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NT11-19 Cruise Report

September 24 (Naha) – September 27 (Naha), 2011
R/V Natsushima and *ROV Hyper-Dolphin*

Glycomics of chemosynthetic communities & Hg dynamics in deep-sea hydrothermal vents

Iheya North: Dives #1324-1326

Photo: Sampling squat crabs

Hokkaido University / National Institute of Advanced Industrial Science and Technology (AIST) / Japan Agency for Marine-Earth Science & Technology (JAMSTEC) / University of Tsukuba / Kagoshima University / Enoshima Aquarium

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1. Expedition Overview

Satoshi Nakagawa

We performed the NT11-19 cruise at the Iheya North hydrothermal field, from 24th to 27th of September, 2011. The cruise was done for microbiological, biogeochemical, and mineralogical studies in deep-sea hydrothermal fields. The survey was conducted by means of ROV *Hyper Dolphin* and R/V *Natsushima*.

Four out of seven dives were cancelled because of bad weather. However, sampling and onboard analyses of hydrothermal vent fluids, fluids surrounding animal colonies, sediments, animals, and sulfides, were successfully performed. One of our major research foci was “symbiosis”. 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 chemoautotrophic 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 both symbiosis and pathogenicity. For example, the attachment of *Helicobacter pylori*, a pathogenic 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 chemoautotrophs have an ability to form unique *N*-linked glycans. These support to the hypothesis that the capacity to synthesize diverse glycan structures may have arisen in part from the need of both host and symbionts to both evade pathogenic relationships and to coevolve symbiotic relationships with non-pathogenic resident microorganisms. During this cruise, we prepared both the serum from squat crabs and cells of epi-symbionts. In addition, habitats of crabs were both physically and chemically characterized, since our previous study indicated that glycan structures were quickly changed depending on physicochemical conditions. In our shore-based study, we will analyze glycan profiles of both host and epibionts in order to figure their functions out.

Second, we successfully collected various hydrothermal samples in order to

isolate chemoautotrophs in pure cultures. It has been increasingly recognized that microbial genomes are dynamically changing in response to natural selection pressures. Some of variable genes have obvious roles in determining the relative fitness of the ecotypes in response to key environmental variables, and hence in regulating their distribution and abundance in the oceans. Genomic variability among closely related microorganisms has increasingly become of particular interest for better understanding the microbial ecology and evolution. MLSA (multilocus sequence analysis) has become a universal and unambiguous method for strain genotyping, population genetics, and molecular evolutionary studies. However, to date, there has been no study published that uses this powerful technique to reveal geno- and eco-types of deep-sea vent microbes. In our shore-based study, we will isolate chemoautotrophs in pure cultures, and compare them with strains from other hydrothermal fields.

2. Acknowledgement

We are grateful to all crew and captain Nakamura 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 the Mid-Okinawa Trough with safe and accurate operations. We also thank Mr. Itoh (Nippon Marine Enterprise, Ltd) for their heartfelt supports to our works. We thank all the JAMSTEC personnel who have 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.

3. NT11-19 Participants

3.1 Shipboard Scientists

Chief scientist

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Associate Professor

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Hokkaido University

Vice chief scientist

Dr. Katsumi Marumo

Director and Senior Researcher

National Institute of Advanced Industrial Science and Technology (AIST)

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Subsurface Geobiology Advanced Research (SUGAR) Project,
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Technical Assistant

Mr. Masashi Itoh

Marine Technician

Marine Science Department, Nippon Marine Enterprises, Ltd.

3.2 ROV Hyper-Dolphin Operation Team

Operation Manager	Kazuya MITSUFUJI
1st Submersible Staff	Kazuki IIJIMA
1st Submersible Staff	Mitsuhiro UEKI
2nd Submersible Staff	Homare WAKAMATSU
2ndSubmersible Staff	Shigeru KIKUYA
2nd Submersible Staff	Yudai SAKAKIBARA

3.3 R/V NATSUSHIMA Crews

Captain	Yoshiyuki NAKAMURA
Chief Officer	Akihisa TSUJI
2nd Officer	Isao MAEDA
3rd Officer	Kazuki MIYAKE
Chief Engineer	Hiroyuki SHIBATA
1st Engineer	Wataru KUROSE
2nd Engineer	Saburo SAKAEMURA
3rd Engineer	Shota NAGANO
Chief Radio Operator	Masamoto TAKAHASHI
2nd Radio Operator	Michiyasu KATAGIRI
3rd Radio Operator	Tatsuhiro TAKAKUWA
Boat Swain	Kozo YATOGO
Able Seaman	Takao KUBOTA
Able Seaman	Hideo ISOBE
Able Seaman	Naoki IWASAKI
Able Seaman	Takuya MIYASHITA
Sailer	Hirotaka SHIGETA
Sailer	Jun SHINODA
No.1 Oiler	Hiroyuki Oishi
Oiler	Katsuyuki MIYAZAKI
Oiler	Shinya Sugi
Oiler	Yuji HIGASHIKAWA
Oiler	Ryo MATSUUCHI
Chief Steward	Ryuei TAKEMURA

Steward	Yoshinobu HASATANI
Steward	Hideo FUKUMURA
Steward	Tatsuya YAMAMOTO
Steward	Takumi YAMADA

4. *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*.

4.1 General information about R/V Natsushima

Length: 67.4m Bow thruster: 1 Width: 13.0m Maximum speed: 12kt

Depth: 6.3m Duration: 8400 mile Max capacity: 55 persons

Gross Tonnage: 1553t Main prop: 2 axes, 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, Milli-Q SP TOC). And seawater 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.

4.2 General information about ROV Hyper-Dolphin

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

Principal specification

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

(1) Manipulator capability

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

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

Hand opening width: right 77mm, left 195mm

(2) TV camera

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

(3) Digital photo camera

Type : Seamax DPC7000 (DSSI)

(4) Obstacle escape sonars

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

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

(5) Altitude sonar

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

(6) Depth sensor (with temperature sensor)

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

Range of measuring temperature: -2-40deg.

