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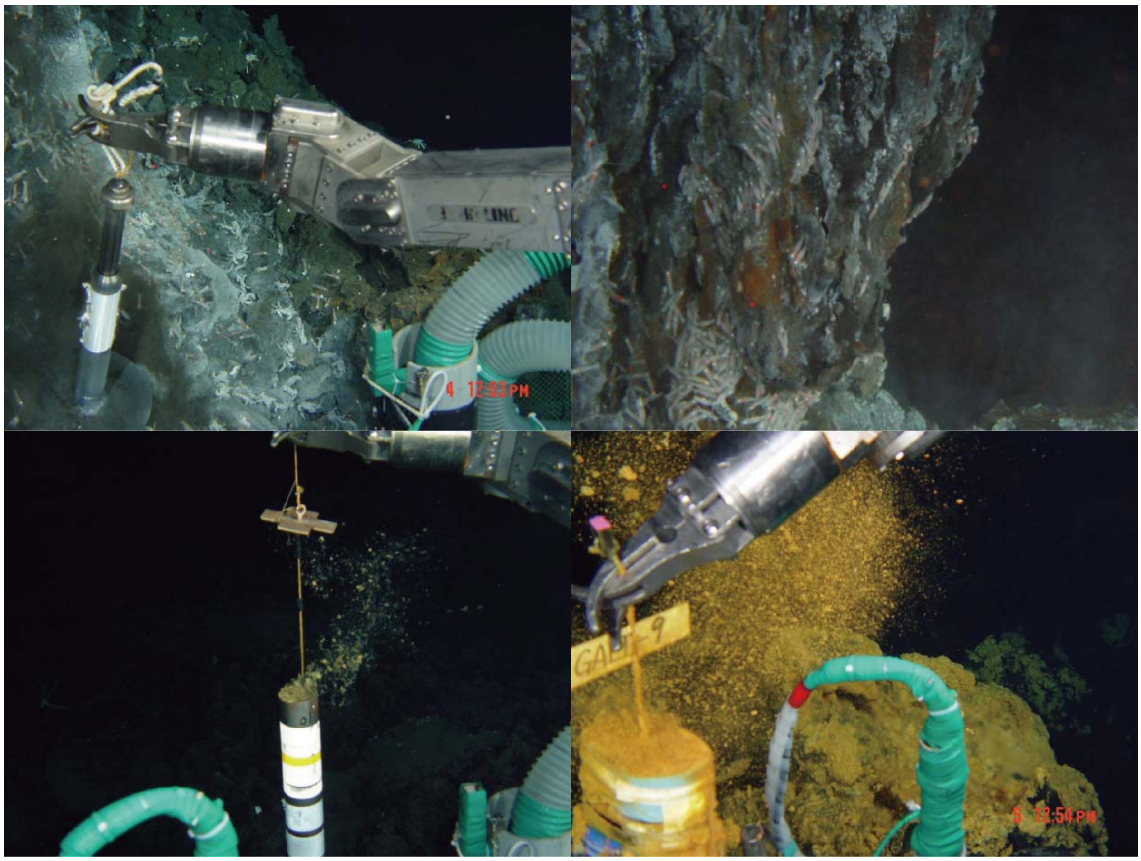
Yokosuka Cruise Report

YK10-13 Leg1

Shinkai6500 exploration in the Southern Mariana Trough

Are there HyperSLiMEs in the subseafloor on basaltic hydrothermal field?

Direct verification of subseafloor microbial ecosystem utilizing high temperature torelance biosampler.



3 October 2010, Guam – 7 October 2010, Guam

Japan Agency for Marine-Earth Science & Technology

(JAMSTEC)

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Acknowledgements

We are grateful to Captain Mr. S. Susami, Chief Officer Mr. T. Aoki, Chief Engineer Mr. T. Kimura and all of crews for their safe navigation and their skillful handling of “S/V Yokosuka”. Great thanks are due to Commander Mr. T. Sakurai and “Shinkai6500” operation team for their operations in sampling. We also thank Mr. S. Machida, Nippon Marine Enterprise, Ltd., for his attentive supports.

