamples and compare each library associated with physicochemical data.

- **Evaluation of Fe(III) reducing microbes**

For the evaluation of Fe(III) reducers in the environment, we will culture the strains obtained in MPN method in medium supplemented with various form of Fe(III) as the sole electron acceptor.

Electron microscopy of hydrothermal samples.

T. Yoshitomi

Electron Microscope (EM) enables us to acquire high resolution image of object with rough surface and chemical composition analysis. Although EM has several advantages, it's difficult to distinguish microbes from the asperity of non-microbial materials. Therefore it is important to improve the method of microbial detection from solid sample using molecular biology based approach. With this method we will try to survey geosphere-biosphere interaction.

The chimney samples, obtained in the dive #712 and #713, have clear laminated structure. Three sub-samples were picked up from each chimney structure:

(1) inner chimney structure: which is mostly made of pyrite and accumulated on hydrothermal channel surface.

(2) old outer layer: mainly includes black granules which component material is not analyzed yet.

(3) intermediate layer: which is mainly made of silica.

These sub-samples were fixed with 3.8% formaldehyde and/or 2.5% glutaraldehyde immediately after sample retrieval. Formalin fixed samples were stored in the refrigerator at 4oC for approximately 12hrs. and preserved at -80oC untile laboratory FISH analysis. Gultaraldehyde fixed samples were also stored at 4oC, 12hrs, and preserved at -80oC after the fixative solution was displaced by 70% EtOH.

These samples will be stained using gold nanoparticle SEM-ISH (electron staining), observed microbial cells under SEM and their chemical composition in micro region will be analyzed with EDS.

Metagenomics of lateral gene transfer factors in hydrothermal field

T. Nunoura

Lateral gene transfers are often observed in microbial genome sequences. Hydrothermal environments are one of the most appropriate environments for dynamic lateral gene transfer such as between Bacteria and Archaea, and mesophile and hyperthermophile. In fact, genes from Archaea and Epsilonproteobacteria (mesophile) are observed in hyperthermophilic Bacteria Thermotoga and Aquifex, respectively. When organisms take in genes from other linage of organisms, gene transfer factors; such as phages, plasmids and extra cellular nucleic acids, and gene uptake systems such as infection, confusion and natural competency must be required, but these systems in natural environments have not been revealed yet.

Recently, metagenomic surveys for prokaryotic and phage genomes have been conducted in various environments in order to know the microbial or phage genomic diversity without isolation. However, metagenomic surveys for plasmids and extra cellular nucleic acids have not been reported yet. The first objective of this study is to know the potential of lateral gene transfer in hydrothermal environments exhaustively. This project is the first comprehensive study for lateral gene transfer factors in specific environments.

Methods

1. Phages

Phage like particle is purified by density gradient ultra centrifuge using cesium chloride. Phage genome is amplified using whole genome amplification methods.

2. Plasmids

Plasmids are purified from microbial cells using alkali-SDS method and further purified using plasmid safe exonuclease. Purified plasmid is amplified by plasmid DNA amplification kit.

3. Extracellular DNA

Extracellular DNA was separated from phage fraction using 0.02μm filters on board. Extracellular DNA will be purified by ethanol precipitation method and amplified using whole genome amplification methods.

4. Sequencing

Amplified DNA from phage, plasmid and extracellular DNA will be analyzed by both Sanger and pyrosequencing methods.

深海生物の観察および長期飼育への取り組み

足立　文

目的：

- 化学合成生態系の環境に棲息する生物を採集し、飼育下での長期間維持みる。
- 深海（特に化学合成生態系の環境）の世界の様子を水族館の展示を通して広く一般に紹介する

材料と方法：

- ハイパードルフィンにより、潜航中に現れる深海生物および生息環境の目視観察を行ない、画像データを収集する。
- 熱水噴出域周辺の化学合成生態系大型生物の観察および、スラープガン等を用いての生物採集
- 採集生物の、船内での飼育維持
- 水族館への輸送（生残率を高く維持するための工夫）

結果：

- 大型採集生物（下船日までの生残数／採集数）

ゴエモンコシオリエビ　*Shinkaia crosnieri*　（95／239）

シンカイヒバリガイ　*Bathymodiolus japonicus*（22／23）

オハラエビのなかま　　*Alvonocaris brevitelsonis*（25／25）
ウロコムシのなかま　　*Polynoidae* sp.　　（5／5）
イトエラゴカイのなかま *Paralvinella* sp.　　（30／30）

・船内での飼育環境

水槽：600×450×450（mm）水量約 100ℓ　3 本
ろ過：密閉型ろ過器（エーハイム 2213・2215）
水温：冷蔵コンテナ（４℃設定）内に水槽設置
飼育水：密閉型ろ過器（ろ材はサンゴ砂）によりろ過した海水を使用
（１～2／日、１／２～１／３換水）
給餌：なし

・輸送：

下船日の早朝より開始。
200×400（mm）程度の大きさのビニール袋を二重にし、ゴエモンコシオリエビは 3～5 個体ずつを小分けにして入れ、若干の空気を入れて輪ゴム　　で口を閉じた。450×350×350（mm）ほどの発泡スチロールの箱に詰めて、１箱に保冷材 6 個を入れ、合計 6 箱の梱包となった。　奄美空港から羽田空港まで手荷物として預け、羽田空港からは車で輸送した。生物の袋詰めから水槽収容までの所要時間は約 10 時間であったが、保冷も効いており、生物の状態も良好であった。

今後の研究：

・採集された生物を新江ノ島水族館の化学合成生態系水槽（3000L×1000W×1000H(mm)）に収容し、長期飼育を試みる。微生物レベルの環境において、どんな変化が起こるのかも合わせて研究したい。
・ゴエモンコシオリエビが集まる場所と、様々な環境条件の関連性を明らかにしたい。また、脱皮や、抱卵個体の継続的観察も行ないたい。

5.2 Biogeochemistry

Hydrogen gas

S. Kawagucci and T. Narita

Hydrogen gas (H_2) is one of most important component for biological processes in

submarine hydrothermal systems because of its redox state that can make organic component without solar radiation through both abiotic reaction and biological methanogenesis. Lately, an innovative gas-tight fluid sampler, named *WHATS*, and also a simple method for simultaneous analysis of both H_2 concentration and its isotope ratio (δD) were developed. In this cruise, we try to understand behavior of H_2 by determining both H_2 concentrations and their δD values. Moreover, in order to investigate hydrogen isotope behavior on biological consumption of H_2, we also quantify H_2 concentrations and δD_{H2} values in hydrothermal plume and sediment pore water around hydrothermal systems, in addition to venting fluid.

