NT07-11 Cruise Report

June 18 (Naha) – June 26 (Naha), 2007 *R/V Natsushima* and *ROV Hyper-Dolphin*

Geomicrobiology of hydrothermal fields

in Mid-Okinawa Trough

Minami-Ensei: Dives #697-700, 703-704 Iheya North: Dives #696, 701-702



Photo: "Goshintai" chimney at the Iheya North

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

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1.1 Expedition Overview

Satoshi Nakagawa

We performed NT07-11 cruise in two hydrothermal fields, Iheya North and Minami-Ensei Knoll, in the Mid-Okinawa Trough, from 18th to 27th of June, 2007. 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 areas 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. We added radioactive tracer (^{14}C ; < 9MBq in total) or stable isotope (^{15}N) into bags prior to dives. 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



RI-Bag deployed on Hyper-Dolphin.

scintillation, MAR-FISH, and geochemical analysis. This 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 NT07-11 Participants

2.1.1 Shipboard Scientists

Chief scientist

Dr. Satoshi Nakagawa

Research scientist Subground Animalcule Retrieval (SUGAR) Program, Extremobiosphere Research Center Japan Agency for Marine-Earth Science and Technology (JAMSTEC)

Co-chief scientist Dr. Takuro Nunoura Research scientist Subground Animalcule Retrieval (SUGAR) Program, Extremobiosphere Research Center Japan Agency for Marine-Earth Science and Technology (JAMSTEC)

Dr. Ken Takai

Program Director Subground Animalcule Retrieval (SUGAR) Program, Extremobiosphere Research Center

Dr. Hiroyuki Imachi

Research Scientist Subground Animalcule Retrieval (SUGAR) Program, Extremobiosphere Research Center, Japan Agency for Marine-Earth Science & Technology (JAMSTEC),

Dr. Masahiro Yamamoto

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Research scientist Research Program for Marine Biology and Ecology Extremobiosphere Research Center Japan Agency for Marine-Earth Science and Technology (JAMSTEC)

Mr. Katsunori Yanagawa

Graduate student Department of Earth and Planetary Science University of Tokyo

Mr. Shinsuke Kawagucci

Graduate student Ocean Research Institute, University of Tokyo

Mr. Taku Narita

Graduate student Ocean Research Institute, University of Tokyo

Ms. Akiko Makabe

Ph.D student Department of Environmental Science and Technology Interdisciplinary Graduate School of Science and Engineering Tokyo Institute of Technology (Tokyo Tech)

Ms. Yuka Masaki

Research Student Japan Agency for Marine Science & Technology

Ms. Sayaka Ohno Graduate Student Tokyo Institute of Technology

Ms. Yukiko Wada

Administrative staff Public Relations Division Marine-Earth Data and Information Department (MEDID) Japan Agency for Marine-Earth Science and Technology (JAMSTEC)

Technical Assistant

Mr. Satoshi Okada

Marine Technician

Marine Science Department, Nippon Marine Enterprises, Ltd.

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 |
|-----------------------------------|-------------------|
| 2 st Submersible Staff | Mitsuhiro UEKI |
| 2 st Submersible Staff | Tomoe KONDO |
| 3 nd Submersible Staff | Katsushi CHIBA |
| 3 nd Submersible Staff | Atsushi TAKENOUCH |
| 3 nd Submersible Staff | Teppei KIDO |
| 3 rd Submersible Staff | Yuudai SAKAKIBARA |

2.1.3 R/V NATSUSHIMA Crews

| Captain | Masayoshi ISHIWATA |
|---------------------------|---------------------|
| Chief Officer | Koji SAMESHIMA |
| 2 nd Officer | Tokuro KOBAYASHI |
| 3 rd Officer | Yuki FURUKAWA |
| Chief Engineer | Minoru TSUKADA |
| 1 st Engineer | Kouji FUNAE |
| 2 nd Engineer | Yoshinobu HIRATSUKA |
| 3 rd Engineer | Daisuke GIBU |
| Chief Electronic Operator | Fukuo SUDA |

2nd Electronic Operator 3rd Electronic Operator **Boat Swain** Able Seamen Able Seamen Able Seamen Able Seamen Able Seamen Able Seamen No.1 Oiler Oiler Oiler Oiler Oiler Chief Steward Steward Steward Steward Steward

Yuichi INOUE Yohey YAMAMOTO Mikio ISHIMORI Kozo YATOGO Washiro OHSAKO Kuniharu KADOGUCHI Ikunori IWASAKI Yoshiaki MATSUO Tomohiro KIMURA Kiyoshi YAHATA Kazuo ABE Takeshi FUKUBARA Keiya TANIGUCHI Masanori SATOH Takeshi MIYAUCHI Shigeto ARIYAMA Shinobu OYU Toshiharu KINOSHITA Futoshi HATAKEYAMA



700th memorial dive of ROV, "Hyper-Dolphin"

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: 67.4mBow thruster: 1Width: 13.0mMaximum speed: 12ktDepth: 6.3mDuration: 8400 mileMax capacity: 55 personsGross Tonnage: 1553tMain 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
(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 : $330kHz \pm 1kHz$

(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 I | .og & Ship Trac | ek(NT07-11 07/06/18 - 07/06/27) | | Position/Weather/Wind/Sea |
|-------------|-----------------|---|----------------------------|---------------------------|
| Date | Time | Description | Remark | condition (Noon) |
| 18,Jun,07 | 9:00 | embarkation science group | | 6/18 12:00 |
| | 11:00 | departure from NAHA Ko | | 26-22.5N, 127-35.0E |
| | 13:00~14:00 | on board seminar | for safety NATSUSHIMA life | over cast |
| | 14:30~15:00 | on board seminar | for HPD operation | S-4(Moderate breeze) |
| | 16:40~17:00 | pray safety cruise to KONPIRASAN | - | Sea smooth |
| | 20:30 | ariived at research area | | |
| | 20:34 | released XBT | | |
| 19,Jun,07 | 7:30 | ariived at research area | | 6/19 12:00 |
| | 8:36 | launched HPD | | 27-47.4N, 126-54.0E |
| | 8:51 | started HPD#696 dive | | cloudy |
| | 9:32 | arrived at bottom | D=1054m | SE-2(Light breeze) |
| | 15:06 | leave the bottom | D=865m | Sea smooth |
| | 15:31 | surfaced HPD | | |
| | 15:45 | recovered HPD | | |
| | 15:51 | commenced proceeding to MINAMI ENSEI area | | |
| 20.Jun.07 | 6:39 | released XBT | | 6/20 12:00 |
| | 8:16 | launched HPD | | 28-23.5N, 127-38.4E |
| | 8:31 | started HPD#697 dive | | fine |
| | 9:15 | arrived at bottom | D=709m | SE-4(Moderate breeze) |
| | 14:28 | leave the bottom | D=709m | Sea smooth |
| | 14:52 | surfaced HPD | | |
| | 15.09 | recovered HPD | | |
| 21.Jun.07 | 8:18 | launched HPD | | 6/21 12:00 |
| | 8:34 | started HPD#698 dive | | 28-23.5N, 127-38.4E |
| | 9.17 | arrived at bottom | D=703m | fine |
| | 11:01 | leave the bottom | D=693m | SE-3(Gentle breeze) |
| | 11:01 | surfaced HPD | 2 0,011 | Sea smooth |
| | 11:38 | recovered HPD | | |
| | 13.15 | launched HPD | | |
| | 13:30 | started HPD#699 dive | | |
| | 13:30 | arrived at bottom | D=701m | |
| | 16:23 | leave the bottom | D=707m | |
| | 16:46 | surfaced HPD | D=/0/m | |
| | 16:57 | recovered HPD | | |
| 22 Jun 07 | 7:30 | ariiyed at research area | | 6/22 12:00 |
| | 8.11 | launched HPD | | 28-23 5N 127-38 4F |
| | 8:26 | started HPD#700 dive | | cloudy |
| | 9:06 | arrived at bottom | D=708m | SSW-4(Moderate breeze) |
| | 15:51 | leave the bottom | D=693m | Sea slight |
| | 16.13 | surfaced HPD | | |
| | 16:24 | recovered HPD | | |
| | 16:30 | commenced proceeding to IHEYA KITA area | | 1 |
| 23 Jun 07 | 6.00 | ariived at research area | | 6/23 12:00 |
| | 8.04 | launched HPD | | 28-47 4N 126-54E |
| | 8.18 | started HPD#701 dive | | fine |
| | 9.06 | arrived at bottom | D=986m | SSW-5(Fresh breeze) |
| | 9.10 | leave the bottom | D=986m | Sea moderate |
| | 9.30 | surfaced HPD | | |
| | 9.50 | recovered HPD | | 1 |
| | 11.48 | launched HPD | | 1 |
| | 12.40 | started HPD#702 dive | | 1 |
| | 12.03 | arrived at bottom | D-1017m | |
| | 12.30 | leave the bottom | D-979m | 1 |
| | 14.02 | surfaced HPD | | 1 |
| | 14.30 | recovered HPD | - | 1 |
| | 14:49 | commenced proceeding to MINAMI ENSEL area | | |
| 1 | 10:30 | commenced proceeding to MINAMI ENSEL area | 1 | 1 |

| Shipboard L | og & Ship Trac | k(NT07-11 07/06/18 - 07/06/27) | | Position/Weather/Wind/Sea |
|-------------|--|-------------------------------------|--------------------|---------------------------|
| Date | Time | Description | Remark | condition (Noon) |
| 24,Jun,07 | 6:00 | ariived at research area | | 6/24 12:00 |
| | 8:14 | launched HPD | | 27-47.4N, 126-54.0E |
| | 8:28 | started HPD#703 dive | | fine |
| | 9:02 | arrived at bottom | D=708m | SSW-5(Fresh breeze) |
| | 16:04 | leave the bottom | D=709m | Sea moderate |
| | 16:26 | surfaced HPD | | |
| | 16:40 | recovered HPD | | |
| 25,Jun,07 | 8:22 | launched HPD | | 6/25 12:00(JST+1.5h) |
| | 8:37 | started HPD#704 dive | | 35-58N, 154-20E |
| | 9:15 arrived at bottom 13:58 leave the bottom 14:21 surfaced HPD | | D=705m | fine |
| | | | D=700m | WNW-5(Fresh breeze) |
| | | | | Sea rough |
| | 14:35 | recovered HPD | | |
| | 14:40 | left research area for NAHA SHINKO | | |
| 26,Jun,07 | 8:00 | arrived at NAHA SHINKO | | |
| | 12:00 | left the ship and concluded NT07-11 | NT07-11 scientists | |