Finally, we would like to appreciate all the people who supported directly or indirectly this cruise.

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Sub-Chief Submarsible Staff	Satosh Ogura

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2st Submarsible Staff	Takuma Ohnishi
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3rd Submarsible Staff	Hitomi Ikeda
3rd Submarsible Staff	Masaya Katagiri

Shipboard Log of YK10-13 Leg1

Date Time Log

2010/10/02
Position: 13-27.7N, 144-40.0E / Weather: fine but cloudy / Wind direction: ESE/ Wind force: 3/ Wave: 1 m/ Swell: 0 m/ Visibility: 8 nautical miles (12:00 JST+1h)
22:00 Onboard

2010/10/03
Position: 13-27.7N, 144-40.0E / Weather: fine but cloudy / Wind direction: East/ Wind force: 2/ Wave: 1 m/ Swell: 0 m/ Visibility: 8 nautical miles (12:00 JST+1h)
08:30 Scientific meeting, setup the laboratory
09:00 Briefing about ship's life and safety
11:00 Briefing about Shinikai6500
14:00 Departure from APRA
15:30 Obtaining water
16:00 Proceeding to survey area(Archaea site)

2010/10/04
Position: 12-56.2N, 143-37.8E / Weather: fine but cloudy / Wind direction: East/ Wind force: 4/ Wave: 3m/ Swell: 2 m/ Visibility: 8 nautical miles (12:00 JST+1h)
07:30 Arrived at survey area(Archaea site)
09:55 Launch Sinkai6500 (6K#1237dive)
11:16 6K lands (3,083m)
15:53 6K leaves the bottom (2,970m)
17:28 6K on deck
18:00 Proceeding to survey area(Snail site)
19:00 Scientific meeting

2010/10/05
Position: 12-57.3N, 143-37.1E / Weather: fine but cloudy / Wind direction: East/ Wind force: 4/ Wave: 3m/ Swell: 2 m/ Visibility: 8 nautical miles (12:00 JST+1h)
07:30 Arrived at survey area(Snail site)
09:55 Launch Sinkai6500 (6K#1238dive)

11:18 6K lands (2,854m)

15:57 6K leaves the bottom (2,827m)

17:24 6K on deck

18:00 Proceeding to APRA

19:00 Scientific meeting

2010/10/06

Position: 13-27.7N, 144-40.0E / Weather: fine but cloudy / Wind direction: East/ Wind force: 4/ Wave: 1m/ Swell: 0 m/ Visibility: 8 nautical miles (12:00 JST+1h)