Hydrothermal fluid samples are collected by using *WHATS* equipped to Hyper Dolphin. Sample gasses dissolved in hydrothermal fluid samples are extracted and subsampled into approximately 50 cm^3 of stainless bottles on board for chemical and isotopic measurements at the onshore laboratory. Detail of gas extraction from *WHATS* fluid is described in other Section in this report. Hydrothermal plume samples are collected by Niskin-type water sampler equipped to Hyper Dolphin. Water sampling from Niskin sampler is conducted in manner of a dissolved oxygen analysis. Water sample is subsampled into 120cm^3 glass vial using Teflon tube attached to stop cock of Niskin bottle overflowing more than twice, carefully avoiding bubble injection that may extract dissolved gasses from sample water. Then, we add 500μl $HgCl_2$ saturated solution into a vial and cap a vial using Teflon-coated Butyl-rubber septum in order to avoid escape and consumption of H_2 during sample storage. Seafloor sediment is collected using a MBARI corer. An approximately 30cm^3 sediment is subsampled from MBARI sampler into a 50cm^3 disposal syringe, and is then pressed for extracting pore water. Extracted pore water sample is introduced through 0.45μm filter into 5cm^3 vial provided $HgCl_2$ and amidosulfate powders respectively for poison and acidifying.

Both H_2 concentration and its δD value in the samples are simultaneously quantified using a continuous flow isotope ratio mass spectrometer system onshore laboratory. The gas sample from *WHATS* is injected from the stainless bottle into the analytical system through vacuum line while those from Niskin and MBARI are injected using a gas-tight syringe in manner of a Head-space method. By ultra pure carrier gas and three gas chromatograph columns in the analytical system, H_2 in the introduced gas is separated from the other gas species, concentrated for high sensitivity analysis, and introduced into a mass spectrometer (Thermo Finnigan, DELTA^{XP}) for quantification of both H_2

concentration and δD_{H2} value.

Measurements of nitrification rate and characterization of stable isotopes of nitrogen compounds in mixing area between hydrothermal vent and sea water

A. Makabe

Introduction

Hydrothermal vent is a treasury of energy for chemoheterotrophic bacteria, and biomass in hydrothermal area is larger than that in sea water. Ammonia oxidation is one of the major microbial reactions in hydrothermal area, and the activity will be high in mixing area that hydrothermal water including a large amount of ammonium meets deep sea water including an abundance of dissolved oxygen. An activity of ammonia oxidation is expected high in area more far from hydrothermal vent compared with hydrogen, sulfur, and methane consumption. It is important to measure rates of ammonia oxidation in several distances from hydrothermal vent for understanding hydrothermal ecosystems. In this study, we tried incubation experiments in deep sea water that could be incubated in situ pressure.

Natural stable isotopes can have information about sources of materials and a history of reactions however, there is little measurements of stable isotopes of nitrogen compounds in hydrothermal area. We will be able to characterize stable isotopes of nitrogen compounds derived from hydrothermal vent and biochemical processes in this study.

Sampling and Analysis

There are many hydrothermal vents in Iheya North, and geochemical and microbiological studies have done. According to the past studies in that area, microbial biomass is large in fluids surrounding vent animals, and it is expected that precedence of microbial species and activities would shade with distance from hydrothermal vent. Therefore animals living around hydrothermal vents also spatially shade, we sampled water surrounding vent animals and incubated both in situ and in ship. In Iheya North, we picked up samples landed on sea floor during NT07-11, and then also sampled and in situ incubated water surrounding the polychaete colony and Control water. We also incubated water surrounding vent animals in ship with shorter incubation period than in

situ, because ammonium concentration showed that rates of ammonium consumption were very high even it is not equal to nitrification.

We use both ^{15}N additional method and natural stable isotopes to measure nitrification rates. In ^{15}N additional method, we calculate nitrification rates from increase of ^{15}N nitrite and nitrate in the batch added ^{15}N labeled ammonium. However, the rates could be overestimate because of increase of substrate. Although it is difficult to estimate quantitative flows in complicated nitrogen cycle using snapshot of stable isotopes, it is useful to analyze after quantification processes using ^{15}N tracer. We tried incubation experiments of sample water added nothing(control), low amount of ^{15}N ammonium, high amount of ^{15}N ammonium, and ^{15}N nitrate to count consumption of nitrate. We will measure concentrations and stable isotopes of ammonium, nitrate, and nitrite in each batch.

In this study, we can characterize stable isotopes of nitrogen compounds, ammonium, nitrate, nitrite, and nitrous oxide, derived from hydrothermal vent and biochemical processes. They will be the first hydrothermal data of stable isotopes of nitrogen compounds associated with oxygen isotopes of dissolved oxygen and water that form nitrogen oxidants. Concentrations of materials around hydrothermal vent have high heterogeneity, so information of stable isotopes is expected to be useful to distinguish between hydrothermal fluid and influence of organismal activities. We will analyze the water samples including hydrothermal vents, hydrothermal plumes, and water surrounding vent animals in Iheya North.

We sampled mainly hydrothermal using WHATS sampler and took subsamples for nitrous oxide, dissolved oxygen, ammonium, nitrate, and nitrite. We sampled mainly hydrothermal plumes using Niskin sampler and took subsamples for ammonium, nitrate, nitrite, dissolved organic nitrogen (DON), nitrous oxide, dissolved oxygen, and water. We sampled mainly hydrothermal fluid surrounding vent animals using 20L plastic bags and took subsamples for ammonium, nitrate, nitrite, DON, and nitrous oxide. Subsamples for ammonium and DON were filtrated with 0.45 μ m and stored in freezing. Subsamples for nitrate and nitrite were filtrated with 0.45 μ m and stored in pH 12 with added NaOH. Subsamples for nitrous oxide and dissolved oxygen were stored with added $HgCl_2$.

