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R/V Yokosuka & DSV Shinkai 6500 Cruise Report

YK13-05

Geochemical, Geomicrobiological and Biogeographical Investigation of Deep-Sea Hydrothermal Activities in the Mid Cayman Ridge, the Caribbean

June 17, 2013 from San Juan, Puerto Rico –

July 3, 2013 to Chistbal, Panama

Japan Agency for Marine-Earth Science and Technology

(JAMSTEC)

Acknowledgements

We are grateful to Captain Mr. H. Tanaka, Chief Officer Mr. H. Masujima and Chief Engineer Mr. K. Kaneda for their safe navigation and their skillful handling of “R/V Yokosuka”. Great thanks are due to Submersible Operation Manager Mr. T. Sakurai and “Shinkai 6500” operation team for their operations in sampling. We also thank Mr. H. Iwamoto, Nippon Marine Enterprise, Ltd., for his attentive supports. In this cruise, all the ship crews, Shinkai 6500 operation team and onboard scientists tried to broadcast all the day of Shinkai 6500 survey onboard and in the deep-sea to public peoples in Japan by way of a satellite communication. It was really successful as the result of everyone’s efforts and endeavors and was a great landmark challenge of JAMSTEC. We thank all the JAMSTEC peoples who have supported us and this cruise. Finally, we would like to appreciate all the persons who have encouraged directly or indirectly this cruise.

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Cruise information

Cruise ID: YK13-5

Vessel: YOKOSUKA

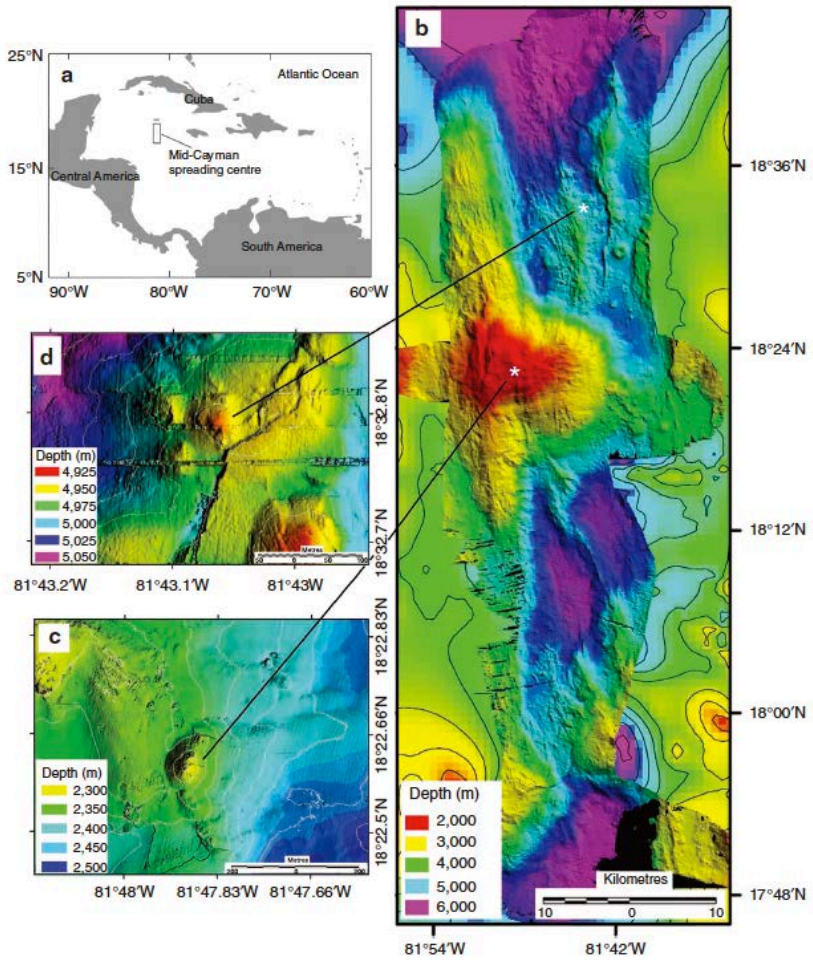
Title of the cruise: Quelle 2013 Quest 4: Geochemical, Geomicrobiological and Biogeographical Investigation of Deep-Sea Hydrothermal Activities in the Mid Cayman Ridge, the Caribbean

Title of proposal:
Proposal 1) Clarification of the diversity of geo- and bio-systems and of the potential principles linking the systems in Central Indian Ridge hydrothermal activities
Proposal 2) Ship Observation of Global CO2 Concentration for Atmosphere-Ocean Carbon Exchange Estimation from GOSAT Data (Researchers not attended)

Representative of the science parties [Affiliation]:
Proposal 1) Ken Takai [JAMSTEC]
Proposal 2) Kei Shiomi [JAXA] (Not attended)

Cruise period: June 17 – July 3, 2013

Ports of call: San Juan, Puerto Rico – Chistbal, Panama



Research area:
1. Mid Cayman Rise (Ridge)

Research map:

Figure (A) Location and of the of the Mid-Cayman Spreading Centre (MCSC), (B):ship-borne multibeam bathymetry of the MCSC, (C) highresolution (2m) bathymetry of the ultra-deep vent site and (D) of the Mt Dent vent site. (taken from Murton, Copley, Connelly and the Ship party of JC44).

List of Participant

Scientific party

Chief Scientist

Dr. Ken Takai

Program Director (Geomicrobiology)

Subsurface Geobiology Advanced Research (SUGAR) Program & Precambrian Ecosystem Laboratory, Submarine Hydrothermal System Research Group, Japan Agency for Marine-Earth Science & Technology (JAMSTEC)

Vice Chief Scientist

Dr. Jonathan Timothy Peter Copley

Lecturer (Biology)

Ocean and Earth Science, National Oceanography Centre Southampton, University of Southampton Waterfront Campus, European Way, Southampton SO14 3ZH, UK

Staff Scientists

Dr. Hiromi Watanabe

Research Scientist (Biology)

Marine Biodiversity Research Program, Institute of Biogeoscience, Japan Agency for Marine-Earth Science & Technology (JAMSTEC)

Dr. Junichi Miyazaki

Research Scientist (Microbiology)

SUGAR Program & Precambrian Ecosystem Laboratory, Japan Agency for Marine-Earth Science & Technology (JAMSTEC)

Dr. Katsunori Yanagawa

Postdoctoral Research Fellow (Microbial Ecology)

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

Dr. Shinsuke Kawagucci
Research Scientist (Geochemistry)
SUGAR Program & Precambrian Ecosystem Laboratory, Japan Agency for Marine-Earth Science & Technology (JAMSTEC)

Dr. Norio Miyamoto
Research Scientist (Biology)
Marine Biodiversity Research Program, Institute of Biogeoscience, Japan Agency for Marine-Earth Science & Technology (JAMSTEC)

Mr. Takuya Yahagi
Ph. D. Student (Biology)
School of Fronteir Sciences, University of Tokyo

Mr. Tadashi Yoshizawa
Public Relation Administrator
Public Relations Division, Advanced Research and Technology Promotion Department, Japan Agency for Marine-Earth Science & Technology (JAMSTEC)

Ms. Verity Ellen Nye
Ph.D. Student (Biology)
Ocean and Earth Science, National Oceanography Centre Southampton, University of Southampton Waterfront Campus, European Way, Southampton SO14 3ZH, UK

Ms. Diva Joan Amon
Ph. D Student (Biology)
Ocean and Earth Science, National Oceanography Centre Southampton, University of Southampton Waterfront Campus, European Way, Southampton SO14 3ZH, UK

Dr. Priya Narasingarao
Postdoctoral Research Fellow (Microbiology)
Scripps Institution of Oceanography, University of California, San Diego, La Jolla, 92037, USA

Dr. Daniella Morgan Smith
Postdoctoral Research Fellow (Microbiology)
East Carolina University, Department of Biology, Howell Science Complex, Rm. S301B, Greenville, NC 27858, USA

Mairne Technicians

Mr. Hisanori Iwamoto
Marine Science Department, Nippon Marine Enterprises, LTD.

Captain and crew of the R/V YOKOSUKA

Captain	**Hitoshi Tanaka**
Chief Officer	**Hiroaki Masujima**
2nd Officer	**Takeshi Egashira**
3rd Officer	**Yumihiko Kobayashi**
Chief Engnieer	**Kazuhiko Kaneda**
1st Engnieer	**Takashi Ohta**
2nd Engnieer	**Kenta Ikeguchi**
3rd Engnieer	**Shota Nagano**
Chief Radio Officer	**Tokinori Nasu**
2nd Radio Officer	**Hiroki Ishiwatari**
3rd Radio Officer	**Yoshikazu Kuramoto**
Boat Swain	**Kazuo Abe**
Able Seaman	**Masanori Ohhata**
Able Seaman	**Ikunori Iwasaki**
Able Seaman	**Takuya Miyashita**
Able Seaman	**Daiki Shigeta**
Able Seaman	**Yuta Motooka**
Able Seaman	**Sumihiro Ogawa**
Sailor	**Kouzo Miura**
Sailor	**Toshinori Matsui**
Sailor	**Masayuki Fujiwara**
Sailor	**Yuji Azumagawa**
Oiler	**Ryo Sato**
Steward	**Ikuo Mastumoto**
Steward	**Hideo Fukumura**
Steward	**Yoshio Okada**
Steward	**Kazuma Sonoda**
Stewerd	**Tadanori Kanda**

“Shinkai 6500” Operation Team

Operation Manager	**Toshiaki Sakurai**
Assistant Operation Manager	**Yoshitaka Sasaki**
1st Submersible Staff	**Kazuki Iijima**
1st Submersible Staff	**Mituhiro Ueki**
1st Submersible Staff	**Keita Matsumoto**
2nd Submersible Staff	**Hitomi Ikeda**
2nd Submersible Staff	**Hirofumi Ueki**
2nd Submersible Staff	**Keigo Suzuki**
2rd Submersible Staff	**Takuma Onishi**
2rd Submersible Staff	**Akihisa Ishikawa**
3rd Submersible Staff	**Masaya Katagiro**
3rd Submersible Staff	**Yudai Tayama**

I. CRUISE SUMMARY

In YK13-05 cruise, we have totally conducted 9 dives of Shinkai6500 in two hydrothermal fields (5 dives in Beebe Field and 4 dives in Von Damm Field) in the Mid Cayman Ridge (MCR). One of the significant events during the cruise, we have successfully attained a live streaming of full-time scientific dive survey of a day (from the preparation of Shinkai6500's dive early morning to the completion of Shinkai6500 onboard). This was the world's first example of a live streaming of a manned submersible's scientific dive survey and was the second example of a successful live streaming of a manned submersible survey after James Cameron's Titanic exploration. More than 300,000 peoples in the world (of course, mainly in Japan) watched the Shinkai6500's day including fantastic images of 5000 m deep seafloor, hydrothermal vents and dense animals there. This challenge highly energized and fostered the deep curiosity and interest of public peoples, particularly younger generations who had never seen or known about the deep-sea world and the unique ecosystem, the yet-unexplored world in this planet. The widespread curiosity in public society will be the most powerful encouragement of the future scientific exploration and investigation of manned submersible that has been quickly replaced by unmanned vehicles such as ROV and AUV.

We have obtained lots of and diverse samples from two of the MCR deep-sea hydrothermal environments. We have confirmed that the world's deepest hydrothermal system (Beebe Field) has about 400 ˚C of endmember hydrothermal fluid and that the hydrothermal fluids probably immediately after effluent from the newly opened conduits represent the supercritical state by direct observation of human eyes and by recorded video images. The hydrothermal fluids from both Beebe and Von Damm fields were characterized by extraordinary high concentrations of H_2, which would be the world's highest concentrations or among the highest levels. The H_2-enrichment in the fluids are attributed to the association of subseafloor serpentinization somewhere in the overall hydrothermal circulation. However, different concentrations of CH4 in each of the two systems clearly indicated that the hydrothermal recharge and reaction processes of the two systems are quite different. In Von Damm field, CH_4 is highly abundant while Beebe hydrothermal fluid contains a concentration level comparable to magmatic input. The different CH4 concentrations in these hydrothermal systems point to the different host rock compositions and hydrothermal reaction processes: serpentinization of mantle peridotite and rapidly proceeding Fisher-Tropsch Type (FTT) reaction in Von Damm field and serpentinization of dunite and/or gabbroic rocks and slaggish proceeding Fisher-Tropsch Type (FTT) reaction in Beebe field. However, it is still a big mystery why quite low concentration of CO_2 is present in Von Damm hydrothermal fluids. This is a important subject for future onshore investigation.

According to a proposition proposed by Takai & Nakamura (2010;2011), the H_2-enriched hydrothermal fluid vents would host HyperSLiME-like microbial communities in the proximity of the hydrothermal fluid discharges. Prior to this YK13-05 cruise, we predicted that both Beebe and Von Damm hydrothermal systems harbored extraordinary populations of (hyper)thermophilic H_2-trophic methanogens and chemolithotrophic primary production by diverse H_2-trophs. However, based on the onboard experiments of microbial H_2 consumption using the very fresh chimney samples obtained from both fields, the microbial H_2 consumption was found to be much lower than that in the Kairei field in the CIR and the (hyper)thermophilic H_2-trophic methanogen populations seemed to be less abundant that those in CIR Kairei field and MAR Raibow field. This preliminary results are quite interesting, and we are going to pursue why (hyper)thermophilic H_2-trophic methanogens are less abundant although H2 concentration in the fluids represents the highest ever known. We wish that chemolithotrophic H_2-trophs other than (hyper)thermophilic H_2-trophic methanogens would serve as the primary producers of these hydrothermal vent microbial communities.

Most of the hydrothermal vent-endemic animal species in two hydrothermal fields were sampled. In tight collaboration between JAMSTEC and University of Southampton, species composition of chemosynthetic animal communities will be completely characterized and the biogeographic and dispersal characteristics of animal components will be pursued based on population genetics, and evolutionary and developmental research.

Finally, during YK13-05 cruise, we have nurtured good international relationship and possible future international collaborations between JAMSTEC, University of Southampton and American microbiology groups. Of course, we will develop much bigger international framework of MCR hydrothermal vent research with our friends who have worked the same time in MCR with R/V Falkor.

The new findings and knowledge from the MCR hydrothermal systems and ecosystems will provide great new aspects to understand the deep-sea hydrothermal systems and associating microbial and biological interactions in this planet.

II. INTRODUCTION

1. General backgrounds

The 110km-long Mid-Cayman spreading center (MCSC) or Mid Cayman Rise (MCR) is an ultraslow ridge, located in the Cayman Trough of the Caribbean Sea, and thereby isolated by 4,000km from the contiguous mid-ocean ridge systems. The MCR has a full-spreading rate of 15mm per year and has been active for 49My (Leroy et al., 2000). The spreading axis of the MCR presently consists of two second-order segments, separated by a small non-transform offset at 18°20'N. The depth of the spreading axis of the MCR ranges from 4,200 to >6,000 m, which makes it the world's deepest seafloor spreading center (ten Brink et al., 2002).

On the western flank of the axial valley of the MCR, there is an off-axis topographic feature known as Mount Dent, which rises 2,700m above the spreading center axis. This feature has been interpreted as an oceanic core complex (OCC) (Hayman et al., 2011), where gabbroic lower crustal and peridotite upper mantle rocks have been exposed during seafloor spreading. OCCs are now recognized as common features of slow- and ultraslow-spreading ridges, which are possibly responsible for ~50% of the extension along such ridges (Escartin et al., 2008). The mode and tempo of hydrothermal activities, the geochemical diversity of hydrothermal fluids and development and functions of chemosynthetic microbial communities associated with hydrothermal systems in these slow- and ultraslow-spreading ridges have been conducted only in limited hydrothermal fields of the Mid Atlantic Ridge (MAR).

Discoveries of hydrothermal vent fields around the world have so far revealed six biogeographic provinces for vent fauna (Bachraty et al., 2009; Van Dover et al., 2002; Vrijenhoek et al., 2010), but the origin and maintenance of these provinces are poorly understood (Tunnicliffe & Fowler, 1996; Tyler et al., 2002). At present, the only mid-ocean ridge connection between the biogeographic provinces of the eastern Pacific and northern Mid-Atlantic occurs at high latitudes, via the Pacific-Antarctic and Indian Ocean ridge systems (Van Dover et al., 2002). But before the complete closure of the Isthmus of Panama by ~3.1 Ma, there was a low-latitude deep- ocean gateway between the Pacific and Atlantic via the Caribbean (Burton et al., 1997). Chemosynthetic fauna may have dispersed across this gateway, as indicated by similarities at genus level between cold seep communities on the Oregon Margin and in the Gulf of Mexico, and a high level of COI (cytochrome oxidase subunit I) gene sequence similarity among siboglinid polychaete species at cold seeps in the Gulf of Mexico and the Gulf of California. Given this regional history, the MCR has been therefore identified by the international Census of Marine Life as a priority target for hydrothermal exploration, to investigate the influences of hydrography, geological history, isolation and bathymetry on global patterns of vent biogeography (Tyler et al., 2002; Van Dover et al., 2002).

References

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Vrijenhoek, R. C. Genetic diversity and connectivity of deep-sea hydrothermal vent metapopulations. Mol. Ecol. 19, 4391–4411 (2010).

2. Objectives of the cruise

A primary scientific goal of this expedition is to understand the geochemical, geomicrobiological and biogeographical diversity of deep-sea hydrothermal activities in the recently explored province in the Mid Cayman Rise, the Caribbean. The detail objective is to re-visit the recently discovered hydrothermal fields on the MCR in the EEZ of the UK, the Beebe and Von Damm hydrothermal fields, by means of the UK-USA joint explorations since August, 2009, and to characterize the geological settings, the hydrothermal fluid chemistry and the associating microbial and macrofaunal communities throughout the seafloor observation, the onboard measurements and experiments of the shore-base analyses and experiments.