5. Ship Operation Log

Masashi Itoh

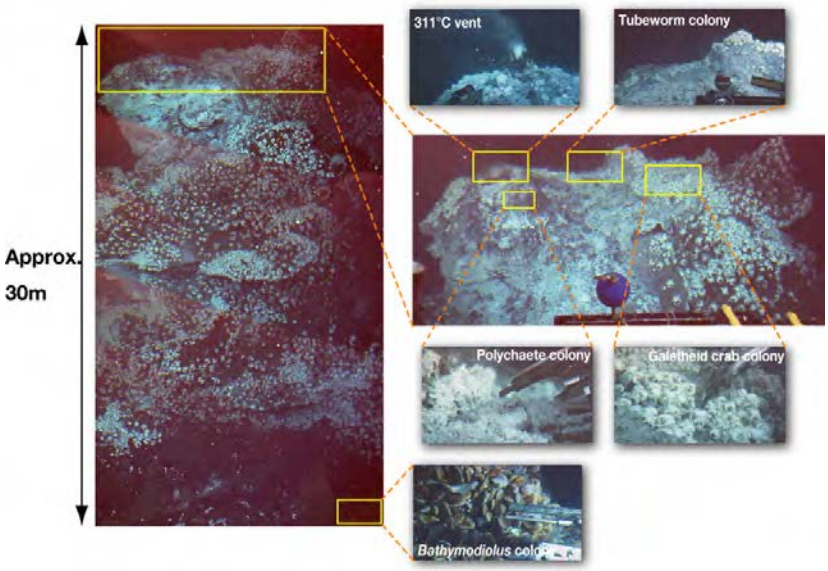
Date	Local Time	Note	Description	Position/Weather/Wind/Sea condition
24,Sept,2011		**Sail out and transit**		
	15:00	Let go all shore line,left NAHA		09/24 12:00(UTC+9h)
	15:30	Onboard education and safety training		26-14.2N 127-40.8E
				Overcast
				SE-2(Light breeze)
				1(Calm)
				1(Low swell sea)
				Visibly:7
25,Sept,2011		**HPD#1324**		
	4:00	Arrived at research area		09/25 12:00(UTC+9h)
	5:55	Released XBT at 27-47.7148N 126-54.0217E		27-47.4N 126-53.8E
	8:13	Hoisted up H.P.D.		cloudy
	8:18	Launched H.P.D. on the surface		East-5(Fresh breeze)
	8:28	H.P.D. dove & started her operation #1324		3(Sea slight)
	9:26	H.P.D. launded on sea bottom.(D=1015m)		2(Low swell long)
	11:35	H.P.D. left the sea bottom(D=977m)		Visibly:7
	12:23	H.P.D. floated		
	12:33	Hoisted up H.P.D.		
	12:38	Recovered H.P.D & finished above operation		
		HPD#1325		
	14:08	Hoisted up H.P.D.		
	14:12	Launched H.P.D. on the surface		
	14:22	H.P.D. dove & started her operation #1325		
	15:09	H.P.D. launded on sea bottom.(D=1000m)		
	17:20	H.P.D. left the sea bottom(D=1044m)		
	17:58	H.P.D. floated		
	18:09	Hoisted up H.P.D.		
	18:14	Recovered H.P.D & finished above operation.		
	20:00	Stop'd eng,then commenced drifting.		
26,Sept,2011		**HPD#1326**		
	5:30	Finished drifting.		09/26 12:00(UTC+9h)
	8:09	Hoisted up H.P.D.		27-47.5N 126-53.8E
	8:13	Launched H.P.D. on the surface		cloudy
	8:24	H.P.D. dove & started her operation #1326		East-3(Gentle breeze)
	9:13	H.P.D. launded on sea bottom.(D=1025m)		3(Sea slight)
	11:53	H.P.D. left the sea bottom(D=1001m)		5(Moderate long)
	12:38	H.P.D. floated		Visbly:8
	12:51	Hoisted up H.P.D.		
	12:58	Recovered H.P.D & finished above operation		
	13:15	Com'ced proceeding to NAHA.		
27,Sept,2011		**Finished NT11-19**		
	8:50	Sent out 1st shore line,then arrived at NAHA.		

6. Introduction of the Iheya North hydrothermal field

Satoshi Nakagawa

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

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

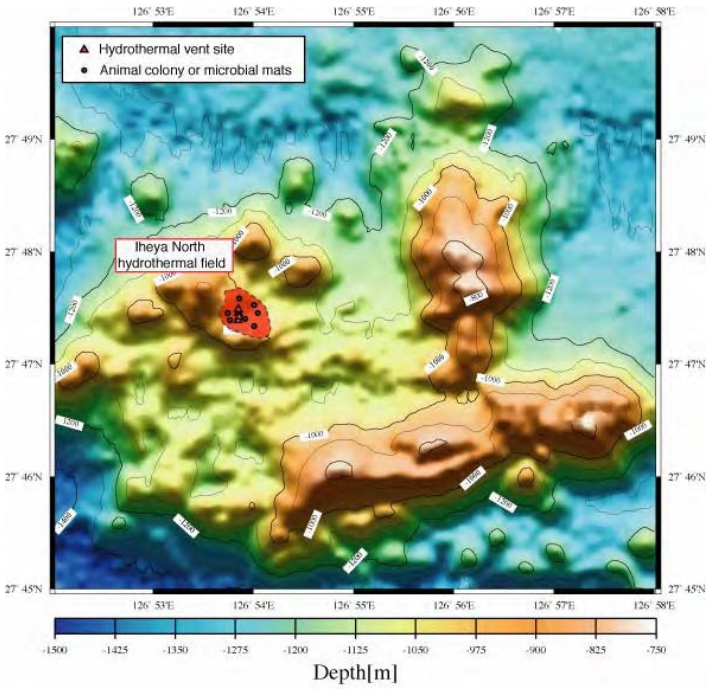


Mixing zones at the NBC in the Iheya North hydrothermal field. From Nakagawa et al. (2007).

microbes.

On the basis of our previous microbiological studies using samples obtained during NT02-06, NT05-03, YK06-09, NT07-11, and NT07-13, members of the *Proteobacteria*, especially *Epsilonproteobacteria* and *Gammaproteobacteria*, commonly represent the numerously abundant microbial populations in a variety of mixing zones. The ratio of the free-living *Epsilonproteobacteria* to total cell numbers was found to decrease with increasing distance between vent emission and habitats studied. We demonstrated *Epsilonproteobacteria* members had an extensive metabolic repertoire, including hydrogen- and sulfur-compounds-oxidation, coupled with the reduction of oxygen, nitrate (denitrification and ammonification), and sulfur compounds. In addition, we determined whole genome sequences of two epsilonproteobacterial 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, molecular mechanisms through which chemoautotrophs interact with other micro- and macro-organisms remain to be further investigated.

During this cruise, we collected samples from each of the mixing zones. Together with the geochemical and microbiological analysis, we will perform the glycobiological analysis. This 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

7. Preliminary Results

7.1 Microbiology

S. Nakagawa

During the NT11-19, we collected various hydrothermal samples including vent fluids, chimney structures, and hydrothermal vent animals from the Iheya North hydrothermal field. Immediately after the recovery, all samples were prepared for the multidisciplinary shore-based microbiological study (described below).

For cultivation, samples were kept anaerobically under 4 deg C. For molecular analyses, which target microbial DNA, RNA, enzymes, and glycoconjugates, microbial cells were harvested either on 0.2 μm pore size filters or by centrifugation, and then immediately stored at –80ºC.

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

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

7.2 Mineralogy and geochemistry

K. Marumo

We collected sulfide samples, sediments, and water for shore-based studied on their mercury concentrations and mercury isotope ratios. On-board qualitative chemical analysis on sulfide samples were performed using an energy dispersive X-ray fluorescence spectrometer (EDXRF).

Hg^0 and Hg^{2+} concentration in water samples is measured by cold vapor atomic absorption spectrometry (CVAAS) on-board. Hg^{2+} concentration in water was also monitored using an electrochemical sensor attached to the “ROV Hyper-Dolphin”.

8. Shore-based study
8.1 Microbiology

S. Nakagawa

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 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 various hydrothermal vents, and has succeeded in cultivation of more than 10% of the members that were detected in culture-independent analyses in each habitat.

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

MPN analysis: This is a method to enumerate culturable populations of microbes. Hydrothermal samples were diluted in 10-fold steps into liquid media, which should support the growth and putative population of specific physiological types of

microorganisms. The isolates obtained from the highest positive dilutions will be characterized since they are probably dominant in the habitat.

• Culture –independent molecular ecological surveys

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

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

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

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

Glycan-profiling provides key insights into the molecular interactions between symbiont and host animals. We will purify glycans using enzymatic and chemical methods, and then analyze the structure using MALDI-TOFMS and 2D-HPLC.