3.1. Iheya North hydrothermal field (Dives 696, 701-702; Map 1)

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 high contain 30m 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 epsilonand 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

3.2 Minami-Ensei Knoll (Dives 697-700, 703-704; Map 2)

Takuro Nunoura

The Minami-Ensei Knoll located about 140 km west from the Amami Island. Hydrothermal activity at the Minami-Ensei Knoll was discovered by the combination of the Deep-tow camera and the 'Shinkai 2000' in 1988 to 1989 as the third hydrothermal active field in the Okinawa Trough (Hashimoto et al. 1990; Aoki et al. 1993). Biological and geochemical surveys followed the discovery (Hashimoto et al. 1990; Nezu et al. 1992; Chiba et al. 1993; Nakashima et al. 1993). However, scientific survey using ROV and manned submersible had been prohibited after the ROV 'Dolphin 3K' had trapped by fishing rope in 1993. In March 2005, the ROV 'Hyper Dolphin' confirmed the safety of survey in this area and NT07-11 cruise is the first scientific survey after the accident. During the previous expeditions, hydrothermal activities are observed in four small basins on the Minami-Ensei Knoll and hydrothermal vents are only discovered in the 'C basin'. In C basin, there are two hydrothermal vent sites; 'the center of the basin (CB) site' and 'the chimney hill (CH) site'. The CH site locates about 200 m north from the CB site. In CB site, three chimney structures are observed and the temperatures of the vent emissions were above 230°C. On the other hand, more than five chimney structures crowd in very narrow area in the CH site. Several emissions in CH site are almost boiling and the temperatures were above 270°C. Previous geochemical analyses of hydrothermal fluids from both CB and CH sites indicate that these hydrothermal fluids have single origin and do not differentiate each other (Chiba et al. 1993). The discussion is based on just three vent emissions and seems to contradict the observation of liquid carbon dioxide in this area. If the hypothesis is correct, it is the very rare case in the studies of hydrothermal activity in the Okinawa Trough. In addition, previous microbiological researches show the importance of phase separation of hydrothermalism in diversification of microbial ecosystem associated with hydrothermal activity (Nakagawa et al. 2005; Takai et al. 2007). Thus, the intra field comparison of vent emissions in geochemistry and microbiology is the major objective of this cruise.



Map 2. Minami-Ensei Knoll

4. Preliminary Results4.1. Microbiology

S. Nakagawa

During the NT07-11, we collected various hydrothermal samples including hydrothermal plumes, vent fluids, chimney structures, and vent animals from Iheya North hydrothermal field and Minami-Ensei Knoll. 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 μ m pore size filters, and then immediately stored at -80°C. 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 $^{\circ}$ 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 NT07-11, we collected various hydrothermal samples from Iheya North hydrothermal field and Minami-Ensei Knoll using WHATS, Bag, and Niskin samplers. Several geochemical parameters have been analyzed onboard to avoid chemical alterations during sample storage as follows.

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 SiO₂ and NH₄, water samples filtered by 0.2µm were used. Using a colorimeter (Hach, DR2010), concentrations of dissolved silica (SiO₂) and ammonium ion (NH₄) were analyzed following classical methods; molybdenum blue method (λ =812nm) for SiO₂ and indo-phenol method (λ =640nm) for NH₄. 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 (SiO₂) and of interference by specific species (NH₄).

| Dive No. | Sampler | | Depth | pH | Alk. | SiO2 | NH4 |
|-------------|------------|------------------------------|-------|------|------|-------|------|
| | | | m | | | ug/L | uM |
| #696 | B1 | Galetheid colony | 980 | 7.29 | 2.47 | 125 | 11 |
| (6/19/2007) | B2 | Polychaete colony | 980 | 7.39 | 2.27 | 254 | <1 |
| | N1 | Reference | 1054 | 7.38 | 2.30 | 114 | 7 |
| | N2 | | | 7.58 | 2.45 | 110 | <1 |
| | W1 | Galetheid colony | 980 | N.D. | 2.37 | 146 | 2 |
| | W4 | Polychaete colony | 980 | 6.6 | 3.09 | 430 | <1 |
| #697 | B1 | Bathymodiolus colony | 691 | 7.51 | 2.82 | 65 | <1 |
| (6/20/2007) | B3 | Bathymodiolus colony | 691 | 7.31 | 2.60 | 66 | <1 |
| | B4 | Bathymodiolus colony | 705 | 7.31 | 2.59 | 68 | 1 |
| | N1 | Bathymodiolus colony | 705 | 7.56 | 2.51 | 63 | <1 |
| | Takai | | | 5.6 | 3.82 | 10283 | 611 |
| | W1 | 250deg vent | 691 | 5.98 | 3.33 | 10168 | 482 |
| | W3 | Bathymodiolus colony | 705 | 6.01 | 3.39 | 9638 | 524 |
| #698 | B 1 | Reference | 698 | 4.76 | 3.03 | N.D. | N.D. |
| (6/21/2007) | B 3 | Just above vent | 700 | 5.43 | 2.68 | 1793 | 85 |
| | B 4 | Just above vent | 700 | 5.18 | 2.86 | 1821 | 89 |
| | N1 | Reference | 698 | N.D. | N.D. | 4794 | 156 |
| | W1 | H697-2 marker | 700 | 5.37 | 3.38 | 4909 | 188 |
| | W3 | chimney 4 vent | 692 | 5.36 | 3.27 | 63 | 2 |
| #699 | B1 | Bathymodiolus with Galetheid | 700 | N.D. | N.D. | 65 | <1 |
| (6/21/2007) | W1 | H699-1 marker | 700 | 4.94 | 3.35 | 12046 | 524 |
| | W3 | Morinaga site | 707 | 5.15 | 3.20 | 9853 | 534 |
| #700 | WI | Diffuse flow | 707 | 5.42 | 3.08 | 290 | 16 |
| (6/22/2007) | W3 | Diffuse flow | 706 | 5.67 | 2.92 | 99 | 9 |
| #702 | W1 | Bathymodiolus colony | 995 | 5.95 | 2.58 | 129 | <1 |
| (6/23/2007) | B1 | Bathymodiolus colony | 995 | 5.86 | 2.50 | 121 | <1 |
| #703 | WI | Bathymodiolus colony | 690 | 7.18 | 2.51 | 86 | <1 |
| (6/24/2007) | W3 | Bathymodiolus with Galetheid | 700 | 7.05 | 2.91 | 76 | <1 |
| #704 | B2 | Paralvinella vent | 709 | 5.96 | 2.43 | 372 | 14 |
| (6/25/2007) | N1 | Paralvinella vent | 709 | 7.38 | 2.71 | 70 | <1 |
| | N2 | Vent fluids | 700 | N.D. | N.D. | 79 | <1 |
| | W1 | Paralvinella vent | 709 | 5.44 | 2.99 | 1606 | 85 |
| | W3 | Vent fluids | 700 | 5.44 | 3.38 | 11071 | 829 |

4.2.3. Result of onboard analysis

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 °C |
| Resolution | 0.001 °C |
| 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.

| | dive | date | bottom | penetrat | pull | latitude | lontitude |
|-------------|-------|-----------|----------|----------|----------|--------------------|-------------|
| | ui ve | uutt | temp | e | P an | | 10111111111 |
| 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 | 16:22:31 28-23.286 | |
| 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 |
| ongoi ghf4 | 704 | 2007 6 25 | 11.20.27 | 11.52.50 | 12.11.00 | concentrated | nearby |
| enser_sm4 | 704 | 2007.0.23 | 11.39.27 | 11.55.58 | 12.11.00 | obserbation | shf2 |
| ongoi ghf5 | | | | 12.16.44 | 12.24.27 | concentrated | nearby |
| enser_sm5 | | | | 12.10.44 | 12.34.27 | obserbation | shf2 |
| anaai ahf6 | | | | 12.29.06 | 12.10.21 | concentrated | nearby |
| enser_smo | | | | 12.38.00 | 15.10.21 | obserbation | 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 |

Fig2. Operation of SAHF

4. Results

Fig3. All data









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, and 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 sulfur oxidizers Epsilonproteobacteria, autotrophic such as Aquificales, Alphaproteobacteria, Gammaproteobacteria and Epsilonproteobacteria, nitrate or nitrite reducers such as Aquificales, Deferribacteriales, 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.

FISH (Fluorescence In Situ Hybridization) is a method for microscopic observation of cells. This technique can visualize the results of clone analysis of 16S rDNA. Hydrothermal plume harbored chemolithoautotrophic microbes and the microbes were detected as elevated microbial population compared to surrounding non-hydrothermal deep-sea water.

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 ¹³C-labelled CO_2 will be incorporated into cellular molecules including DNA. The ¹³C-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 to collect try concentrated bacterial cells from a large of We quantity seawater. are constructing "in situ filtration system", which consists of an oil pressure pump, tandem connected filter membranes, and flow meter. We examine the system in this cruse.





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 ¹⁴CO₂ and clarify the phylogenetic affiliation of the autotrophic microbial cells in the hydrothermal environment at a single cell level.

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.

5.2 Biogeochemistry

Hydrogen gas

S. Kawagucci, T. Narita

Hydrogen gas (H₂) 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 H₂ concentration and its isotope ratio (δ D) were developed. In this cruise, we try to understand behavior of H₂ by determining both H₂ concentrations and their δ D values. Moreover, in order to investigate hydrogen isotope behavior on biological consumption of H₂, we also quantify H₂ concentrations and δ D_{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 120cm³ 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 HgCl₂ saturated solution into a vial and cap a vial using Teflon-coated Butyl-rubber septum in order to avoid escape and consumption of H₂ during sample storage. Seafloor sediment is collected using a MBARI corer. An approximately 30cm³ sediment is subsampled from MBARI sampler into a 50cm³ disposal syringe, and is then pressed for extracting pore water. Extracted pore water sample is introduced through 0.45µm filter into 5cm³ vial provided HgCl₂ and amidosulfate powders respectively for poison and acidifying.

