## NATSUSHIMA Cruise Report NT09-11

## Hatoma Knoll (as a substitute area for Iheya North)

July 27 (Naha) – August 4 (Yokosuka), 2009

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

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## 1. Cruise Information

#### 1.1. Cruise number:

NT09-11

#### 1.2. Name of vessel:

R/V Natsushima ROV Hyper-Dolphin

#### 1.3. Title of the cruise:

'Hyper-Dolphin' deep-sea dive research

#### 1.4. Titles of proposals:

- In-situ electrochemical analysis of sulfur compounds in deep-sea hydrothermal field

- Ecological study of primary producers utilizing methane in the deep-sea: "How much do *Bathymodiolus* mussels eat?"

#### 1.5. Cruise period:

July 27 - August 4, 2009

#### 1.6. Ports of call:

Naha (daparture) – Yokosuka (arrival)

#### 1.7. Research area:

**Iheya North** within a square from 27° 46.5'N 126° 53.0'E to 27°49.0'N 126°55.3'E (Extra area) **Hatoma Knoll** within a radius of 1.5 miles at 24°51.5'N 123°50.5'E

## 1.8. Research map:





See '3.4. Dive information' for the details.

## 2. Researchers

## 2.1. Chief scientist:

Masahiro Yamamoto [JAMSTEC]

## 2.2. Representatives of the science party:

Masahiro Yamamoto [JAMSTEC] Hisako Hirayama [JAMSTEC]

#### 2.3. Science party:

| Names              | Affiliations                        |
|--------------------|-------------------------------------|
| Masahiro Yamamoto  | JAMSTEC                             |
| Hisako Hirayama    | JAMSTEC                             |
| Ken Takai          | JAMSTEC                             |
| Takuro Nunoura     | JAMSTEC                             |
| Tomoo Watsuji      | JAMSTEC                             |
| Hiroko Makita      | JAMSTEC                             |
| Yukari Yoshida     | JAMSTEC                             |
| Ryuichi Aoyagi     | JAMSTEC                             |
| Satoshi Nakagawa   | Hokkaido University                 |
| Michinari Sunamura | Tokyo University                    |
| Mitsuru Tanaka     | Tokyo University                    |
| Yuichiro Ueno      | Tokyo Institute of Technology       |
| Kazuhiro Inoue     | Tokyo Institute of Technology       |
| Toshishige Itoh    | Enoshima Aquarium                   |
| Hironori Akashi    | Okayama University                  |
| Kaya Hamamoto      | Kyusyu University                   |
| Soichiro Kato      | Japan Science and Technology Agency |

## 3. Observation

#### 3.1. Observation

#### 3.1.1. Overview

We had two major objectives shown in titles of proposals, 1) In-situ electrochemical analysis of sulfur compounds in deep-sea hydrothermal field, 2) Ecological study of primary producers utilizing methane in the deep-sea: "How much do *Bathymodiolus* mussels eat?". Both objectives were contained in a magnificent purpose, 'to elucidate flux of materials and energy in deep-sea hydrothermal fields'. For the attainment of our goal, we had studied on composition of hydrothermal fluids, inhabiting microorganisms, functional genes and metabolic pathways, interaction between the environmental chemical features and the ecosystems, and so on. In this cruise, we especially focused on the concentration of sulfide, and the consumption of methane by *Bathymodiolus* mussels. To measure concentration of sulfide, we developed an electrochemical analyzing system. This system was examined in this cruise for the first time. We aimed for the real-time and pinpoint observation of sulfide. То research the methane-eating mussels, we collected *Bathymodiolus* mussels living in the hydrothermal field by using a slurp gun. We extracted fresh gill tissues from the mussels, and prepared s series of experimental mixture to measure methane consumption rates at the shore laboratory. Moreover, we collected several samples of fluids, plumes, rocks, and animals from the deep-sea hydrothermal field. We will investigate various factors in the ecosystem by chemical and biological methods to reinforce our previous results and understanding. At first of the cruise, we were planning to carry out these projects at the Iheya North hydrothermal field. However, several bad conditions, such as a tropical low pressure, a rapid tidal current, and a conflict with fishing, prevented us from staying at the Iheya North field. Therefore, we accomplished above projects at the Hatoma Knoll, where we had previously designated as the substitute area for the Iheya North.

#### 3.1.2. In-situ electrochemical analysis of sulfur compounds in deep-sea

#### hydrothermal field

#### Masahiro Yamamoto (JAMSTEC)

Sulfide is one of the most important compounds for the ecosystem in hydrothermal environments. Various sulfide-oxidizing microorganisms have been reported as both free-living and symbiotic cells. We have collected the hydrothermal fluids and seawater in the mixing zone, and measured the concentration of sulfide by a chemical method. However, sulfide is easily oxidized under the oxidative conditions, and it is difficult to know actual concentration of sulfide in the environment. We developed an electrochemical analyzing system which available in deep-sea. This system was examined in this cruise for the first time. We detected some species of sulfides in the deep-sea hydrothermal environments.