To verify epibiotic structures controlled by hydrothermal environments and elastic property of setae of *Shinkaia crosnieri*

T. Watsuji & A. Yamamoto

Many species of invertebrates dwelling in deep-sea hydrothermal vents and cold seeps are known to host bacteria (epibionts) on the surface of specialized tissues such as the dorsal setae of *Alvinella pompejana*, the gill chamber of *Rimicaris exoculata*, the setae of *Shinkaia crosnieri*, the setae of *Kiwa hirsuta*, and the sulfide-coated scales of scaly-foot snails. The epibiotic microbial communities on the host animals mainly included the phylotypes affiliated with the genus *Sulfurovum* within

Epsilonproteobacteria and the Marine epibiont group I within *Gammaproteobacteria*. The fluorescence in situ hybridization (FISH) analysis reveled that most of the epibionts belonging to *Epsilonproteobacteria* and *Gammaproteobacteria* were filamentous in the epibiotic communities. Molecular approaches have revealed that the dominant epsilon-proteobacterial epibionts in *A. pompejana* expressed a gene encoding ATP citrate lyase, a key enzyme in the reverse tricarboxylic acid (rTCA) cycle, a CO_2 fixation pathway. Additionally, a metagenomic analysis of the epibiotic community in *A. pompejana* revealed the presence of genes involved in the complete reductive tricarboxylic acid (rTCA) cycle and sulfur oxidation. Actually, the epibiotic communities of *S. crosnieri* assimilated ^{13}C-labeled bicarbonate. Moreover, the incorporation of $H^{13}CO_3^-$ into the epibiotic microbial community was enhanced with the addition of potentially thioautotrophic substrates such as sulfide and thiosulfate. These results suggested that the dominant filamentous epibionts were capable of chemolithoautotrophic growth by sulfur oxidation. However, the filamentous epibiont affiliated with *Epsilonproteobacteria* and *Gammaproteobacteria* have not been isolated from the epibiotic communities. In this cruise, we are going to collect in situ colonization devices deployed in *Bathymodiolus* colony, *S. crosnieri* colony, *Paralvinella hessleri* colony and non-hydrothermal area in Iheya North to cultivate the filamentous epibionts at the actual site without depending on the host. We will find the colonization of the filamentous epibionts by SEM observations, phylogenetic analysis, and FISH analysis of the devices.

Structure analysis of deep-sea vent symbionts lipopolysaccharides. ~To elucidate deep-sea host-symbionts interactions~

A. Kando

In deep-sea hydrothermal vent, most invertebrates get their nutrients by establishing a symbiotic relationship with chemoautotrophic bacteria. Invertebrates, especially those inhabiting near vent emissions, acquire their specific symbionts from environments. However little is known how host invertebrates and symbionts recognize with each other.

Recently, the genomic analysis of two cultivatable species of deep-sea vent bacteria, *Sulfurovum* sp. NBC37-1 and *Nitratiruptor* sp. SB155-2, had done and revealed that

they are the origins of pathogenic microorganisms, such as *Helicobacter* and *Campylobacter*. So, study of deep-sea symbiotic system would be an important key to reveal the pathogenic bacterial evolution.

Gram-negative bacterial lipopolysaccharides (LPSs), crucial components of outer membrane, have a potential to be responsible for these interaction. So, we try to analyze *Sulfurovum* sp. NBC37-1 and *Nitratiruptor* sp. SB155-2 LPS structure. As they are cultivatable, we can get their cells easily but it is not sure whether they are really the symbionts. So, in this cruise, we got a lot of *Shinkaia crosnieri* and cut their hair with symbionts.

In the future, we will try to elucidate these symbionts LPS structure after establishing the way of *Sulfurovum* sp. NBC37-1 and *Nitratiruptor* sp. SB155-2 LPS structure analysis.

Population genetics of deep-sea hydrothermal vent chemoautotrophs.

S. Mino

It has been increasingly recognized that microbes have extremely high genomic diversity. Some studies of microbial variable genes clearly indicated the geographic isolation of terrestrial bacteria, although the concept "everything is everywhere; but the environment selects" has been widely accepted in microbiology.

Genomic variability among closely related microorganisms has become of particular interest for better understanding the microbial ecology and evolution. MLSA (multilocus sequence analysis) is the powerful tool understanding to the strain genotyping, population genetics, and molecular evolutionary. However, there has been no study that uses this technique to reveal genomic features of deep-sea free-living microbes.

The class *Epsilonproteobacteria* is an important phylogenetic group because this group contains non-pathogenic deep-sea chemolithoautotrophs and human pathogens, i.e. *Helicobacter pylori* (causative agent of gastric ulcer and cancer) and *Campylobacter jejuni* (causative agent of gastroenteritis and neuromuscular paralysis). Previous population genetic studies about these epidemic *Epsilonproteobacteria* have revealed that they have extremely high rates of genetic mutation and recombination, which generate genomic diversity. However, little is known about genetic

characteristics of deep-sea epsilonproteobacterial population. In addition, it has not been cleared why deep-sea *Epsilonproteobacteria* could be dominant spices in hydrothermal fields around the world. The main goals of this study are 1) to clarify whether there are biogeographic barriers in the deep-sea microbial populations, 2) to determine the characteristic of population genetic structure about them, and 3) to reveal the genetic difference between *Epsilonproteobacteria* and other chemoautotrophic populations.

During this cruise, we could collect a variety of hydrothermal samples. In our shore-based study, we will isolate mesophilic *Epsilonproteobacteria* and thermophilic *Persephonella* in pure cultures. Then, compare them with strains from other hydrothermal fields.

Shore-based study includes:

Enrichment -> Isolation of bacteria (in several different media and cultivation conditions by using dilution-to-extinction method.)

DNA extraction

PCR amplification (several house-keeping genes), Sequencing

Performing population genetic analyses including estimation of recombination / mutation rates, construction of phylogenetic trees, molecular variance and linkage equilibrium analysis.

Purification and Characterization of Lectin, Sugar binding Proteins as a Tool of Deep-Sea Host-Symbionts Recognition

S. Fujiyoshi

Lectin, a protein that recognizes cell sugar chains, is found in many plants and animals.

Lectin is essential for invertebrate immune system, because of the absence of immunoglobulin. Lectin is used for finding out self and non-self.

The galatheid crab, *Shinkaia crosnieri* living in hydrothermal vents, has setae covered with filamentous epibiotic microorganism. Symbionts give nutrients to their host there. However, little is known about properties and role of invertebrate lectin in symbiosis.

In this cruise, we are collected a plenty of *Shinkaia crosnieri* serum samples

containing lectin. In the future, we are going to purify these samples to extract lectin and find out its target carbon hydrates. The study is expected to give us the role of this protein in host-symbionts recognition.

Structure analysis of saccharide-chains of symbiotic microbes in deep-sea vents
~To clarify the symbiotic interactions and mechanism~

T. Arai

In deep-sea vents, invertebrates make symbiont association with chemoautotrophic bacteria. However, Little is known about the symbiotic relationships in detail at molecular level.

All creatures (including microbes) have saccharide-chains on the surface of their cells. These saccharide-chains could work as molecular communication tools between livings. In symbiotic relationships, the host may use their saccharide-chains as a communication tool to find and identify proper symbiotic partner.