In summer 2009, an UK-USA joint expedition using American R/V Atlantis and ROV Nereus found physical and chemical signatures of several hydrothermal activities in the MCR, the Caribbean (German et al., 2010). The expedition found at least three distinct hydrothermal vent fields (tentatively, named as Piccard, Walsh and Europa fields, respectively) in only 110 km distance along the MCR. Then, in spring 2010, an UK-USA joint expedition using British R/V James Cook and ROV HY-BIS observed seafloor hydrothermal vents in the previously named Piccard field and many hydrothermal vent-endemic animals around the hydrothermal field. It

was the deep-sea hydrothermal activity at the deepest seafloor ever known and was named as Beebe field (Connely et al, 2012). In addition, the expedition found another deep-sea hydrothermal field near the location of the previously named Europa field. Around the newly found seafloor hydrothermal activity, tubeworms that had been believed to live only in the Pacific were found (Connely et al, 2012). The second hydrothermal field was named as Von Damm field and provided important clues to understanding evolution and propagation histories of hydrothermal vent-endemic animals by using a short cut of the Isthmus of Panama during the last several million years against the ground theory from the eastern Pacific to the Atlantic oceans (Connely et al, 2012). In addition, in January 2012, an UK-USA joint expedition using American R/V Atlantis and ROV Jason was conducted to obtain more samples from these two deep-sea hydrothermal vent fields in the MCR.

The explorations of deep-sea hydrothermal systems in the MCR have provided new and important findings as reported by Nye et al. (2013). However, there is still undiscovered the hydrothermal activities that are probably equivalent to the hydrothermal plume previously named as Walsh and even true Europa. In addition, the detail geochemical, geomicrobiological and biogeographical characteristics of the deep-sea hydrothermal systems in the MCR remain to be investigated.

Thus, here, we, Japanese and British scientific team, are planning to re-visit the Beebe and Von Damm hydrothermal fields located in the UK EEZ of the MCR and to characterize the geological settings, the hydrothermal fluid chemistry and the associating microbial and macrofaunal communities throughout the seafloor observation by the SHINKAI6500 and the research vessel (RV) YOKOSUKA.

References:

Connelly, D. P. et al. Hydrothermal vent fields and chemosynthetic biota on the world's deepest seafloor spreading centre. Nature Comm. 3, 620, DOI:10.1038/ncomms1636 (2012).

German, C. R. et al. Diverse styles of submarine venting on the ultraslow spreading Mid-Cayman Rise. Proc. Natl. Acad. Sci. USA 107, 14020-14025 (2010).

Nye, V., Copley, J. T., & Tyler, P. A. Spatial variation in the population structure and reproductive biology of Rimicaris hybisae (Caridea: Alvinocarididae) at hydrothermal vents on the Mid-Cayman Spreading Centre. PLoS One 8, e60319 (2013).

III. EXPLANATORY NOTE

1. Manned Research Submersible "Shinkai 6500"

Mission of "Shinkai 6500"

"Shinkai 6500" is able to operate surveys and observations down to the depth 6500 meters with one scientist and two pilots. During the operation, "Shinkai 6500" finds her position by two ways; Long Base Line system (LBL) and Super Short Base Line system (SSBL). The LBL system needs three bottom-mounted transponders to be deployed in the survey area. "Shinkai 6500" locates her own position by herself in real time and the mother ship determines the position of "Shinkai 6500" based on the position of transponders. The SSBL system does not require any transponder but the accuracy of the position is inferior to LBL system and "Shinkai 6500" cannot determine her own position.

Specifications

Length:	9.5m
Width:	2.7m
Height:	3.2m
Weight in air:	25.8t
Maximum operation depth:	6500m
Complement:	3 (2 pilots and 1 researcher)
Inner radius of pressure vessel:	2.0m
Normal dive time:	8 hours
Life support duration:	129 hours
Payload:	150kg (weight in air)
Under water speed:	0-2.0 knots (Emergency: 2.5 knots)
Observation instruments:	Pan-tilt-zoom color video camera
	Fixed-view color video camera
	35mm still camera
	CTD sensors
	Gamma ray spectrometer
	CTFM sonar
	Video-image transmission system
Operating devices:	2 manipulators
	2 retractable baskets

2. Research Vessel "Yokosuka"

Mission of "Yokosuka"

1) Operate submersible "Shinkai 6500"
2) Operate underway-geophysical equipments;

Multi Narrow Beam Echo Sounder (Sea Beam 2112.04)
Gravity meter (Type S-63)
Ship-borne three-components magnetometer (Type SFG-1212)
Proton magnetometer (Typ STC10)

Research Facilities

In wet laboratory, a fumigation chamber, Milli-Q water purification system, -80°C and -20°C freezer, incubator and rock saw are equipped. In addition, "Yokosuka" has on-board video editing system for DVCAM, S-VHS and VHS.

Specifications

Length:	105.22m
Breadth:	16.0m
Height:	7.3m
Draft:	4.5m
Gross tonnage:	4439t
Cruising speed:	about 16kts
Cruising range:	about 9000mile

3. Geochemistry

WHATS fluid sampler

WHATS-II (Water Hydrothermal Atsuryoku Tight Sampler II) was developed for collecting hydrothermal fluid samples without any loss and atmospheric contamination of gas species. Since it overflows its sample bottle with sampling fluids, it is rather easy to collect fluids close to the endmember. This sampling system consists of inlet tubing, 4 pressure-resistant sample bottles with ball valves at both ends (volume of one bottle: 150 ml), an arm to open and shut the valve and a deep-sea compatible pump. Usually WHATS is installed just below the shell of Shinkai 6500 and a sample inlet is handled with a manipulator (Fig. 3-8). Operation is controlled from inside the shell. At the time of each sampling, fluid temperature can be monitored using a thermometer attached to the top of the inlet tube. It takes about 7 minutes to fill up one sample bottle of 150 ml capacity. Detailed description of the system is shown bellow.

Description

Dimension of frame:	600 mm × 660 mm
Weight:	35.2 kg in air
	28.0 kg in seawater
Depth range:	4000 m
Sample volume:	150 ml × 4

Sampling rate:	75-300 ml / min
Electricity:	DC24 V / 1.0 A

Cheap WHATS sampler

Cheap WHATS is a newly developed gas-tight fluid sampler (Fig.3-9) by Ken Takai, after several modification of WHATS-II, which much less cost than WHATS-II. The whole sampling scheme is very similar with that of WHATS-II but the manipulation of valve open/close is not operated by electric motor but by the submersible's or the ROV's manipulator. This improvement ensures the successful valve operation. However, in some cases, the successful valve operation is inhibited by messy payload and is affected by the unexpected physical misuse.

Description	
Dimension of frame:	Flexible
Weight:	Flexible (>4 kg in air, 2 kg in water)
Depth range: 3	500 m
Sample volume:	Flexible
Sampling rate:	150 ml / min
Electricity:	DC24 V / 1.0 A

Treatment and onboard analyses of WHATS-II & Cheap WHATS samples for gas chemistry

In general, for vent fluid sampling, the WHATS-II sample bottles were in pairs, with one of the bottles used for the analysis of soluble compoenents chemistry and the other for gas chemistry. The bottle devoted to gas chemistry was processed on board using a high vacuum line specifically designed for this purpose using the following procedure. After the WHATS bottle is connected to the vacuum line, all of the connecting lines are evacuated to high vacuum using an oil diffusion pump. When sufficient vacuum is achieved, the vacuum line is closed in a static condition, and the valve on the bottle is opened and the water plus gas is allowed to drop into an evacuated flask. Sulfamic acid or cadmium chloride is added to the flask prior to the extraction in order to acidify the sample and aid in the extraction of carbon dioxide or to precipitate H2S gas and dissolved sulfide in the hydrothermal fluid as CdS for the subsequent sulfur isotope analysis. The water in the extraction flask is then agitated by stirring bar. The gas phase was transferred to a total of 150 cm3 evacuated stainless steel and glass container (for 34 compositional and isotope measurements of gas components). After the gas phase was obtained, the liquid phase was taken into a 50 cm3 polypropylene bottle (for major cation and anion measurements). The obtained gas sample in the glass container with a butyl rubber stopper was balanced with ultrapure He gas and the gas pressure was monitored by a manometer. Then, gas components were quantified by GC-PID system on board.

Onboard analyses for dissolved species

The bottle devoted to fluid chemistry was shared with microbiological study. After sample for pH, Alkalinity, and H2S determination was drawn, the rest of the fluid was filtered with a 0.2μm disk filter. The filtrate was provided for chemical analysis of major elements, nutrients (NH4, SiO2), and trace metals. The filtrate aliquot for trace metals was acidified with nitric acid to avoid hydroxide precipitation during storage. Because some chemical species such as nutrients and pH are difficult to be conserved during storage, we therefore analyzed these species onboard. In this cruise, colorimetric methods and titration were employed for onboard analyses as described below. Using the same apparatus, some conservative species were also analyzed. Most of these analytical methods are conventional ones and summarized in Gieskes et al. (1991).

pH and alkalinity

Determination of pH at room temperature was conducted with a pH meter with a combined glass electrode (Metrohm, 794 Basic Titrino). Measurements were done within an hour after sample distribution from the WHATS bottle. Calibration was conducted daily using JIS standard 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 (Metrohm, 794 Basic Titrino).

Colorimetric method

Using a colorimeter (Shimazu, UV mini 1240), concentrations of dissolved silica (SiO2), ammonium ion (NH4), and hydrogen sulfide (H2S) were analyzed following classical methods; molybdenum blue method (λ=812nm) for SiO2 and indo-phenol method (λ=640nm) for NH4 and methylene blue method (λ=670nm) for H2S. Analytical precision is usually better than 3% for seawater analysis, although sometimes the precision is somewhat worse for the case of hydrothermal fluids due to wide range of concentrations (SiO2 and H2S) and interference by specific species (NH4).

4. Microbiology and macrobiology

Sample preparation

For cultivation, water samples collected by the Niskin bottle and WHATS were immediately poured into sterilized glass vials under the atmosphere of nitrogen gas. Chimney samples were subsampled into several portions (e.g. vent orifice surface, inside structure, middle-inside structure). Each piece of chimney structure was slurried with filter-sterilized seawater under N2 for cultivation. For molecular analysis, the rest of pieces was kept under -80 °C. Hydrothermal vent animals were dissected, incubated, or fixed once onboard ship.

Individuals or their tissues were applied to various onboard experiments (e.g. enzyme activity measurement and incubation under the H2-containing atmosphere), or kept under -80 °C. Some individuals of hydrothermal vent animals were frozen under -80 °C or fixed with ethanol or formalin.

Larval and reproductive characteristics of vent animals

Planktonic larval stage is important for benthic animals to disperse and settle on the new population, as well as maintain their source population. In addition, some vent animals acquire their symbionts during the metamorphosis from planktonic larvae to juvenile (e.g., Nussbaumer et al. 2006). To figure out the ecological characteristics of the larvae and juvenile, we developed the larval sampler named Yousei Hoihoi to collect larva and juveniles at vent fields. The collected larva and juveniles were observed under compound microscope on board for brief identification. The details of the morphology of the larva and juveniles will be observed with SEM. The relationships between symbiont and larvae or juveniles will be studied with FISH and stable isotopic analyses.

References:

Nussbaumer, A.D., Fisher, C.R., Bright, M., 2006. Horizontal endosymbiont transmission in hydrothermal vent tubeworms. Nature 441, 7091.

IV. DIVE REPORTS

#1349 DIVE (Beebe Field)	**Dr. Jon Copley**
#1350 DIVE (Beebe Field)	**Dr. Ken Takai**
#1351 DIVE (Von Damm Field)	**Dr. Verity Nye**
#1352 DIVE (Von Damm Field)	**Dr. Hiromi Watanabe**
#1353 DIVE (Von Damm Field)	**Dr. Diva Amon**
#1354 DIVE (Beebe Field)	**Dr. Shinsuke Kawagucci**
#1355 DIVE (Beebe Field)	**Dr. Katsunori Yanagawa**
#1356 DIVE (Von Damm Field)	**Dr. Norio Miyamoto**
#1357 DIVE (Beebe Field)	**Dr. Daniella Smith**

Dive Report: Shinkai6500 Dive #1349
Date: June 21, 2013
Site: Beebe Hydrothermal Field (MCSC)
Landing: 11.29, 18° 32.9840' N 81° 43.2557' W D=5099m
Leaving: 15.08, 18° 32.9079' N 81° 43.2296' W D=4917m
Observer: Jon Copley (University of Southernpton)
Pilot: Yoshitaka Sasaki **Co-Pilot**: Yudai Tayama

Objectives:
Dive #1349 planned to visit the Hashtag+Beebe125 chimneys, and Anemone Field area, of the Beebe Vent Field on the Mid-Cayman Spreading Centre to: (1) collect high-temperature hydrothermal fluids from two sets of chimneys (Hashtag and Beebe125) for geochemical studies; (2) collect fresh sulfides from one set of chimneys for microbiological studies, including *in situ* preservation of chimney material; (3) collect quantitative faunal samples and measure the environmental parameters of sampled assemblages, using "mystery circle" area sampler and DO+temperature sensor.

Dive summary:
After trim adjustment, at 11.12 we triggered the Niskin bottle at vehicle depth 4997 m and altitude 72 m above the seafloor, to collect a reference water sample away from the vent area. At 11.29 we landed on pelagic-sediment seafloor at 5099 m deep. Shinkai6500 was then directed towards the Hashtag+Beebe125 chimneys, approximated 400 m distant on a heading of 135. We initially traversed pelagic-sedimented seafloor with occasional basalt outcrops. Sparse fauna observed in this area consisted of deposit-feeding echinoderms (holothurians, an echinoid, and an ophiuroid) and scavenger fish. At 11.40 we started to climb a slope of basalt fragments. At 11.49, approximated 150 m to the target chimneys, we encountered sulfide blocks and sulfidic sediment on the slope, at depth 5082 m. By 11.50, sulfide blocks dominated the seafloor, with occasional *Munidopsis* sp. decapod crustaceans, indicating that we had arrived at the base of the sulfide mound of the Beebe Vent Field. The steep slope and blocky nature of the substratum, however, precluded collection of pushcores from the vent field area. At 11.53 and depth 5043 m, we altered heading of the Shinkai6500 to 150, and at 11.58 and depth 5000 m we started to follow the contours of the mound to the south. At 12.05 we turned to heading 090 to approach the target chimneys, and the Hashtag chimneys were sighted at 12.10, depth 4969 m. The Jason-2 marker "X-19" was observed next to the Hashtag chimneys.
At 12.24 we positioned the submersible at the base of the Hashtag chimneys and deployed the "mystery circle" on a flat area of seafloor, with visible actinostolid anemones (*Maractis* sp.) and *Rimicaris hybisae* shrimp, for faunal sampling. The DO+temperature sensor was deployed in the centre of the "mystery circle" between 12.33:23 and 12.37:32, during which time close-up

images were recorded with HD and stills cameras. At 12.39 fauna within the "mystery circle" were suctioned into Chamber 1 of the suction sample, which was rotated to Chamber 2 afterwards.

At 13.05 we collected chimney material from the top of an active chimney at Hashtag into the Chimney Box, which was then pumped full of preservative. During sampling of further material, the submersible dislodged a neighbouring chimney at Hastag, which fell across the Starboard sample basket. This additional chimney material was stowed into the normal sample box and spare space in the Starboard sample basket, to complete the chimney sampling requirements of the dive. During this process the metal-edged yellow tray fell to the seafloor at the base of the Hashtag chimneys, beyond reach of the manipulators.

At 13.33 we deployed the intake for the D-WHATS fluid sampler into the vent orifice exposed by our chimney sampling at Hashtag. Temperature data were not available from the probe within the nozzle, and fluids were pumped into bottles 1+2 for 5 minutes, while observing "black smoke" venting from the exit valve. While closing the valve for bottles 1+2 of the D-WHATS and opening the valve to bottles 3+4, the Shinkai6500 was dislodged from the seafloor and rose to an altitude of 55 m, drifting away from the chimney area. Directions were requested from RV Yokosuka to return to the target chimneys. At 14.01 we observed the UK wood package on the seafloor, and turned to the south to observe the Hashtag chimneys. We moved immediately upslope from Hashtag to observe the Beebe125 chimneys at 14.14. We observed ~0.35 m of new chimney growth on chimney #1 of Beebe125 since February 2013.

"Black smoke" from the Hashtag chimneys obscured our initial view at Beebe125 from 14.16, and the submersible repositioned to continue sampling tasks from a different approach to Beebe125. At 14.45 we deployed the intake for the D-WHATS sampler at chimney #1 of Beebe125, having removed chimney material to improve access of the intake to high-temperature fluids. Fluids were pumped from chimney #1 of Beebe125 into bottles 3+4 of D-WHATS for 5 minutes from 14.47. During this period, we also collected *Rimicaris hybisae* shrimp from the side of the chimneys at Beebe125 into Chamber 2 of the suction sampler, which was rotated after sampling but may have not stopped rotation fully on Chamber 3. We left the Beebe Vent Field at 14.58, and begin ascent to the surface at 15.08.