6. Sample list

NT07-13 Sample distribution (Microbiology)

	Sample		Depth (m)	Time (WHATS)	Temp (av.) (WHATS)	Description
#710 (7/4/2007) Iheya North	6B-1	*Bathymodiolus* colony (deployed during dive #702)	995			530ml Scintillation (SUGAR), 160ml MAR-FISH (Univ. of Tokyo)
	6B-2		995			5350 (3500+1850)ml Scintillation (SUGAR), 160ml MAR-FISH (Univ. of Tokyo)
	6B-3		995			2500ml Mol (SUGAR)
	6B-4		995			2500ml Mol (SUGAR)
	6B-5		995			1700ml Mol (SUGAR)
	6B-6		995			100ml Mol (SUGAR)
#711 (7/4/2007) Iheya North	B1	control (deployment)	1054			25ml CLT (SUGAR), 16000ml Mol (SUGAR), 100ml FISH (Univ of Tokyo)
	6B-1	control (deployment)	1054			
	6B-2	control (deployment)	1054			
	6B-3	control (deployment)	1054			
	6B-4	control (deployment)	1054			
	N1	plume?	1030			25ml CLT (SUGAR), 100ml FISH (Univ of Tokyo)
	B2	Galetheid colony	979			25ml CLT (SUGAR), 16000ml Mol (SUGAR), 100ml FISH (Univ of Tokyo)
	Galetheid crab		979			approx. 100 individuals (SUGAR)
	W1-4	*Paralvinella* colony	979	14:04-14:20	4.8-9.4 (6.7)	All for biogeochemistry (600ml)
	6B-5	*Paralvinella* colony	979			
	6B-6	*Paralvinella* colony	979			
#712 (7/5/2007) Iheya North	N1	Alt=10m	1045			25ml CLT (SUGAR), 100ml FISH (Univ of Tokyo)
	W1	control	1054	9:30-9:35	4.0-4.1 (4.1)	25ml CLT (SUGAR)
	B1	control	1054			25ml CLT (SUGAR), 15000ml Phage (SUGAR), 100ml FISH (Univ of Tokyo)
	B2	*Bathymodiolus*	1000			25ml CLT (SUGAR), 16000ml Mol (SUGAR), 100ml FISH (Univ of Tokyo)
	KT1-4	Galetheid colony	980			1300ml CLT (SUGAR)
	Chimney	NBC top vent	979			30g CLT (SUGAR), 80g Mol (SUGAR), 10g FISH&EM (Univ of Tokyo) 30g CLT (Kyoto Univ), 80g Mol (Kyoto Univ)
	B3	1m above NBC vent	979			25ml CLT (SUGAR), 15000ml Phage (SUGAR), 100ml FISH (Univ of Tokyo)
	B4	just above polychaetes	980			25ml CLT (SUGAR), 16000ml Mol (SUGAR), 100ml FISH (Univ of Tokyo)
	Filtration	just above polychaetes	980	13:33-14:10		420L (SUGAR)
#713 (7/6/2007) Iheya North	W1	NBC top vent	980	9:35-9:42	300.2-308.0 (305.9)	25ml CLT (SUGAR)
	B1	polychaetes	980			25ml CLT (SUGAR), 16000ml Mol (SUGAR), 100ml FISH (Univ of Tokyo)
	B2	Galetheid colony	980			25ml CLT (SUGAR), 16000ml Mol (SUGAR), 100ml FISH (Univ of Tokyo)
	6B-1	deployed during #711	1054			1700ml Mol (SUGAR)
	6B-2					1700ml Mol (SUGAR)
	6B-3					1700ml Mol (SUGAR)
	6B-4					1700ml Mol (SUGAR)
	6B-5					1700ml Mol (SUGAR)
	6B-6					1700ml Mol (SUGAR)
	Filtration	*Bathymodiolus*	990			400L (SUGAR)
	Chimney	Pseudo SBC	1006			30g CLT (SUGAR), 80g Mol (SUGAR), 10g FISH&EM (Univ of Tokyo) 30g CLT (Kyoto Univ), 80g Mol (Kyoto Univ)
	W3	Pseudo SBC	1006	12:00-12:07	272.3-288.7 (275.5)	25ml CLT (SUGAR)
	MBARI (yellow)	Pelagic clay	1079			700ml Mol (SUGAR), 50ml FISH (Univ of Tokyo)
	MBARI (green)	Pelagic clay	1079			500ml Mol (SUGAR)

和名	Species	Inds No.	Locality	Dive No.	Lat.			Long.			Fixation
ゴエモンコシオリエビ	*Shinkaia crosnieri*	95	North Iheya	HD#711	27 °	47.455 '	N	126 °	53.796 '	E	alive
ゴエモンコシオリエビ	*Shinkaia crosnieri*	139	North Iheya	HD#711	27 °	47.455 '	N	126 °	53.796 '	E	Freeze
ゴエモンコシオリエビ	*Shinkaia crosnieri*	5	North Iheya	HD#711	27 °	47.455 '	N	126 °	53.796 '	E	実験用
オハラエビの仲間	*Alvinocaris brevitelsonis*	9	North Iheya	HD#711	27 °	47.455 '	N	126 °	53.796 '	E	alive
ウロコムシの仲間	*Polynoidae* sp.	5	North Iheya	HD#711	27 °	47.455 '	N	126 °	53.796 '	E	alive
イトエラゴカイの仲間	*Paralvinella* sp.	20	North Iheya	HD#711	27 °	47.455 '	N	126 °	53.796 '	E	alive
シンカイヒバリガイ	*Bathymodiolus japonicus*	22	North Iheya	HD#712	27 °	47.409	N	126 °	53.859 '	E	alive
シンカイヒバリガイ	*Bathymodiolus japonicus*	1	North Iheya	HD#712	27 °	47.409	N	126 °	53.859 '	E	Freeze
オハラエビの仲間	*Alvinocaris brevitelsonis*	16	North Iheya	HD #713	27 °	47.423	N	126	53.811 '	E	alive
イトエラゴカイの仲間	*Paralvinella* sp.	10	North Iheya	HD #713	27 °	47.423	N	126	53.8 '	E	alive