In this cruise, we got the setae, gut and serum of Shinkaia crosnieri. This crab has symbiotic microbes at their setae. So we will get saccharide-chains of the symbiont microbes from the setae. And we will analyse the structure of symbiont saccharide-chains and finally get clear the molecular mechanisms in symbiosis between Shinkaia crosnieri and its symbiont microbes.

Development of to longtime feeding technology of *Shinkaia closnieri*

M. Sugimura

Galatheid crab, *Shinkai closnieri* (Decapoda: Galatheidae) specifically inhabits Hydrothermal vent in the Okinawa Trough. The Galatheid crab depends for food on chemosynthetic bacterium attached to ventral carapace of that.

We aim for the longtime feeding of the Galatheid crab by two approaches. One of processes is reproducing the sulfide concentration same in a tank as environment of a habitat of the Galatheid crab. Another object is giving the common food to the Galatheid crab.

These experiments will prove habitation conditions, and he dependence to chemosynthetic bacteria of Galatheid crab.

In this cruise, we try to measure sulfide concentration and nitrogen compounding (NH_3-N, NO_2-N, NO_3-N) in Water around colony of the Galatheid crab. This result will be applied to the breeding experiment.

These researches will be opened to visitors as joint research with JAMSTEC in Enoshima Aquarium.

Study of Enoshima Aquarium

1. A comparative experiment breed Shinkaia crosnieri .
   a. Add to H_2S of experiment breed in watertank .
   b. Not add to H_2S of experiment breed in watertank .
      Shinkaia crosnieri give other foods (shrimps and Shellfishes)
   c. *Shinkaia crosnieri* don't give not at all .
2. Public information depend on display of *Shinkaia crosnieri*.
3. This dive analyzed water (NH_3-N, NO_2-N, NO_3-N, H_2S) make use of deep sea living things breed.

8.2 Geochemistry and mercury isotope geochemistry

K. Marumo

We will obtain $\delta^{202}Hg/^{198}Hg$, $\delta^{202}Hg/^{199}Hg$ and $\delta^{202}Hg/^{200}Hg$ values of the sulfide chimney samples, squat crabs and gastropods collected from Iheya-Kita seafloor hydrothermal area in the Okinawa Trough, using a high resolution multicollector inductively coupled plasma mass spectrometer (MC-ICP-MS). If a large range of $\delta^{202}Hg/^{198}Hg$ variation is obtained in these sulfide and biological samples than might be expected for such a heavy element as Hg, it will be due to a predominance of kinetic effects. Light Hg isotope in the vapor will be mixed with oxygenated seawater near seafloor during the mineralization, meanwhile, heavy Hg isotope in liquid is expected to be deposited as Hg sulfide in chimney samples.

The major elements and some minor elements of the sulfide chimney samples will be determined using an X-ray fluorescence spectrometer (XRF), and other minor and trace elements were determined by inductively coupled plasma optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS). Sample decomposition for ICP-OES and ICP-MS analysis were performed by fusion and digestion with mixed acid. Mercury concentration was determined using a Flow Injection Mercury System.

Hg analysis

T. Tomiyasu, H. Kodamatani, and Y. Kono

The mercury concentration in sea water and sediment samples taken around geothermal vents will be determined. After the measurement, the dispersion and change of chemical forms of mercury will be discussed.

Water: Hg^0 in water sample is collected on porous gold collector by bubbling the water samples with N_2 gas. The porous gold collector is heated and atomic absorption of released mercury is measured. After the Hg^0 measurement, Hg^{2+} concentration in water samples is measured by cold vapor atomic absorption spectrometry (CVAAS) by using $SnCl_2$ as a reducing agent. For the total concentration of mercury, the water sample is filtered with 0.45 μm membrane filter. After the filtration, BrCl solution is added into the water sample to oxidize mercury compounds to form Hg^{2+}. After the oxidation, NH_2OHHCl solution is added to decompose the excess BrCl and the

mercury concentration in water samples is measured by CVAAS.

Sediment: Total concentration of mercury is measured by CVAAS after acid digestion at 230^{o}C by using nitric acid, perchloric acid and sulfuric acid. For the measurement of methylmercury concentration, sediment sample is shaken with hydrochloric acid and methylmercury eluted into the hydrochloric acid is extracted into tluen layer. Methylmercury in toluene layer is back extracted into sodium sulfide solution and measured by CVAAS after acid digestion.

Development of the mercury sensor in deep-sea

M. Yamamoto

It is important for the investigation of mineral deposit under deep-sea and for the metal poisoning of animals to know the mercury dynamics in deep-sea. I have been developed an electrochemical sensor, namely D-POTE, for several chemicals in deep-sea using voltanmetry method. We made a gold disc electrode with propeller screw, to detect ionic mercury in seawater. In this cruise, I use the D-POTE with this electrode to monitor mercury in deep-sea hydrothermal field in Iheya North, Okinawa Trough. Last year, it was reported that there is a large-scale mineral deposit under the hydrothermal field in Iheya North. My analytical technique and data of mercury dynamics in Iheya North should be useful for estimate of the market value of the deposit and of the environmental pollution of mercury with exploiting.

Mineralogy

K. Komuro

In the course of development of an exploration tool for submarine hydrothermal deposits by using mercury isotopes, samples for elemental and mercury isotopic analysis will be prepared, and mode of occurrences of mercury in modern submarine hydrothermal deposits will be examined. Samples in this project are collected in this cruise.

Sulfides ore and associated rock samples around the hydrothermal chimney are collected in submarine survey of the cruise (Figs. 1-3). In the laboratory in Natsushima, modes of occurrences of minerals in the collected samples were observed under a binocular microscope and were selected and prepared for portable X-ray fluorescence

analysis (XRF).

The plan in the future will be (1) sample preparation for elemental and mercury isotopic analysis, and (2) petrographic works for understanding the mode of occurrences of mercury. With using these data, behavior of mercury isotopes and its potential for exploration tool for submarine hydrothermal deposits will be discussed.

Fig. 1. Sample #1324R01 taken from a chimney in the cruise #1324. The sample is composed of fine muddy sulfides with outer white part probably of quartz.

Fig. 2. Samples #1325R01-1 (upper, left), 1325R01-2 (upper right) and 1325R02 (lower) taken from a base of chimney in the cruise #1325. The sample is composed of

two parts, black part mainly of sulfides and orange part probably of goethite.