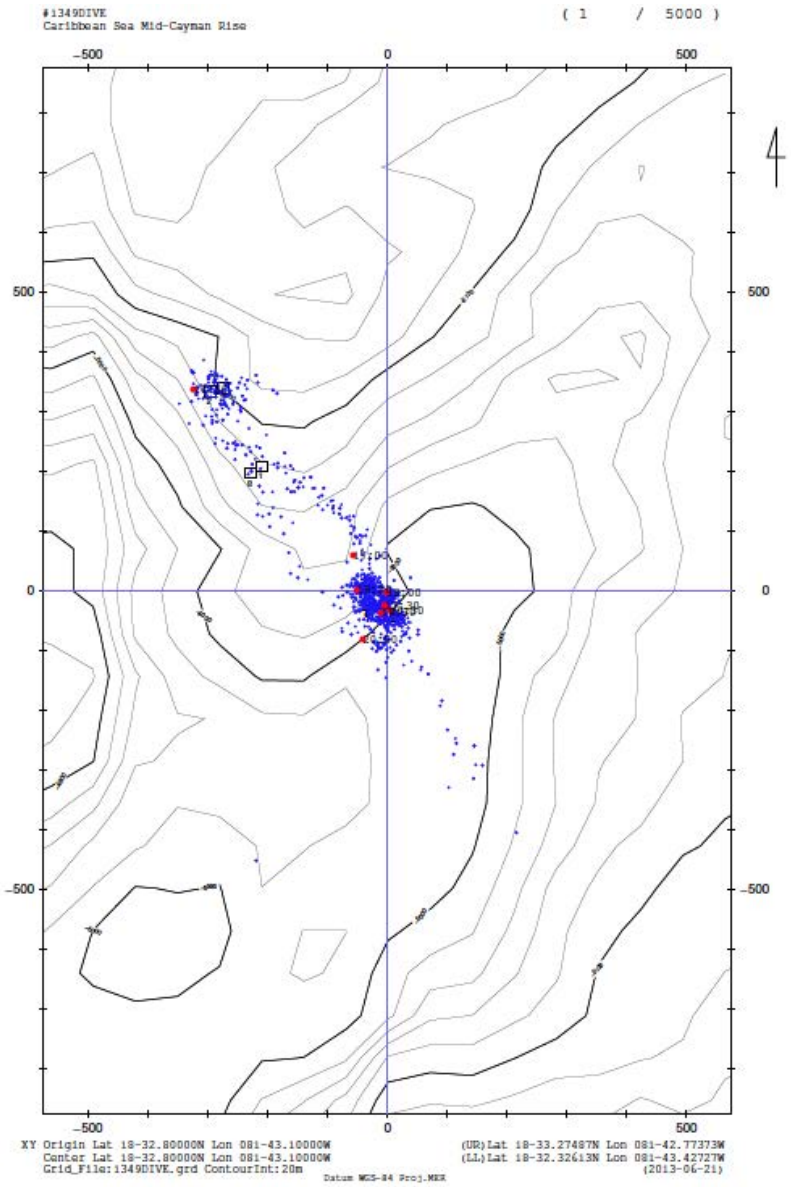
Payloads:

1) Niskin sampler
2) 6 bottle Canister sampler
3) D-WHATs with 4 bottles
4) Miyazaki Chimney Box
5) Normal Box
6) DO+temperature sensor
7) "Mystery circle" faunal sample area delimiter
8) 2 pushcores

Event list:

1. 11.12, Niskin sample immediately following trim adjustment: D=4997m alt=72m 18° 32.9818’ N 81° 43.2686’ W
2. 11.29, landed at seafloor: 18° 32.9840’ N 81° 43.2557’ W D=5099m
3. 12.14, Marker “X19” observed at Hashtag chimneys: 18° 32.7881’ N 81° 43.1184’ W D=4960m
4. 12.41, Mystery Circle DO+temperature measurement & suction sample into Canister #1, at Hashtag chimneys: 18° 32.7881’ N 81° 43.1184’ W D=4964m
5. 13.48, Chimney samples collected into Miyazaki Chimney Box, normal Sample Box, and Starboard basket space, then high-temperature fluid sample collected into D-WHATS bottles 1+2, at Hashtag chimneys: 18° 32.7881’ N 81° 43.1184’ W D=4964m
6. 14.53, high-temperature fluid sample collected into D-WHATS bottles 3+4, and shrimp sample collected by suction sampler into Canister #2, Beebe 125 chimney: 18° 32.7774’N 81° 43.0981’ W D=4957m
7. 15.08, left seafloor: 18° 32.9079’ N 81° 43.2296’ W D=4917m

Dive Track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Caribbean Sea Mid-Cayman Rise
(1 / 5000)
-500
0
500
XY Origin Lat 18-32.80000N Lon 081-43.10000W
Center Lat 18-32.80000N Lon 081-43.10000W
Grid_File:1349DIVE.grd ContourInt:20m
(UR)Lat 18-33.27487N Lon 081-42.77373W
(LL)Lat 18-32.32613N Lon 081-43.42727W
(2013-06-21)
Datum WGS-84 Proj.MER

Dive Report: Shinkai 6500 Dive# 1350
Date: June 22, 2013
Site: Beebe Hydrothermal Field (MCSC)
Landing: 11:32; 18˚32.7541N, 81˚43.0886W, D= 4967m
Leaving: 15:15; 18˚32.8643N, 81˚43.2622W, D=4959m
Observer: Ken Takai (JAMSTEC)
Pilot: Kazuki Iijima **Co-Pilot**: Hitomi Ikeda

Objectives:
Main objective of the dive #1350 is to make live streaming of the seafloor view as long as possible. The scientific objective is to collect high temperature fluid from 'Beebe Woods' chimney site and recovery of in situ colonization system (ISCS that was deployed at 'Beebe Woods' chimney site at February, 2013 during the James Cook cruise. The other objectives are to collect vent-endemic animal samples such as shrimps, anemone and gastropods and to recover the pig and whale bone that were deployed around 'Beebe Woods' chimney site at February, 2013 during the James Cook cruise.

Dive summary:
First, we made a trim position at ~100 m above the seafloor at ~300 m south of 'Beebe Woods' chimney site. After quick operation for trim position, we headed north with slight down falling. There is a big W-E fault (cliff with ~10 m down at the northern side) and immediately after the fault, we observed two small hydrothermal vent sites with small shrimp colonies. Several meters north of these small vents, we observed "Beebe Woods' site. First we landed southern foot area of 'Beebe Woods' and deployed a mystery circle (smaller one) and collected benthic animals by suction sampler (box #1). Then, we climbed a little (several meters) of 'Beebe Woods' and stopped at position where we obtained the Rimicaris shrimps. We collected Rimicaris shrimps by suction sampler into boxes #2 & 3. During the animal sampling, we found the ISCS almost buried in the newly growing chimney. So, next, we moved to the front of the ISCS deployment and tried to collect the ISCS. However, the ISCS was heavily rusted and only the top part of ISCS was recovered. To recover the substratum part of ISCS, we grabbed the ISCS together with chimney portion. Fortunately we succeeded in obtaining both chimney samples and ISCS main body. The ISCS was settled in the recovery box and the recovery box was replaced by 0.5% of neutralized sodium sulfide solution.

After finishing the ISCS recovery and the chimney sample collection, we tried to collect the high-temperature hydrothermal fluid from 'Beebe Woods' site. All the chimneys in 'Beebe Woods' site were 'Bee-Hive' structures and it was difficult to find obvious hydrothermal conduits. Anyway, we broken one of the chimney in the middle and took high temperature hydrothermal

fluid by D-WHATS sampler into #1 - #4 bottles. The maximal temperature during the sampling was observed (need to check the temperature record) to be 318 ˚C but the average temperature semmed to be 250-280 ˚C (need to check the temperature record).

After leaving 'Beebe Woods' site, we looked for the pig carcass that was deployed around 'Beebe Woods' chimney site at February, 2013 during the James Cook cruise. It was a bit difficult to find the pig carcass and the marker, but finally found the Whale Bone and Wood deployment site near 'Beebe Woods' site. By detail observation by camera, the whole bone was little consumed and one galetherid colonized there. In both whale bone and wood, small white amphipods were observed. So, we collected the surface colonizer of whale bone by suction sampler (box #4) and recovered the whale bone into the sample box. After that, the sediments beneath the whale bone were collected by suction sampler (box #6). We deployed a marker (6K#151) here.

After the bone collection, we headed to 'Hashtag' or 'Beebe 125' site for the temperature measurement. Although we found 'Anemone garden', we did not arrive at 'Hashtag' or 'Beebe 125' site. Finally, we came back to 'Beebe Woods' site after struggling. Thus, we quited the our dive at the end. We left the bottom at 300 m northwest from the Beebe field'.

Payloads:

- Deep-WHATS sampler (D-WHATS)
- Suction sampler with multi-bottle (6-series) collectors (No. 1-3 bottles for large animals and No. 4-6 bottles for plankton)
- DO sensor
- Recovery box for ISCS
- Sample Boxes (as many as possible)
- Marker x 2

Event list:

1) 11:32, Landing in front of 'Beebe Woods' site and collecting of benthic animals
18˚32.7541N, 81˚43.0886W, D=4967 m

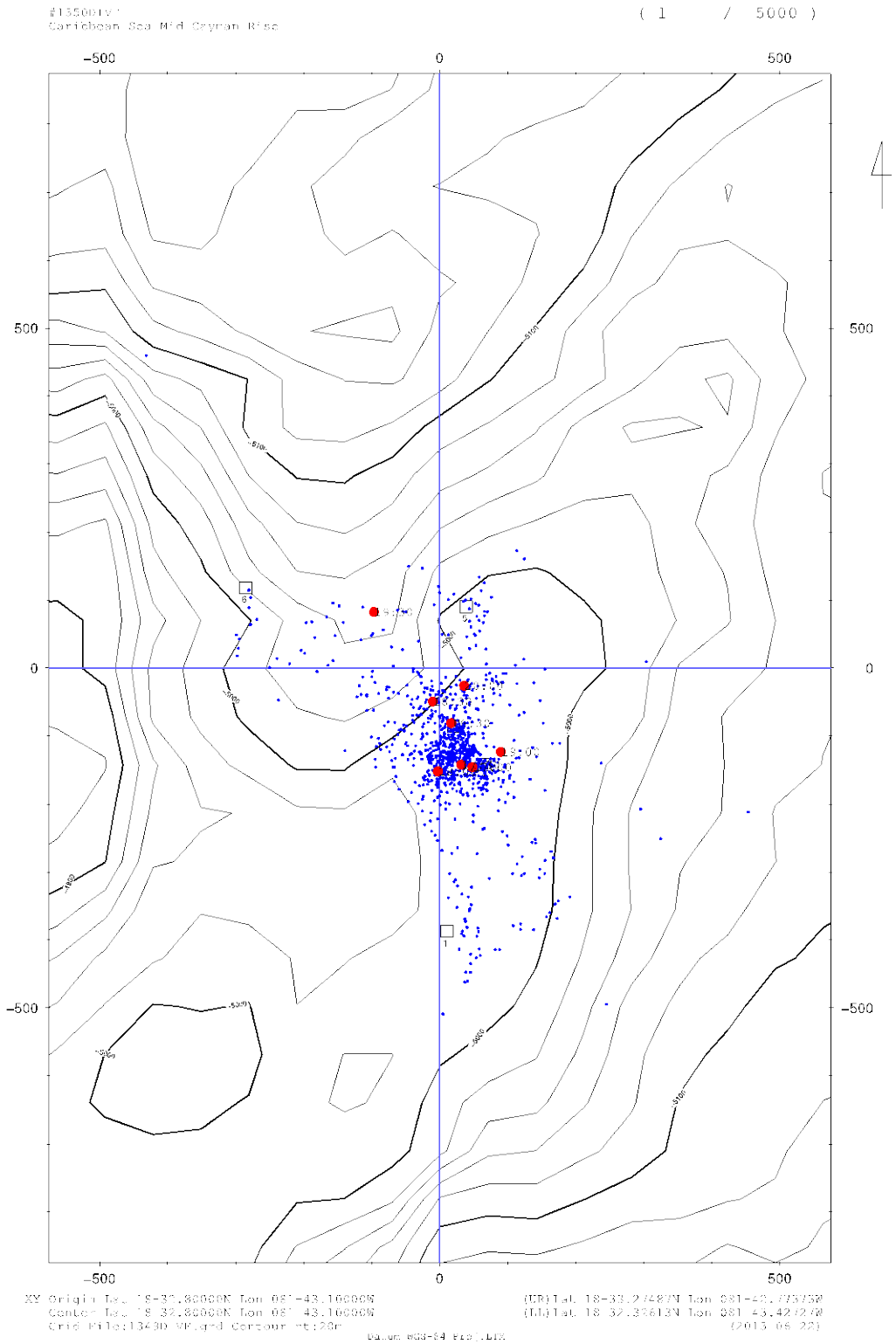
2) 12:56, Shrimp sampling, recovery of ISCS, chimney sampling, hydrothermal fluid sampling
18˚32.7541N, 81˚43.0886W, D=4963 m

3) 14:17, Recovery of whale bone, sampling animals and sediments around there, deployment of marker (6K#151)
18˚32.7230N, 81˚43.0625W, D=4978 m

4) 14:35, Finding deployed wood at the anemone field
18˚32.8499N, 81˚43.0780W, D=4958 m

5) 15:15, left bottom
   18-32.8643N, 81-43.2622W, D=4959 m

Dive Track


#1550div'
Caribbean Sea Mid Cayman Rise
(1 / 5000)
-500
0
500
500
0
-500
-500
0
500

Dive Report: Shinkai 6500 Dive# 1351
Date: June 23, 2013
Site: Von Damm Hydrothermal Field (MCSC)
Landing: 11:29; 18º 22-5205'N 18º 48.0552'W, D=2374m
Leaving: 16:04; 18º 22-4759'N 81º 47.7541'W, D=2299 m
Observer: Verity Nye (University of Southampton)
Pilot: Keita Matsumoto **Co-Pilot**: Takuma Onishi

Objectives:
Dive #1351 planned to locate a pockmark in the sediment to the west of the main mound to collect pushcores from inside, on the edge and outside the pockmark. The dive also planned to visit the Spire, Hole to Hell and Hotter than Hole to: (1) collect high-temperature hydrothermal fluids from two locations (Spire and Hole to Hell) for geochemical studies; (2) collect material from the main 'chimney' (Hole to Hell) for microbiological studies, including *in situ* preservation of 'chimney' material; (3) collect quantitative faunal samples and measure the environmental parameters of samples assemblages, using "mystery circle" area sampler and DO+temperature sensor; (4) collect quantitative plankton sample, using suction sampler.

Dive summary:
At 11.29, we landed on pelagic-sediment seafloor at 2374 m deep. Shinkai6500 was then directed towards the main mound, approximated 350 m distant on a heading of 093. We initially traversed pelagic-sediment seafloor with occasional scours. Sparse fauna observed on this area consisted of deposit-feeding holothurians and solitary shrimp. At 11:49 we located a pockmark, at depth 2335 m, suitable for taking push cores. We collected six pushcores at the pockmark (two on the edge, two inside and two outside) and deposited a marker (152). Shinkai6500 then continued on the same heading towards the main mound. At 12:32 we encountered rubble at the base of the main mound, at depth 2442. We proceeded up the steep slope of the mound, which was blocky in nature, towards the Spire. At 12:39 we encountered a dense patch of shrimp (*Rimicaris hybisae*) around a small, active vent at 2303 m depth. At 12:41, 2297 m depth, we sighted Hole to Hell as we continued up the steep, blocky slope towards the Spire.
At 12:44 we reached the target area and positioned the submersible just below the Spire at 2289 m depth. At 13:01 we deployed the intake for the D-WHATS fluid sampler into the vent orifice. The maximum temperature recorded from the probe was 118°C. Fluids were pumped into bottle 1 (978 ml) and bottle 2 (928 ml). At 13:25 we deployed the Mystery Circle on a flat area of the Spire, in close proximity to the vent orifice. The DO+temperature sensor was deployed in the centre of the Mystery Circle between 13.26:29 and 13.30:304, during which time close-up images were recorded with HD and stills cameras. At 13.31 fauna within the Mystery Circle were suctioned into Chamber 1 of the suction sampler, which was rotated to Chamber 2

afterwards. The DO+temperature sensor was deployed in an area of dense shrimp (*Rimicaris hybisae*) with visible small or juvenile individuals between 13.37:00 and 13.40:00, during which time close-up images were recorded with HD and stills cameras. At 13:42 a sample of shrimp and other fauna were suctioned from the area of dense shrimp into Chamber 2 of the suction sampler, which was subsequently rotated to Chamber 3.

At 13:46 we left the Spire and descended towards Hole to Hell. At 13:51 we positioned the submersible at the base of Hole to Hell, 2295 m depth. At 14.04 we deployed the intake for the D-WHATS fluid sampler into the vent orifice. The maximum temperature recorded from the probe was 226°C. Fluids were pumped into bottle 3 (915 ml) and bottle 4 (936 ml). At 14:30 we started to collect chimney material from the right side of the active chimney into the Chimney Box, which was then pumped full of preservative. During sampling of further material, dislodged material fell onto the main Sample Box. This additional chimney material was stowed in the normal Sample Box. At 14:41 a sample of fauna, including shrimp and gastropods (*Rimicaris hybisae* and *Iheyaspira bathycodon*) were suctioned from the chimney side into Chamber 3 of the suction sampler, which was subsequently rotated to Chamber 4. The DO+temperature sensor was deployed in the sampled area between 14.48:00 and 14.51:13, during which time close-up images were recorded with HD and stills cameras. The sheer slope precluded quantitative sampling of fauna.

At 14:58 we repositioned the submersible approximately two meters from the base of Hole to Hell, at 2295 m depth. At 15:03 we deployed the Mystery Circle in an area of relatively flat blocky substratum. The DO+temperature sensor was deployed in the centre of the Mystery Circle between 15.05:00 and 15.08:00, during which time close-up images were recorded with HD and stills cameras. At 15.09 fauna within the Mystery Circle were suctioned into Chamber 4 of the suction sample, which was rotated to Chamber 5 afterwards. Between 15:14 and 15.19, seawater several meters above the surface of the mound was suctioned into Chamber 5 of the suction sampler, which was rotated to Chamber 6 afterwards. At 15:23 we deployed the Mystery Circle in another area of relatively flat blocky substratum. The DO+temperature sensor was deployed in the centre of the Mystery Circle between 15.27:00 and 15.30:00, during which time close-up images were recorded with HD and stills cameras. At 15.31 fauna within the Mystery Circle were suctioned into Chamber 6 of the suction sample.