Sample distribution of extracted gas						
Sample ID	Field	Site	T max	TGC	Packing pressure	Distribution
HPD#712-W4	Iheya North	NBC	309.5 ˚C	231.50 mM	95.03 kPa	ORI-H2, 50 ml bottle
						TITECH, 100 ml glass
						SUGAR, 50 ml bottle
						ORI-He, 50 ml Pb glass
					95.90 kPa	TITECH, 100 ml glass
HPD#711-W1	Iheya North	NBC Paralvinella's colony 20˚C, 0 hour	10.4 ˚C	5.20	8.99	ORI-H2, 50 ml bottle
						TITECH, 100 ml glass
						ORI-He, 50 ml Pb glass
					7.32	SUGAR, 50 ml bottle
					5.69	TITECH, 100 ml glass
HPD#711-W2	Iheya North	NBC Paralvinella's colony 20˚C, 5 hour	10.4 ˚C	6.21	8.67	ORI-H2, 50 ml bottle
						TITECH, 100 ml glass
						ORI-He, 50 ml Pb glass
					6.66	SUGAR, 50 ml bottle
					4.83	TITECH, 100 ml glass
HPD#711-W3	Iheya North	NBC Paralvinella's colony 20˚C, 15.5 hour	10.4 ˚C	7.45	8.99	ORI-H2, 50 ml bottle
						TITECH, 100 ml glass
						ORI-He, 50 ml Pb glass
					4.75	SUGAR, 50 ml bottle
					5.87	TITECH, 100 ml glass
HPD#711-W4	Iheya North	NBC Paralvinella's colony 20˚C, 48 hour	10.4 ˚C	6.25	9.43	ORI-H2, 50 ml bottle
						TITECH, 100 ml glass
						ORI-He, 50 ml Pb glass
					5.88	SUGAR, 50 ml bottle
					5.88	TITECH, 100 ml glass
HPD#712-W2	Iheya North	bottom seawater	4.1 ˚C	1.75	6.18	ORI-H2, 100 ml glass
						TITECH, 100 ml glass
						SUGAR, 50 ml bottle
HPD#713-W2	Iheya North	NBC	309.3 ˚C	144.32 mM	100.27 kPa	ORI-H2, 50 ml bottle
						SUGAR, 50 ml bottle
					95.99 kPa	TITECH, 100 ml glass
HPD#713-W4	Iheya North	East SBC	288.3 ˚C	49.70 mM	49.49 kPa	ORI-H2, 50 ml bottle
						TITECH, 100 ml glass
						SUGAR, 50 ml bottle
						ORI-He, 50 ml Pb glass
					19.32 kPa	TITECH, 100 ml glass

Water Sample distribution (geochemistry)

Sampler	Analyte	Analyst	volume (ml)	
WHATS				
	Anion	ORI	10	*
	Cation	ORI	20	*
	pH, Alk., Halogen	ORI	10	
	dD-H2O	ORI	5	*
	Org. acid	(Okayama)	15	*
	d15N-DIN	titech	10	*
Bag				
	Anion	ORI	10	*
	Cation	ORI	20	*
	pH, Alk., Halogen	ORI	10	
	Metals and REEs	ORI	200	
	N2O	tittech	250	
	d15N-NH4	tittech	100	
	d15N-DON	tittech	50	
	d15N-NO3	tittech	50	
	TN	tittech	50	
	d18O-H2O	tittech	10	
	H2S	tittech	120	
	(for incubation)	tittech	10,000	
Niskin				
	Anion	ORI	10	*
	Cation	ORI	20	*
	pH, Alk., Halogen	ORI	10	
	H2, dDH2, CO	ORI	500	
	N2O	tittech	200	
	O2	tittech	200	
	d15N-NH4	tittech	100	
	d15N-DON	tittech	50	
	d15N-NO3	tittech	50	
	TN	tittech	50	
	d18O-H2O	tittech	10	
	CH4	tittech	100	
Takai sampler II				
	Anion	ORI	10	*
	Cation	ORI	20	*
	pH, Alk., Halogen	ORI	10	
	H2S	titech	30	
	CH4	titech	50	
	d15N-NH4, NO3	tittech	20	

** filtered*

Geochemistry

	Sampler	Description
#711 (7/4/2007)	B1	240ml(ORI), 630ml(Titech)
	B2	240ml(ORI), 630ml(Titech)
	N1	540ml(ORI), 760ml(Titech)
#712 (7/5/2007)	B1	240ml(ORI), 630ml(Titech)
	B2	240ml(ORI), 630ml(Titech)
	B3	240ml(ORI), 630ml(Titech)
	B4	240ml(ORI), 630ml(Titech)
	N1	540ml(ORI), 760ml(Titech)
	W1	60ml (ORI), 10ml(Titech)
#713 (7/6/2007)	W1	60ml (ORI), 10ml(Titech)
	B1	240ml(ORI), 630ml(Titech)
	B2	240ml(ORI), 630ml(Titech)
	W3	60ml (ORI), 10ml(Titech)
	M直上	10ml(ORI&Titech)
	MU	10ml(ORI&Titech)
	MM	10ml(ORI&Titech)
	ML	10ml(ORI&Titech)

7. Dive Report

7-1. Dive Report #710

Satoshi Nakagawa

Date: July 4, 2007

Site: Iheya North

Landing: 9:25; 27°47.451'N, 126°53.805'E, 997m

Leaving: 9:32; 27°47.451'N, 126°53.805'E, 997m

Objectives:

The objective is to recover the RI-bag sampler deployed during the dive #702 (June 23).

Dive Summary:

We successfully recovered the RI-bag sampler. Samples were prepared immediately for shore-based microbiological and biogeochemical analyses.

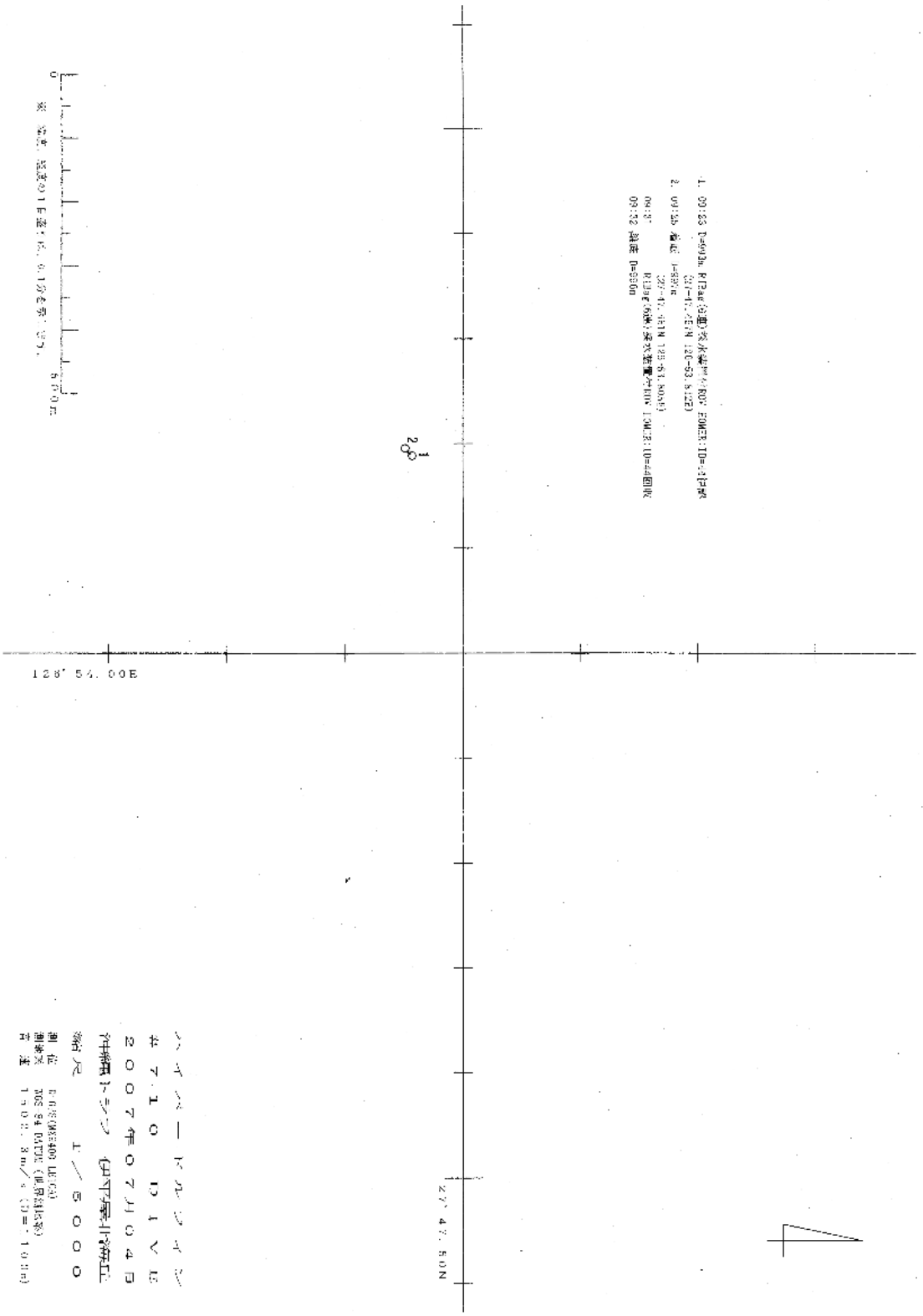
Payloads:

1) WHATS with a temperature probe
2) Bag pomp sampler (20L x 2)
3) Sample box
4) Niskin bottles (x 2)
5) DO meter
6) Turbidity meter
7) Slurp gun
8) SAHF

Event List:

9:30 27-47.451N, 126-53.805E D=997m Recovery of RI bag

Dive track:



7-2. Dive Report #711 *Satoshi Nakagawa*

Date: July 4, 2007
Site: Iheya North
Landing: 13:03; 27°47.422'N, 126°53.939'E, 1054m
Leaving: 14:32; 27°47.467'N, 126°53.799'E, 998m

Objectives:

The major objectives are 1) to deploy the RI-bag sampler, 2) to measure heat flow, and 3) to take hydrothermal samples including sediments, chimney structures, hydrothermal plumes, hydrothermal vent animals, and fluids surrounding vent animals.

Dive Summary:

We landed on seafloor at 100m east of event mark 10. At the landing point, we collected seawater using 20L bag (x1) and 6L bag (x4). Then, we headed to WNW. At event mark #10 (Alt=10m), we sampled seawater using a Niskin bottle (red).

At the top of NBC mound, we took a lot of galetheid crabs. In addition, we took fluids surrounding the *Shinkaia crosnieri* (20L bag x1). Then, we collected fluids immediately above polychaete colony with WHATS (x4) and 6L bag (x2). After that, we deployed 6L bags at the base of NBC mound. We had to left bottom much earlier than expected because of a fisherman's boat.

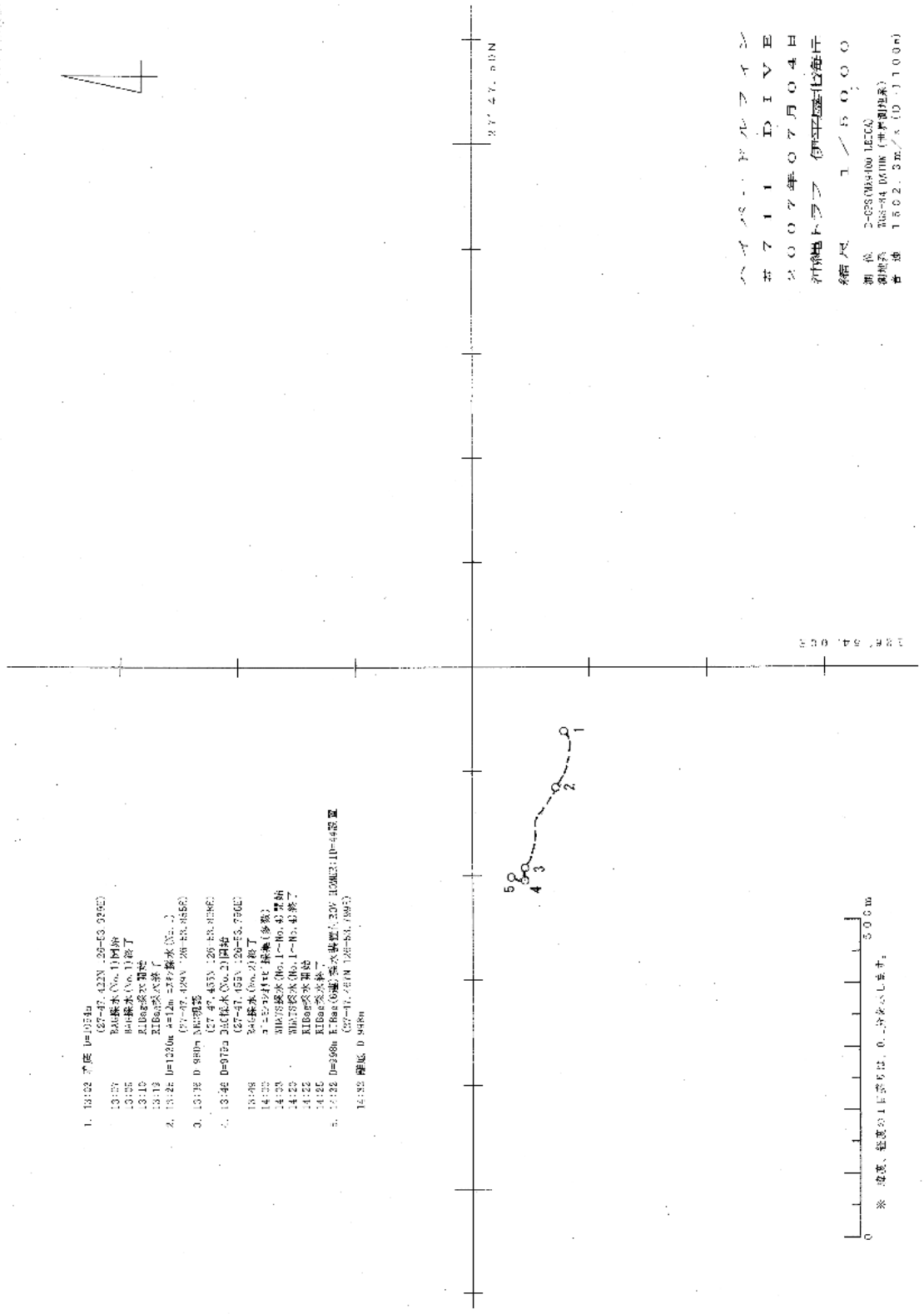
Payloads:

1) WHATS with a temperature probe
2) Bag pomp sampler (20L x 2)
3) Sample box
4) Niskin bottles (2 bottles)
5) DO meter
6) Turbidity meter
7) Slurp gun
8) SAHF
9) Bag pomp sampler (6L x 6)

Event List:

13:07	27-47.422N, 126-53.939E	D=1054m	Bag sampling (20L x1)
13:10	27-47.422N, 126-53.939E	D=1054m	Seawater sampling (6L Bag 1-4)
13:25	27-47.429N, 126-53.985E	D=1030m	Seawater sampling (Niskin 1)
13:46	27-47.455N, 126-53.796E	D=979m	Bag sampling (20L x1)
14:00	27-47.455N, 126-53.796E	D=979m	Sampling galetheid
14:03	27-47.455N, 126-53.796E	D=979m	WHATS sampling (1-4)
14:22	27-47.455N, 126-53.796E	D=979m	Bag sampling (6L Bag 5-6)
14:32	27-47.467N, 126-53.799E	D=998m	Leaving bottom

Dive track:



7-2. Dive Report #712 *Yuichiro Ueno*

Date: July 5, 2007
Site: Iheya North
Landing: 9:22; 27°47.412'N, 126°54.053'E, 1054m
Leaving: 15:46; 27°47.680'N, 126°53.972'E, 1043m

Objectives:

The major objectives are 1) to place 5 IODP markers, 2) to measure heat flow, and 3) to collect reference bottom seawater, vent fluid, plume water, colony water, chimney, and mussels.

Dive Summary:

Before landing, bottom seawater was collected by NISKIN 10m above the bottom. The ROV landed on seafloor, where bottom seawater was collected by WHATS-1, 2 and BAG-1. Then, ROV headed to Calyptogena colony site. At the site, we placed the IODP Marker (INA-5A). Then, the ROV moved to the "bubbling site". At this site, we placed the marker INA-4A within Galetheid and Mussel colony, and collected mussels. After that, the ROV moved through the CBC, to SBC, where we placed the marker INH-3A. Then, the ROV moved to the other Mussel colony, basal part of the NBC mound, where colony water was sampled by the BAG-2. After that, the ROV climbed the NBC mound.

At the top of the NBC, we conducted followings: (1) sampling for incubation experiment by four different TK samplers at Galetheid colony (TK1-4; for H_2, CH_4, S_2O_3, and NH_4); (2) placing the marker INH-1A; (3) sampling chimney 1m away from the vent; (4) sampling vent fluid (max-T: 309.3˚C) by WHATS-4 from the destroyed chimney; (5) sampling shimmering water just 1m above the vent; (6) sampling water of Polychaete colony 20cm away from pink shrimp; and (7) in-situ filtration (300L+100L) at the same Polychaete colony. Unfortunately, we were unable to use WHATS-3 due to trouble of the controller.

Then, the ROV headed to NEC mound, where the marker INH-2A was deposited. After that, the ROV moved to north east. We conducted two SAHF measurements.

Payloads:

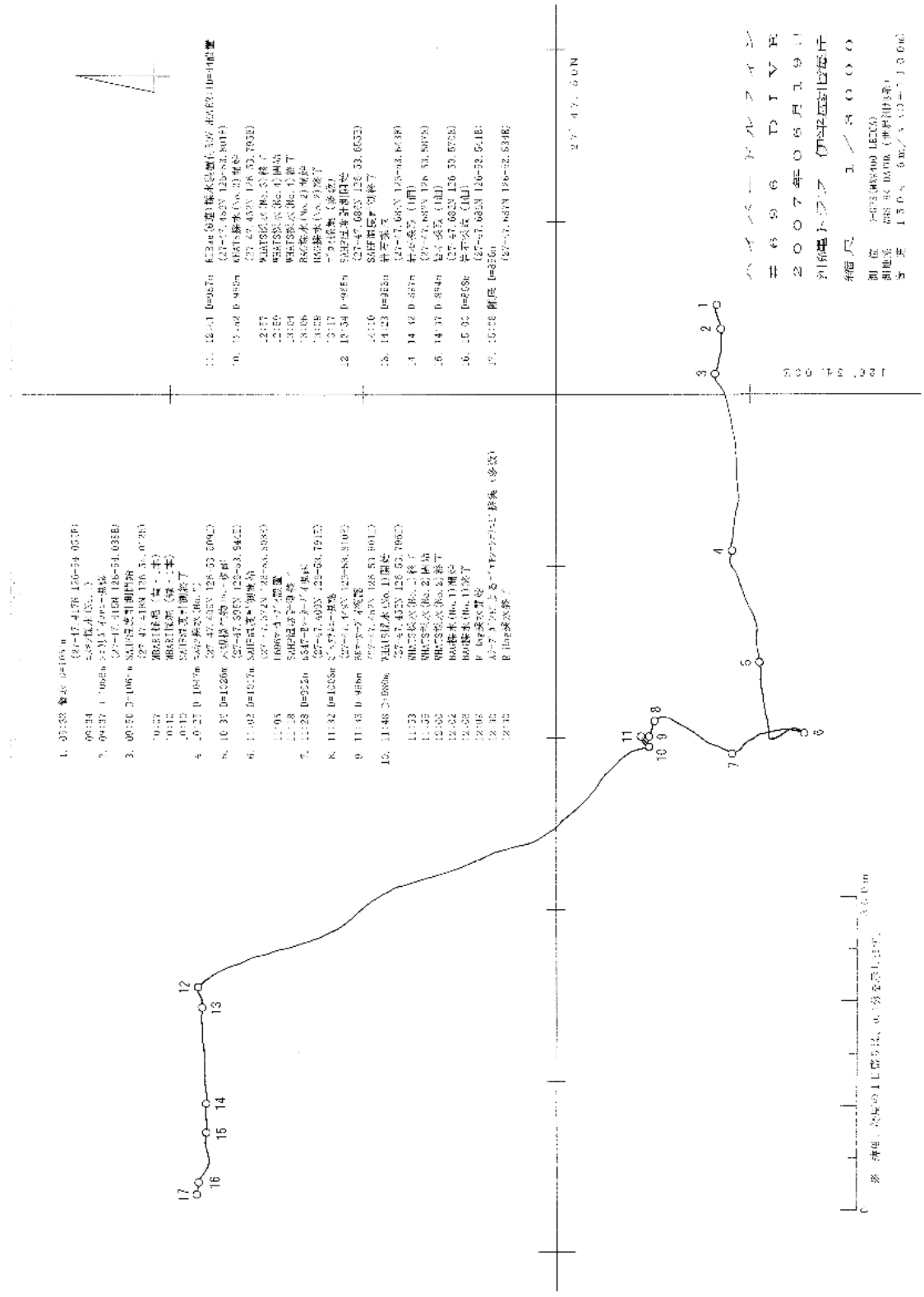
1) WHATS with a temperature probe
2) Bag pomp sampler (20L x 4)
3) Sample box
4) Niskin bottles (1 bottle)
5) DO meter
6) Turbidity meter
7) Vacuum sampler (x4)
8) SAHF
9) In-situ filtration sampler