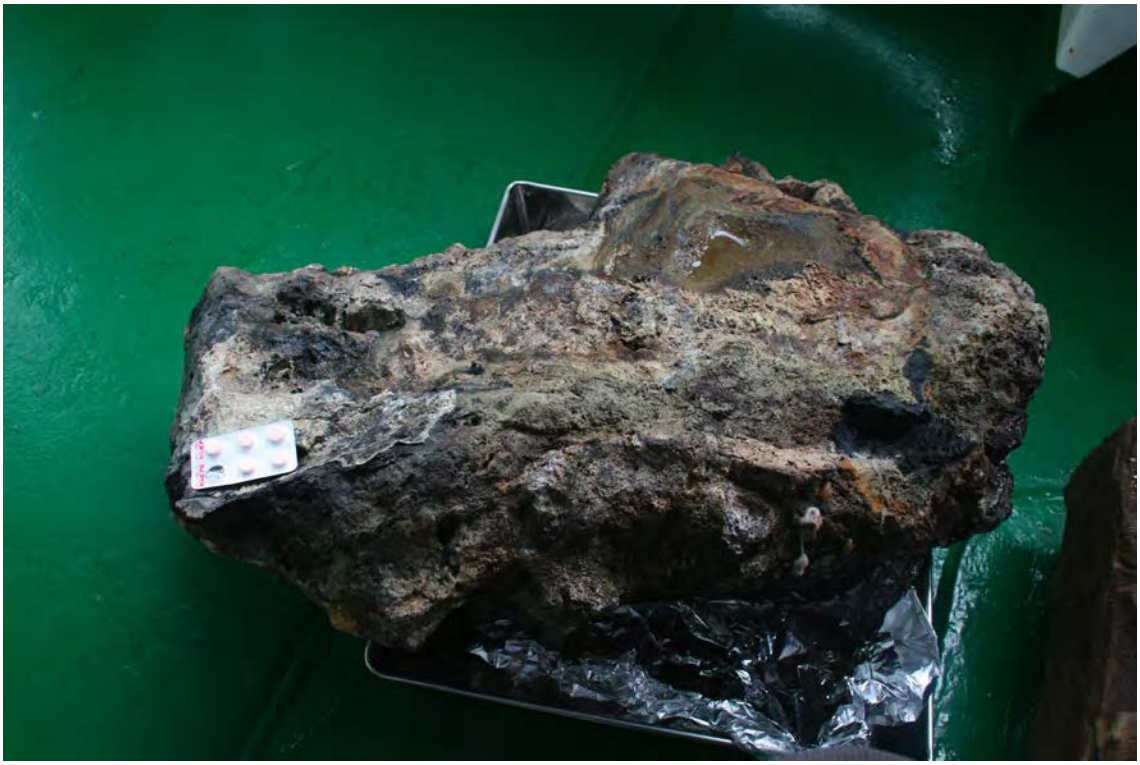


Fig. 3. Samples #1326R01 and #1326R02 taken from a base of chimney in the cruise #1326. The sample #1326R01 is loose black part of sulfides with orange part

probably of goethite. The sample #1326R02 is composed of two parts, black part mainly of sulfides and yellow part mainly of native sulfur.

9. Sample list

NT11-19 #1324-1326

Sample code	Description	Site	Date	Latitude	Longitude	Depth	Total	Dsistribution (amount)
HPD#1324-R01	rock	NBC	Sep 25th	27-47.459N	126-53.796E	981	1500g	Tsukuba univ.(1500)
HPD#1324-R02	chimney structure	NBC	Sep 25th	27-47.459N	126-53.796E	978	3060g	AIST(1000), Tsukuba univ.(1000), JAMSTEC(60), Hokkaido univ.(1000)
HPD#1324-N01	Niskin red	NBC	Sep 25th	27-47.466N	126-53.839E	1016	1140ml	JAMSTEC(100), Enoshima(30) Kagoshima univ.(1000), Hokkaido univ.(10)
HPD#1324-W01	WHATS(1-4)	NBC	Sep 25th	27-47.463N	126-53.795E	981	20ml	JAMSTEC(20)
HPD#1324-BA01	Bag 1	NBC	Sep 25th	27-47.463N	126-53.795E	981	3140ml	JAMSTEC(100), Enosui(30), Kagoshima(3000), Hokkaido(10)
HPD#1324-BA02	Bag 2	NBC	Sep 25th	27-47.459N	126-53.796E	978	1240ml	JAMSTEC(100), Enosui(30), Kagoshima(1100), Hokkaido(10)
HPD#1324-B01	squat crab	NBC	Sep 25th	27-47.463N	126-53.795E	981	321 individuals	AIST(10), JAMSTEC(120), Hokkaido univ.(203)
HPD#1325-R01	chimney structures	ESBC	Sep 25th	27-47.422N	126-53.817E	1006	1740g	Tsukuba univ.(1500), JAMSTEC(120), Hokkaido univ.(120)
HPD#1325-R02	rock	ESBC	Sep 25th	27-47.422N	126-53.817E	1006	1560g	Tsukuba univ.(1500), Hokkaido univ.(60)
HPD#1325-W01	WHATS(1-4)	ESBC	Sep 25th	27-47.422N	126-53.817E	1006	20ml	JAMSTEC(20)
HPD#1325-BA01	Bag 1	ESBC	Sep 25th	27-47.422N	126-53.817E	1006	2890ml	JAMSTEC(100), Enosui(30), Kagoshima univ.(2750), Hokkaido univ.(10)
HPD#1325-BA02	Bag 2	ESBC	Sep 25th	27-47.506N	126-53.943E	1043	3040ml	JAMSTEC(100), Enosui(30), Kagoshima univ.(2900), Hokkaido univ.(10)
HPD#1325-N01	Niskin red	ESBC	Sep 25th	27-47.506N	126-53.943E	1043	1140ml	JAMSTEC(100), Enosui(30), Kagoshima(1000), Hokkaido univ.(10)
HPD#1325-N02	Niskin green	ESBC	Sep 25th	27-47.526N	126-53.941E	780	1540ml	JAMSTEC(100), Enosui(30), Kagoshima(1400), Hokkaido univ.(10)
HPD#1325-B01	squat crabs	ESBC	Sep 25th	27-47.422N	126-53.817E	1005	287 individuals	AIST(10), JAMSTEC(30), Enosui(34), Kagoshima univ.(10), Hokkaido univ.(210)
HPD#1325-B02	Bathymodiolus japonicus	ESBC	Sep 25th	27-47.422N	126-53.817E	1005	30 individuals	AIST(20), Kagoshima univ.(10)
HPD#1325-B03	Alvinocarididae	ESBC	Sep 25th	27-47.422N	126-53.817E	1005	24 individuals	Enosui(16)
HPD#1325-M01	MBARI 1	ESBC	Sep 25th	27-47.506N	126-53.943E	1043	22cm	Kagoshima univ.(22)
HPD#1326-R01	rcok (with Bathymodiolus japonicus)	NBC EAST	Sep 26th	27-47.419N	126-53.985E	1059	2030g	Tsukuba univ.(2000), Hokkaido univ.(30)
HPD#1326-R02	rock	NBC	Sep 26th	27-47.456N	126-53.816E	1001	1120g	Tsukuba univ.(1000), JAMSTEC(60), Hokkaido univ.(60)
HPD#1326-N01	Niskin red	NBC EAST	Sep 26th	27-47.356N	126-54.185E	1025	1540ml	JAMSTEC(100), Enosui(30), Kagoshima univ.(1400), Hokkaido univ.(10)
HPD#1326-W01	WHATS(1-4)	NBC	Sep 26th	27-47.456N	126-53.816E	1001	20ml	JAMSTEC(20)
HPD#1326-BA01	Bag 1	NBC	Sep 26th	27-47.456N	126-53.816E	1001	3040ml	JAMSTEC(100), Enosui(30), Kagoshima univ.(2900), Hokkaido univ.(10)
HPD#1326-N02	Niskin green		Sep 26th	27-47.456N	126-53.816E	600	1490ml	JAMSTEC(100), Enosui(30), Kagoshima univ.(1350), Hokkaido univ.(10)
HPD#1326-B01	squat crabs	NBC EAST	Sep 26th	27-47.443N	126-53.919E	1043	96 individuals	AIST(10), Enosui(35), Hokkaido univ.(66)
HPD#1326-B02	squat crabs	NBC	Sep 26th	27-47.456N	126-53.816E	1002	7 individuals	JAMSTEC(7)
HPD#1326-M01	MBARI 1	NBC EAST	Sep 26th	27-47.356N	126-54.185E	1025	26cm	Kagoshima univ.(26)
HPD#1326-M02	MBARI 2	NBC EAST	Sep 26th	27-47.435N	126-53.963E	1060	18cm	Kagoshima univ.(18)
HPD#1326-M03	MBARI 3	NBC EAST	Sep 26th	27-47.440N	126-53.893E	1042	25cm	Kagoshima univ.(25)

10. Dive Reports

10-1. 1st dive

Satoshi Nakagawa

Dive No.: 1324
Date: September 25th, 2011
Site: Iheya North
Landing: 9:26; 27°47.474'N, 126°53.841'E, 1015m
Leaving: 11:35; 27°47.459'N, 126°53.796'E, 977m

Objectives:

The objectives include 1) to collect hydrothermal samples including vent animals, vent fluids, chimney structures, and bottom seawater, and 2) to recover Watsuji-type colonizers deployed before.