At 15:35 Shinkai6500 was directed around the west side of the main mound to Hotter than Hole on the northeast slope. At 15:41 we reached Hotter than Hole. We positioned the vehicle at the edge of Hotter than Hole, at 2305 m depth. At 15:57 we collected further rock samples from underneath a dense aggregation of *Rimicaris hybisae* that surrounded the vent orifice into the main sample box. At 15:58 we deployed a marker (153) at the edge of Hotter than Hole. We left the Von Damm Vent Field at 15.59 and began ascent to the surface at 16.06.

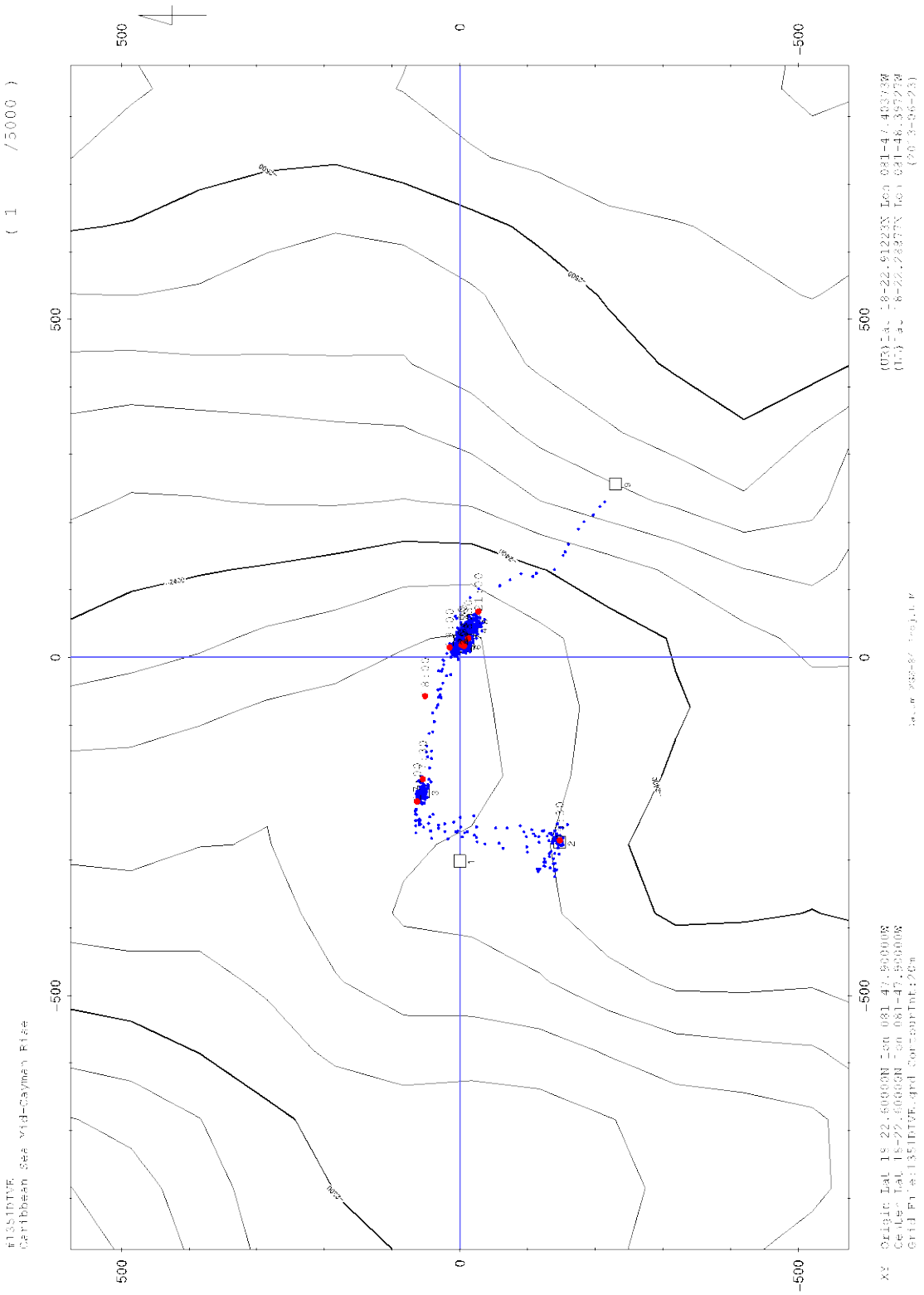
Payloads:

1) WHATs with 4 bottles
2) Miyazaki Chimney Box
3) Normal Box
4) 6 bottle Canister sampler
5) DO+temperature sensor
6) “Mystery circle” faunal sample area delimiter
7) 6 push cores
8) Yellow metal-edged tray
9) Markers

Event list:

1) 11:29, landed at seafloor: 18º 22-5205’N 18º 48.0552’W, D=2374m
2) 11:49, at pockmark: 18º 22-6296’N 18º 48.0128’W, D=2335 m
   Push cores 1-6, set #152 Marker.
3) 12:44, Spire, 18º 22-5904’N 81º 48.8763’W, D=2291m
   High-temperature fluid sample collected into D-WHATS bottles 1+2, Mystery Circle DO+temperature measurement & suction sample into Canister #1, shrimp sample DO+temperature measurement & suction sampler into Canister #2.
4) 13:51, Hole to Hell, 18º 22-6007’N 81º 47.8909’W, D=2295m
   High-temperature fluid sample collected into D-WHATS bottles 3+4, chimney samples collected into Miyazaki Chimney Box and normal Sample Box, fauna DO+temperature measurement & suction sampler into Canister #3. 15:33, Hole to Hell, 18º 22-5951’N 18º 47.8903’W, D=2295 m
5) 15:03, Hole to Hell, 18º 22-5948’N 81º 47.8820’W, D=2295 m
   Mystery Circle DO+temperature measurements & suction samples into Canister #4, plankton suction sample into Canister #5.
6) 15:23, Hole to Hell, 18º 22-5948’N 81º 47.8820’W, D=2295 m
   Mystery Circle DO+temperature measurements & suction samples into Canisters #6.
7) 15:41, Hotter than Hole, 18º 22-5890’N 81º 47.8690’W, D=2306 m
   Rock samples collected into normal Sample Box, set #153 Marker.
8) 15:59m left seafloor: no position data.
9) 16:06, started ascent: 18º 22-4759’N 81º 47.7541’W, D=2299 m, A=170 m.

Dive Track



Dive Report: Shinkai 6500 Dive# 1352
Date: June 24, 2013
Site: Von Damm Hydrothermal Field
Landing: 11:12; 18º 22.3909'N 81º 47.7446 E, D = 2481m
Leaving: 15:52; 18º 22.5008'N 81º 47.7075'E, D = 2349m (A = 120m)
Observer: Hiromi Watanabe (JAMSTEC)
Pilot: Hirofumi Ueki **Co-Pilot**: Masaya Katagiri

Objectives:
The dive #1352 was planned to observe south-north transect of the tail to the main mound of Von Damm vent field, and take some quantitative faunal sampling with environmental measurement (DO and temperature). On the way, recovery of in situ cultivation (ISCS) and water sampling at the same site was also planned.

Dive summary:
Shinkai landed at the south of the tail of the Von Damm Vent Field (D =2481m). The muddy sea bottom was covered with dead shells of pteropods, and holothroids and nests of some deposit feeders could be observed. Shinkai headed to the area where tubeworms and dead mussels were observed, and climbed steep slope completely covered by fine ivory-colored sediment without any shells. According to sonar signals, Shinkai traveled to a bit west and find a small rocky tower. The slope became almost flat at the depth of 2396m, and dead mussel shells were found among the rocky bottoms at the depth of 2377m. The dead shells and some sediments were collected into MORINAGA box in the left basket. DO/Temperature measurement was also conducted above the dead shells (12:04:25-12:07:53). *Lamellibrachia* tubeworms were scattered among rocks. Shinkai traveled to north and found "X-18" marker which was deployed by Jason. *Lamellibrachia* tubeworms, *Munidoosis* squat lobser, *Lebbeus* and *Alvinocaris* shrimps, and dead shells of vesicomyid clams were observed in this area. Shinkai moved up a steep wall in the western side of the tail, and some dead *Lamelibrachia* tubeworms surrounded by a white-colored sediment (or bacterial mat?) were observed. At rocky bottom, we could find many tubeworm aggregations among rocks and collected into the left large box. Shinkai headed to south and reached at "X-16" marker and retrieved ISCS at a hydrothermal vent and filled the vent fluid into two bottles of WHATS sampler (#1 and 2; max temperature was 63°C). Beside the vent, where *Rimicaris* and *Lebbeus* shrimps, *Iheyaspira* gastropod, and *Pachycara* fish are distributed, we conducted "mystery circle" procedure with DO/temperature measurement (14:15:47-14:18:40). We could find INDEEP colonization table, which will be collected at the next dive, and deploy #154 marker for the next diver. Shinkai headed to north and again reached at exactly the same site as where we collected the tubeworms, conducted DO/temperature measurement (14:34:00-14:37:12) on the tubeworm colony and collected a rock. During the

dive, we misunderstood that this site was located at the tail, but actually, at the middle mound. Shinkai continued to travel north, climbed rocky steep wall, and got to find #153, which was deployed at "Hotter than Hole" site by the former dive. We filled the vent fluid into two bottles of WHATS sampler (#3 and 4; max temperature was 103°C). Shinkai headed to east to find "Fumerole" site, but we could not find it before leaving the bottom. Shinkai left the bottom due to battery limitation.

Payloads:

1) WHATs with 4 bottles
2) ISCS recovery case with reduced chemicals
3) Normal Box (x 2)
4) Slurpgun
5) 6-bottle Canister sampler
6) Mystery ring (for quantitative sampling)
7) DO/temperature sensor

Event list:

1) 11:12, Sea bottom, 18º 22.3909'N, 81º 47.7446' W, D=2481m

2) 12:11, the Tail, 18º 22.4536'N, 81º 47.8517'W, D=2377 m
Sampling dead mussel shells into left small box, DO measurement

3) 12:22, Jason's "X-18" marker, 18º 22.4807'N, 81º 47.8432'W, D=2375m
Sampling dead clam shells and shrimps in slurpgun bottle#1

4) 13:07, the middle mound, 18º 22.5113'N, 81º47.8688'W, D=2362m
Sampling *Lamellibrachia* tubeworm in left large box

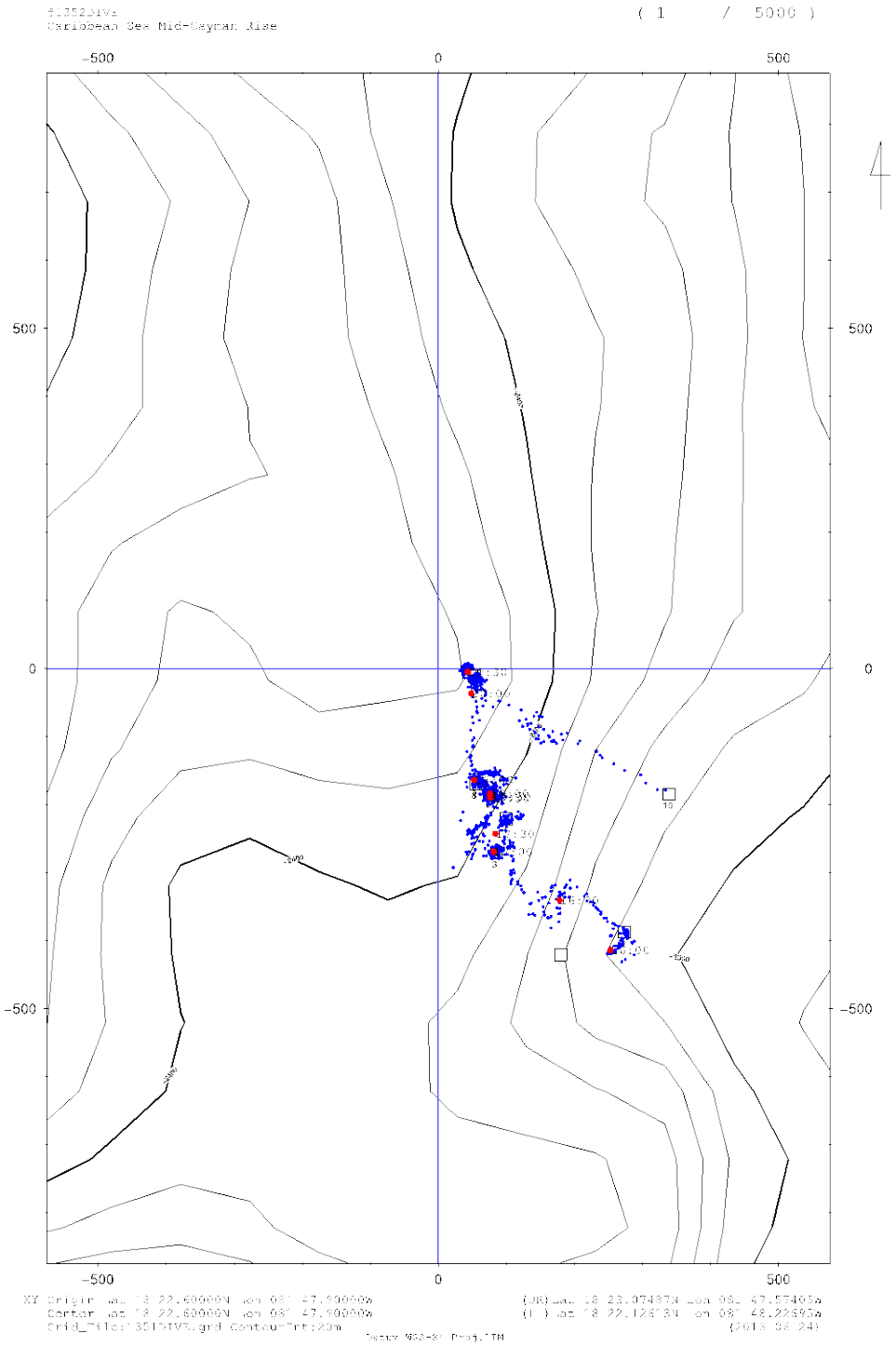
5) 13:34, Jason's "X-16" marker, 19º 33.3935'N, 65º 50.8753'E, D=2614m
Retrieval of ISCS, WHATS sampling (#1-2), deploying #154 marker, and "mystery circle" procedure

6) 14:42, the middle mound (exactly the same point as 4)), 18º 22.5080'N, 81º47.8691'W, D=2360m
DO measurement, Sampling rocks

7) 15:29, #153 marker at "Hotter than Hole", 18º 22.5940'N, 81º47.8703'W, D=2306m
WHATS sampling (#3-4)

8) 15:52, left the bottom, 18º 22.5008'N, 81º47.7075'W, D=2349m, A=120m

Dive Track


Caribbean Sea Mid-Cayman Rise
(1 / 5000)
-500
0
500
500
0
-500
-500
0
500

Dive Report: Shinkai 6500 Dive# 1353
Date: June 25, 2013
Site: Von Damm Hydrothermal Field (MCSC)
Landing: 11:29; 18º 22.4283'N 18º 47.8796'W, D=2382m
Leaving: 16:10; 18º 22.4923'N 81º 47.7448'W, D=2272 m
Observer: Diva Amon (University of Southampton)
Pilot: Yoshitaka Sasaki **Co-Pilot**: Akihisa Ishikawa

Objectives:
Dive #1353 planned to collect an experimental deployment (INDEEP02) at the X16 marker area. There a quantitative 'mystery circle' faunal sample as well as environmental-variable measurements would also be collected. The dive then planned to visit the sedimented area SSW of the main spire to locate, observe and collect the experimental deployment INDEEP01 and the pig victim. The third target area was an area of venting (a fumarole) on the way to the main spire. Here, two samples of high-temperature hydrothermal fluids would be collected for geochemical studies, another quantitative 'mystery circle' faunal sample with environmental-variable measurements would be taken and marker left. The fourth sampling area would be the main spire where two samples of high-temperature hydrothermal fluids and rocks in contact with those fluids would be collected for geochemical studies, as well as another quantitative 'mystery circle' faunal sample with environmental-variable measurements would be taken. The fifth and final sampling area would be down the slope from 'Hole to Hell' where one quantitative 'mystery circle' faunal sample with environmental-variable measurements would be collected.

Dive summary:
At 11.29, we landed on pelagic-sediment seafloor at 2382 m deep. *Shinkai6500* was then directed towards the X16 site where an INDEEP02 colonization experiment had previously been deployed (18º 22.5035'N, 81º 47.8716'W) (Target Area 1). During transit to this site, we traversed sedimented seafloor that had occasional scour marks containing phytodetritus. There were also many animals including many purple holothurians *Benthodytes* sp., tripod fish *Bathypterois* sp., *Nematocarcinus* sp. shrimp, fish, a dumbo octopus and a seastar. We then began to move up a steep rocky slope with many areas of *Bathymodiolus* sp. shells. At the top of this slope at 12:03 we located the X16 marker and INDEEP02 package (18º 22.5035'N, 18º 47.8716'W). This package was collected and placed in the port tool tray at 12:04 at a depth 2372 m. *Shinkai6500* moved slightly forward from the INDEEP02 site, where at 12:13 we deployed the 'mystery circle' in close proximity to the vent orifice. The DO + temperature sensor was deployed in the centre of the 'mystery circle' between 12:21:00 and 12.24:00, during which time close-up images were recorded with HD and stills cameras. At 12.28, fauna within the 'mystery

circle' were suctioned into Chamber 1 of the suction sampler, which was rotated to Chamber 2 afterwards.