## 3.1.3. Ecological and physiological study of methane-utilizing *Bathymodiolus* mussels

Hisako Hirayama (JAMSTEC)

#### 3.1.3.1. Objective

Deep-sea *Bathymodiolus* mussels are among the dominant chemosynthetic animals found at hydrothermal vents and cold seeps worldwide. Previous studies have revealed the phylogenies of both host mussels and their symbionts (methanotrophs and/or thiotrophs) inhabiting various deep-sea sites; however little is still known of the mussels' energy metabolism. Most of mussels found in Okinawa Trough including the Hatoma Knoll are known to house methanotrophic symbionts within the gills. I would like to know how much methane is consumed by symbionts at the natural habitat, how much methane they can consume at the maximum, and also what factors control their methanotrophy.

During this cruise, we collected a lot of *Bathymodiolus* mussels, and I immediately estimated methane consumption rates of mussels under a wide range of methane concentration on board the ship. The effect of the *in situ* level of hydrostatic pressure upon methane consumption rates was also examined. The following methane analysis by GC will be conducted after the samples will be brought back to JAMSTEC. In addition, a cultivation experiment of methanotrophic symbionts will be tried by using a continuous flow cultivation system.

## 3.1.4. Research in viral ecology in the deep-sea hydrothermal vent environments

Yukari Yoshida, Takuro Nunoura (JAMSTEC) Deep-sea hydrothermal activity provides steep physical and chemical gradients by mixing of reductive high temperature hydrothermal fluids and cold oxidative seawater. Along these gradients, metabolically diverse microorganisms inhabit in their own micro-niches.

Viruses are abundant and ubiquitous components in marine microbial ecosystems, and it is reveled that they play an important role in the ecosystem of surface waters. In surface water ecosystems, viruses are recognized to be important mortality agents of microbes that can regulate the biomass production, global carbon and nutrient cycles, and microbial community structure. Furthermore, they mediate lateral gene transfer among microorganisms and affect genomic co-evolution with their both host organisms. However, viral impacts on the deep ocean ecosystems, especially on hydrothermal vent environment have not been revealed yet.

In this study, we investigate the role of viruses in the deep-sea hydrothermal vent ecosystem by multiple approaches. To do these experiments, we collected a variety of samples as described above by means of HYPER-DOLPHIN deployed in Natsushima.

#### 3.1.5. Research in episymbiotic Galatheid crab in hydrothermal field

Tomoo Watsuji (JAMSTEC) Galatheid crab, *Shinkaia crosnieri* (Decapoda: Galatheidae) having numerous setae covered with filamentous epibiotic microorganisms forms dense colonies in deep-sea hydrothermal vent fields in the Okinawa Trough. The external symbiosis between *S. crosnieri* and the epibionts was expected. Therefore, in this investigating cruise, I studied the uptake experiments using the <sup>13</sup>C-labeled tracers to find out if H<sup>13</sup>CO<sub>3</sub><sup>-</sup> and <sup>13</sup>CH<sub>4</sub> were assimilated into the setae associating the epibiotic microbial communities, and the epibiont-free tissue of living *S. crosnieri*. I conducted another experiment focused on Attached property of epibionts to the setae of *S. crosnieri*. I hypothesized the epibionts have a character that they attach on flexible filaments such as setae. I prepared artificial setae, set in *S. crosnieri* colony and collected it after 2 days. I will investigate attached microbes to it.

# 3.1.6. Ecological study for primary producers which utilizing Iron and concerned rock alteration in the deep-sea hydrothermal fields.

Hiroko Makita (JAMSTEC)

The purpose of this cruise is to obtain rusty rocks and dead chimney samples to examine the associations between endlithic microorganisms and rock alteration processes at deep-sea hydrothermal fields of the Hatoma knoll.

Recent studies have demonstrated a diverse and abundant epi- and endo-lithic microbial community on seafloor basalts and in fluid emanating from ridge-flank crust. And, rarefaction analyses show that the alteration basalt biome appears to harbor bacterial diversity and richness levels comparable to some of the most diverse identifies so far on Earth. On the other culture -depend and -independent microbiological hand, characterization demonstrated that the has zeta-proteobacteria "Mariprofundus ferrooxidans", which utilizing ferrous iron choemolithoautotrophic microorganism, commonly observed in some deep-sea low-temperature hydrothermal fields; rocks alteration regions and iron mat site. This kind of iron utilizing chemolithooutotroph microorganisms has the most significant ecological roles, such as iron and carbon cycling, in microbial communities occurring in deep-sea low-temperature hydrothermal field. However, little is known about these iron-utilizing chomolithomicroorganisms, how many types existing, what is dominant species in each site, what exactly do they role in natural habitats, and how do they interact with other microorganisms and rocks. Objectives of our microbiological studies include, 1) the evaluation of microbial diversity and distribution, 2) the measurement of microbial activity by using cultivation-, enzymatic-, DNA and RNA approaches, and metabolic product

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analysis.