Both H₂ 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, H₂ in the introduced gas is separated from the other gas species, concentrated for high sensitivity analysis, and introduced into a mass spectrometer (Thermo Fisher Scientific, DELTA^{XP}) for quantification of both H₂ 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

Akiko 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 Minami Ensei, 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 sampled water surrounding the colony of galetheid crab and landed on sea floor (Dive#696) and picked up samples (Dive#701), and then also sampled water surrounding the Bathymodiolus colony and landed on sea floor (Dive#702). In Minami Ensei, we sampled water surrounding the Bathymodiolus colony and landed it in ship.

We use both ¹⁵N additional method and natural stable isotopes to measure nitrification rates. In ¹⁵N additional method, we calculate nitrification rates from increase of ¹⁵N nitrite and nitrate in the batch added ¹⁵N 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 ¹⁵N tracer. We tried incubation experiments of sample water added nothing(control), low amount of ¹⁵N ammonium, high amount of ¹⁵N ammonium, and ¹⁵N 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 and Minami Ensei.

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 HgCl₂.

6. Sample list

NT07-11 Sample distribution (Microbiology)

| | Sample | | Depth (m) | Time (WHATS) | Temp (av.) (WHATS) | Description |
|------------------------|-----------------|------------------------------|--------------|-----------------|---------------------|---|
| #696 | Niskin1 | Reference | 1054 | (111115) | Temp (av.) (WIIITI) | 25ml CLT (SUGAR) 100ml FISH (Univ of Tokyo) |
| (6/19/2007) | MBARI | licitite | 1064 | | | 100ml CLT (SUGAR), 50ml FISH (Univ of Tokyo) |
| Iheva North | W1 | Galetheid colony | 980 | 11.48-11.54 | 48-56(52) | 25ml CLT (SUGAR) |
| | R1 | Galetheid colony | 980 | 11.40 11.54 | 4.0 5.0 (5.2) | 18000ml Phage&Bacteria (SUGAR) 100ml FISH (Univ of Tokyo) |
| | Galetheid crah | Saletherd colony | 980 | | | 200 individual (SUGAR) |
| | W4 | Polychaete colony | 980 | 12.48-13.05 | 5 4-23 1 (12 5) | 25ml CLT (SUGAR) |
| | R2 | Polychaete colony | 980 | 12.40 15.05 | 5.4 25.1 (12.5) | 18000ml Phage&Bacteria (SUGAR) |
| | Polychaete nest | r oryenaete colony | 980 | | | 200g CLT&MOL (SUGAR) 50g (Ueno) |
| #697 | Chimney? | 697-1 marker | 691 | | | 10g CLT (SUGAR) 20g Mol (SUGAR) |
| (6/20/2007) | W1 | 250deg vent | 691 | 11.32-11.34 | 189 4-253 9 (230 6) | 25ml CLT (SUGAR) |
| Minami- | B1 | Bathymodiolus colony | 691 | | | 25ml CLT (SUGAR), 15000ml Phage (SUGAR), 100ml FISH (Univ of Tokyo) |
| Ensei | B3 | Bathymodiolus colony | 691 | | | 25ml CLT (SUGAR), 4000ml Mol (SUGAR), 100ml FISH (Univ of Tokyo) |
| | W3 | Bathymodiolus colony | 705 | 13:11-13:16 | 185.5-261.4 (231.3) | 25ml CLT (SUGAR) |
| | B4 | Bathymodiolus colony | 705 | 13:43 | | 25ml CLT (SUGAR), 8500ml Mol (SUGAR), 100ml FISH (Univ of Tokyo) |
| | NI | Bathymodiolus colony | 705 | | | 25ml CLT (SUGAR), 100ml FISH (Univ of Tokyo) |
| | Chimnev1 | | | | | 50g CLT (SUGAR), 150g Mol (SUGAR), 10g FISH (Univ of Tokyo) |
| #698 | NI | Reference | 698 | 9:17 | | 25ml CLT (SUGAR), 100ml FISH (Univ of Tokyo) |
| (6/21/2007) | B1 | Reference | 698 | 9:22 | | 25ml CLT (SUGAR), 15000ml Phage (SUGAR), 100ml FISH (Univ of Tokyo) |
| Minami- | Chimnev3 | H697-2 marker | 700 | 9:34 | | 50g CLT (SUGAR), 150g Mol (SUGAR), 10g FISH (Univ of Tokyo) |
| Ensei | W1 | H697-2 marker | 700 | 9:43-9:48 | 83.3-140.1 (113.4) | 25ml CLT (SUGAR) |
| | B3 | Just above vent | 700 | 10:00 | | 25ml CLT (SUGAR), 15000ml Phage (SUGAR), 100ml FISH (Univ of Tokyo) |
| | B4 | Just above vent | 700 | 10:05 | | 25ml CLT (SUGAR), 15000ml Phage (SUGAR), 100ml FISH (Univ of Tokyo) |
| | Chimney4 | | 692 | | | 10g CLT (SUGAR), 30g Mol (SUGAR), 2g FISH (Univ of Tokyo) |
| | W3 | chimney 4 vent | 692 | 10:43-10:47 | 92.1-134.6 (110.2) | 25ml CLT (SUGAR) |
| #699 | B1 | Bathymodiolus with Galetheid | 700 | | | 25ml CLT (SUGAR), 15000ml Phage (SUGAR), 100ml FISH (Univ of Tokyo) |
| (6/21/2007) | B2 | Bathymodiolus with Galetheid | 700 | | | 25ml CLT (SUGAR), 15000ml Phage (SUGAR), 100ml FISH (Univ of Tokyo) |
| Minami- | Galetheid crab | | 700 | | | 10 individuals (SUGAR) |
| Ensei | W1 | H699-1 marker | 700 | 14:47-14:51 | 233-281 (264.2) | 25ml CLT (SUGAR) |
| | W3 | Morinaga site | 707 | 15:28-15:33 | 248.2-262.6 (259.0) | 25ml CLT (SUGAR) |
| | N1 | Morinaga site | 707 | 15:42 | | 25ml CLT (SUGAR), 100ml FISH (Univ of Tokyo) |
| #700 | W1 | Diffuse flow | 707 | 11:14-11:19 | 7.3-11.8 (9.5) | 25ml CLT (SUGAR) |
| (6/22/2007) | W3 | Diffuse flow | 706 | 13:16-13:21 | 8.8-11.9 (10.2) | 25ml CLT (SUGAR) |
| #701 | 6B-1 | Deployed during dive 696 | 986 | | | 2300ml Scintillation (SUGAR), 160ml MAR-FISH (Univ. of Tokyo) |
| (6/23/2007) | 6B-2 | Immediately above galetheid | 986 | | | 400ml Scintillation (SUGAR), 160ml MAR-FISH (Univ. of Tokyo) |
| Iheya North | 6B-3 | crabs | 986 | | | 2500ml Mol (SUGAR) |
| | 6B-4 | | 986 | | | 2500ml Mol (SUGAR) |
| | 6B-5 | | 986 | | | 2500ml Mol (SUGAR) |
| | 6B-6 | | 986 | | | 2500ml Mol (SUGAR) |
| #702 | W1 | Bathymodiolus colony | 995 | 13:05-13:10 | 4.4-4.7 (4.6) | 25ml CLT (SUGAR) |
| (6/23/2007) | B1 | Bathymodiolus colony | 995 | 13:22-13:25 | | 25ml CLT (SUGAR), 15000ml Phage (SUGAR), 100ml FISH (Univ of Tokyo) |
| Ineya North | Filtration | Galetheid colony | 980 | | | 135L (SUGAR) |
| #705 | W1 | Bathymodiolus colony | 690 | 13:41-13:46 | 7.3-7.8 (7.5) | 25ml CLT (SUGAR) |
| #704 | W3 | Bathymodiolus with Galetheid | 700 | 14:00-14:05 | 7.3-7.5 (7.5) | 25ml CLT (SUGAR) |
| #704 | BI | Paralvinella vent | 709 | | | 25ml CLT (SUGAR), 15000ml Phage (SUGAR), 100ml FISH (Univ of Tokyo) |
| (0/25/2007) Minami- | D2 | Paralvinella vent | 709 | | | 25ml CL1 (SUGAR), ISOUOMI Phage (SUGAR), 100ml FISH (Univ of Tokyo) |
| Ensei | NI W1 | Paraivinella vent | 709 | 0.50 10.04 | 96.0 116.0 (100.2) | 25mi CL1 (SUGAR), 100ml FISH (Univ of Tokyo) |
| Liisei | W I | Paraivinella vent | 709 | 9:59-10:04 | 80.9-110.9 (100.3) | 25ml CL1 (SUGAR) |
| | W 3 | vent fluids | 700 | 12:54-12:59 | 251.4-275.6 (262.3) | 25ml CL1 (SUGAK) |
| | INZ | vent fluids | 700 | | | 25mi CL1 (SUGAR), 100mi FISH (Univ of Tokyo) |
| | MDAKII | | 700 | | | SUG MOL (SUGAR), SUG (UNIV OF 10KYO) |
| | D2 | Pathumodialus with Colet-1 | 700 | | | 201 Mal (SUGAR) |
| | DJ | Bathymoatolus with Galetheid | 700 | | | 20L (NUCAR) 260L (SUCAR) |
| | ringation | bainymoatotus with Galetheid | 700 | | | JUUL (SUGAR) |