Shinkai6500 then continued on to the INDEEP01 deployment and Petunia pig victim SSW of the main spire on the sedimented plain (Target Area 2). During the transit, we passed over many more areas of *Bathymodiolus* shells and sparse colonies of live *Lamellibrachia* tubeworms on the rocky slope to the right of the submersible. On the left, the sedimented seafloor with many *Benthodytes* sp. and scour marks continued. At 12:47, we spotted the INDEEP02 deployment and pig victim. A large fish was noted swimming away from the pig victim. The INDEEP02 deployment was collected and placed in the port tool tray at 12:56 at 18º 22.5738'N, 81º 47.9195'W at a depth of 2344 m. At 12:58 we moved closer to the pig victim and took some time to observe and record still and video images. Many animals were noted around a small depression containing a few rib and vertebrae bones covered in bacterial mats. Some of the bones were also black in colour. Five *Munidopsis* squat lobsters were noted, as well as, a small eel like fish, a *Sergestes* shrimp and a *Nematocarcinus* shrimp observed feeding. At 13:09 at 2343 m at 18º 22.5772'N, 81º 47.9240'W, the large fauna surrounding the bone deployment were suctioned into Chamber 2 of the suction sampler. The chamber was rotated to Chamber 6 after. The pig bones were then collected at 13:11 using the manipulator and placed in starboard sample box. Then the sediment below where the pig victim had been sitting was suctioned to collect macrofauna into Chamber 6 (fine mesh) at 13:26. The chamber was rotated to Chamber 3. The lead weight and marker from the pig victim were also collected using the manipulators and placed in the starboard tool tray at 13:31.

At 13:36, *Shinkai6500* began to transit to the Main Spire. At 13:39, transiting up the steep rocky slope, an area of high-temperature venting was located at 2299 m, 18º 22.5904'N, 81º 47.8902'W (Target Area 3). Here at 13:56, we deployed the intake for the WHATS fluid sampler into the vent orifice. The maximum temperature recorded from the probe was 25.4°C. At 14:05, fluids were pumped into Bottle 1. During this time, *Shinkai6500* moved position and so WHATS fluid sampling had to begin again after repositioning. This was at 14:09 and Bottle 1 was filled (1234 ml). Bottle 2 was also filled here with a maximum temperature recorded of 84.9°C. The 'mystery circle' and DO + temperature sensor was deployed in close proximity to the vent orifice between 14.35:00 and 14.40:00, during which time close-up images were recorded. At 14:40, a sample of shrimp and other fauna were suctioned from the area into Chamber 3 of the suction sampler, which was subsequently rotated to Chamber 4. It should be noted that the quantitative 'mystery circle' samples taken on the rocky mound of Von Damm Vent Field may be underestimates due to fauna living between and under loose rocks. Many *Iheyaspira bathycodon* could not be suctioned due to their awkward positioning around rocks within the 'mystery circle'. During this sampling event, many ovigerous and brooding females of the shrimp species *Lebbeus virentova* were observed in the periphery. Two rusty metal pipes were also observed in the area. It was also during this sampling event, that the suction sampler tube was broken into

two pieces by being crushed while in use when the ROV moved unexpectedly. Subsequent to this event, the suction sampler could no longer be used. The thermometer was also bent. Marker #155 was deployed at this sampling site at 14:49.

At 14:53 we began transit to the Main Spire (Target Area 4). *Shinkai6500* arrived at the Main Spire (18º 22.6011'N, 81º 47.8896'W) at a depth of 2290 m at 14:58. *Shinkai6500* did not position itself until 15:20 however. At 15:26, we deployed the intake for the WHATS fluid sampler into the vent orifice. The maximum temperature recorded from the probe was 128°C. Fluids were not pumped into bottle 3 (900 ml) and bottle 4 (900 ml) until 15:34 and 15:46 respectively. At 16:01, chimney material from in direct contact with the high-temperature vent fluid was collected and placed in the port sample box. This was difficult to collect however, as the rock crumbled easily. At this point, there was no more time for sampling so Target Area 5 could not be reached. At 16:10, *Shinkai6500* left the seafloor and began ascent.

Payloads:

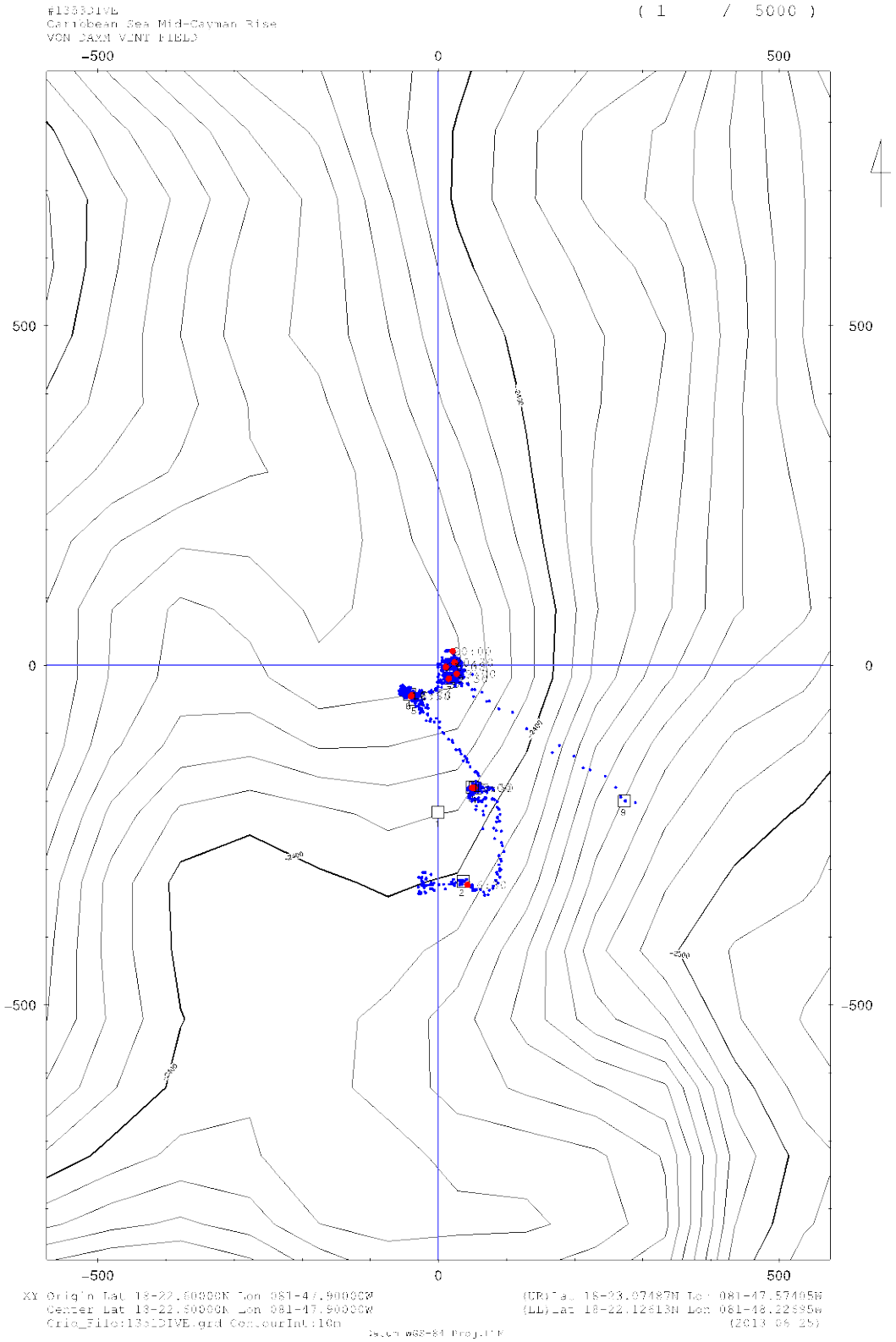
1. WHATs with 4 Bottles
2. Normal Sample Box
3. Normal Sample Box
4. 6-bottle Canister Suction Sampler
5. DO + Temperature Sensor
6. "Mystery circle" Faunal Sample Area Delimiter
7. Markers

Event list:

1. 11:29, Landed at seafloor: 18º 22.4283'N 81º 47.8796'W, D=2382m
2. 12:04, At X16, retrieve colonization experiment: 18º 22.5035'N 18º 47.8716'W, D=2372 m
   INDEEP02 placed in port tool tray.
3. 12:31, Left X16: 18º 22.5024'N 81º 47.8693'W, D=2372m
   'Mystery circle' DO + temperature measurement & suction sample into Canister #1
4. 12:56, Arrived SSW of main mound, retrieve colonization experiment: 18º 22.5738'N 81º 47.9195'W, D=2344m
   INDEEP01 placed in port tool tray.
5. 13:34, Left SSW of main mound, retrieve colonization experiment: 18º 22.5772'N 81º 47.9240'W, D=2343m
   Observed the pig victim. Suction sampled the large fauna around pig victim into Canister #2. Picked up pig bones and placed in starboard sample box. Then suction sampled sediment where bones had been to collect macrofauna into Canister #6 (fine mesh).

6. 14:51, Left fumarole area found on main mound on transit to main spire: 18º 22.5904'N 81º 47.8902'W, D=2299 m
   High-temperature fluid samples collected into WHATS bottles 1+2, 'mystery circle' DO + temperature measurement & suction sample into Canister #3. Set #155 Marker.
7. 16:02, Left the Main spire: 18º 22.6011'N 81º 47.8896'W, D=2290 m
   High-temperature fluid samples collected into WHATS bottles 3+4. Sampled rocks from in high-temperature venting and placed in port sample box.
8. 16:10, Left seafloor: 18º 22.4923'N 81º 47.7448'W, D=2272 m.

Dive Track


#1333DIVE
Caribbean Sea Mid-Cayman Rise
VON DAMM VENT FIELD
(1 / 5000)
-500
0
500
XY Origin Lat 18-22.50000N Lon 081-47.90000W
Center Lat 18-22.50000N Lon 081-47.90000W
Grid_File:13slDIVE.grd ContourInt:10m
(UR)Lat 18-23.07487N Lon 081-47.57405W
(LL)Lat 18-22.12613N Lon 081-48.22595W
(2013 06 25)

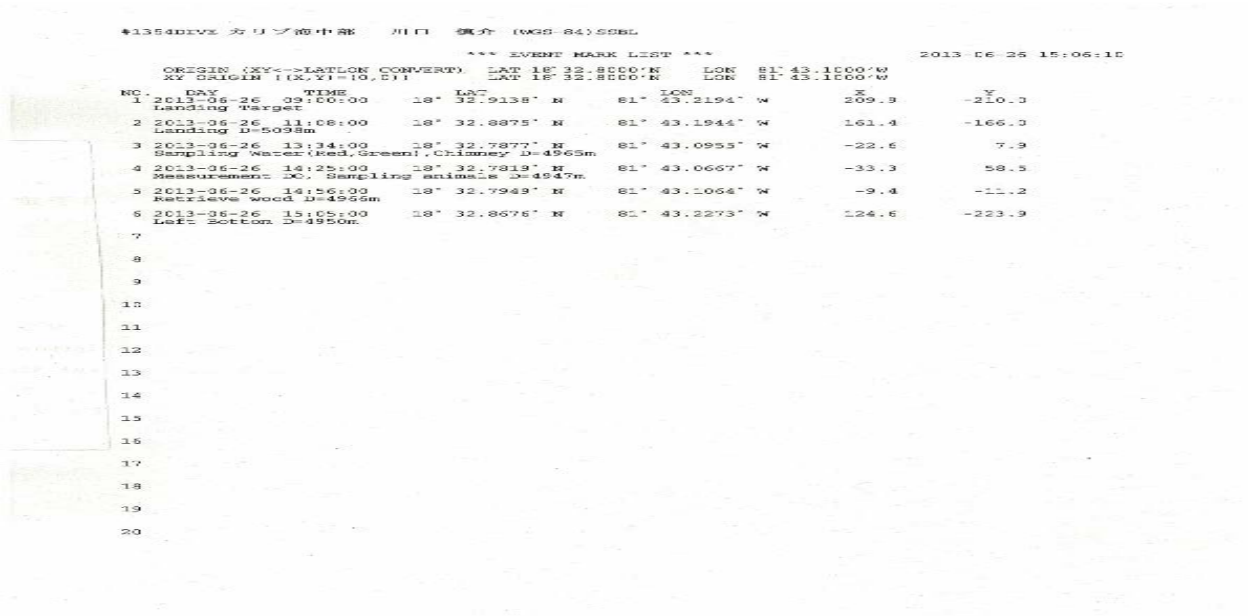
Dive Report: Shinkai 6500 Dive# 1354
Date: June 26, 2013
Site: MCR Beebe Hydrothermal Field
Landing: 11:08; 18º 32.8875'N 81º 43.1944' W, D = 5098m
Leaving: 15:05; 18º 32.8676'N 81º 43.2273'W, D = 4950m
Observer: Shinsuke Kawagucci (JAMSTEC)
Pilot: Kazuki Iijima **Co-Pilot**: Takuma Ohnishi

Objectives:
The dive #1354 was planned to: (1) visit Hashtag and Beebe125 chimneys to make temperature measurement and collect high-temperature hydrothermal fluids; (2) collect faunal samples with T/DO measurement around Anemone field; and (3) recover the UK-wood colonization device deployed in Feb. 2013.

Dive summary:
Shinkai landed at the west of the Beebe Vent Field and directed toward the Hashtag chimneys. At 11:08 we reached Hashtag chimneys and made temperature measurement of black smoker fluid there, recording 396oC as maximum value. We tried to collect high-temperature hydrothermal fluid into the bottles No.1-2 (red) and No.3-4 (green) of Deep-WHATS sampler (13:34), but failed to sampling due to filling of the fluid inlet tube by particles. Then, we moved to Anemone field, made T/DO measurement at the edge of Rimicaris colony, and sucked animals (14:25). Finally, we retrieved the UK-wood colonization device (14:56) and left the seafloor (15:05).

Payloads:
1) Deep-WHATS with 4 bottles
2) Normal Chimney Box
3) Black Box
4) Slurp gun
5) Single Canister sampler
6) Mystery ring (for quantitative sampling)
7) T/DO sensor
8) MORINAGA Box
9) Markers

Event list:



♦1354DIVZ 方リブ南中部　川口　佩介 (WGS-84)SSBL

*** EVENT MARK LIST ***　2013-06-26 15:06:10

ORIGIN (XY<->LATLON CONVERT)　LAT 18°32.8000'N　LON 81°43.1000'W
XY ORIGIN [(X,Y)=(0,0)]　LAT 18°32.8000'N　LON 81°43.1000'W

NO.	DAY	TIME	LAT		LON		X	Y
1	2013-06-26	09:00:00	18° 32.9138'	N	81° 43.2194'	W	209.3	-210.3
	Landing Target							
2	2013-06-26	11:08:00	18° 32.8875'	N	81° 43.1944'	W	161.4	-166.3
	Landing D=5098m							
3	2013-06-26	13:34:00	18° 32.7877'	N	81° 43.0955'	W	-22.6	7.9
	Sampling Water(Red,Green),Chimney D=4965m							
4	2013-06-26	14:25:00	18° 32.7819'	N	81° 43.0667'	W	-33.3	58.5
	Measurement DO, Sampling animals D=4947m							
5	2013-06-26	14:56:00	18° 32.7949'	N	81° 43.1064'	W	-9.4	-11.2
	Retrieve wood D=4955m							
6	2013-06-26	15:05:00	18° 32.8676'	N	81° 43.2273'	W	124.6	-223.9
	Left Zotton D=4950m							
7								
8								
9								
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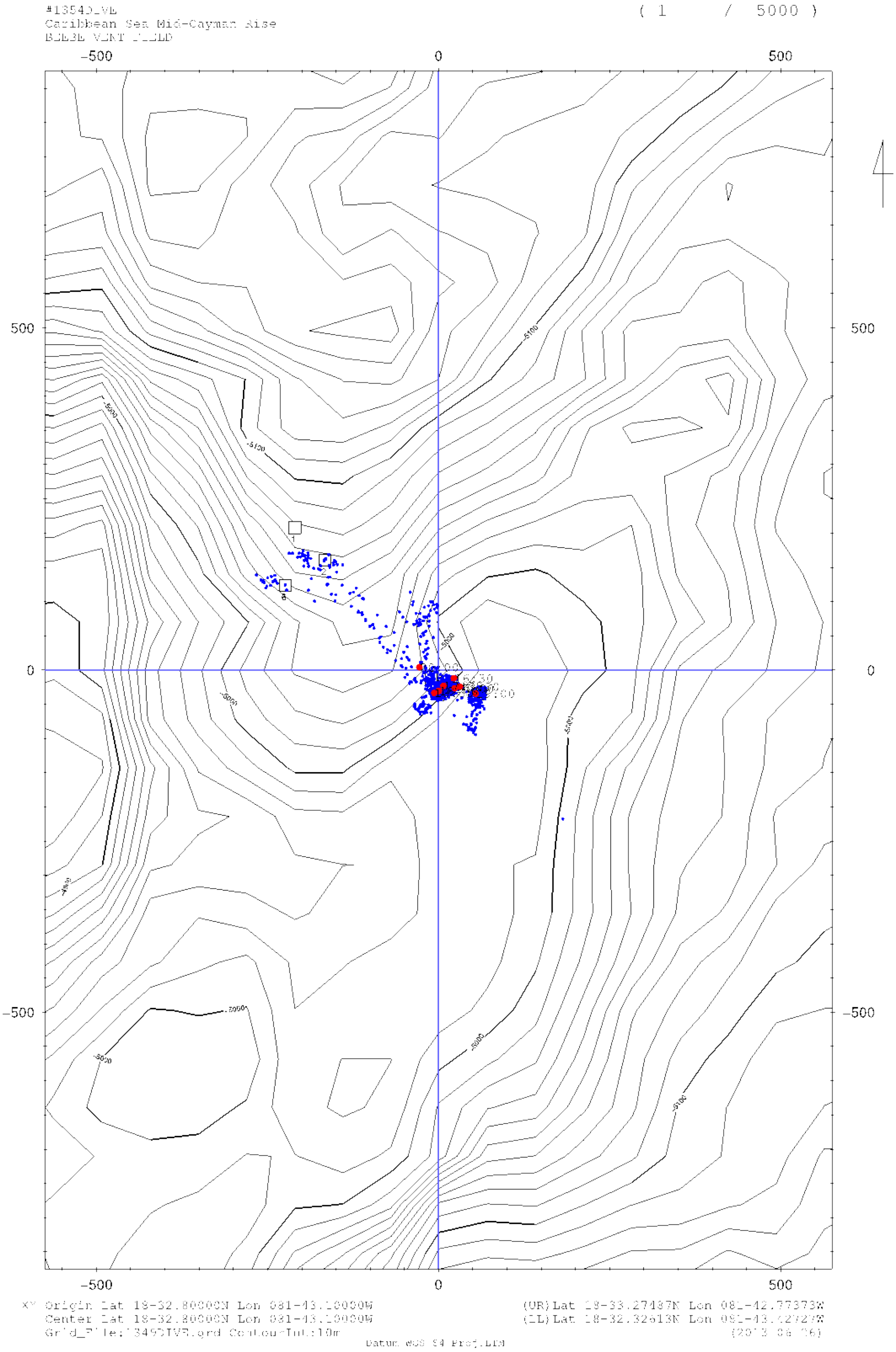
13								
14								
15								
16								
17								
18								
19								
20								

Dive Track



Caribbean Sea Mid-Cayman Rise
(1 / 5000)
-500
0
500
-500
0
500
XY Origin Lat 18-32.80000N Lon 081-43.10000W
Center Lat 18-32.80000N Lon 081-43.10000W
(UR)Lat 18-33.27437N Lon 081-42.77373W

Dive Report: Shinkai 6500 Dive# 1355
Date: June 27, 2013
Site: Beebe Hydrothermal Field (MCSC)
Landing: 11:10; 18˚32.9428N, 81˚43.2053W, D= 5105m
Leaving: 15:12; 18˚32.8932N, 81˚43.2053W, D=4888m
Observer: Katsunori Yanagawa (JAMSTEC)
Pilot: Keita Matsumoto **Co-Pilot**: Masaya Katagiri

Objectives:
In the Dive #1355, we aimed to measure the temperature of hydrothermal fluids at Beebe125 and Hashtag sites in the Beebe Vent Field in the Mid-Cayman Rise, and to collect hydrothermal fluids from two sites with D-WHATS sampler for geochemical analysis. Second objective is to visit the Chimlets site, which is hitherto unexplored in this cruise. At that site, we planed to measure the DO and temperature in the vicinity of faunal colony, to collect animal samples such as shrimps and gastropods, and to recover the chimney samples or rocks for geomicrobiological studies.