We have collected some dead chimney and rusty rock samples during NT09-11. Samples were onboard prepared for future studies. Results of the analyses will provide insights into contribution of microorganisms to alteration of oceanic rocks, and iron utilizing microorganism's diversity.

#### 3.1.7. Glycomics in deep-sea vents

Satoshi Nakagawa (Hokkaido University) Deep-sea vents are the light-independent, highly productive ecosystems fueled primarily by chemoautotrophic microorganisms. Most of the invertebrates thrive in the ever-changing physical and chemical gradients through their relationship with proteobacterial symbionts. Deep-sea vent invertebrates inhabiting near the vent emission, e.g. shrimps, squat crabs and gastropods, are hypothesized to acquire their endo- or epi-symbiotic bacteria from the environment each generation. However, little is known about the molecular mechanism through which host-microbe recognize with each other.

Recently, glycoconjugates have been recognized as legislators of host-microbial interactions including symbiosis and pathogenicity. For example, the attachment of *Helicobacter pylori*, a member of Epsilonproteobacteria, to fucosylated or sialylated glycans produced by various gastric epithelial lineages and their progenitors skews the destiny of colonization toward pathogenicity. Our previous work indicated symbiotic deep-sea vent Epsilonproteobacteria have characteristic N-linked glycans. These lend support to the hypothesis that the capacity to synthesize diverse carbohydrate structures may have arisen in part from the need of both host and symbionts to both evade pathogenic relationships and to coevolve symbiotic relationships with non-pathogenic resident microorganisms.

During this cruise, we prepared both the serum from lots of squat crabs and cells of symbionts. In our shore-based study, we will analyze glycan profiles of both host and epibionts.

# 3.1.8. Determination and imaging of growing microbial cells in hydrothermal mixing zone

Mitsuru Tanaka & Michinari Sunamura (Univ. Tokyo)

In this decade, dominant members of microbial community in hydrothermal area have been determined using cultivation, gene analysis, and cell analysis. The microbial community consisted of chemolithoautotrophs, heterotrophs, and mixotrophs. The major members of chemolitoautotrophs in the mixing zone between seawater and hydrothermal fluids belong to gamma and epsilon proteobacteria, which can utilize reduced sulfur species, methane, hydrogen, and reduced metals as an energy source. However, the population of each microbial members, which responsible for each energy source and carbon source in natural environment, have not been determined yet. In this cruise, we planned to detect the carbon and energy source of microbes in the hydrothermal mixing zone at a single cell level. For this purpose, we use two types of instruments for in situ microbial incubation combined with radio / stable isotope as tracers for labeling of microbial cells and specific inhibitors.

#### 3.1.9. Electrochemical cultivation of microorganisms

Souichiro Kato (JST)

My research theme is 'electrochemical cultivation of microorganisms that can utilize crystalline iron oxides/sulfides for their metabolisms'. Our group has found that some iron reducing bacteria can utilize (semi)conductive crystalline iron oxides as an electron acceptor without redox reaction of iron. My object in this cruise is to get sediment/chimney samples containing crystalline iron oxides/sulfides. Using such iron-rich samples as microbial sources, we will conduct enrichment culture in an electrochemical cell with working electrode (poised at appropriate potential), in order to enrich microorganisms that can utilize crystalline iron sulfides/oxides as electron donor or acceptor.

#### 3.1.10. Analysis in carbon and hydrogen isotopic compositions in

#### Bathymodiolus mussels

Kaya Hamamoto (Kyushu University) To investigate bacterial activities in Okinawa Trough, we will analyze lipid biomarkers of Bathymodiolus mussels and chimney. The carbon and hydrogen isotopic compositions of the biomarkers will be determined with respect to the carbon and hydrogen sources associated with their metabolic pathways and ecosystems.

#### 3.1.11. Cultivation of animals from deep-sea hydrothermal fields

Toshishige Itoh (Enoshima Aquarium)

#### 3.1.11.1. Respective proposals

In Enoshima Aquarium, we have been trying to rear some of the deep-sea animals inhabiting in hydrothermal vent and seep, and establishing a cultivation system to raise these animals (Fig. 3.1.11-1).

During this cruise, we are going to collect and raise vent-specific animals using the suction sampler system and sorted all the samples in North Iheya Knoll (1000m) and Hatoma Knoll(1500m). And we are going to release the "My traveling diaries" from Enoshima Aquarium's web-page everyday (Fig. 3.1.11-2).