Dive Summary:

We successfully collected hydrothermal samples, i.e. vent animals (squat crabs), vent fluids, chimney structures, and rocks. Samples were prepared immediately for shore-based microbiological and biogeochemical analyses. In addition, we successfully recovered Watsuji-type colonizers deployed in the colony of squat crabs (NBC mound).

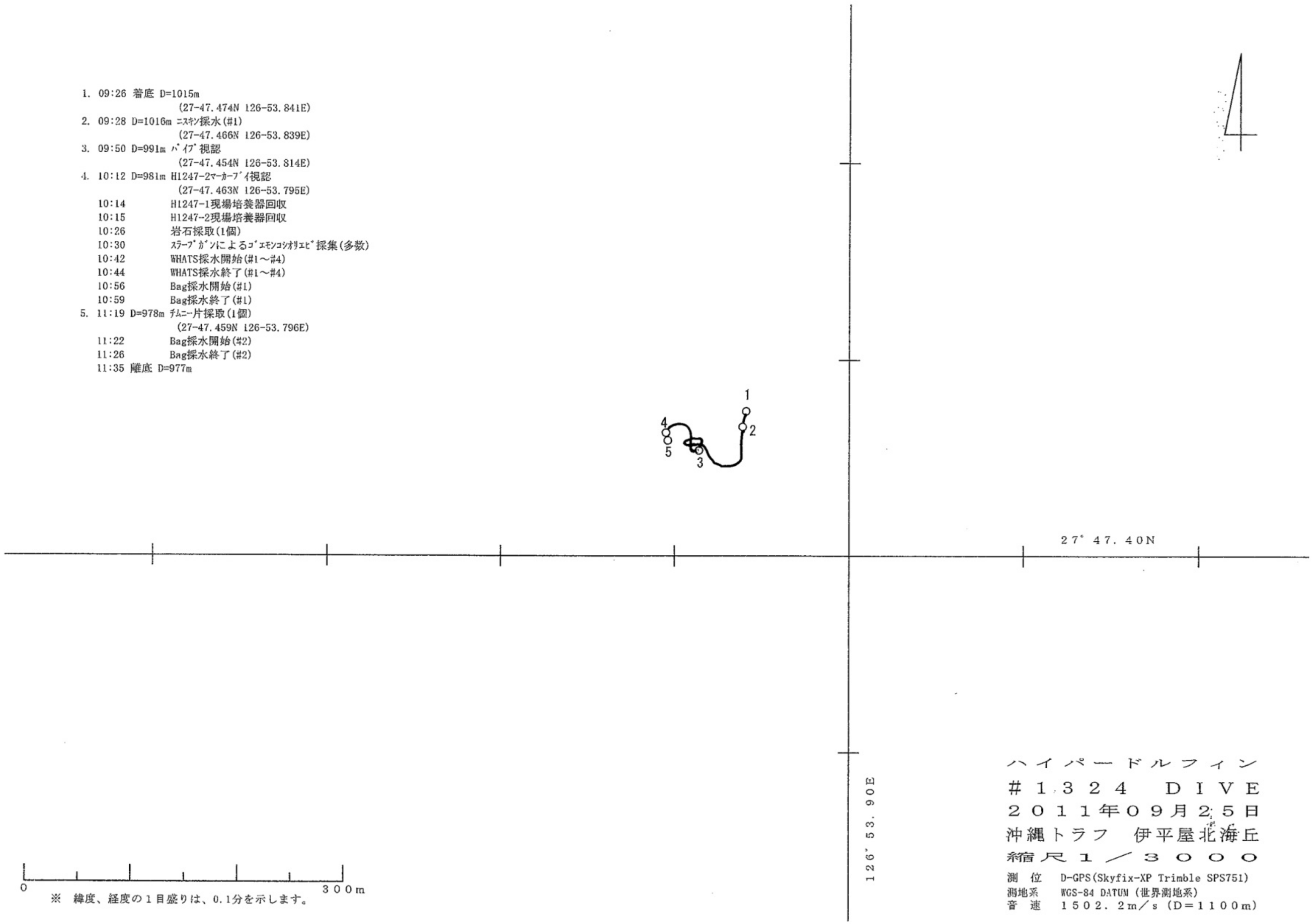
Payloads:

1) WHATS with a temperature probe
2) Bag pump sampler (6L x 2)
3) Sample box (x 2)
4) Niskin bottles (x 2)
5) DO meter
6) D-pote
7) Slurp gun (2 canisters)
8) MBARI-type corer (x 3)
9) Turbidity meter

Event List:

9:26	27-47.474N, 126-53.841E	D=1015m	Landing bottom
9:28	27-47.466N, 126-53.839E	D=1016m	Sampling seawater (Niskin 1)
10:14	27-47.463N, 126-53.795E	D=981m	Recovering colonizer (1247-1)
10:15	27-47.463N, 126-53.795E	D=981m	Recovering colonizer (1247-2)
10:26	27-47.463N, 126-53.795E	D=981m	Sampling a rock
10:30	27-47.463N, 126-53.795E	D=981m	Sampling squat crabs
10:42	27-47.463N, 126-53.795E	D=981m	Sampling seawater (WHATS1-4)
10:56	27-47.463N, 126-53.795E	D=981m	Sampling seawater (Bag 1)
11:19	27-47.459N, 126-53.796E	D=978m	Sampling chimney structure
11:22	27-47.459N, 126-53.796E	D=978m	Sampling seawater (Bag 2)

Dive track:


1. 09:26 着底 D=1015m
(27-47.474N 126-53.841E)
2. 09:28 D=1016m ﾆｽｷﾝ採水(#1)
(27-47.466N 126-53.839E)
3. 09:50 D=991m ﾊﾟｲﾌﾟ視認
(27-47.454N 126-53.814E)
4. 10:12 D=981m H1247-2ﾏｰｶｰﾌﾞｲ視認
(27-47.463N 126-53.795E)
10:14 H1247-1現場培養器回収
10:15 H1247-2現場培養器回収
10:26 岩石採取(1個)
10:30 ｽﾗｰﾌﾟｶﾞﾝによるｺﾞｴﾓﾝｺｼｵﾘｴﾋﾞ採集(多数)
10:42 WHATS採水開始(#1～#4)
10:44 WHATS採水終了(#1～#4)
10:56 Bag採水開始(#1)
10:59 Bag採水終了(#1)
5. 11:19 D=978m ﾁﾑﾆｰ片採取(1個)
(27-47.459N 126-53.796E)
11:22 Bag採水開始(#2)
11:26 Bag採水終了(#2)
11:35 離底 D=977m
1
2
3
4
5
27° 47. 40N
126° 53. 90E
0
300m
※ 緯度、経度の1目盛りは、0.1分を示します。
ハイパードルフィン
#1324 DIVE
2011年09月25日
沖縄トラフ 伊平屋北海丘
縮尺1／3000
測 位 D-GPS(Skyfix-XP Trimble SPS751)
測地系 WGS-84 DATUM (世界測地系)
音 速 1502. 2m／s (D=1100m)

10-2. 2nd dive *Satoshi Nakagawa*

Dive No.: 1325
Date: September 25th, 2011
Site: Iheya North
Landing: 15:09; 27°47.433'N, 126°53.820'E, 1000m
Leaving: 17:20; 27°47.506'N, 126°53.943'E, 1043m

Objectives:

The objectives include 1) to collect hydrothermal samples including vent animals, vent fluids, chimney structures, and bottom seawater, and 2) to recover Watsuji-type colonizers deployed before.