08:40 Arrival at APRA

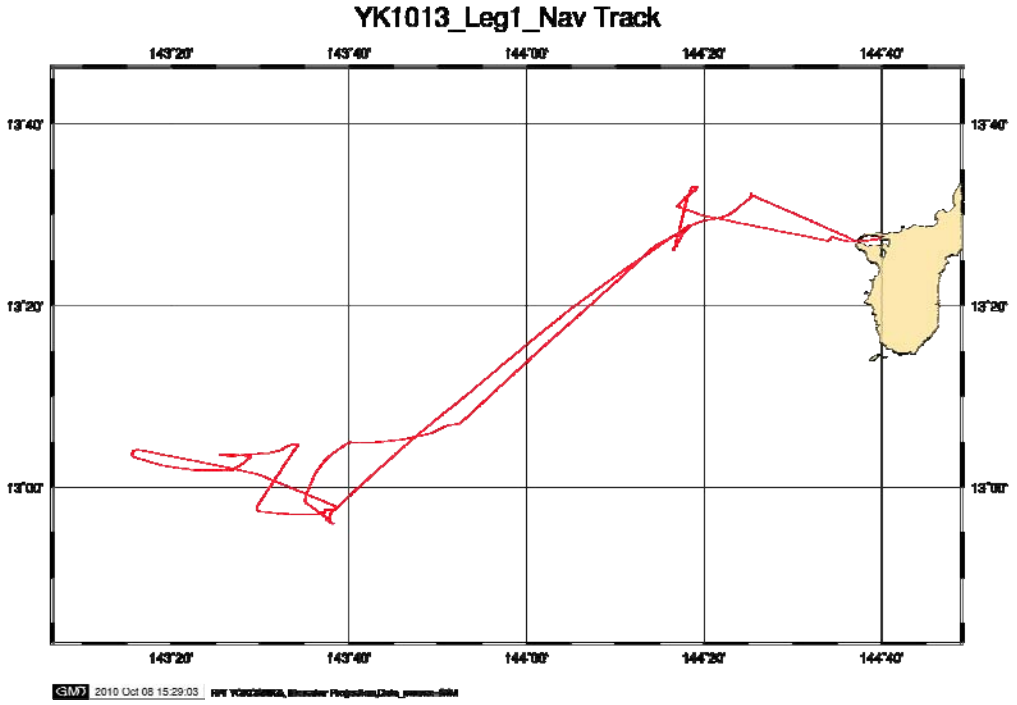
13:00 Scientific meeting

14:00 Cleaning at lab

2010/10/07

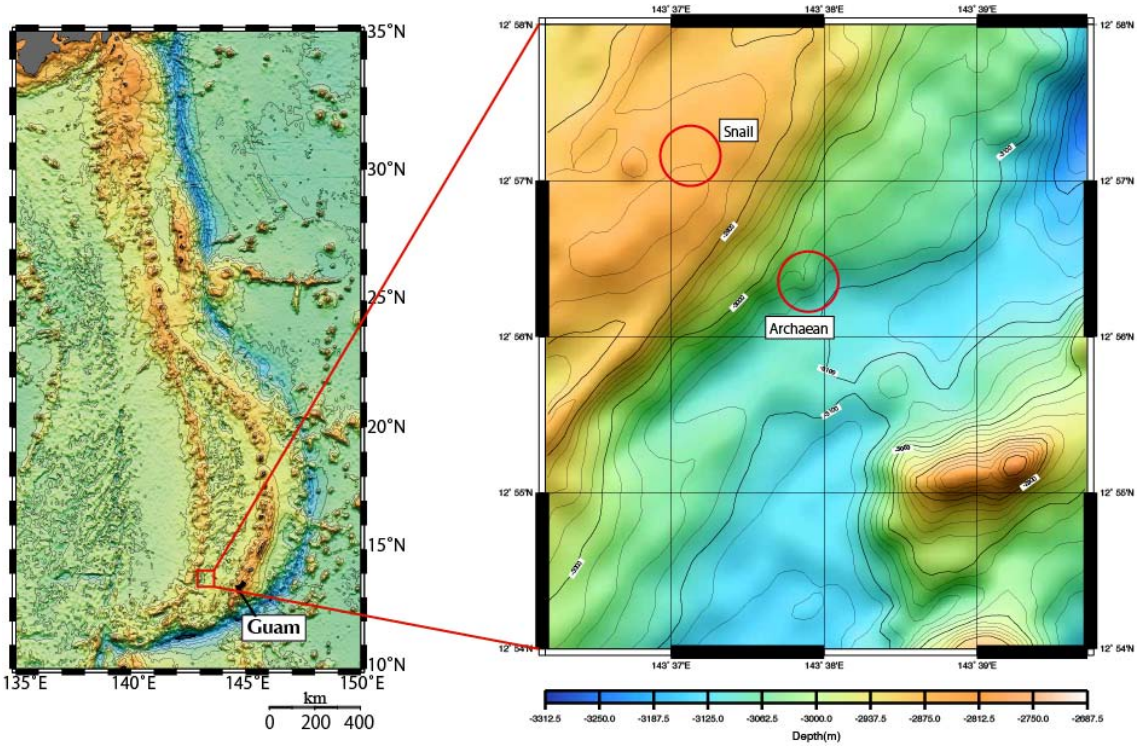
10:00 Cleaning at lab

12:30 YK10-13 Leg.1 finish and disembarkation



I. CRUISE SUMMARY

In this YK10-13 Leg1 cruise, we conducted 2 Shinkai 6500 dives and we successfully recovered 4 *in situ* colonization systems (ISCS) which were deployed into the vent or casing-inserted borehole to detect subsurface microbial ecosystem one month ago (YK10-10 cruise). And also we obtained very valuable samples to analyze microbial ecosystem, fluid chemistry, and rock composition. It is expected that many achievements will be generated in shore-based studies.