Fig.3.1.11-1. Cultivation system to raise vent



Fig.3.1.11-2. "My traveling diaries" from Enoshima Aquarium's web-page

#### 3.1.11.2. Respective results

We have collected Goemon-Koshiori-Ebi (*Shinkaia crosnieri*), Alvinocaridid shrimps (*Alvinocaris* spp.), *Bathymodiolus* spp., *Provanna* sp. in vent areas,

Hatoma Knoll to cultivate and display at our aquarium.

*S. crosnieri, A.* spp. and *B.* spp. were dominant and high population density species in the vent field. Especially, population density of *S. crosnieri* was higher than other above species.

These animals are being cultivated in Enoshima Aquarium (Fig. 3.1.11-3). The tank is displayed in the image of Knoll hydrothermal vent areas with real chimney and estimated particular system. In this system, water temperate is kept about  $3^{\circ}$ C, in addition, hot water including sulfide is ejected from inside one of the displayed chimney and CO<sub>2</sub> bubble is added in the tank. We've been trying to make the observation of these animal behaviors for a long time. In the future, we would like to have these animals breed in our aquarium.

And we released nine stories of "My traveling diaries" from Enoshima Aquarium's web-page everyday. When many customers accessed the web contents, they can study and understand for fun our institutes and researches on board.



Fig.3.1.11-3. Deep sea animals exhibition in Enoshima Aquarium to raise vent animals.

#### 3.1.12. Biogeochemistry alliance

Ken Takai (JAMSTEC)

All the fluid samples taken from the hydrothermal vents and animal colonies will be analyzed by following scheme:

Major cations; Toki, Ryukyu University Major anions; Toki, Ryukyu University Gas concentrations; Takai, JAMSTEC DOC and organics; Yamanaka & Akashi, Okayama University δD(H2); Kawagucci, JAMSTEC δ15N; Nishizawa, JAMSTEC δ13C(CH4&C2H6&CO2), δD(CH4&C2H6); Inoue & Ueno, TITEC Multiple sulfur isotopes; Ueno, TITEC

These data will be combined with the onboard data taken by Akashi & Hamamoto, and then will be thoroughly discussed among the alliance. Probably, characterization of high temperature of hydrothermal fluids in the Hatoma Knoll will be wrapped up by Toki, and the biogeochemical processes of microorganisms in the mixing hydrothermal fluid habitats will be reported by Ueno.

### 3.1.13. Organic geochemical study of hydrothermal fluid and plume emitted from Hatoma knoll hydrothermal field

Hironori Akashi (Okayama Univ.) Dissolved organic matter (DOM) is expected to play an important role of global carbon cycle as one of carbon reservoirs, however, the behavior of DOM is still unclear. Although the seawater contains around a hundred µmol/kg of DOC (dissolved organic carbon), it is insufficiently understood that the hydrothermal activity at seafloor work as organic carbon source or sink against to the ocean reservoir. In addition, the recent knowledge about sub-vent biosphere implies that the microbial activities of subsurface, especially sub-vent is not negligible with respect to the surface carbon cycles. However, it is not sufficiently clear interaction between DOM in the hydrothermal fluid and the sub-vent biosphere.

In this study, we will be analyzing DOC concentrations and low-molecular weight volatile fatty acids in the hydrothermal fluids and the associated water samples (plume, shimmering water, etc.). Furthermore, we will also determine the carbon isotopic compositions of the DOC.

#### 3.1.14. Multiple sulfur isotope biogeochemistry

Yuichiro Ueno (Tokyo Institute of Technology)

The aim of this study is to understand biogeochemical cycling of sulfur around deep-sea hydrothermal vent using quadruple sulfur isotopes (<sup>32</sup>S/<sup>33</sup>S/<sup>34</sup>S/<sup>36</sup>S). Sulfur biogeochemical processes can be traced by stable isotopes of sulfur. Previously, <sup>34</sup>S/<sup>32</sup>S ratio has been widely used for monitoring sulfate reduction processes. Recently, analysis of rare isotopes <sup>33</sup>S and <sup>36</sup>S has been found to be additional new tracer.

During this cruise, we collect various sulfur compounds including H<sub>2</sub>S in hydrothermal vent fluid, diffusive flow around biological colony, elemental sulfur, organic sulfur and seawater sulfate. In subsequent shore-based study, stable isotopic compositions of these samples will be analyzed by newly-developed fluorination technique.

## 3.1.15. Characterization of stable isotopes of carbon compounds and determination of isotopic fractionation factors of methane oxidation in mixing zones

Kazuhiro Inoue (Tokyo Institute of Technology) Carbon dioxide and methane are the major materials supplied from seafloor hydrothermal systems, and there are microorganisms that utilize them to synthesize organic matters in the Okinawa Trough. Therefore, it is important to understand behaviors of carbon compounds, and information of stable isotope ratios is useful. The purposes of my study are to characterize stable isotopic compositions of gaseous carbon compounds in the Okinawa Trough and to determine isotopic fractionation factor for methane oxidation by incubation experiments in deep-sea water that be incubated in situ pressure.