Event List:

9:20	27°47.412'N, 126°54.053'E, 1045m	Seawater sampling (Niskin)
9:22	27°47.412'N, 126°54.053'E, 1054m	Landing
9:30	27°47.412'N, 126°54.053'E, 1054m	WHATS sampling (1)
9:41	27°47.412'N, 126°54.053'E, 1054m	Bag sampling (1)
9:46	27°47.412'N, 126°54.053'E, 1054m	WHATS sampling (2)
9:56	27°47.414'N, 126°54.035'E, 1057m	Finding #857-1 marker
10:01	27°47.414'N, 126°54.053'E, 1057m	Putting marker INH-5A
10:17	27°47.416'N, 126°53.914'E, 1048m	Finding mussel colony
10:26	27°47.409'N, 126°53.859'E, 1034m	Bubbling
10:32	27°47.409'N, 126°53.859'E, 1034m	Putting marker INH-4A
10:45	27°47.409'N, 126°53.859'E, 1034m	Collecting mussel
10:56	27°47.420'N, 126°53.815'E, 1005m	Finding hydrothermal vent
11:00	27°47.411'N, 126°53.804'E, 997m	Finding the SBC
11:04	27°47.411'N, 126°53.793'E, 993m	Putting marker INH-3A
11:25	27°47.455'N, 126°53.811'E, 1001m	Bag sampling (2)
11:37	27°47.454'N, 126°53.798'E, 980m	Arriving top of the NBC
11:55	27°47.454'N, 126°53.798'E, 980m	Vacuum sampling (1-4)
11:37	27°47.454'N, 126°53.798'E, 980m	Arriving top of the NBC
12:29	27°47.454'N, 126°53.798'E, 980m	Putting marker INH-1A

12:54	27°47.454'N, 126°53.798'E, 980m	Sampling chimney
13:04	27°47.454'N, 126°53.798'E, 980m	WHATS sampling (4)
13:33	27°47.454'N, 126°53.798'E, 980m	In-situ filtration (slurp gun)
14:32	27°47.498'N, 126°53.779'E, 964m	Arriving the NEC
14:35	27°47.498'N, 126°53.779'E, 964m	Putting marker INH-2A
15:04	27°47.684'N, 126°53.895'E, 1022m	SAHF measurement
15:28	27°47.680'N, 126°53.972'E, 1044m	SAHF measurement
15:46	27°47.680'N, 126°53.972’E, 1043m	Leaving

Dive track:



7-3. Dive Report #713 *Tomoo Watsuji*

Date: July 6, 2007
Site: Iheya North
Landing: 9:18; 27°47.453'N, 126°53.799'E, 986m
Leaving: 14:20; 27°47.394'N, 126°54.748'E, 1113m

Objectives:

The major objectives are 1) to collect hydrothermal samples including chimney and sediment, 2) to collect the RI-bag sampler on NBC mound, and 3) to perform the Slurp gun filtration system at *Bathymodiolus* site.

Dive Summary:

We landed on seafloor near the marker 348-N on event no. 7 (North Big chimney [NBC]). We tried to collect chimney at the site, but our attempt was a total failure. Then, we climbed the mound. We collected hydrothermal fluids from hydrothermal vent on the top of NBC by using WHATS sampler (2 bottles).The temperature of fluids was approximately 308˚C. After we headed to other hydrothermal vent on NBC, we collected fluids surrounding polychaete and galetheid crab colony by using Bag pomp sampler (20L x 2; each 1st bag and 2nd bag was fluids of surrounding polychaete and galetheid crab colony.) Then, we picked up RI-bag samplers and rocks that organisms like cotton attached at the basement of NBC mound. We headed to Bathymodiolus colony on NBC and we filtrated water just above them using the slurp gun filtration system. We filtrated 300 L seawater and had about 5 min intervals until the second operation. Finally, 400 L seawater was filtrated.

We headed to event no. 11. We collected a whole chimney and hydrothermal fluids from the vent picked up the chimney by using WHATS sampler (2 bottles). The temperature of fluids was approximately 270˚C.

We headed to east. We performed SAHF measurement three times and collected sediments in the seafloor by using MBARI corer (2 bottles).