Research Area of the YK10-13 Leg1 Cruise

II. INTRODUCTION

General background and objectives:

The primary scientific objective of this research project is to clarify whether there are HyperSLiME (Takai, K., *et al*. 2004) in subvent on basaltic hydrothermal field, Archaean site in Southern Mariana hydrothermal field. To clarify this question, we will use a short Bio Sampler (Miyazaki11's Bio Sampler) which is *in situ* colonization system pressure-tightly closed in deployed place to detect subvent microbial ecosystem without sea-water contamination.

We have hypothesized that hyperthermophlic methanogen is close to origin of life and its surrounding ecosystem called HyperSLiME (Hyperthermophilic Subsurface Lithoautotrophic Microbial Ecosystem) is most primitive ecosystem on the Earth (Takai, K., *et al*. 2004). So that we need to know how geological and geochemical backgrounds the HyperSLiME is habitable. We obtained one answer for this question that HyperSLiME exists in hydrogen-rich environment. The mother rock of most of hydrogen-rich hydrothermal field is ultlamafic rock for example Kaiei field in CIR (Central Indian Ridge), Raibow, Logatchev, Ashadze hydrothermal fields in MAR (Mid-Atlantic Ridge). Actually, from chimneys from in hydrothermal fields, we detected methanogen by culture-dependent and –independent anlysis. So we proposed that Ultramafics-Hydrothermalism-Hydrogenesis-HyperSLiME (UltraH_3) linkage is necessary for maintain the methanogen and the surrounding microbial ecosystem.

However, recently we obtained information that there are hydrogen-rich hydrothermal fields whose host rock of hydrothermal activity is not ultlamafic rocks but basalt. It is generally said that basaltic rock does not generate much hydrogen enough to support methanogenic activity. But there actually are hydrothermal field erupting hydrogen-rich hydrothermal fluids. On of them is Archaean site in Southern Mariana Hydrothermal Field. In the DSV Shinkai 6500 dive #903 in YK05-09 Leg2, about 7-m height chimney structure was found around the 6k Marker #12. From the top of the chimney, black smoker (343°C) was erupted. On the other hand, from the foot of this chimney, clear smoker (117°C) was shimmered. The distance between two smoker vents was only about 2 m. Previous geochemical study by Dr. T. Toki showed that methane concentration of the clear smoker was 8 times higher than that of black smoker and the carbon isotopic ratio of methane of the clear smoker vent fluid in Archaean site was much lighter than that of the black smoker fluid. And also hydrogen concentration of

clear smoker was slightly higher than that of Black smoker. Moreover, carbon dioxide concentration of the clear smoker was slightly lower than that of black smoker. These results suggested that there is the subvent biosphere supported by methanogen (HyperSLiME) around the clear smoker stream. The lighter methane and large amount of hydrogen become strong evidence for the existence of methanogen. As a example for this evidence, we could actually detect and cultivate methanogenic archaea from DODO field in Central Indian Ridge whose mother rock is also basalt. So that Archaean site in Southern Mriana hydrothermal field will become the second example not applying to UltraH^3 linkage.

To accomplish the primary objective, we deployed the Miyazaki11's Bio Sampler directly into the vents in YK10-10 cruise on August. In this YK10-13 Leg1 cruise, the Bio Sampler will be recovered. The sample will be used for incuvation and PCR detection of both 16S rRNA genes and *mcrA* genes against extracted DNA and RNA from the sample detection to obtain direct evidences for subvent methanogens.