Hydrothermal fluids and water samples were collected by using several samplers. At the onshore laboratory, I will measure concentrations and isotopic compositions of carbon dioxide, methane and ethane with using a continuous-flow isotope ratio mass spectrometer system.

#### 3.2. List of observation instruments:

| Place       | Instruments                                    |  |  |  |  |  |
|-------------|--|--|--|--|--|--|
| ROV payload | Deep-sea potentio/galvanostat system (Deepote) |  |  |  |  |  |
|             | HWATS  |  |  |  |  |  |
|             | Bag pomp sampler                               |  |  |  |  |  |
|             | Niskin sampler                                 |  |  |  |  |  |
|             | Vacuum water sampler                           |  |  |  |  |  |
|             | Syringe sampler                                |  |  |  |  |  |
|             | Sampling box                                   |  |  |  |  |  |
|             | Slurp gun                                      |  |  |  |  |  |
|             | DO meter                                       |  |  |  |  |  |
|             | Turbidity meter                                |  |  |  |  |  |
| Laboratory  | Potentio/galvanostat                           |  |  |  |  |  |
|             | Pressure cultivation capsule                   |  |  |  |  |  |
|             | Water tank                                     |  |  |  |  |  |
|             | HPLC   |  |  |  |  |  |
|             | pH meter                                       |  |  |  |  |  |
|             | Gas extraction system                          |  |  |  |  |  |
|             | Spectrophotometer                              |  |  |  |  |  |

## 3.3. Cruise log:

| Date (2009)   | Vessel             | Area         | Work         |
|---------------|--------------------|--------------|--------------|
| Jul. 27 (Mon) | Departure          | Naha         | Embarkation  |
| 28 (Tue)      | Cruising           | Iheya North  |              |
| 29 (Wed)      | Dive #1035         | Hatoma Knoll | Research     |
| 30 (Thu)      | Dives #1036 & 1037 |              | •Sampling of |
| 31 (Fri)      | Dives #1038 & 1039 |              | animals      |

| Aug. 1 (Sat) | Cruising |             |           |
|--------------|----------|-------------|-----------|
| 2 (Sun)      | Cruising | Iheya North |           |
| 3 (Mon)      | Cruising |             |           |
| 4 (Tue)      | Arrival  | Yokosuka    | Disembark |

#### 3.4. Dive information:

3.4.1. #1035

Masahiro Yamamoto

Date: July 29, 2009 Site: Hatoma Knoll Landing: 10:49; 24°51.530'N, 123°50.462'E, 1474m Leaving: 12:38; 24°51.511'N, 123°50.462'E, 1475m

#### **Objectives**:

The major objectives are 1) to confirm the working of the <u>Deep</u>-sea <u>potentio/galvanostat</u> system (Deepote) in the deep-sea, and take data of voltammetory analysis in the hydrothermal environments, 2) to take hydrothermal samples including, hydrothermal plumes, hydrothermal vent animals (galetheid crab), fluids, and blocks of chimney, 3) to set 'mimic setae of galetheid crab' on colonies of galetheid crab.

#### Dive Summary:

ROV landed on the water, and we passed a check of data communication between the Deepote loaded on the ROV and PC in the control room. We started diving. Water sample was collected using Niskin bottle (#1, red) at 1,000m depth of the event no. 1. Before landing on seafloor, we rechecked and failed the data communication of Deepote. We could not repair the connection error during the dive. ROV landed on seafloor at the event no. 2. Then, we moved dozens of meter to southwest. At the event no. 3 (Big chimney C-2), we destroyed one of chimneys, and collected several blocks of the chimney in a sample box. Then, we collected fluid of the vent using WHATS sampler (bottle of #1 and 2). The temperature of the fluid was approximately 320 °C Next we tried to collect fluid surrounding colonies of galetheid crab using WHATS, but the collection was failed because of disablement of a valve of #3. Water collection using two bottles of RI-vacuum sampling were cancelled, because this sampler shared the pomp and sampling line with WHATS. We collected fluid surrounding colonies of galetheid crab using 20L-bag pomp sampler. We set 'mimic setae of galetheid crab' on colonies of galetheid crab. We collected water sample just above the vent fluid using Niskin bottle (#2, green). Lastly, we collected a lot of galetheid crabs using a slurp gun.