| Species | Inds No. | Locality | Dive No. | Lat. | | Long. | | Fixation |
|------------------------------|----------|--------------|----------|------|-----------|-------|----------|-------------------|
| Shinkaia crosnieri | ca.100 | North Iheya | HD#696 | 27 ° | 47.452 'N | 126° | 53.8 'E | alive |
| Shinkaia crosnieri | ca.100 | North Iheya | HD#696 | 27 ° | 47.452 'N | 126° | 53.8 'E | Freeze |
| <i>Lebbeus</i> sp. | 9 | Minami-Ensei | HD#697 | 28 ° | 23.498 'N | 127° | 38.37 'E | 99.5%Ethanol |
| Alvinocaris brevitelsonis | 21 | Minami-Ensei | HD#697 | 28 ° | 23.498 'N | 127° | 38.37 'E | 99.5%Ethanol |
| Shinkaicaris leurokolos | 30 | Minami-Ensei | HD#697 | 28 ° | 23.498 'N | 127° | 38.37 'E | 99.5%Ethanol |
| Unidentifies Echinodermata | 2 | Minami-Ensei | HD#697 | 28 ° | 23.498 'N | 127° | 38.37 'E | 99.5%Ethanol |
| Unidentifies Nemertini | 2 | Minami-Ensei | HD#697 | 28 ° | 23.498 'N | 127° | 38.37 'E | 99.5%Ethanol |
| Paralomis jamsteci | 1 | Minami-Ensei | HD#697 | 28 ° | 23.498 'N | 127° | 38.37 'E | −30 deg. C freeze |
| Bathymodiolus japonicus | 23 | Minami-Ensei | HD#697 | 28 ° | 23.498 'N | 127° | 38.37 'E | alive |
| Alvinocaris brevitelsonis | 11 | Minami-Ensei | HD#699 | 28 ° | 23.48 'N | 127° | 38.4 'E | 99.5%Ethanol |
| <i>Provanna</i> sp. | 15 | Minami-Ensei | HD#699 | 28 ° | 23.48 'N | 127° | 38.4 'E | 99.5%Ethanol |
| Cyathermia sp. | 4 | Minami-Ensei | HD#699 | 28 ° | 23.48 'N | 127° | 38.4 'E | 99.5%Ethanol |
| Bathymodiolus japonicus | 8 | Minami-Ensei | HD#699 | 28 ° | 23.48 'N | 127° | 38.4 'E | alive |
| Shinkaia crosnieri | 4 | Minami-Ensei | HD#699 | 28 ° | 23.48 'N | 127° | 38.4 'E | Freezed |
| Alvinocaris brevitelsonis | 17 | Minami-Ensei | HD#700 | 28 ° | 23.347 'N | 127° | 38.41 'E | 99.5%Ethanol |
| Lebbeus sp. | 5 | Minami-Ensei | HD#700 | 28 ° | 23.347 'N | 127° | 38.41 'E | 99.5%Ethanol |
| <i>Olgasolaris</i> sp. | 4 | Minami-Ensei | HD#700 | 28 ° | 23.347 'N | 127° | 38.41 'E | 99.5%Ethanol |
| Bathymodiolus japonicus | 15 | Minami-Ensei | HD#700 | 28 ° | 23.347 'N | 127° | 38.41 'E | alive |
| Unidentified gastropod | 1 | Minami-Ensei | HD#700 | 28 ° | 23.301 'N | 127° | 38.38 'E | 10% Formalin |
| Unidentified Scorpeniformis | 1 | Minami-Ensei | HD#700 | 28 ° | 23.313 'N | 127° | 38.38 'E | 10% Formalin |
| Unidentified Scorpeniformis | 1 | Minami-Ensei | HD#700 | 28 ° | 23.313 'N | 127° | 38.38 'E | 10% Formalin |
| Zoarcidae gen. sp. | 1 | Minami-Ensei | HD#700 | 28 ° | 23.367 'N | 127° | 38.41 'E | alive |
| Zoarcidae gen. sp. | 1 | Minami-Ensei | HD#700 | 28 ° | 23.367 'N | 127° | 38.41 'E | −30 deg. C freeze |
| Austinograea yunohana | 1 | Minami-Ensei | HD#703 | 28 ° | 23.37 'N | 127° | 38.4 'E | −30 deg. C freeze |
| Zoarcidae gen. sp. | 3 | Minami-Ensei | HD#703 | 28 ° | 23.37 'N | 127° | 38.4 'E | alive |
| Zoarcidae gen. sp. | 1 | Minami-Ensei | HD#703 | 28 ° | 23.37 'N | 127° | 38.4 'E | −30 deg. C freeze |
| Bathymodiolus japonicus | 3 | Minami-Ensei | HD#703 | 28 ° | 23.37 'N | 127° | 38.4 'E | −30 deg. C freeze |
| Bathymodiolus japonicus | 2 | Minami-Ensei | HD#703 | 28 ° | 23.37 'N | 127° | 38.4 'E | 10% Formalin |
| Bathymodiolus japonicus | 10 | Minami-Ensei | HD#703 | 28 ° | 23.37 'N | 127° | 38.4 'E | alive |
| Lebbeus sp. | 16 | Minami-Ensei | HD#703 | 28 ° | 23.37 'N | 127° | 38.4 'E | 99.5%Ethanol |
| Alvinocaris brevitelsonis | 17 | Minami-Ensei | HD#703 | 28 ° | 23.37 'N | 127° | 38.4 'E | 99.5%Ethanol |
| Unidentified caridean shrimp | 1 | Minami-Ensei | HD#703 | 28 ° | 23.37 'N | 127° | 38.4 'E | 99.5%Ethanol |
| <i>Lepetodrilus</i> sp. | 21 | Minami-Ensei | HD#703 | 28 ° | 23.37 'N | 127° | 38.4 'E | 99.5%Ethanol |
| <i>Olgasolaris</i> sp. | 7 | Minami-Ensei | HD#703 | 28 ° | 23.37 'N | 127° | 38.4 'E | 99.5%Ethanol |
| Solemydae gen. sp. | 1 | Minami-Ensei | HD#703 | 28 ° | 23.55 'N | 127° | 38.47 'E | 99.5%Ethanol |
| Polynoidae gen. sp. | 1 | Minami-Ensei | HD#703 | 28 ° | 23.37 'N | 127° | 38.4 'E | 10% Formalin |
| Unidentified polychaete | 1 | Minami-Ensei | HD#703 | 28 ° | 23.55 'N | 127° | 38.47 'E | 10% Formalin |
| Cantrainea jamsteci | 4 | Minami-Ensei | HD#703 | 28 ° | 23.484 'N | 127° | 38.4 'E | 10% Formalin |

| Sample distribution of ext | racted gas | | | | | |
|----------------------------|--------------------|---------------------------|----------|-------------------------|-----------------------|------------------------|
| Sample ID | Field | Site | T max | TGC | Packing pressure | Distribution |
| HPD#696 W2 | Iheya North | NBC Galetheid's colony | 6.6 °C | 2.71 mM | 5.05 kPa (100.5 kPa) | ORI-H2, 50 ml bottle |
| | • | - | | | | ORI-He, 50 ml Pb glass |
| | | | | | | TITECH, 100 ml glass |
| HPD#696-W3 | Iheya North | NBC Paralvinella's colony | 30.2 °C | 17.21 mM | 15.08 kPa (100.5 kPa) | ORI-H2, 50 ml bottle |
| | | | | | | ORI-He, 50 ml Pb glass |
| | | | | | | TITECH, 100 ml glass |
| | | | | | 14.37 kPa (100.5 kPa) | SUGAR, 50 ml bottle |
| HPD#697-W2 | Minami Ensei Knoll | Marker 697-3 chimney | 236.6 °C | 41.56 mM | 58.52 kPa (100.5 kPa) | ORI-H2, 50 ml bottle |
| (Heavy air contamination) |) | | | (pure hydrothermal gas) | | TITECH, 100 ml glass |
| | | | | | | SUGAR, 50 ml bottle |
| HPD#697-W4 | Minami Ensei Knoll | Yajiri chimney | 274.1 °C | 82.93 mM | 80.21 kPa (100.5 kPa) | ORI-H2, 50 ml bottle |
| | | | | | | ORI-He, 50 ml Pb glass |
| | | | | | | TITECH, 100 ml glass |
| HPD#697-TakaII-Red | Minami Ensei Knoll | Yajiri chimney | 274.1 °C | 84.47 mM | 51.89 kPa | ORI-H2, 50 ml bottle |
| | | | | | | TITECH, 100 ml glass |
| | | | | | | SUGAR, 50 ml bottle |
| HPD#698-W2 | Minami Ensei Knoll | Marker 697-2 chimney | 280.4 °C | 61.75 mM | 63.70 kPa | ORI-H2, 50 ml bottle |
| | | | | | | TITECH, 100 ml glass |
| | | | | | | SUGAR, 50 ml bottle |
| HPD#698-W4 | Minami Ensei Knoll | Jizo chimney | 266.2 °C | 58.74 mM | 58.74 kPa | ORI-H2, 50 ml bottle |
| | | | | | | TITECH, 100 ml glass |
| | | | | | | SUGAR, 50 ml bottle |
| HPD#699-W2 | Minami Ensei Knoll | Marker 699-1 chimney | 281.5 °C | 114.39 mM | 94.95 kPa | ORI-H2, 50 ml bottle |
| (Filtrate lost no Mg data) | | | | | | TITECH, 100 ml glass |
| | | | | | | SUGAR, 50 ml bottle |
| HPD#699-W4 | Minami Ensei Knoll | Taimatsu chimney | 266.4 °C | 83.19 mM | 94.85 kPa | ORI-H2, 50 ml bottle |
| | | | | | | TITECH, 100 ml glass |
| | | | 110.00 | 2 00 36 | | SUGAR, 50 ml bottle |
| HPD#/00-W2 | Minami Ensei Knoll | 12 °C diffusing flow | 14.9 °C | 7.09 mM | 12.65 kPa | ORI-H2, 50 ml bottle |
| | | | | | | TITECH, 100 ml glass |
| | | | 10.0.00 | (= 0) (| 0.00 L D | SUGAR, 50 ml bottle |
| HPD#/00-W4 | Minami Ensei Knoll | shoboshobo flow | 12.9 °C | 4.78 mM | 8.80 kPa | ORI-H2, 100 ml glass |
| | | | | | | TITECH, 100 ml glass |
| | | | | | 0.00 L D | SUGAR, 50 ml bottle |
| HPD#/01-W2 | Iheya North | NBC mussel's colony | | 2.27 mM | 8.80 kPa | ORI-H2, 100 ml glass |
| | | | | | | TITECH, 100 ml glass |
| | | | | | | SUGAR, 50 ml bottle |

| 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 | | | | |
| U | 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 sam | pler II | | | |
| | Anion | ORI | 10 | * |
| | Cation | ORI | 20 | * |
| | nH Alk Halogen | ORI | 10 | |
| | pri, mix., maiogen | | | |
| | H2S | titech | 30 | |
| | H2S CH4 | titech titech | 30 50 | |