Dive Summary:

We successfully collected hydrothermal samples, i.e. vent animals (squat crabs), vent fluids, chimney structures, and rocks. Samples were prepared immediately for shore-based microbiological and biogeochemical analyses. In addition, we successfully recovered Watsuji-type colonizers deployed at event #15 (control site).

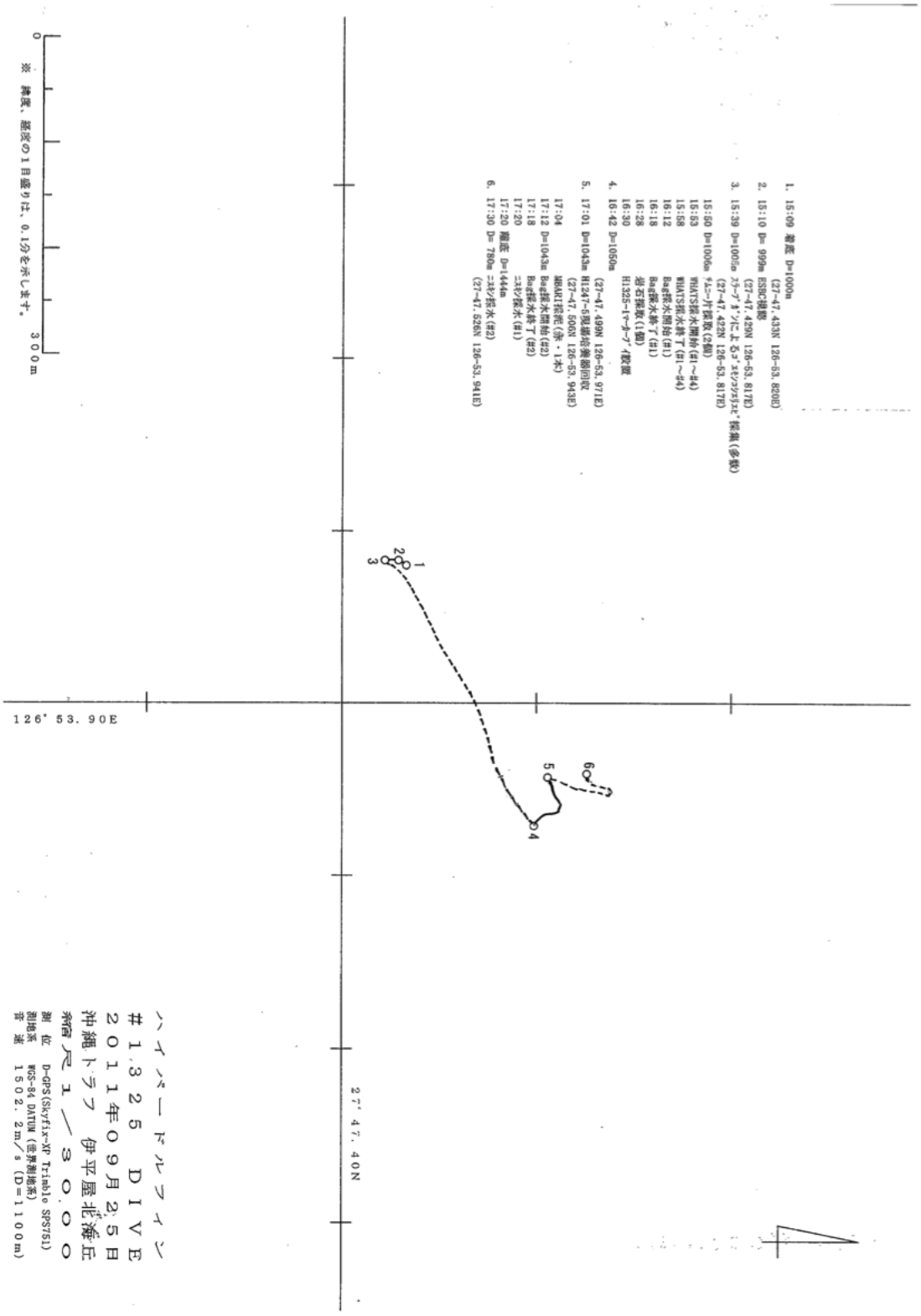
Payloads:

1) WHATS with a temperature probe
2) Bag pump sampler (6L x 2)
3) Sample box (x 2)
4) Niskin bottles (x 2)
5) DO meter
6) D-pote
7) Slurp gun (2 canisters)
8) MBARI-type corer (x 3)
9) Turbidity meter

Event List:

15:09	27-47.433N, 126-53.820E	D=1000m	Landing bottom
15:39	27-47.422N, 126-53.817E	D=1006m	Sampling squat crabs
15:50	27-47.422N, 126-53.817E	D=1006m	Sampling chimney structures
15:53	27-47.422N, 126-53.817E	D=1006m	Sampling seawater (WHATS1-4)
16:12	27-47.422N, 126-53.817E	D=1006m	Sampling seawater (Bag 1)
16:28	27-47.422N, 126-53.817E	D=1006m	Sampling a rock
16:30	27-47.422N, 126-53.817E	D=1006m	Marker deployment (H1325-1)
17:01	27-47.506N, 126-53.943E	D=1043m	Recovering colonizer (1247-5)
17:04	27-47.506N, 126-53.943E	D=1043m	Sampling sediments (MBARI1)
17:12	27-47.506N, 126-53.943E	D=1043m	Sampling seawater (Bag 2)
17:20	27-47.506N, 126-53.943E	D=1043m	Sampling seawater (Niskin 1)
17:20	27-47.506N, 126-53.943E	D=1043m	Leaving bottom
17:30	27-47.526N, 126-53.941E	D=780m	Sampling seawater (Niskin 2)

Dive track:


1. 15:09 着底 D=1000m
(27-47.433N 126-53.820E)
2. 15:10 D= 999m ESSC採取
(27-47.429N 126-53.817E)
3. 15:39 D=1005m ﾌﾗｰﾌﾞﾈｯﾄ及びｽﾎﾟﾝｼﾞｽﾀｰﾗｲﾄ採集(多数)
(27-47.422N 126-53.817E)
15:50 D=1006m ﾊﾋﾆｰ片採取(2個)
15:53 WHATS採水開始(#1～#4)
15:58 WHATS採水終了(#1～#4)
16:12 Bag採水開始(#1)
16:18 Bag採水終了(#1)
16:28 岩石採取(1個)
16:30 H1325-ﾄﾚｰﾅｰ1枚回収
4. 16:42 D=1050m
(27-47.499N 126-53.971E)
5. 17:01 D=1043m H1247-5現場投棄器回収
(27-47.506N 126-53.943E)
17:04 MMARI採泥(泳・1本)
17:12 D=1043m Bag採水開始(#2)
17:18 Bag採水終了(#2)
17:20 ﾆｽﾝ採水(#1)
17:20 着底 D=1444m
17:30 D= 780m ﾆｽﾝ採水(#2)
6. (27-47.526N 126-53.941E)
1
2
3
4
5
6
126° 53. 90E
27° 47. 40N
0
300m
※ 横軸、縦軸の1目盛りは、0.1分を示します。
ハイパードルフィン
#1325 DIVE
2011年09月25日
沖縄トラフ 伊平屋北海丘
縮尺 1／3000
測 位 D-GPS(SkyFix-XP Trimble SPS751)
測地系 WGS-84 DATUM (世界測地系)
音 速 1502.2m/s (D=1100m)

10-3. 3rd dive *Masahiro Yamamoto*

Dive No.: 1326

Date: September 26th, 2011

Site: Iheya North

Landing: 9:13; 27°47.356'N, 126°54.185'E, 1025m

Leaving: 11:53; 27°47.456'N, 126°53.816'E, 1001m

Objectives:

The objectives include 1) to collect deep-sea samples including hydrothermal vent animals, sediments, rocks, and bottom seawater, and 2) to collect measurement data using sensors of turbidity, dissolved oxygen, and ionic mercury.

Dive Summary:

Through the dive was shortened because of bad sea condition, we successfully collected deep-sea samples, i.e. vent animals (squat crabs), vent fluids, chimney structures, and rocks, and data, i.e. turbidity, dissolved oxygen, and ionic mercury. Samples were prepared immediately for shore-based microbiological and biogeochemical analyses.

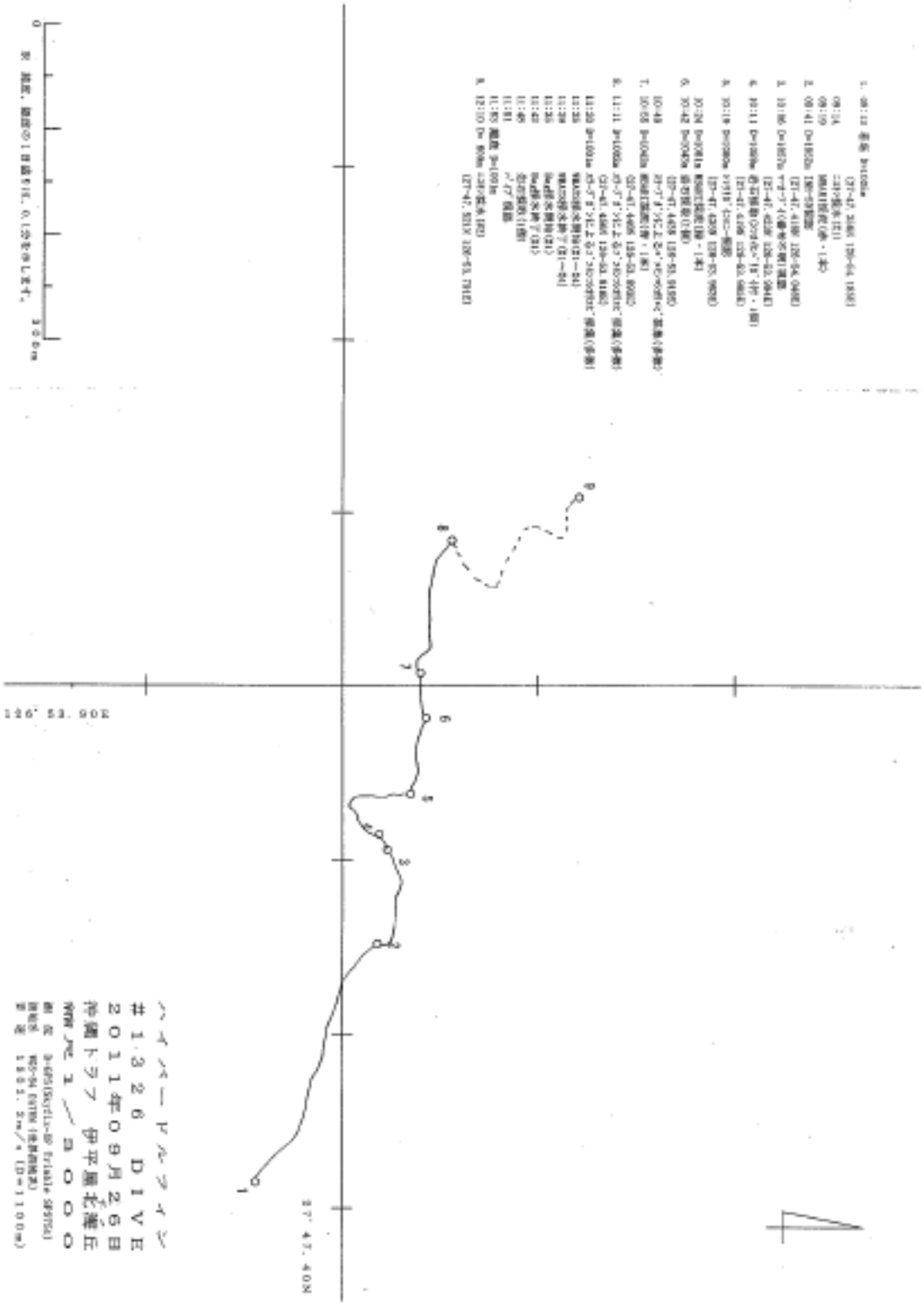
Payloads:

1) WHATS with a temperature probe
2) Bag pump sampler (6L x 2)
3) Sample box (x 2)
4) Niskin bottles (x 2)
5) DO meter
6) D-pote
7) Slurp gun (2 canisters)
8) MBARI-type corer (x 3)
9) Turbidity meter

Event List:

8:13		D=0m	Landing on the water
9:13	27-47.356N, 126-54.185E	D=1025m	Landing bottom
9:14			Sampling seawater (Niskin 1)
9:19			Sampling sediments (MBARI 1)
10:11	27-47.419N, 126-53.985E	D=1059m	Sampling a rock with bivalves
10:24	27-47.435N, 126-53.963E	D=1060m	Sampling sediments (MBARI 2)
10:42	27-47.443N, 126-53.919E	D=1043m	Sampling a rock
10:49			Sampling squat crabs
10:55	27-47.440N, 126-53.893E	D=1042m	Sampling sediments (MBARI 3)
11:11	27-47.456N, 126-53.816E	D=1002m	Sampling squat crabs
11:20		D=1001m	Sampling squat crabs
11:25			Sampling seawater (WHATS 1-4)
11:35			Sampling seawater (Bag 2)
11:48			Sampling a rock
11:53			Leaving bottom
12:10		D=600m	Sampling seawater (Niskin 2)
12:38		D=0m	Surfacing

Dive track:


126° 53. 90E
27° 47. 40N
ハイパードルフィン
#1326 DIVE
2011年09月26日
沖縄トラフ 伊平屋北東丘
深度 2000