#### Payloads:

- 1) WHATS with a temperature probe
- 2) RI-Vacuum bottle sampler
- 3) Bag pomp sampler (20L x 4)
- 4) Niskin bottles (2 bottles)
- 5) Slurp gun
- 6) Sample box
- 7) DO meter
- 8) Turbidity meter
- 9) Deepote
- 10) Mimic setae

#### Event List:

 10:32
 24-51.527N, 123-50.467E D=1000m
 Water sampling (Niskin [red])

 10:49
 24-51.530N, 123-50.462E D=1474m
 Landing on seafloor

 11:15
 24-51.511N, 123-50.473E
 D=1474m
 Chimney

 sampling (samle box)
 Chimney
 Sampling (samle box)

| 11:35    | D=1473m | WHATS sampling (1st)    |          |         |
|----------|---------|-------------------------|----------|---------|
| 11:40    |         | WHATS sampling (2nd)    |          |         |
| 12:02    | D=1475m | Bag sampling (20L x 1)  |          |         |
| 12:14    |         | Mimic setae setting     |          |         |
| 12:17    | D=1471m | Water                   | sampling | (Niskin |
| [green]) |         |                         |          |         |
| 12:32    | D=1475m | Galetheid crab sampling |          |         |
| 12:38    |         | Leaving b               | ottom    |         |



Dive track:

#### 3.4.2. #1036

#### Hisako Hirayama

Date: July 30, 2009 Site: Hatoma Knoll Landing: 9:20; 24°51.491'N, 123°50.507'E, 1475m Leaving: 10:15; 24°51.488'N, 123°50.492'E, 1492m

#### **Objectives**:

The major objectives are to take samples including, *Bathymodiolus* mussels, colony water of *Bathymodiolus* mussels, hydrothermal plumes, vent fluids, and chimneys.

#### Dive Summary:

Before landing, hydrothermal plumes were taken at 1250 m (N-1; red) and 1400 m (N-2; green) by Niskin samplers, and also taken at 1399 m by the syringe sampler. HD landed near the #189-2M (Oritori) site and approached there. We could see a lot of *Bathymodiolus* mussels and galetheid crabs around the hydrothermal chimney. We started sampling of Bathymodiolus mussels by a slurp gun. Next, we took colony water at the *Bathymodiolus* mussel colony by WHATS (2 bottles; W-1 & W-2) and Bag sampler. A temperature at the mussel colony was 3.7°C. After that, HD moved to the hot vent at the top of the chimney, and the inlet of the water sampler connected with a thermometer was inserted into the vent, where the temperature of 197°C was recorded. We tried to take the hot vent fluid by WHATS, but a valve of the #3 bottle didn't open because of the discord between valve and pin. We gave up to take the vent water, then collected the active chimney blocks at the vent. After the chimney sampling, HD moved northwest by 30 m and found dead chimneys. We took a big dead chimney there. Finally, HD left the bottom.

#### Payloads:

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

#### Event List:

```
9:08
      24-51.494N, 123-50.496E D=1251m Niskin water sampling (N-1;
red)
9:12
      24-51.496N, 123-50.502E D=1400m Niskin water sampling (N-2;
green)
9:14
      24-51.499N, 123-50.496E D=1399m Syringe water sampling
9:20
      24-51.491N, 123-50.507E D=1475m Landing
9:27
      24-51.482N, 123-50.505ED=1479m Sampling of Bathymodiolus
mussels
9:35
                             D=1479m WHATS water sampling (#1)
9:40
                             D=1479m WHATS water sampling (#2)
9:49
                             D=1479m Bag water sampling
                             D=1478m Failed
                                                    WHATS
9:57
                                               in
                                                              water
sampling (#3)
10:06
                             D=1477m Active chimney sampling
      24-51.488N, 123-50.492E D=1492m Dead chimney sampling
10:14
                             D=1473m Left the bottom
10:15
```



Dive track:

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#### Michinari SUNAMURA

Date: July 30, 2009 Site: Hatoma Knoll Landing: 14:13; 24°51.515'N, 123°50.459'E, 1473m Leaving: 15:42; 24°51.487'N, 123°50.463'E, 1475m

#### **Objectives**:

The objectives in this dive are: 1.) High & Low temperature hydrothermal fulid sampling with WHATS, 2.) Mixing water sampling and incubation with RI vacuum bottle sampler, 3.) Galetheid crab sampling, 4.)Plume sampling with Niskin bottle sampler, 5.) Chimney sampling.

#### Dive Summary:

Hyper Dolphin landed on the water at the west side of the main vents and plunged in the direction to the north of EM#11. On the way to the seafloor, we visibly detected turbid water at from 1410 to 1450 m in water depth. We collected hydrothermal plume water at the depth of 1410 and 1430 m by Niskin bottle sampler. The HD went through the EM#15 vent and arrived at the EM#11.