Water Sample distribution (geochemistry)

* filtered

| | Sampler | | Depth (m) | 11me (WHA1S) | Temp (av.) (WHATS) | Description |
|-------------|---------|------------------------------|-----------|--------------|---------------------|---------------------------|
| #696 | NI | Reference | 1054 | | | 540ml(ORI), 760ml(Titech) |
| (6/19/2007) | MBARI | | 1064 | | | |
| | W1 | Galetheid colony | 980 | 11:48-11:54 | 4.8-5.6 (5.2) | 60ml (ORI), 10ml(Titech) |
| | B1 | Galetheid colony | 980 | | | 240ml(ORI), 630ml(Titech) |
| | W4 | Polychaete colony | 980 | 12:48-13:05 | 5.4-23.1 (12.5) | 60ml (ORI), 10ml(Titech) |
| | B2 | Polychaete colony | 980 | | | 240ml(ORI), 630ml(Titech) |
| #697 | W1 | 250deg vent | 691 | 11:32-11:34 | 189.4-253.9 (230.6) | 60ml (ORI), 10ml(Titech) |
| (6/20/2007) | B1 | Bathymodiolus colony | 691 | | | 240ml(ORI), 630ml(Titech) |
| | B3 | Bathymodiolus colony | 691 | | | 240ml(ORI), 630ml(Titech) |
| | W3 | Bathymodiolus colony | 705 | 13:11-13:16 | 185.5-261.4 (231.3) | 60ml (ORI), 10ml(Titech) |
| | B4 | Bathymodiolus colony | 705 | 13:43 | | 240ml(ORI), 630ml(Titech) |
| | N1 | Bathymodiolus colony | 705 | | | 540ml(ORI), 760ml(Titech) |
| #698 | N1 | Reference | 698 | 9:17 | | 540ml(ORI), 760ml(Titech) |
| (6/21/2007) | B1 | Reference | 698 | 9:22 | | 240ml(ORI), 630ml(Titech) |
| | W1 | H697-2 marker | 700 | 9:43-9:48 | 83.3-140.1 (113.4) | 60ml (ORI), 10ml(Titech) |
| | B3 | Just above vent | 700 | 10:00 | | 240ml(ORI), 630ml(Titech) |
| | B4 | Just above vent | 700 | 10:05 | | 240ml(ORI), 630ml(Titech) |
| | W3 | chimney 4 vent | 692 | 10:43-10:47 | 92.1-134.6 (110.2) | 60ml (ORI), 10ml(Titech) |
| #699 | BI | Bathymodiolus with Galetheid | 700 | | | 240ml(ORI), 630ml(Titech) |
| (6/21/2007) | B2 | Bathymodiolus with Galetheid | 700 | | | 240ml(ORI), 630ml(Titech) |
| | W1 | H699-1 marker | 700 | 14:47-14:51 | 233-281 (264.2) | 60ml (ORI), 10ml(Titech) |
| | W3 | Morinaga site | 707 | 15:28-15:33 | 248.2-262.6 (259.0) | 60ml (ORI), 10ml(Titech) |
| | N1 | Morinaga site | 707 | 15:42 | | 540ml(ORI), 760ml(Titech) |
| #700 | W1 | Diffuse flow | 707 | 11:14-11:19 | 7.3-11.8 (9.5) | 60ml (ORI), 10ml(Titech) |
| (6/22/2007) | W3 | Diffuse flow | 706 | 13:16-13:21 | 8.8-11.9 (10.2) | 60ml (ORI), 10ml(Titech) |
| #702 | W1 | Bathymodiolus colony | 995 | 13:05-13:10 | 4.4-4.7 (4.6) | 60ml (ORI), 10ml(Titech) |
| (6/23/2007) | B1 | Bathymodiolus colony | 995 | 13:22-13:25 | | 240ml(ORI), 630ml(Titech) |
| #703 | W1 | Bathymodiolus colony | 690 | 13:41-13:46 | 7.3-7.8 (7.5) | 60ml (ORI), 10ml(Titech) |
| (6/24/2007) | W3 | Bathymodiolus with Galetheid | 700 | 14:00-14:05 | 7.3-7.5 (7.5) | 60ml (ORI), 10ml(Titech) |
| #704 | B1 | Paralvinella vent | 709 | | | 240ml(ORI), 630ml(Titech) |
| (6/25/2007) | B2 | Paralvinella vent | 709 | | | 240ml(ORI), 630ml(Titech) |
| | N1 | Paralvinella vent | 709 | | | 540ml(ORI), 760ml(Titech) |
| | W1 | Paralvinella vent | 709 | 9:59-10:04 | 86.9-116.9 (100.3) | 60ml (ORI), 10ml(Titech) |
| | W3 | Vent fluids | 700 | 12:54-12:59 | 231.4-275.6 (262.3) | 60ml (ORI), 10ml(Titech) |
| | N2 | Vent fluids | 700 | | | 540ml(ORI), 760ml(Titech) |
| | MBARI1 | | 700 | | | 5ml(ORI) |
| | MBARI2 | | 700 | | | 5ml(ORI) |
| | B3 | Bathymodiolus with Galetheid | 700 | | | 240ml(ORI), 630ml(Titech) |

Geochemistry

Satoshi Nakagawa

7. Dive Report

7-1. Dive Report #696

Date: June 19, 2007 Site: Iheya North Landing: 9:32; 27°47.417'N, 126°54.052'E, 1054m Leaving: 15:06; 27°47.687'N, 126°53.534'E, 856m

Objectives:

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

Dive Summary:

We landed on seafloor near the event no. 4 (*Calyptogena* colony). Then, ROV headed to west. At the middle of event no. 4 and 6, we performed the first SAHF measurement. At this area, we also collected two sediment cores using MBARI samplers (labeled by yellow and green tapes). After the sediment sampling, we headed to event no. 12. By the way to event no 12, we thus performed seawater collection by using Niskin bottle, since previous survey using URASHIMA suggested that hydrothermal fluids are emanated from seafloor there. At the 20m east from event no. 12, we did 2nd SAHF measurement and set #696 marker. Then, we headed to event no. 7 (North Big Chimney [NBC]).

First, we collected fluids surrounding galetheid crab by using WHATS sampler (2 bottles). The temperature of fluids was approximately 5 °C. At the same point, we performed pump sampling using 20L bag (x1), and 6L bag (x6). In addition, we collected a lot of galetheid crabs by using slurp gun. The RI-bag sampler was deployed at the basement of NBC mound (depth = 987m). Then, we climbed the mount again, and collected fluids surrounding polychaete colony by using WHATS sampler (2 bottles) and 20L bag (x1). We also collected the nest of polychaete.

After the sampling at NBC, we headed to NW. By the way to climb up the mound, we found lots of shells of *Bathymodiolus* or *Calyptogena*. We performed SAHF

measurement and collected rocks.

Payloads:

- 1) WHATS with a temperature probe
- 2) Bag pomp sampler (20L x 4)
- 3) Sample box
- 4) Niskin bottles (2 bottles)

5) DO meter
6) Turbidity meter
7) Bag pomp sampler (6L x 6)
8) MBARI corer x 2

Event List:

| 9:34 | 27-47.417N, 126-54.052E | D=1054m | Seawater sampling (Niskin [red]) |
|-------|-------------------------|---------|-------------------------------------|
| 9:50 | 27-47.415N, 126-54.038E | D=1064m | SAHF measurement |
| 10:07 | 27-47.415N, 126-54.038E | D=1064m | MBARI (yellow) |
| 10:09 | 27-47.415N, 126-54.038E | D=1064m | MBARI (green) |
| 10:25 | 27-47.409N, 126-53.909E | D=1046m | Niskin (green; 170m east of SBC) |
| 11:01 | 27-47.372N, 126-53.803E | D=1017m | SAHF measurement (2 nd) |
| 11:48 | 27-47.452N, 126-53.795E | D=980m | WHATS sampling (1 st) |
| 11:55 | 27-47.452N, 126-53.795E | D=980m | WHATS sampling (2nd) |
| 12:02 | 27-47.452N, 126-53.795E | D=980m | Bag sampling (20L x 1) |
| 12:09 | 27-47.452N, 126-53.795E | D=980m | RI-Bag sampling |
| 12:30 | 27-47.452N, 126-53.795E | D=980m | Galetheid crab sampling |
| 12:41 | 27-47.456N, 126-53.801E | D=987m | RI-Bag deployment |
| 12:52 | 27-47.452N, 126-53.795E | D=980m | WHATS sampling (3 rd) |
| 12:59 | 27-47.452N, 126-53.795E | D=980m | WHATS sampling (4th) |
| 13:06 | 27-47.452N, 126-53.795E | D=980m | Bag sampling (20L x 1) |
| 13:17 | 27-47.452N, 126-53.795E | D=980m | Polychaete nest sampling |
| 13:54 | 27-47.686N, 126-53.655E | D=965m | SAHF measurement (3 rd) |
| 14:23 | 27-47.684N, 126-53.643E | D=897m | Rock sampling (pumice?) |
| 14:32 | 27-47.682N, 126-53.587E | D=898m | Rock sampling |
| 14:37 | 27-47.682N, 126-53.570E | D=883m | Rock sampling (large piece) |
| 15:00 | 27-47.686N, 126-53.541E | D=868m | Rock sampling |
| 15:06 | 27-47.687N, 126-53.534E | D=856m | Leaving bottom |



Dive track:

Takuro Nunoura

7-2. Dive Report #697

Date: June 20, 2007 Site: Minami-Ensei Knoll Landing: 9:15; 28°23.282'N, 127°38.351'E, 709m Leaving: 14:28; 28°23.361'N, 127°38.396'E, 710m

Objectives:

The major objectives are 1) to explore hydrothermal vents, 2) to collect hydrothermal fluids, chimney structure, hydrothermal plumes, vent animals and sediments.