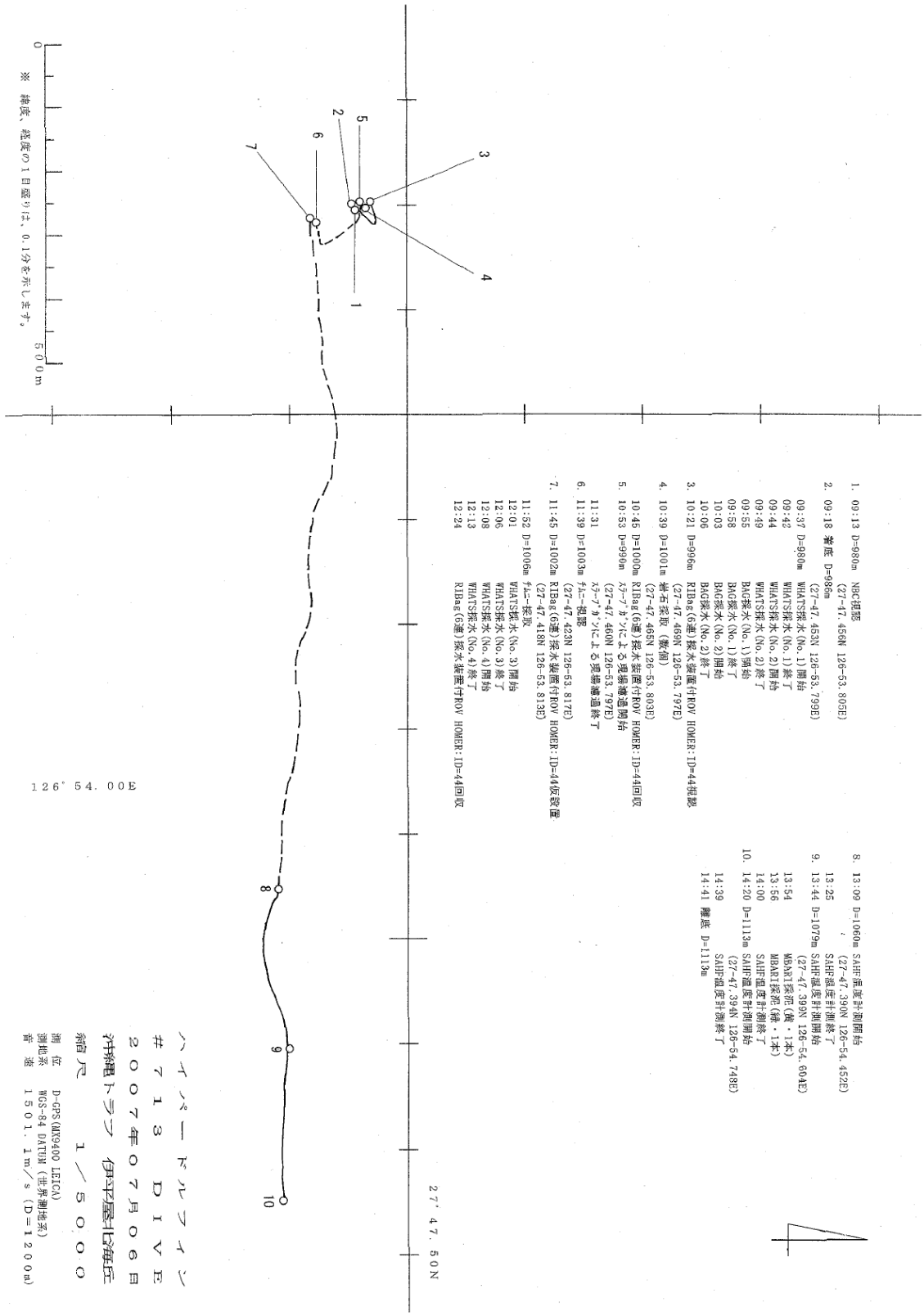
Payloads:

1) WHATS with a temperature probe
2) Slurp gun filtration system
3) Turbidity meter
4) DO meter
5) Bag pomp sampler (20L x 2)
6) Niskin bottles x 2
7) Venial bottle having cap
8) SAHF
9) MBARI corer x 2

Event List:

9:18	27°47.453N, 126°53.799E	D=986m	Landing
9:37	27°47.453N, 126°53.799E	D=980m	WHATS sampling (1st)
9:44	27°47.453N, 126°53.799E	D=980m	WHATS sampling (2nd)
9:55	27°47.453N, 126°53.799E	D=980m	Bag sampling (1st)
10:03	27°47.453N, 126°53.799E	D=980m	Bag sampling (2nd)
10:21	27°47.469N, 126°53.797E	D=996m	RI-bag collection
10:39	27°47.465N, 126°53.803E	D=1001m	rock sampling
10:53	27°47.460N, 126°53.797E	D=990m	slurp gun filtration
11:52	27°47.418N, 126°53.813E	D=1006m	chimney sampling
12:01	27°47.418N, 126°53.813E	D=1006m	WHATS sampling (3rd)
12:08	27°47.418N, 126°53.813E	D=1006m	WHATS sampling (4th)
13:09	27°47.390N, 126°54.452E	D=1060m	SAHF measurement (1st)
13:54	27°47.399N, 126°54.604E	D=1079m	MBARI sampling (1st yellow)
13:56	27°47.399N, 126°54.604E	D=1079m	MBARI sampling (2nd green)
13:44	27°47.399N, 126°54.604E	D=1079m	SAHF measurement (2nd)
14:20	27°47.394N, 126°54.748E	D=1113m	SAHF measurement (3rd)
14:41	27°47.394N, 126°54.748E	D=1113m	left the bottom

Dive track:


1. 09:13 D=980m NBC揚収 (27-47.456N 126-53.805E)
2. 09:18 着底 D=988m (27-47.453N 126-53.799E)
09:37 D=980m WHATS採水(No.1)開始
09:42 WHATS採水(No.1)終了
09:44 WHATS採水(No.2)開始
09:49 WHATS採水(No.2)終了
09:55 BAG採水(No.1)開始
09:58 BAG採水(No.1)終了
10:03 BAG採水(No.2)開始
10:06 BAG採水(No.2)終了
3. 10:21 D=996m RIBag(6連)採水装置付ROV HOMER:ID=44揚収 (27-47.469N 126-53.797E)
4. 10:39 D=1001m 岩石採取(敷領) (27-47.465N 126-53.803E)
10:45 D=1000m RIBag(6連)採水装置付ROV HOMER:ID=44回収
5. 10:53 D=990m スラープガンによる堆積物採取開始 (27-47.460N 126-53.797E)
11:31 スラープガンによる堆積物採取終了
6. 11:39 D=1003m チムニー揚収 (27-47.423N 126-53.817E)
7. 11:45 D=1002m RIBag(6連)採水装置付ROV HOMER:ID=44仮設置 (27-47.418N 126-53.813E)
11:52 D=1006m チムニー採取
12:01 WHATS採水(No.3)開始
12:06 WHATS採水(No.3)終了
12:08 WHATS採水(No.4)開始
12:13 WHATS採水(No.4)終了
12:24 RIBag(6連)採水装置付ROV HOMER:ID=44回収
8. 13:09 D=1060m SAHF温度計測開始 (27-47.390N 126-54.452E)
13:25 SAHF温度計測終了
9. 13:44 D=1079m SAHF温度計測開始 (27-47.399N 126-54.604E)
13:54 MBART採泥(東・1本)
13:56 MBART採泥(南・1本)
14:00 SAHF温度計測終了
10. 14:20 D=1113m SAHF温度計測開始 (27-47.394N 126-54.748E)
14:39 SAHF温度計測終了
14:41 離底 D=1119m
0
※ 緯度、経度の1目盛りは、0.1分を示します。
500m
126° 54.00E
27° 47.50N
ハイパードルファイン
#713 DIVE
2007年07月06日
沖縄トラフ 伊平屋北海丘
縮尺 1／5000
測位 D-GPS(MX9400 LEICA)
測地系 WGS-84 DATUM (世界測地系)
音速 1501.1m/s (D=1200m)