Dive summary:
We adjusted a trim position at 62 m above the seafloor, collected seawater sample into the Niskin bottle and landed at ~200 m northwest of Beebe125 and Hashtag chimney sites. The seafloor was composed of pelagic sediment, and bottom water temperature was 4.5°C. We headed southeast towards the Beebe125/Hashtag chimney sites, and followed almost the same route as Dive #1350 and #1354. Faunal colonies of anemones and shrimps in the shimmering diffuse flow were observed at "Anemone field" located near the center of sulfide mound. After running through Anemone field, we could find the Beebe125 site. First, we extended the orifice of hydrothermal conduit by using a stick and measured temperature of hydrothermal fluid. After several trials, we successfully recorded the maximum temperature of 396.7°C. Then we collected the hydrothermal fluid from Beebe125 with D-WHATS sampler into green bottles (#3 and #4). The average temperature during the sampling was calculated to be 374°C, suggesting hydrothermal fluid was unlikely mixed with ambient seawater. However, because the operating system of D-WHATS sampler might improperly pump up ambient seawater before closing the valve, we have to consider the possibility of seawater intrusion in onshore analysis. After the fluid sampling at Beebe125, we moved to Hashtag site and collected the high-temperature hydrothermal fluid with D-WHATS sampler into red bottles (#1 and #2). The average temperature during the sampling was 380°C. Then we looked for "Chimlets" site in northeast slope of the mound, where the Jason-2 marker #23 was landmark object. There, we planed to collect small chimney sample, diffuse flow fluid and Provannid gastropods. However we could not find the marker and Chimlets site. Finally we gave up Chimlets and went to Anemone field

for the sampling of animals and rocks. We landed at the Rimicaris shrimp colony, deployed red-colored mystery circle at the periphery of the colony, conducted DO+temperature measurement in the center of the mystery circle and collected faunal samples with a suction sampler (chamber #1). Then, the same step was repeated at the inside of the colony (chamber #2). At the same position, we also recovered rock samples into both MJ sample box and sample box. For RNA preservation, the seawater in the MJ sample box was replaced by RNA protective solution. Finally we went back to the vicinity of landing position and left the bottom at 200 m northwest from the Beebe field.

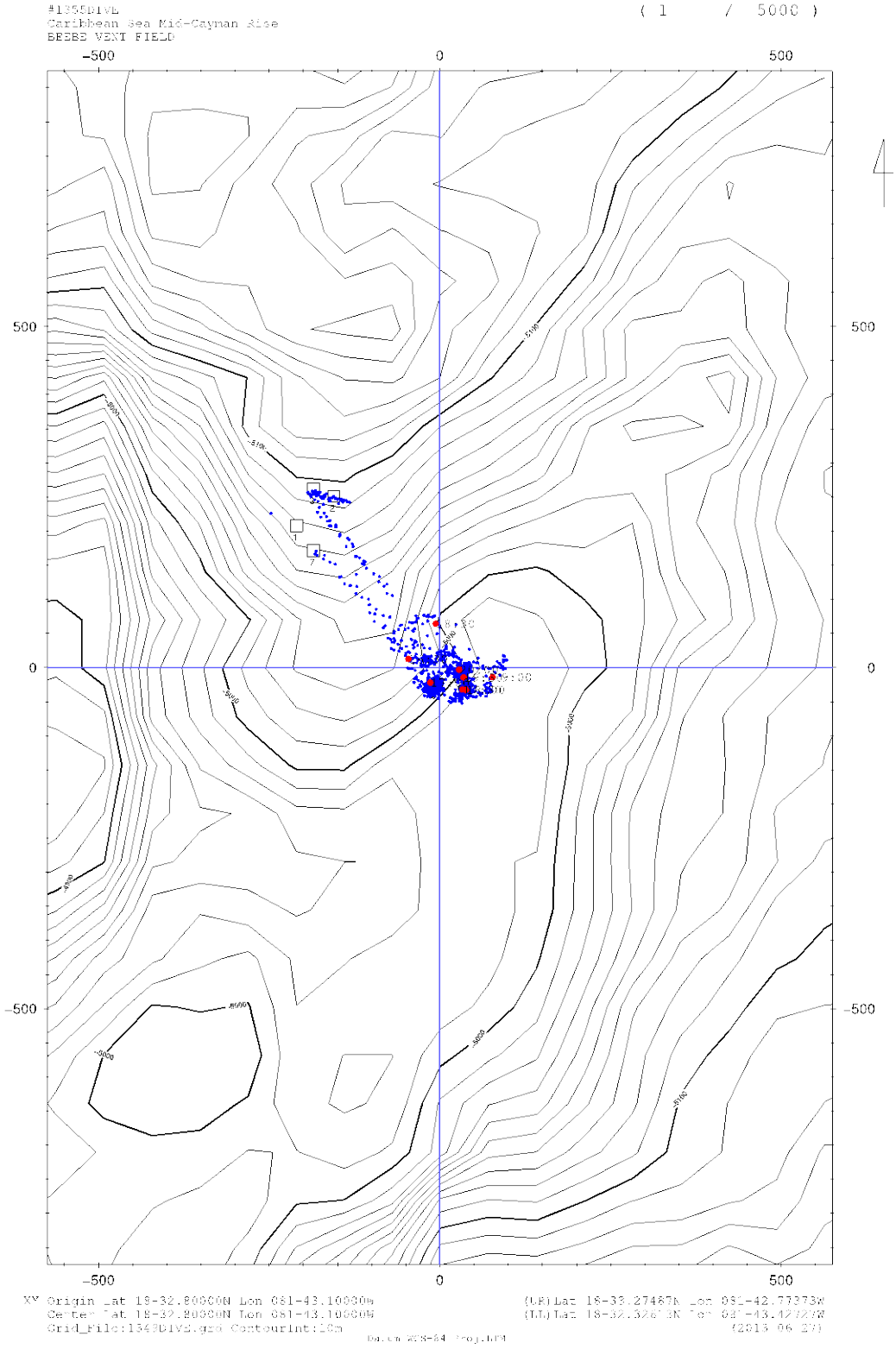
Payloads:

- Deep-WHATS sampler (D-WHATS)
- Chimney box (MJ box with RNA fixation reagent)
- Niskin x 1
- DO+temperature sensor
- Mystery circle for faunal sampling (Red)
- Suction sampler with multibottle (6 series) collectors (No. 1-5 bottles for large animals and No. 6 bottle for meiofauna)
- Sample Box
- Marker x 2

Event list:

1. 11:04, Niskin Sampling after trim adjustment at 62 m above the seafloor
   18°32.9366N, 81°43.1883W, D=5042 m
2. 11:10, Landing at ~200 m northwest of Beebe 125
   18°32.9428N, 81°43.2053W, D=5105 m
3. 12:29, Hydrothermal fluid sampling with D-WHATS sampler (green) at Beebe 125
   18°32.7870N, 81°43.1042W, D=4958 m
4. 13:00, Hydrothermal fluid sampling with D-WHATS sampler (red) at Hashtag
   18°32.7870N, 81°43.1042W, D=4965 m
5. 14:59, DO+Temperature measurement, suction sampling of animals in the Mystery circle and rock sampling at Anemone field
   18°32.7939N, 81°43.0822W, D=4965 m
6. 15:12, left bottom
   18°32.8932N, 81°43.2053W, D=4888 m

Dive Track


#1355DiVE
Caribbean Sea Mid-Cayman Rise
BEEBE VENT FIELD
(1 / 5000)
-500
0
500
-5100
-5000
-4900
-4800
-4700
-4600
XY Origin Lat 18-32.800600N Lon 081-43.100300W
Center Lat 18-32.800600N Lon 081-43.100600W
Grid_File:1343DiVE.grd Contourint:10m
(LR)Lat 18-33.27487N Lon 081-42.77373W
(LL)Lat 18-32.326 3N Lon 081-43.427 2W
(2013 06 27)
Datum WGS-84 Proj:UTM

Dive Report: Shinkai 6500 Dive# 1356
Date: June 28, 2013
Site: Von Damm Hydrothermal Field
Landing: 11:08; 18º 22.4457'N 81º 47.7675 E, D = 2451m
Leaving: 15:45; 18º 22.4753'N 81º 47.7751'E, D = 2349m (A = 10m)
Observer: Norio Miyamoto (JAMSTEC)
Pilot: Hirofumi Ueki **Co-Pilot**: Akihisa Ishikawa

Objectives:
The dive #1356 was planned to: (1) visit Main Spire and Hole to Hell of the main mound of Von Damm vent field to collect high temperature fluids and chimney materials; (2) collect faunal samples around the chimney; (3) visit diffuse flow area, collect faunal samples and measure the environmental parameters; (4) and visit tubeworm site to collect tubeworms and rocks.

Dive summary:
Shinkai landed at the south east of the main mound of the Von Damm Vent Field (D =2451m). Sparse fauna observed on this area consisted of deposit-feeding holothurians and solitary shrimp. Shinkai6500 was directed toward the top of the main mound and proceeded up the steep slope of the mound. During the way to the top of the main mound, we found several small vents where *Rimicaris* shrimps colonized.
At 11:45 we reached Hole to Hell and collected high temperature fluid into the bottle No.1 and No.2 of WHATS fluid sampler. Then, we collected chimney material and rocks around the chimney into the MJ box and the Chimney box. The seawater in the MJ box was replaced with fixative. Fauna around the chimney were suctioned into chamber 1 of the suction sampler, which was rotated to chamber 2 afterward. Next, we deployed the Mystery Circle on a flat area. The DO+temperature sensor was deployed in the center of the Mystery Circle for three minutes. Fauna within the Mystery Circle were suctioned into Chamber 2 of the suction sampler. When we tried to recover the Mystery Circle, the circle fell to a cleft of a rock and failed to recover.
We moved to a diffuse flow area where *Rimicaris* colonized. We collected fluid into the bottle No. 3 and No. 4 of WHATS sampler. Next, we measured DO and temperature of the middle of the colony, the periphery of the colony and the outside of the colony where *Lebbeus* shrimp were sparsely distributing. *Lebbeus* shrimps were suctioned into chamber 3 of the suction sampler.
The submersible was directed toward the tubeworm site where we visited in the #1352 dive. In the tubeworm site, we measured DO and temperature and collected tubeworms and rocks by manipulator into the black box.
We left the von Damm vent site and landed on muddy sea floor east side of the vent area. We collected several holothurians into chamber 4 and 5 of the suction sampler. Then, we left the bottom.

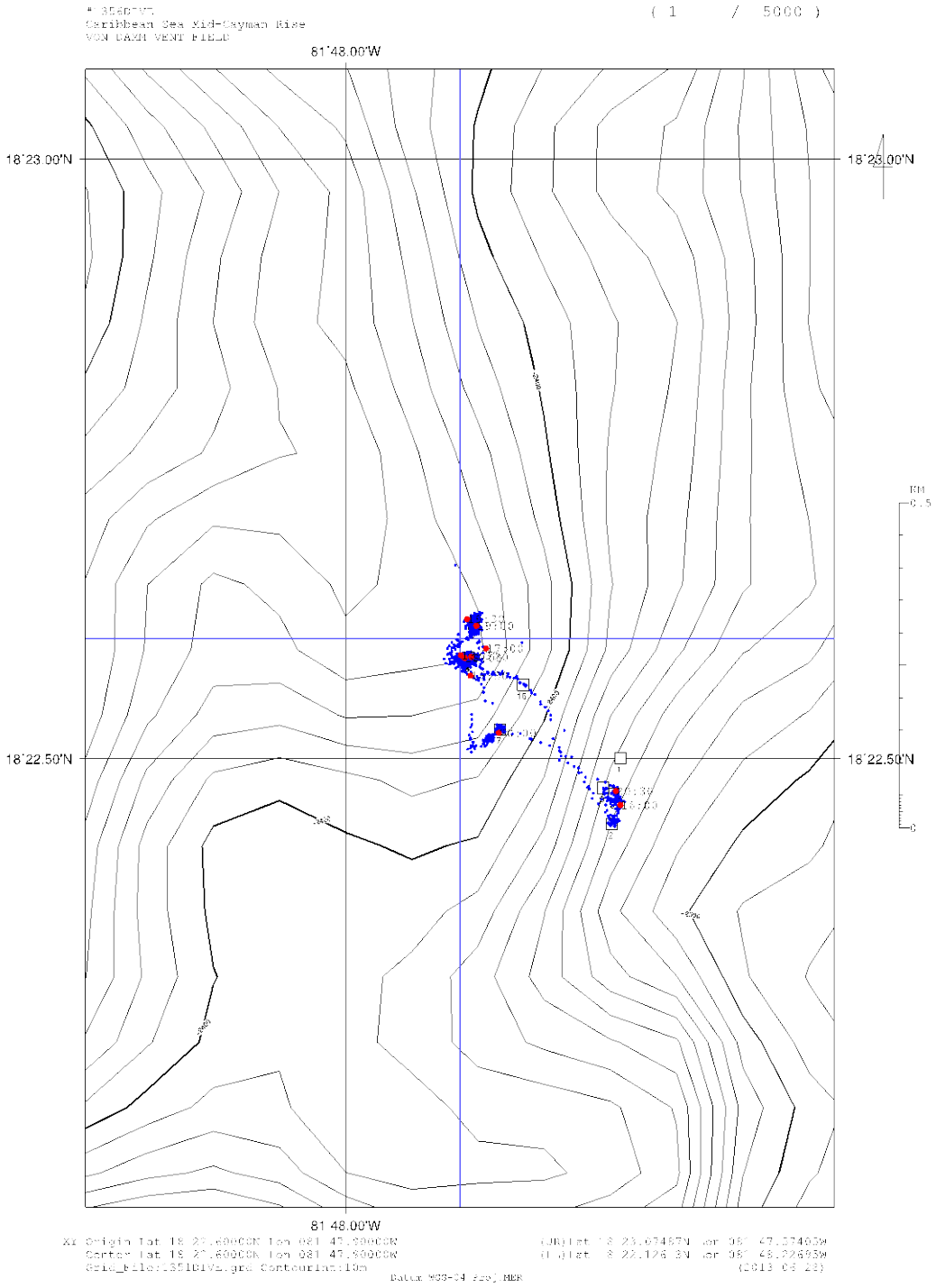
Payloads:

1) WHATS with 4 bottles
2) MJ Chimney Box
3) Normal Chimney Box
4) Black Box
5) Slurp gun
6) 6-bottle Canister sampler
7) Mystery ring (for quantitative sampling)
8) DO/temperature sensor
9) MORINAGA Box
10) Markers

Event list:

NO.	DAY	TIME	LAT	LON	X	Y
1	2013-06-28	10:00:00	18° 22.5000' N	81° 47.7600' W	-184.4	246.5
	Landing Target					
2	2013-06-28	11:08:00	18° 22.4457' N	81° 47.7675' W	-284.6	233.3
	Landing D=2451m					
3	2013-06-28	11:55:00	18° 22.5841' N	81° 47.8924' W	-29.3	13.3
	Sampling WHATS(2 bottle) D=2295m					
4	2013-06-28	12:45:00	18° 22.5841' N	81° 47.8924' W	-29.3	13.3
	Sampling Rocks D=2295m					
5	2013-06-28	13:26:00	18° 22.5841' N	81° 47.8924' W	-29.3	13.3
	Measur.DO , Samp.animals , Set Mystry circle D=2996m					
6	2013-06-28	14:31:00	18° 22.6126' N	81° 47.8879' W	23.2	21.3
	Measur.DO, Sampling WHATS(2 bottle),shrimp D=2308m					
7	2013-06-28	15:16:00	18° 22.5241' N	81° 47.8652' W	-140.0	61.2
	Measurement DO , Sampling Tubeworm,Rock D=2349m					
8	2013-06-28	15:43:00	18° 22.4702' N	81° 47.7660' W	-239.4	235.9
	Sampling Sea cucumber D=2448m					
9	2013-06-28	15:45:00	18° 22.4753' N	81° 47.7751' W	-230.0	219.9
	Left Bottom D=2437m A=10m					

Dive Track


Caribbean Sea Mid-Cayman Rise
VON DAMM VENT FIELD
(1 / 5000)
81°48.00'W
18°23.00'N
18°22.50'N
81 48.00'W

Dive Report: Shinkai 6500 Dive# 1357
Date: July 1, 2013
Site: Beebe Hydrothermal Field (MCSC)
Landing: 09:11; 18˚32.9630N, 81˚43.2027W, D= 5135m
Leaving: 13:05; 18˚32.7400N, 81˚43.0310W, D=4930m
Observer: Daniella Morgan Smith (East Carolina University)
Pilot: Yoshitaka Sasaki **Co-Pilot**: Keigo Suzuki

Objectives:
The main objective of this dive was to recover fiber optic cable left on the seafloor during previous dives, which covered several hydrothermal venting sites. The scientific objectives were to sample high-temperature fluid and chimney from the Hashtag site, as well as fluid from the Beebe Woods site.