Dive Summary:

We landed on sandy sediments about 30 m southwest from the end of the hydrothermal vent area. We soon found a tiny hydrothermal vent and measured fluids temperature (>90°C). Then, we continued to go northeast. The sediments of hydrothermal field were covered by numbers of white sponges that look like deep-sea coral. About 150 m northwest from the first hydrothermal vent, we arrived at the CB site and found a nice chimney structure on a hydrothermal mound. A chimney stood on the center of a hydrothermal mound that diameter was about 10 m. There were no vent animals on this mound and the mound was surrounded by *Bathymodiolus* colonies, but *Galatheid* crab colonies were not distributed with *Bathymodiolus*. That was the typical distribution of chemosynthetic communities in this hydrothermal field. We found a buried blue sample box that was recorded in the event map before 1993. Therefore, the chimney structure was identified as the 'Taimatsu chimney'. In addition, sedimentation rate of sulfide was about 15-20cm / 15 years. We deployed no. 697-1 marker and left the vent. Then, the ROV went to the CH site.

In the CH site, we soon found a chimney structure that was similar to the 'Taimatsu chimney'. We dropped a marker no. 697-2 and continued to observe vent distribution in this area. We turned to northwest and found more than 5 active chimneys. In order to determine the CH site area, the ROV head to east. We soon arrived at the edge of the CH site and found *Calyptogena* colony. The CH site was smaller than 50 m square. In this site, we took a small chimney structure, vent emissions (max temp. 251°C) by

WHATS, plume water on *Bathymodiolus* colony (temp. $10.6 - 10.9^{\circ}$ C) by a bag water sampler and *Bathymodiolus* by suction sampler. A marker (no. 697-3) was placed in front of the vent. Then, we went back the CB site. On the edge of the CH site, large gas bubbling site covered by white pavements was observed.

In the CH site, a nice chimney was observed 20 m north from the 'Taimatsu chimney'. From the old map, it seemed to be the 'Yajiri chimney'. Two or more old chimney structures were lying by the active chimney on the top of the hydrothermal mound. We sampled pieces of chimney structure, hydrothermal fluids by WHATS (max temp. 274° C) and by vacuum type water sampler, plume water of vent fluids by Niskin sampler, and diffusing fluids on *Bathymodiolus* colony (temp. $10.3 - 10.6^{\circ}$ C) by Bag pomp sampler. After this operation, we started to explore novel vent sites and vent animal colonies for the next dive survey. However, unfortunately, we found standing rope from a sinker on seafloor. Therefore, the ROV cut and retrieved the rope, and left the bottom.

I note here features of this hydrothermal field that I noticed from this dive.

- Height of chimney structures was 50 cm to 2 m.
- No black or gray smoker.
- Apparent activity center vent was not observed.
- Chimney structures were very soft.

•Most of the vent stand on center of sulfide mounds without vent animal community and the mounds were surrounded by *Bathymodiolus* colonies.

- Galathid crab was there but not predominant vent animals.
- Tubeworms were not observed.
- Seafloor was covered by sponges.
- Numbers of *Eptatreus* ('Nuta-unagi) were observed in CB site.

Payloads:

- 1) WHATS with a temperature probe
- 2) Bag pomp sampler (20L x 4)
- 3) Sample box
- 4) Niskin bottles (2 bottles)
- 5) Vacuum water bottles: Takai type (2 bottles)

- 6) DO meter
- 7) Turbidity meter
- 8) Suction sampler
- 9) MBARI corer x 2

Event List:

| 9:15 | 28°23.282N, 127°38.351E | D= 709m | Landing |
|-------|-------------------------|---------|---|
| 9:20 | 28°23.289N, 127°38.364E | D= 710m | Observe a vent (max.90°C) |
| 9:50 | 28°23.282N, 127°38.351E | D= 708m | Marker deployment (H697-1) |
| 10:29 | 28°23.480N, 127°38.396E | D= 699m | Marker deployment (H697-2) |
| 11:08 | 28°23.480N, 127°38.396E | D=691m | Chimney sampling |
| 11:28 | 28°23.480N, 127°38.396E | D=691m | WHATS sampling (1 st) (max 251°C) |
| 11:35 | 28°23.480N, 127°38.396E | D=691m | WHATS sampling (2 nd) (max247°C) |
| 11:54 | 28°23.480N, 127°38.396E | D=691m | Bag sampling (20L x 2) |
| 11:21 | 28°23.480N, 127°38.396E | D=691m | Sampling bivalves |
| 11:22 | 28°23.480N, 127°38.396E | D=691m | Marker deployment (H697-3) |
| 13:03 | 28°23.369N, 127°38.402E | D=705m | Sampling chimney |
| 13:11 | 28°23.369N, 127°38.402E | D=705m | WHATS sampling (3 rd) (max274°C) |
| 13:17 | 28°23.369N, 127°38.402E | D=705m | WHATS sampling (4 th) (max273°C) |
| 13:32 | 28°23.369N, 127°38.402E | D=705m | Vacuum water sampler (500 ml x 2) |
| 13:32 | 28°23.369N, 127°38.402E | D=705m | Bag sampling (20L x 1) |
| 13:49 | 28°23.369N, 127°38.406E | D=708m | Observed a standing rope |
| 14:02 | 28°23.369N, 127°38.406E | D=708m | Observed a standing rope |
| 14:28 | 28°23.369N, 127°38.406E | D=708m | Recovery of a standing rope and left |
| | | | bottom |





7-3. Dive Report #698

Date: June 21, 2007 Site: Minami-Ensei Knoll Landing: 9:17; 28°23.461'N, 127°38.403'E, 703m Leaving: 11:01; 28°23.499'N, 127°38.377'E, 693m

Objectives:

The major objectives are 1) to take hydrothermal samples including chimney, sediments, hydrothermal plumes, and fluids for reference.

Dive Summary:

We landed on seafloor at 50 m south from the marker buoy H697-2 (hydrothermal field). At this area (event no. 1), we collected ambient seawater as reference by the Niskin bottle (red) and the bag pomp sampler (20L x 1). Then, ROV headed to north. At the marker buoy H697-2 (event no. 2), we collected a few of small peaces of a chimney. We also collected hydrothermal fluids from a vent under the broken chimney by using WHATS sampler {2 bottles; approximately 140-170°C (1st), and 280°C (2nd)}. In addition, hydrothermal plumes (30°C) above the vent also collected by using the bag pomp sampler (20L x 2). Although we tried to collect more pieces of broken chimney on seafloor by using the MBARI corer, the chimney was so solid that the corer did not sick into the surface of the chimney. Then, ROV headed to northwest. At the area of event no. 5, we broke a chimney and collected several parts of the chimney. We also collected hydrothermal fluids by WHATS sampler {2 bottles; approximately 140°C (3rd), and 260°C (4th)}. Lastly, we set the marker buoy H698-1 at this spot.

Payloads:

- 1) WHATS with a temperature probe
- 2) Bag pomp sampler (20L x 4)
- 3) Sample box
- 4) Niskin bottles (2 bottles)
- 5) DO meter

- 6) Turbidity meter
- 7) MBARI corer x 1
- 8) Slurp gun
- 9) Marker buoy
- 10) Cutter

Event List:

| 9:18 | 28-23.461N, 127-38.403E | D=703m | Seawater sampling (Niskin [red]) |
|-------|-------------------------|--------|-----------------------------------|
| 9:22 | 28-23.461N, 127-38.403E | D=703m | Bag sampling (20L x 1; no.1) |
| 9:39 | 28-23.483N, 127-38.396E | D=700m | Chimney sampling |
| 9:43 | 28-23.483N, 127-38.396E | D=700m | WHATS sampling (1 st) |
| 9:50 | 28-23.483N, 127-38.396E | D=700m | WHATS sampling (2 nd) |
| 10:00 | 28-23.483N, 127-38.396E | D=700m | Bag sampling (20L x 1, no.3) |
| 10:05 | 28-23.483N, 127-38.396E | D=700m | Bag sampling (20L x 1, no.4) |
| 10:13 | 28-23.483N, 127-38.396E | D=700m | MBARI (green), unsuccessful |
| 10:38 | 28-23.499N, 127-38.377E | D=693m | Chimney sampling |
| 10:43 | 28-23.499N, 127-38.377E | D=693m | WHATS sampling (3 rd) |
| 10:50 | 28-23.499N, 127-38.377E | D=693m | WHATS sampling (4 th) |
| 10:58 | 28-23.499N, 127-38.377E | D=693m | Marker buoy H698-1 setting |



7-4. Dive Report #699

Date: June 21, 2007 Site: Minami-Ensei Knoll Landing: 14:18; 28°23.468'N, 127°38.396'E, 701m Leaving: 16:23; 28°23.286'N, 127°38.377'E, 707m

Objectives:

The major objectives are 1) to take hydrothermal samples including chimney, hydrothermal plumes, fluids surrounding vent animals (galetheid crab), and animals, 2) to measure heat flow tentatively.

Dive Summary:

We landed on seafloor at 15 m south from the marker buoy H697-2. ROV moved to the marking area and landed in front of nest of galetheid crab (event no. 2). We collected fluids around galetheid crabs by the bag pomp sampler (20L x 2). In addition, we collected a couple of galetheid crabs and a couple of fishes by using slurp gun. Then, ROV moved north a little (event no. 3). We broke a chimney, and collected several pieces of the chimney in a tube of the slurp gun. From the vent under the broken chimney, we collected hydrothermal fluids by using WHATS sampler {2 bottles, approximately 280°C (1st and 2nd)}. Marker buoy H699-1 was set on the spot. After this area, ROV headed to 250 m south, and landed in front of a chimney (event no. 4). We collected a large part of the chimney in a sample box. We also collected hydrothermal fluids by using WHATS sampler {2 bottles, approximately 260°C (3rd and 4th)}. Seawater surrounding the vent was collected in the Niskin bottle (green). After the sampling at this area, ROV headed to 150 m south. At the area of event no. 5, we performed the SAHF measurement.