At the EM#11, we started water sampling around galetheid crab colonies using WHATS pump system. Approximately 6L of mixing water sample was collected into a 6L plastic bag, which connected to the RI vacuum bottle sampler. The water in the plastic bag was moved into two RI vacuum bottle samplers by open and close valves of the bottle samplers. Then we collected the same water samples using WHATS sampler (bottle of #1 & 2). Next, The HD moved to EM#2 and approached to the top of the chimney. After broke and collected the chimney, we sampled high temperature hydrothermal fluid using WHATS sampler (bottle of #3 &4). The temperature of the fluid was 320°C. Finally, the HD slightly moved to galetheid crab colonies and collected many individual of galetheid crabs and shrimps using a slurp gun.

#### Payloads:

- 1) WHATS with a temperature probe
- 2) RI vacuum bottle sampler
- 3) Niskin bottles (2 bottles)
- 4) Slurp gun
- 5) Sample box
- 6) DO meter
- 7) Turbidity meter

#### Event List:

| 14:10              | 24-51.529N,                 | 123 <b>-</b> 50.462E | D=1406m | Water sampling (Niskin [red]) |          |             |        |
|--------------------|-----------------------------|----------------------|---------|-------------------------------|----------|-------------|--------|
| 14:11              |                             |                      | D=1435m | Water sampling (N             |          | (Niskin     |        |
| [green]            | )                           |                      |         |                               |          |             |        |
| 14:13              | 24-51.515N,                 | 123-50.459E          | D=1473m | Landin                        | g on sea | afloor      |        |
| 14:44              | 24-51.500N,                 | 123 <b>-</b> 50.463E |         | D=1475                        | óm       | WHATS       | 5      |
| sampli             | ng (#1)                     |                      |         |                               |          |             |        |
| 14:47              |                             |                      |         | Takai                         | type     | vacuum      | bottle |
| sample             | er                          |                      |         | (Yellow, #1)                  |          |             |        |
| 14:49              |                             |                      |         | Takai                         | type     | vacuum      | bottle |
| sampler (Cyan, #2) |                             | #2)                  |         |                               |          |             |        |
| 14:50              | .4:50 WHATS sampling (#2) s |                      | start   |                               |          |             |        |
| 14:54              |                             |                      |         | WHAT                          | S samp   | ling (#2) e | end    |
| 15:11              | 24-51.487N,                 | 123 <b>-</b> 50.463E |         | D=1472                        | 2m       | Chimne      | У      |
| sampli             | ng                          |                      |         |                               |          |             |        |
| 15:16              |                             |                      |         | WHAT                          | S samp   | ling (#3) s | start  |
| 15:20              |                             |                      |         | WHATS sampling (#3) end       |          |             |        |
| 15:23              |                             |                      |         | WHATS sampling (#4) start,    |          | start,      |        |
|                    |                             |                      |         | temp. n                       | nax. = 3 | 320°C       |        |

| 15:25 |         | WHATS sampling (#4) end |
|-------|---------|-------------------------|
| 15:42 | D=1475m | Galetheid crab sampling |
| 15:45 |         | Leaving bottom          |





#### 3.4.4. #1038

Yuichiro Ueno

Date: July 31, 2009 Site: Hatoma Knoll Landing: 9:21; 24°51.502'N, 123°50.477'E, 1478m Leaving: 10:28; 24°51.503'N, 123°50.465'E, 1475m

#### **Objectives**:

The major objectives are 1) to test the <u>Deep-sea potentio/galvanostat system</u> (Deepote) in the deep-sea, and take data of voltammetory analysis in the hydrothermal environments, and 2) to obtain samples including, hydrothermal plumes, vent fluid, animals (galetheid crab), and blocks of chimney.

#### Dive Summary:

Before landing, plume water was sampled at 1382 m (Niskin-1 red) and 1440 m (Niskin-2 green). ROV landed on the seafloor near #11 site. Around Galetheid colony, we took water samples into RI vacuum sampling bottles. The sampling site is near the Artificial Goemon Hair. Then we move 50 cm and collect water samples for incubation experiment by WHATS (1-4) where we can see shimmering water diffused from the chimney wall. The sample temperature is first 26~30°C, then increased from 36 to 40°C. The latter temperature is probably during the sampling. Note that temperature probe was sometimes erroneous (e.g., jumping down to 1°C) today. After sampling, we recovered the Artifical Goemon Hair and move to Hibari-gai colony just below the Galetheid colony, where Hibari-gai with some Ohara-ebi were sampled by slurp gun. Finally, we collect sulfide mound below the Hibari-gai colony. This rock sample is coated by altered brownish (Iron-hydroxide?) rim with blackish internal sulfide. Then we leave the bottom at 10:30.