Dive summary:
We left the sea surface at 07:00 and arrived at an altitude of 68 m above the seafloor (a depth of 5057 m) at 08:58, where we dropped ballast and adjusted trim of the submersible. Initial landing target was the Hashtag site, but we arrived at Anemone Field first, and located the fiber optic cable at 09:50. At this point, the pilot used the manipulator arms to pick up the cable and thread it onto a spool, thereby beginning the process of collecting the optical fiber. The cable broke and had to be re-threaded multiple times before all cable was collected from Anemone Field at 10:49.
We arrived at Hashtag at 10:54, where cable was removed from the top of a chimney structure. Following cable removal at 11:25, two bottles of high-temperature fluid were filled using the D-WHATS sampler. A maximum temperature of 396°C was recorded during sampling. Sampling of chimney using the manipulator arms was then undertaken at 11:33, first with a small piece of friable, black sulfide removed from the top of a narrow chimney. This sample crumbled in the manipulator before reaching the sample box, so a more solid, orange-colored section of chimney was removed from immediately below the first, and placed in the sample box. A sample tray, dropped during a previous dive, was removed from the seafloor of the Hashtag site, and placed in the sample basket before leaving the site at 11:49.
In transit between Hashtag and Beebe Woods, we passed over a field of pillow basalts, and at 12:00 we encountered the fiber optic cable again prior to seeing the Beebe Woods chimneys at 12:04. At 12:13, two bottles of high-temperature fluid were collected, with a maximum temperature of 320°C recorded. Cable spooling operation restarted at 12:30, again breaking and requiring re-spooling during the procedure. At 13:03, we left the seafloor for the surface, and arrived on the deck of the R/V Yokosuka at 15:12.

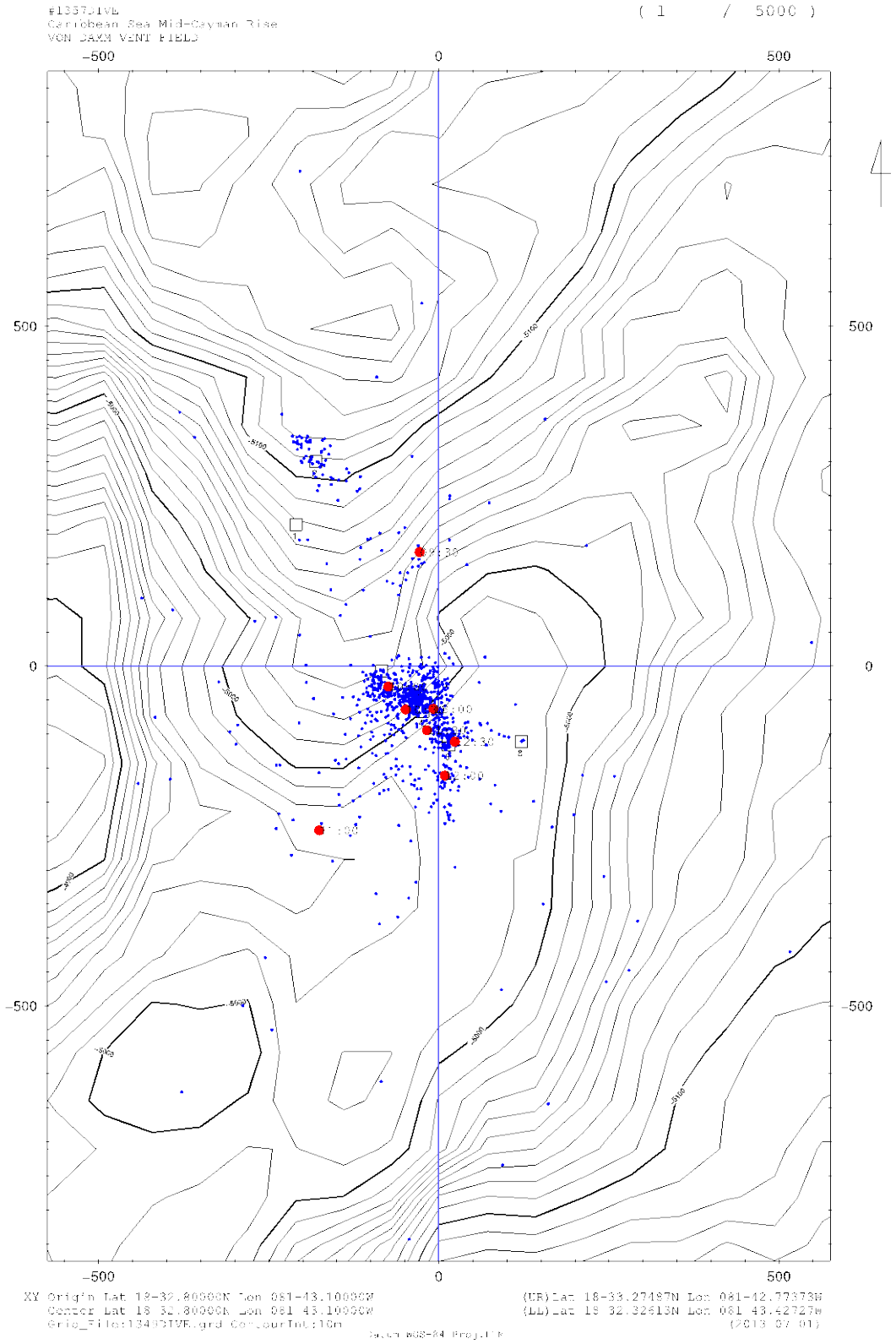
Payloads:

- Deep-WHATS sampler (D-WHATS, 4 bottles)
- Suction sampler with single canister
- DO sensor
- Sample Box
- Spool for cable (x3)
- Plastic tray (return trip only)

Event list:

1. 09:11 Landing at 18-32.9630N, 81-43.2027W, D=5135m
2. 10:00 Retrieval of optical fiber at 18-32.7964N, 81-43.1477W, D=4982m
3. 10:53 Retrieval of optical fiber at 18-32.7642N, 81-43.1136W, D=4963m
4. 11:35 Sampling of hydrothermal fluid (D-WHATS, green bottles), and chimney from Hashtag site at 18-32.7790N, 81-43.1220W, D=4965m
5. 12:27 Sampling of hydrothermal fluid (D-WHATS, red bottles) from Beebe Woods site at 18-32.7375N, 81-43.0920W, D=4961m
6. 12:30 Retrieval of optical fiber at 18-32.7440N, 81-43.0924W, D=4969m
7. 13:05 Left bottom at 18-32.7400N, 81-43.0310W, D=4930m, A=30m

Dive Track


#1337-1VE
Caribbean Sea Mid-Cayman Rise
VON DAMM VENT FIELD
(1 / 5000)
-500
0
500
XY Origin Lat 18-32.80000N Lon 081-43.10000W
Center Lat 18 32.80000N Lon 081 43.10000W
Grid_File:1340?1VE.grd Contour Int:10m
(UR)Lat 18-33.27487N Lon 081-42.77373W
(LL)Lat 18 32.32613N Lon 081 43.42727W
(2013 07 01)

V. Shore base study

Fluid chemistry (Shinsuke Kawagucci)

During YK13-05 cruise in the Mid Cayman Rise, hydrothermal fluids were collected by WHATS (four 150-mL bottles) and Deep-WHATS (four 50-mL bottles) samplers from von Damm vent field and Beebe vent field, respectively. The fluid sampler was loaded on Shinkai6500 and indeed used for sampling in all the dives, although sampling was unfortunately failed in Dives 1354 and 1355. After (Deep-)WHATS was recovered onboard, aliquots of the fluid samples in the bottles were filtered through 0.2-μm pore-size filters to remove suspended particles prior to subsampling for each chemical analysis. For the onshore cation analysis, the filtered subsamples were acidified to pH<2 using 0.1 mL dense hydrochloric acid. For the gas analyses, the fluids from the WHATS bottles were immediately opened to a vacuum line (~1,000 mL) to recover the dissolved gas components, and then reagent-grade solid sulfamic acid ($HOSO_2NH_2$) and cadmium chloride ($CdCl_2$) was added to the fluid through the vacuum line to extract CO_2 and precipitate H2S, respectively. After degassing for 10 min, the gas phase was collected in 120 mL glass and/or 60 mL stainless-steel bottles for subsequent gas analyses.

Fluid pH, H_2S concentration, total gas content (TGC) and gas concentrations (H_2, CH_4, CO, N_2, CO_2, COS, C_2H_6, etc.) were determined onboard by a pH meter, colorimetry, a barometer mounted on a homemade gas extraction device and Gas Chromatography with Helium Ionization Detector. After the back of R/V Yokosuka to JAMSTEC, concentrations of chemical species in the liquid samples will be measured by Ion Chromatography (Cl, SO4, Br, F), Inductively Coupled Plasma Optical Emission Spectrometry (Na, K, Ca, Li, Fe, Mn, Sr, Ba, B, etc.) and Inductively Coupled Plasma Quadrupole Mass Spectrometry (Fe, U, Rb, Cs, Pb, Zn, Cu, Ag, Cr, As, Sb, Tl, W, Cd etc.). Stable isotope ratios of gas species in the gas samples will be measured by Continuous-Flow Isotope Ratio Mass Spectrometry (dD_{H2}, dD_{CH4}, dD_{C2H6}, $d^{13}C_{CH4}$, $d^{13}C_{CO}$, $d^{13}C_{CO2}$, $d^{13}C_{C2H6}$, etc.).

Note: Details about sample processing and analyses have been described elsewhere [e.g., Kawagucci et al., 2013, Chemical Geology].

Microbiology (Katsunori Yanagawa & Junichi Miyazaki)

Hydrogen is the one of the key electron donors for prospering microbial community such as hydrogenotrophic methanogens and hydrogenotrophs. Our previous studies suggested that hydrogen-rich hydrothermal fluids from Kairei field of the Central Indian Ridge harbored abundant hydrogenotrophic methanogens in deep-sea hydrothermal vent chimney structures.

Here, in order to elucidate the importance of hydrogen in the hydrothermal environment of Mid-Cayman Rise, we plan to carry out the geomicobiological experiments as follows;

1. Aerobic or anaerobic incubation experiments of chimney samples and pumice substrate from in situ colonization system (ISCS) with hydrogen and medium at 30, 50, 70, and 100°C. The radiotracer experiments for activity measurement will further help to understand the initial rate of microbial hydrogen consumption in the chimney.
2. DNA extraction from the chimney samples and microbial community structure analysis based on 16S rRNA and functional genes.
3. RNA-based molecular phylogenetic analyses of the chimney samples to evaluate metabolically active microbial population.
4. Quantification of phylogenetically and metabolically specific microbes using CARD-FISH and Q-PCR. In particular, hydrogen consumers are focused in our analyses. If possible, we also carry out mRNA FISH analysis against genes for hydrogen oxidation to clarify the localization of hydrogen-oxidizers in the chimney structure.
5. Isolation of hydrogen-utilizing microbes.

These experiments will be conducted against chimney samples from Beebe and Von Damm Vent Fields to compare the abundance and phylogeny of hydrogen-utilizing microbial populations with those from Kairei field. Onboard preliminary studies did not show the positive evidence of hydrogenotrophic methanogens on the chimney samples in these two fields. Through these combined approach, biogeochemical cycles of hydrogen in deep-sea hydrothermal environment will be discussed quantitatively and qualitatively.

Microbiology (Priya Narasingarao & Daniella Morgan-Smith)

For Priya

Chimney samples were collected from Beebe site on dives 1349, 1350, 1354, and from VonDamnn site on dives 1351, 1353, 1356. The chimney rocks were homogenized and transferred to Mylar pouches and stored at 4C, a small portion was also stored under hydrostatic pressure for isolation of pressure adapted microbes. Multiple tubes containing 0.5g of samples were also fixed with formalin for microscopic observation. Background sea water was collected in Niskin bottles on dives 1349, 1355 and 1356. These were transferred to sterile bulbs and stored under in-situ pressure conditions in stainless pressure vessels. Three animal samples., Amphipods, tube worm and partial sea anemone specimen were collected for culturing of bacterial symbionts.

The fundamental research question that is we are trying to address is whether pressure affects growth and activity of microorganisms. Enrichments covering a wide range of electron donors and acceptors will setup with the aim of culturing microbes adapted to pressure. Long term enrichments under atmospheric pressure will also be setup for comparison. Samples saved for microscopy will be used in future to perform FISH aimed at target populations and to estimate microbial abundance.

For Daniella

Chimney and rock samples from each dive were divided into three sections: 2.0 ml tubes stored at -80°C, Mylar pouches (oxygen impermeable) stored at 4°C and pressurized to the in situ pressure of the sampling site, and Whirl-pak bags (oxygen permeable) stored at 4°C and atmospheric pressure. These three sample types were collected from several sites in the Beebe and Von Damm vent fields. From Beebe, high-temperature chimney was collected from Hashtag (dives 1349 and 1354) and Beebe Woods (dive 1350), and low-temperature rock from Anemone Field (dive 1355). From Von Damm, high-temperature chimney was sampled from Hole to Hell (dive 1351), Hotter than Hole (dive 1351), and the main spire (dive 1353), and low-temperature rock from north of marker X16 (dive 1352).

Upon returning to the lab, samples in 2.0 ml tubes will be used for DNA extraction, to be subsequently sequenced on the 16S gene using Tag (Illumina platform) next-generation sequencing for both Bacteria and Archaea. This will comprise the time-zero dataset, to which later samples will be compared. Samples in Whirl-pak bags will be stored for one year at 4°C and atmospheric pressure, and the sequences of the microbial community from these samples will then be compared to the time-zero sequences to assess the effect of long-term storage of deep-sea rock and chimney samples. This work is being undertaken in support of research on the ability of IODP repository cores to be used for microbiological research after long-term storage under atmospheric pressure at 4°C. A portion of each Whirl-pak sample will also be shared with colleagues for analysis of mineralogical composition to provide context on the microbial habitats and substrates available within the solid rocks or chimneys. Samples which have been pressurized on board in oxygen-impermeable pouches will be used for high-pressure cultivation in the lab at mesophilic temperatures, with the aim of isolating new strains of piezotolerant or piezophilic Bacteria and Archaea.

For joint research

Portions of high-temperature chimney from the Hashtag site were collected during dives 1349 and 1354. Aboard the R/V Yokosuka, 13C-labeled sodium formate or sodium bicarbonate were added to crushed chimney samples in minimal media or sterile-filtered seawater and incubated for 4-6 days. Incubations were carried out at 85 °C and several pressure levels: atmospheric, 50 MPa (in situ), 80 MPa, and 100 MPa. At the end of the incubation period, gas headspace was sampled from each incubation tube.

Gas sampled from incubation tubes described above will be analyzed using Isotope Ratio spectroscopy (GC -IRMS) for 13C-labeled methane. The data will be used to understand the effect of hydrostatic pressure on methanogenic activity at deep sea hydrothermal vents in Mid-Cayman Rise.

Biology (Hiromi WATANABE, Jon COPLEY, Takuya YAHAGI, Diva AMON, Verity NYE)

Deep-sea hydrothermal vent fauna showed high biomass among oceanic biological communities. The distribution of the vent fauna is drastically changed associated with environments. Depth is one of the important environmental factors to affect on the species composition of vent fauna. In Mid-Cayman Rise, the world deepest hydrothermal vent field, Beebe Vent Field (BVF) is located at about 5000 m deep-sea bottom, and the other hydrothermal vent field, Von Damm Vent Field (VDVF) is located at 2300 m in depth (Connelly et al. 2012). In this cruise, we focused on the three general environmental factors, depth, dissolved oxygen (DO) and temperature, as control factors to the faunal composition in hydrothermal vent fields. Totally 16 quantitative faunal sampling with environmental measurements with sensors were operated in this cruise. Based on the species composition and associated environmental factors, we will carry out multivariate analyses to figure out relationships between faunal composition and environmental factors.

Biology (Jon Copley, Verity Nye and Diva Amon)

Samples collected by Shinkai6500 dives during RV Yokosuka YK13-05 will enable further studies in the UK of the reproductive ecology of vent fauna, nutritional sources of vent taxa, and the identification of vent species, summarised below, to continue our collaboration with Japanese colleagues in studying the ecology of Mid-Cayman vent fields. In addition, the dives recovered seafloor experiments deployed by the UK in February 2013: organic substrates for

scavenging and colonisation studies by Diva Amon, and inorganic substrates for colonisation studies by the INDEEP project.

Reproductive ecology

Four samples of the alvinocaridid shrimp Rimicaris hybisae were collected from the Beebe Vent Field, and two samples from the Von Damm Vent Field, for future reproductive study in the UK. One sample of the hippolytid shrimp Lebbeus virentova was also collected from Von Damm for reproductive study. These samples were preserved in 10% formalin to enable analysis of oocyte size-frequency distribution in female specimens, and reproductive maturity of male specimens.