Payloads:

- 1) WHATS with a temperature probe
- 2) Bag pomp sampler (20L x 2)
- 3) Sample box

- 4) Niskin bottles (1 bottle)
- 5) DO meter
- 6) Turbidity meter
- 7) Slurp gun
- 8) SAHF
- 9) Cutter

Event List:

| 14:26 | 28-23.480N, 127-38.396E | D=700m | Bag sampling (20L x 1, no.1) |
|-------|-------------------------|--------|-----------------------------------|
| 14:30 | 28-23.480N, 127-38.396E | D=700m | Bag sampling (20L x 1, no.2) |
| 14:36 | 28-23.480N, 127-38.396E | D=700m | Galetheid crab sampling |
| 14:44 | 28-23.485N, 127-38.395E | D=700m | Chimney sampling |
| 14:47 | 28-23.485N, 127-38.395E | D=700m | WHATS sampling (1 st) |
| 14:53 | 28-23.485N, 127-38.395E | D=700m | WHATS sampling (2 nd) |
| 14:59 | 28-23.485N, 127-38.395E | D=700m | Marker buoy H699-1 setting |
| 15:24 | 28-23.359N, 127-38.406E | D=707m | Chimney sampling |
| 15:29 | 28-23.359N, 127-38.406E | D=707m | WHATS sampling (3 rd) |
| 15:35 | 28-23.359N, 127-38.406E | D=707m | WHATS sampling (4 th) |
| 15:41 | 28-23.359N, 127-38.406E | D=707m | Niskin sampling (green) |
| 16:07 | 28-23.286N, 127-38.377E | D=707m | SAHF measurement |
| | | | |





1. 14:18 着或 D-70in

3. 14:44 D=700m fk:--採取

4. 15:04 D=707m fA=+採取

5. 16:07 D=707m SAUU溫度計經開始

※「繊維、経度の1日感りは、0.1分を示します。

BAG换水 (No. 2) 開始 BAG梁水 (No. 2) 終了

WHATS採水(No.1)開始

WIATS採水(No.1)終了 WATS採水(No. 2) 例后 WATS採水(No. 2) 終了

H699-19-3-774設置

WEATS操木(No.3)開始

WHATS操木(No.3)終了

WHATS探水(No.4) 開始

WHATS採木(No. 4) 終了 =スモン採水(1本)

14:50

14:34 14:36

14:47

14:51

14:53 14:5714:59

15129

15100

15:03

15:40

15:<1

16:22 16:20 隆鹰 I=707m

-----d

Yukiko Wada

Dive Report #700

Date: June 22, 2007 Site: Minami-Ensei Knoll Landing: 9:13; 28°23.338'N, 127°38.399'E, 708m Leaving: 15:51; 28°23.241'N, 127°38.306'E, 693m

Objectives:

JAMSTEC の普及・広報業務に活用するハイビジョン映像の撮影を行いました。 撮影した内容は以下のとおりです。

- 1) HDV カメラによる深海底でのハイパードルフィンの撮影
- 2) 3 種類のエサ(アジ、かまぼこ、ゴーヤ)に集まる生物の観察
- 3) 熱水域の生物の観察および採集
- 4) 圧力実験

Dive Summary:

潜航中に、ドライアイスや数種類のボール、りんご、生卵などの圧力による 変化を観察しました。着底後に熱水噴出孔から少し離れたところへ HDV カメラ を設置し、熱水噴出孔の周りで活動するハイパードルフィンを撮影しました。 撮影終了後、WHATS 採水器による採水を行いました。その後、その近くの生 物群集の中に3種類のエサ(アジ、かまぼこ、ゴーヤ)を設置し、そこに集ま る生物を観察しました。エンセイエゾイバラガニ、イバラモエビ、ヌタウナギ などがエサに集まる様子が観察できました。エサの実験の後、15年前のダイ ブで観察されていた、シロウリガイのコロニーのサイトへ移動しながら、生物 の観察や採集を行いました。ギンザメ、ホウボウのなかま、アナゴのなかまを 撮影し、ヒバリガイ、アカドンコ、エビ、バイガイ、カイメンを採集しました。 結局、シロウリガイのコロニーは見つからず、15:51 に離底しました。

Payloads:

1) WHATS 採水器
 2) 単式スラープガン
 3) エサ (アジ、かまぼこ、ゴーヤ)
 4) 圧力実験
 5) HDV カメラ

Event List:

| 9:13 | 28-23.356N, 127-38.401E | D=705m | H697-1 マーカーブイ視認 |
|-------|-------------------------|--------|------------------|
| 9:21 | | D=706m | HDV カメラ設置 |
| 11:02 | 28-23.359N, 127-38.403E | D=704m | HDV カメラ回収 |
| 11:15 | | D=708m | WHATS 採水(No.1) |
| 11:22 | | D=706m | WHATS 採水(No.2) |
| 11:41 | 28-23.368N, 127-38.402E | D=705m | 熱水噴出孔視認 |
| 11:57 | 28-23.347N, 127-38.407E | D=691m | 餌付マーカーブイ設置 |
| 12:54 | | | スラープガンによる生物採集 (多 |
| | | | 数) |
| 13:08 | 28-23.367N, 127-38.406E | D=706m | 魚採集 (数個体) |
| 13:16 | | | WHATS 採水(No.3) |
| 13:22 | | | WHATS 採水(No.4) |
| 14:54 | 28-23.294N, 127-38.366E | D=711m | 熱水噴出孔視認 |
| 15:19 | 28-23.313N, 127-38.379E | D=714m | 生物採集 |
| 14:54 | | | 貝殻採集(1個体) |
| 15:19 | 28-23.301N, 127-38.375E | D=713m | 生物付岩石採集 |
| | | | |



| 1. | 30:00 | 着臣 D | -708m | | | |
|-----|-------|--------|--|--|--|--------------|
| | | | (26-23.338N 127-38.399E) | | | |
| 2. | 09:13 | D-705m | 16597-1+-1-7-1視節 | | | |
| | | | (28-23, 3565 127-38, 401E) | | | |
| | 09:21 | D=706m | 接影用テレモ「カメラ股雷 | | | |
| 3. | 11:02 | D=709m | 撮影用?>t*** | | | |
| | | | (28-23, 359N 127-38, 403E) | | | |
| | 11:15 | D=708m | FHATS採水(No.1)開始 | | | |
| | 11;20 | | FEATS探水(No.1)終了 | | | |
| | 11:22 | | FEATE標本(No. 2)開始 | | | 4 |
| | 11:27 | | WEATS標木(No. 2) 終了 | | | 3 |
| 4. | 11:41 | D-705m | 熱水噴出孔幌認 | | | |
| | | | (28-23.3685 127-38.402E) | | | 2 3 |
| 5. | 11:57 | D-712m | 餌付==================================== | | | 1 1 5 |
| | | | (28-23, 347N 127-38, 407E) | | | 10 - |
| | 12:54 | | スラーブガンによる生物採集(多数) | | | \sim |
| 6. | 13:08 | D-706a | .魚採環 (数個体) | | | 1 1 |
| | | | (28-23.367N 127-38.406E) | | | _ 48 / |
| | 13:16 | | FEATS探水 (No. 3) 開始 | | | na) / |
| | 13:20 | | FEATS採水 (No. 3) 終了 | | | 1991 |
| | 13:22 | | FEATS採水 (No. 4) 開始 | | | 7911 |
| | 13;26 | | WEATE標本(No. 4)終了 | | | (~ 1) |
| 7. | 13;49 | D=711m | 熱水噴出孔視範 | | | U I |
| | | | (28-23, 2948 127-38, 3668) | | | 7 |
| 8. | 15:26 | D-714m | 生物採集 | | | |
| | | | (26-23.313N 127-38.379E) | | | |
| | 15:27 | | 貝殻誤集(1個体) | | | |
| 9. | 15:33 | D=713m | 生物村岩石採集 | | | ~ / |
| | | | (28-23.301N 127-38.375E) | | | 10 |
| 10. | 15:51 | 醒底 D: | =693m | | | 10 |
| | | | (28-23.241N 127-38.360E) | | | |
| | | | | | | |
| | | | | | | |



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| 200 |)7年(| 063 | 122 | E |
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127°38.50E

Hiroyuki Imachi

7-5. Dive Report #701

Date: June 23, 2007 Site: Iheya North Landing: 9:06; 27-47.454N, 126-53.801E, 986m Leaving: 9:10; 27-47.454N, 126-53.801E, 986m

Objectives:

The major objective is to recover the RI-bag sampler from seafloor.

Dive Summary:

We landed on seafloor near the event no. 10 (dive no. 696) where the RI-bag sampler was deployed. After setting a marker, we immediately started to recover the RI-bag sampler. We successfully picked up the RI-bag sampler.

Payloads:

1) Marker

Event List:

| 9:06 | 27-47.454N, 126-53.801E | D=986m | Marker (red) deployment |
|------|-------------------------|--------|--------------------------------|
| 9:10 | 27-47.454N, 126-53.801E | D=986m | recovery of the RI-Bag sampler |
| 9:10 | 27-47.454N, 126-53.801E | D=986m | Leaving bottom |



Hiroyuki Imachi

7-6. Dive Report #702

Date: June 23, 2007 Site: Iheya North Landing: 12:50; 27°47.441'N, 126°53.829'E, 1017m Leaving: 14:02; 27°47.453'N, 126°53.796'E, 979m

Objectives:

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

Dive Summary:

We landed on seafloor at event no. 4 where the number is assigned in dive #696. At this point, we found *Bathymodiolus* spp. colony and then collected fluids above on them by using WHATS sampler (2 bottles; temperature was approx. 4.5° C). We also collected the fluids at the same point by using the bag pomp sampler (20L x 1). Moreover, we sampled the fluids for RI-bag sampler (6L bag x6) at the same point. The RI- bag sampler was deployed.