#### Payloads:

1) WHATS with a temperature probe

- 2) RI-Vacuum bottle sampler
- 3) Niskin bottles (2 bottles)
- 4) Slurp gun
- 5) Sample box
- 6) DO meter
- 7) Turbidity meter
- 8) Deepote

### Event List:

| 8:15                      | D=0m            | Start to div            | ve          |             |
|---------------------------|-----------------|-------------------------|-------------|-------------|
| 9:12                      | D=1382m         | Sampling                | plume       | (Niskin-1   |
| [red])                    |                 |                         |             |             |
| 9:17                      | D=1440m         | Sampling                | plume       | (Niskin-2   |
| [green])                  |                 |                         |             |             |
| 9:21 24-51.530N, 123-50.4 | 462ED=1478m     | Landing                 | on seafle   | oor (cloudy |
| plume)                    |                 |                         |             |             |
| 9:24                      | D=1469m         | Arriving a              | t #11 site  |             |
| 9:29                      |                 | Sampling                | Goemon      | water into  |
| RI                        |                 |                         |             |             |
| 9:43                      |                 | RI vacuum               | l [blue] op | en          |
| 9:45                      |                 | RI vacuum [yellow] open |             |             |
| 9:58                      |                 | WHATS                   | 1-4         | incubation  |
| sampling                  |                 |                         |             |             |
| shimmer                   | ring water arou | nd Goemon               | site (26-3  | 0C)(36-40C) |
| 10:07                     |                 | closing WF              | IATS valv   | ves         |
| 10:13                     |                 | Retrieved               | Artificia   | al Goemon   |
| Hair                      |                 |                         |             |             |
| 10:16                     |                 | Sampling                | Hiba        | ri-gai +    |
| Ohanra-ebi                |                 |                         |             |             |
| 10:24                     |                 | Sampling                | brown       | rock below  |
| Hibari                    |                 |                         |             |             |
| 10:28                     | D=1470m         | Leaving bo              | ottom       |             |



Dive track:

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#### 3.4.5. #1039

Ken Takai

Date: July 31, 2009 Site: Hatoma Knoll Landing: 10:49; 24°51.530'N, 123°50.462'E, 1474m Leaving: 12:38; 24°51.511'N, 123°50.462'E, 1475m

#### **Objectives**:

The major objectives are 1) to confirm the working of the <u>Deep</u>-sea <u>potentio/galvanostat</u> system (Deepote) in the deep-sea, and take data of voltammetory analysis in the hydrothermal environments, 2) to take colony water of the *Paralvinella* spp. and hydrothermal vent animals.

#### Dive Summary:

HD landed on the Ese-Ese-Gekiatsu chimney. Before landing, seawater was collected by Niskin (red) sampler. After approaching to the polychaetes colonies in the Ese-Ese-Gekiatsu chimney, we started the hydrothermal fluid sampling by WHATS+C-WHATS. The temperature of the water was about 8 °C. After finishing the sampling of paralvinella colony water, we tried to take the same sample by Bag sampler. Then, we collected many individuals of the galetheids from the top of the Ese-Ese-Gekiatsu chimney. Next, we also collected several pieces of polychaetes colonies from the Monk sub-chimney of the Ese-Ese-Gekiatsu chimney. Finally, HD left the bottom and took the water just above the vent.

#### Payloads:

- 1) Directly aligned WHATS with a temperature probe
- 2) Cheap WHATS (C-WHATS) connected with WHATS
- 3) Bag pomp sampler (20L)
- 4) Niskin bottles (2 bottles)
- 5) Slurp gun

- 6) Sample box
- 7) DO meter
- 8) Turbidity meter
- 9) Deepote

### Event List:

24-51.497N, 123-50.459E D=1472m Water sampling (Niskin [red]) 14:22fluid 24-51.494N, 123-50.472E D=1473m Hydrothermal 14:34(Paralivinella colony) sampling (WHATS+C-WHATS, Temp = 8.0) 14:48 24-51.494N, 123-50.472E D=1473m Finish hydrothermal fluid sampling (WHATS+C-WHATS, Temp = 8.0) 14:56 24-51.494N, 123-50.472ED=1473m Hydrothermal fluid (Paralivinella colony) sampling (Bag) 14:59 24-51.494N, 123-50.472ED=1473m Finish hydrothermal fluid (Paralivinella colony) sampling (Bag) 15:13 24-51.492N, 123-50.472E D=1473m Sampling galetheids (governers of the Ese-Ese-Gekiatsu chimney) 15:2124-51.494N, 123-50.472ED=1473m Sampling polychaetes colonies 24-51.494N, 123-50.472ED=1473m Leaving the bottom 15:2824-51.494N, 123-50.472ED=1473m Sampling the Niskin (green) 15:30just above the vent of the Ese-Ese-Gekiatsu chimney





## 4. Notice on Using

This cruise report is a preliminary documentation as of the end of the cruise.

This report may not be corrected even if changes on contents (i.e. taxonomic classifications) may be found after its publication. This report may also be changed without notice. Data on this cruise report may be raw or unprocessed. If you are going to use or refer to the data written on this report, please ask the Chief Scientist for latest information.

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