The data will complement those from previous samples at the vent fields (Nye et al., 2013) to provide new information on temporal variation in reproduction in Rimicaris hybisae – with these first samples collected in summer – and further information on spatial variation, this time with information on microenvironments recorded during sampling as part of a joint UK-Japan study of reproductive ecology.

Samples for future reproductive study in UK:
6K#1349_01 – 66 x Rimicaris hybisae, Beebe Vent Field
6K#1354_03 – 224 x Rimicaris hybisae, Beebe Vent Field
6K#1355_01 – 95 x Rimicaris hybisae, Beebe Vent Field
6K#1355_02 – 84 x Rimicaris hybisae, Beebe Vent Field
6K#1351_01 – 67 x Rimicaris hybisae, Von Damm Vent Field
6K#1351_06 – 78 x Rimicaris hybisae, Von Damm Vent Field
6K#1356_07 – 39 x Lebbeus virentova, Von Damm Vent Field

Nutritional sources

Samples of three taxa were collected and preserved by freezing at -80C for future analysis of nutritional sources in the UK: the hippolytid shrimp Lebbeus virentova, the siboglinid polychaete Lamellibrachia sp., and the holothurian Benthodytes sp. These data will be added to results from previous samples of other taxa at the vents to provide comprehensive information on nutritional sources and resource use among faunal assemblages.

Samples for nutritional source study in UK:
6K#1356_03 – 5 x Lebbeus virentova, Von Damm Vent Field
6K#1352_11 – 2 x Lamellibrachia sp., Von Damm Vent Field
6K#1356_16 – 1 x Benthodytes sp., Von Damm Vent Field

Identification of vent species
Samples of three taxa were collected and preserved for further taxonomic identification in the UK through morphology and molecular phylogenetics, to assist in compiling the total species lists for the vents. At the Von Damm Vent field, samples collected from the Main Spire included an amphipod species not seen in samples from previous UK research cruises, distinct from the lyssianassoid Onesimoides sp. previously collected at the vent. Other samples from Von Damm included a species of capitellid polychaete found in "empty" siboglinid tubes; this taxon was previously sampled in February 2013, but further specimens will aid its final identification, and if necessary description. A specimen of a holothurian resembling Benthodytes sp. was also preserved for confirmation of identification by specialists in the UK.

In addition, specialists at the UK Natural History Museum will assist in the future with identification of any nematodes in pushcore samples (not listed here) collected for meiofaunal analysis from a pockmark site near the Von Damm Vent Field.

Samples for ID studies in UK:
6K#1356_17 – 3 x Amphipoda sp. B, Von Damm Vent Field
6K#1351_09 – 21 x Amphipoda sp. B, Von Damm Vent Field
6K#1356_13 – Capitellid polychaetes, Von Damm Vent Field
6K#1356_15 – Benthodytes sp., Von Damm Vent Field

Scavenging and organic substrate colonisation studies
In February 2013, UK research cruise RRS James Cook JC82 deployed wood and bone packages at Beebe Vent field for a study of colonisation of these organic substrates. Bone and wood packages were recovered from Beebe Vent Field during RV Yokosuka research cruise YK13-05; although scavenging macrofauna were present, there was no evidence of colonisation by wood or bone specialist fauna. One further wood and one further bone package were therefore left in place at the Beebe Vent Field to continue the experiment for a longer duration.

In February 2013, a raw side of pork, with viscera removed, was deployed at each vent field, to study scavenging fauna during UK ROV dives, and for a longer-term study of resource use and colonisation of organic substrates. The experimental package was absent from its deployment area at the Beebe Vent Field during June 2013, and may have been removed completely by scavengers, consistent with higher scavenging rates of food-falls at greater depth.

The experimental package at the Von Damm Vent field in June 2013 consisted of a few remnant pig bones (ribs and a vertebra), with all flesh having been removed. The bones were recovered by Shinkai6500, along with scavenging fauna present. All fauna recovered with the experiment were preserved for future taxonomic identification in the UK using morphology and molecular phylogenetics.

Samples for UK organic substrate colonisation studies:
6K#1350_01 – 1 x Munidopsis sp., from Beebe Vent Field bone deployment
6K#1350_02 – 5 x Rimicaris hybisae, from Beebe Vent Field bone deployment
6K#1350_03 – Partial polychaete, from Beebe Vent Field bone deployment
6K#1350_04 – 2 x amphipods, from Beebe Vent Field bone deployment
6K#1353_31 – 2 x Munidopsis sp., from Von Damm scavenger experiment
6K#1353_32 – 3 x Munidopsis sp., from Von Damm scavenger experiment
6K#1353_33 – 12 x Iheyaspira bathycodon, from Von Damm scavenger expt
6K#1354_34 – 2 x Lebbeus virentova, from Von Damm scavenger experiment
6K#1354_35 – 5 x Amphipoda sp. B, from Von Damm scavenger experiment
6K#1354_36 – 1 x polychaete, from Von Damm scavenger experiment
6K#1354_37 – 19 x amphipods, from Von Damm scavenger experiment
6K#1354_38 – 14 x copepods, from Von Damm scavenger experiment
6K#1354_39 & 57 – residues from Von Damm scavenger experiment
6K#1354_41 to 56 – polychaetes from Von Damm scavenger experiment

INDEEP colonisation studies
In February 2013, two standard INDEEP colonisation packages were deployed at the Von Damm Vent Field, one close to an area of hydrothermal activity and the other in a location

away from any visible hydrothermal activity. These experimental packages, which consist of an array of inorganic substratum blocks mounted in a frame, were recovered by Shinkai6500 and preserved following INDEEP protocols, for future analysis of larval colonisation as part of the international INDEEP project.

Samples for INDEEP colonisation study:
6K#1356_01 to 09, & 15 – colonisation blocks from package INDEEP-1
6K#1356_21 to 30 – colonisation blocks from package INDEEP-2

Biology (Norio Miyamoto)
Organisms in extreme environments, such as deep-sea hydrothermal vents and hydrocarbon seeps, have some unique organs for sensing the environments. Understanding the evolution of these organs should provide important information on how organisms have adapted to new environments. Hydrothermal vent shrimps of the genes Rimicaris have a unique sensory organ on their dorsal side of the brain called the dorsal organ. It is proposed that the dorsal organ senses the week light from hydrothermal vents, however, molecular mechanisms of sensory system and its origin remain to be solved. To uncover the function and origin of the dorsal organ, we are going to analyze the spatiotemporal expression pattern and structure of genes expressing in the dorsal organ.

VI. Appendix

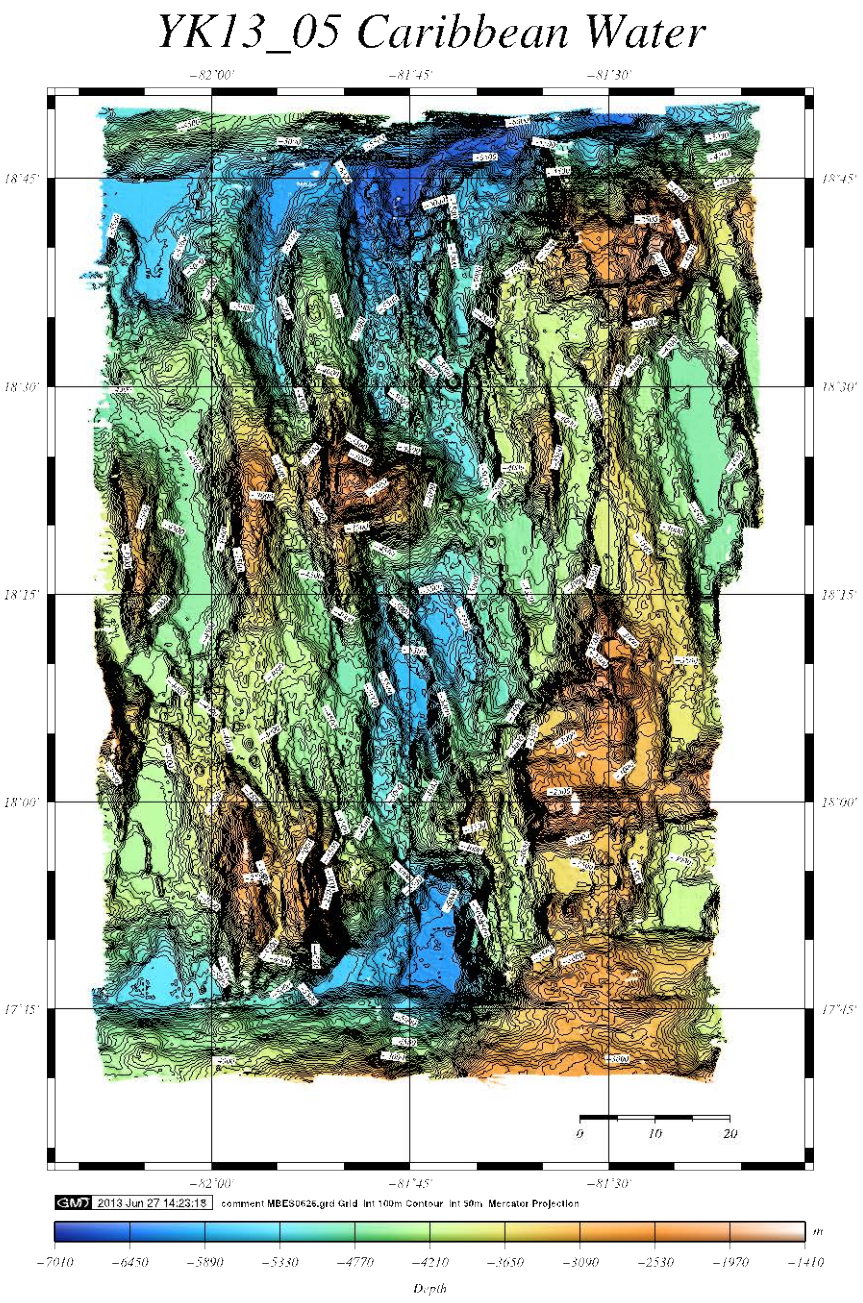


Figure. Bathymetry map of Mid Cayman Rise or Mid Cayman Spreading Center

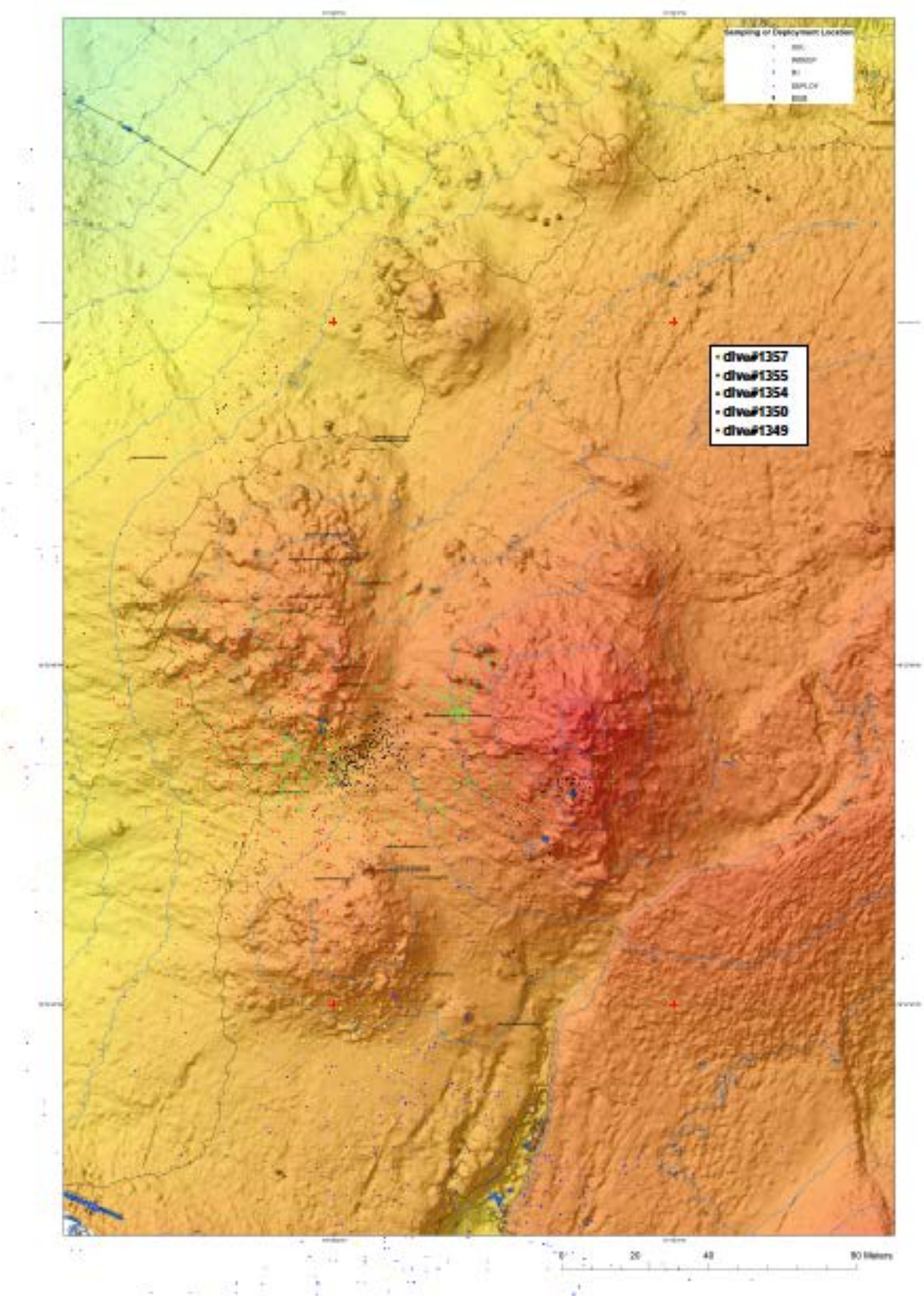


Figure. Tracks of all the dives in Beebe Field

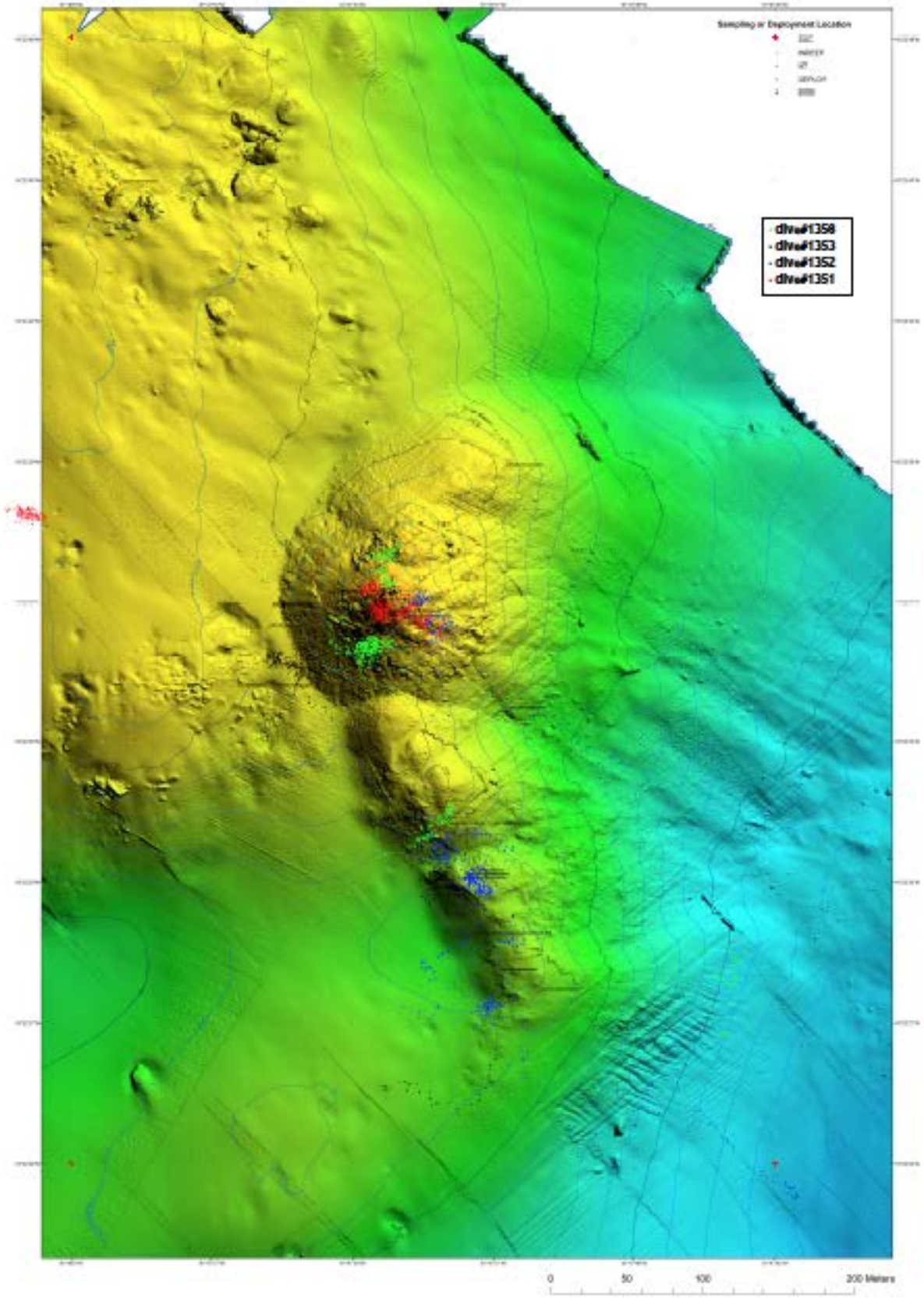


Figure. Tracks of all the dives in Von Damm Field