After sampling the fluids, we climbed North Big Chimney and tested an in situ filtration system above galetheid crab colony. Unfortunately, we stopped the filtration, because fishing implements was coming near this research area.

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) Bag pomp sampler (6L x 6)
- 8) MBARI corer x 2

9) SAHF

10) Slurp gun filtration system

Event List:

| 13:05 | 27-47.451N, 126-53.805E | D=995m | WHATS sampling (1 st) |
|-------|-------------------------|--------|---------------------------------------|
| 13:12 | 27-47.451N, 126-53.805E | D=995m | WHATS sampling (2nd) |
| 13:22 | 27-47.451N, 126-53.805E | D=995m | Bag sampling (20L x 1) |
| 13:29 | 27-47.451N, 126-53.805E | D=995m | RI-Bag sampling (6L x 6) |
| 13:49 | 27-47.451N, 126-53.805E | D=995m | RI-Bag deployment |
| 13:06 | 27-47.453N, 126-53.796E | D=980m | In situ filtration by using slurp gun |
| | | | (approx.135 L) |
| 14:02 | 27-47.453N, 126-53.796E | D=980m | Leaving bottom |
| | | | |





Shinji Tsuchida

7-7. Dive Report #703

Date: June 24, 2007 Site: Minami-Ensei Knoll Landing: 9:02; 28°23.314'N, 127°38.403'E, 709m Leaving: 16:04; 28°23.492'N, 127°38.459'E, 709m

Objectives:

This dive was arranged for the Public Relations Division in JAMSTEC to introduce deep-sea researches in public. Main objectives is to take video images like as,

- 1) Hyper-Dolphin moving on the deep-sea bottom using by a high-definition handy camera.
- Animals attracted by three kinds of baited markers (raw mackerel, steamed fish paste, vegetable, "goya").
- 3) Distribution of hydrothermal vent community.

Dive Summary:

We landed on the bottom about 100 m distance in south from the Marker#H697-1. We deployed the high-definition handy camera on *Bathymodiolus* bed near a chimney actively venting. Video images of the Hyper-Dolphin approaching to the camera and sampling animals (Bathymodiolus japonicus, Lebbeus sp., Austinograea yunohana, zoarchid fish, and so on) were recorded. After the recovery of the handy camera, we deployed three kinds of baited markers, raw mackerel, steamed fish paste, vegetable, "goya". Carnivorous animals, lithodid crab (Paralomis jamsteci), hug fish (unidentified species), caridian shrimp (Lebbeus sp.), alvinocaridid shrimp (probably Alvinocaris brevitelsonis) are attracted to raw mackerel and steamed fish paste, but not vegetable, "go-ya". On the way to Marker#H697-3, we measure the temperature under the muddy bottom near chimney mound. At the Marker#H697-3 and Marker#H697-2 sites, sea-waters above Bathymodiolus and Shinkaia beds were sampled by WHATS. Dead shells of bivalves on muddy bottom were found about 200m distance in northeast from the Marker#H697-2. Unidentified species of Solemydae was collected by the manipulator of Hyper-Dolphin. At the same site, we measured temperature under the bottom by SAHFF.

Payloads:

- 1) WHATS with a temperature probe
- 2) Slurp gun with a single canister
- 3) Baited markers (raw mackerel, steamed fish paste, vegetable, "go-ya")
- 4) Color plate
- 5) SAHF
- 6) High-definition handy camera

Event List:

| 9:28 | 28-23.370N, 127-38.403E | D=704m | Deployed the High-definition handy |
|-------|-------------------------|--------|------------------------------------|
| | | | camera |
| 11:00 | 28-23.370N, 127-38.403E | D=704m | Animals sampling |
| 11:14 | 28-23.370N, 127-38.403E | D=704m | Bythograeid crab sampling |
| 11:36 | 28-23.370N, 127-38.403E | D=704m | Recovered the High-definition |
| | | | handy camera |
| 11:44 | 28-23.370N, 127-38.403E | D=706m | Baited markers deploying |
| 12:57 | 28-23.446N, 127-38.392E | D=700m | SAHF measurement |
| 13:41 | 28-23.498N, 127-38.372E | D=691m | WHATS sampling (1 st) |
| 13:47 | 28-23.498N, 127-38.372E | D=691m | WHATS sampling (2 nd) |
| 13:59 | 28-23.484N, 127-38.397E | D=700m | WHATS sampling (3 rd) |
| 14:05 | 28-23.484N, 127-38.397E | D=700m | WHATS sampling (4 th) |
| 14:17 | 28-23.484N, 127-38.397E | D=700m | Animals sampling |
| 14:54 | 28-23.550N, 127-38.468E | D=695m | Bivalve sampling |
| 15:19 | 28-23.552N, 127-38.491E | D=689m | SAHF measurement |
| | | | |



Takuro Nunoura

7-8. Dive Report #704

Date: June 25, 2007 Site: Minami-Ensei Knoll Landing: 9:15; 28°23.282'N, 127°38.356'E, 705m Leaving: 13:58; 28°23.482'N, 127°38.392'E, 700m

Objectives:

The major objectives are 1) to collect hydrothermal fluids from the south end hydrothermal vent, 2) to collect liquid CO_2 bubbles from the CH site, 3) to determine heat flow and collect hydrothermal sediments and plume around the hydrothermal vent (Marker H703-1) and 4) to test the Slurp gun filtration system at Galetheid crab site by Marker H697-2.

Dive Summary:

We landed on seafloor about 30 m southwest from the southern hydrothermal vent area. We found a small hydrothermal vent area where tree or more little vent emissions were observed. We choose a white chimney covered by polychaete nest and had several operations. First, we took a defusing emission from side of the chimney by Bag pump water sampler and plume water by a Niskin bottle. The temperature was about 15 °C, but we put a water sampler between temperature probe and chimney, therefore, the actual temperature must be higher than 15°C. Then, we sampled chimney structure with polychaete nest and vent emissions by WHATS (2 bottles); the maximum temperature was 200°C.

The ROV left the south end hydrothermal vents site and head north to go bubbling site at the CH site. When arriving at the CH site, we passed through a marker 697-2 &3, and landed on the bubbling site. Bubbling vents were observed in arrow white pavement; 1 m in width and more than 10 m in length, that might be composed by carbonate or sulfur. We insert the inlet of vacuum water sampler into the bubbling vent and sampled liquid CO_2 bubble. Because we did not observe emission of vent water, we did not try liquid sampling by WHATS. The temperature of the bubbling vent was higher than 57° C. Then, we left here and went to hydrothermal vent site that a marker

H703-1 was deployed.

At the marker H703-1, thermal gradient of sulfide mound was measured successfully in the dive 703. Before landing, we observed at least two chimneys except for the one that was marked by the marker H703-1. In addition, there were pink old marker before 1993 was observed at the one of the vent site and we also observed a sinker of a marker at the H703-1site. The vents site seems to be the 'Yon~hon matsu (four pine trees) chimneys' site in the old map. At the marker H703-1 site, we measured thermal gradient of sediments around the hydrothermal vent. Then, took sediments that mainly composed of collapsed chimneys by two MBARI type push corers that thermal gradient was determined in the dive 703. We also took hydrothermal emissions by WHATS and plume water by a Niskin bottle. The maximum temperature of the vent fluids was 274°C.

For the last objective in this dive, we arrived at the Galetheid crab colony by the H697-2 vent site. We filtrated plume water just above the Galetheid crab colony using the slurp gun filtration system. The system was operated for 20 min and had about 5 min intervals until the second operation. In 5 min intervals, a 20L bag of plume water above the Garatheid crab colony. When one min passed after the second operation started, the system vibrated due to the blockage of the first filter and we stopped filtration. Then, we finished the all operations in this dive and left the bottom.

Payloads:

- 1) WHATS with a temperature probe
- 2) Bag pomp sampler (20L x 4)
- 3) Sample box
- 4) Niskin bottles (2 bottles)
- 5) Vacuum water bottles (150 ml): Takai type (2 bottles)
- 6) Slurp gun filtration system
- 7) MBARI corer x 2
- 8) SAHF x 1

Event List:

9:15 28°23.282N, 127°38.356E D= 705m Landing

| 9:33 | 28°23.294N, 127°38.366E | D= 709m | Bag sampling (20L x 2) |
|-------|-------------------------|---------|--|
| 9:44 | 28°23.294N, 127°38.366E | D= 709m | Niskin sampling x 1 |
| 9:55 | 28°23.294N, 127°38.366E | D= 709m | WHATS sampling (1 st) (max 160°C) |
| 10:04 | 28°23.480N, 127°38.396E | D=691m | WHATS sampling (2 nd) (max203°C) |
| 11:16 | 28°23.491N, 127°38.386E | D=697m | Vacuum bottle sampling (1 st) |
| 11:22 | 28°23.491N, 127°38.386E | D=697m | Vacuum bottle sampling (2 nd) |
| 11:54 | 28°23.448N, 127°38.389E | D=699m | SAHF measurement (1 st) |
| 12:16 | 28°23.448N, 127°38.389E | D=699m | SAHF measurement (2 nd) |
| 12:38 | 28°23.448N, 127°38.389E | D=699m | SAHF measurement (3 rd) |
| 12:45 | 28°23.448N, 127°38.389E | D=699m | MBARI sampling x 2 |
| 12:54 | 28°23.448N, 127°38.389E | D=699m | WHATS sampling (3 rd) (max273°C) |
| 13:01 | 28°23.448N, 127°38.389E | D=699m | WHATS sampling (4 th) (max273°C) |
| 13:25 | 28°23.482N, 127°38.392E | D=700m | 1 st filtration of the slurp gun system |
| 13:49 | 28°23.482N, 127°38.392E | D=700m | Bag sampling (20L x 1) |
| 13:56 | 28°23.482N, 127°38.392E | D=700m | 2 nd filtration of the slurp gun system |
| 13:58 | 28°23.482N, 127°38.392E | D=700m | left the bottom |