Miyazaki11's short Bio Sampler is a product of Kandata project which is a post-drilling project supported by JAMSTEC AWARDS for "Observing system research and technological development". The goal of this project is an innovation of tools for post-drilling. The project has two rules. One is that this Kandata system must be conducted only by ROV, although many of post drilling research required a large drilling ship to access bore hole. Another feature is that this Kandata system required a tight system to prevent contaminations from seawater. Because these contaminations cause the error for detecting lower microbial population in subvent biosphere. Now in this project, we developed the tools with high-temperature tolerance. In this YK10-10 cruise, we will test the high-temperature tolerance of Bio sampler to capture microbes in the subvent biosphere under the clear smoker vents.

We have another research interests on this YK10-13 Leg1. We will also investigate habitats of *Alviniconcha hessleri*, a chemosynthetic animal which have endosymbionts in their gill cells. *Alviniconcha hessleri* world-widely inhabits and these are almost the same species. However phylogeny of endosymbionts in the gill cells differ at every hydrothermal field. For example, *Alviniconcha* in Indian Ocean possess ε-*Proteobacteria* in their gill cells. On the other hand, those in Lau Basin possess γ-*Proteobacteria* in their gill cells (Suzuki *et al.*, 2006). We will assess the expression level by qPCR against the cDNA generated by reverse transcription of extracted RNA from gill cells.

References

Takai, K., *et al.*, (2004) Geochemical and microbiological evidence for a hydrogen-based, hyperthermophilic subsurface lithoautotrophic microbial ecosystem (HyperSLiME) beneath an active deep-sea hydrothermal field. *Extremophiles*, **8**:269-282.

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Suzuki, *et al*., (2006) Host-symbiont relationships in hydrothermal vent gastropods of the genus *Alviniconcha* from the Southwest Pacific. *Appl. Environ. Microbiol*. **72:**1388-1393.

III. DIVE REPORTS

Shinkai6500#1237 DIVE (Pika & Urashima site) Dr. J. Miyazaki
Shinkai6500#1238 DIVE (Snail site) Dr. S. Sakai

Report: Shinkai 6500 Dive#1237

Date: October 4, 2010
Site: Archaean site, South Mariana Hydrothermal Field
Landing: 11:16; 12°56.2627'N, 143°37.8816'E, 3083 m
Leaving: 15:53; 12°56.3763'N, 143°37.8403’E, 2982 m
Observer: Junichi Miyazaki (JAMSTEC)
Pilot: K. Matsumoto **Co-Pilot:** Y. Chida

Objectives:

Primary objective of this dive is recovering 4 *in situ* colonization systems (ISCSs) deployed around Sagrada familia chimney in YK10-10 cruise. Other objecteves are collecting hydrothermal vent chimneys, vent fluids and animals.

Dive Summary:

At 11:16, we landed on hydrothermal sediments (Depth 3072 m) and headed to north to go to Sagrada familia chimney (6k#110). A few minutes later, we could see lava on seafloor. But when we reached at depth 3060 m, the slope became steep and we could not observed lava on seafloor. From this position to the top of mound, the seafloor was covered with sulfide rocks. At 12:00, we arrived at Sagrada familia Chimney (depth 3001 m).

First, we recovered Watsuji’s hair ISCS deployed near clear smoker vent and put on the sample box. Next, we tried to recover Miyazaki11’s pressure-tight ISCS. But we could not completely close the ISCS in the deployed vent, so that we gave up closing in the vent. The ISCS was picked up and then closed. Next, we tried to recover the OSL dosimeters deployed at the northern edge of the chimney (western side) in YK10-11 cruise. When we move to the northern edge, we found that the center of chimney was clasped and many sulfide rocks were observed on the foot of the chimney. When I visited this chimney in 6k#1216 of YK10-10 cruise, the chimney was not clasped. And also we found that the southern part of the chimney became higher than that in 6k#1216. We recovered the dosimeters and then we moved to the southern part of the chimney to recover ISCS into the pseudo-black smoker vent. We tried to look for ISCS on the southern part, but we could not find it. Since we observed fresh sulfide rocks on the foot of southern part of the chimney, we thought the ISCS into the pseudo-black smoker vent was buried. So that we gave up recovering ISCS into the pseudo-black smoker vent. We

mover to the northern edge of the chimney (Eastern side) to recover ISCS into the black smoker vent. When the moving, we confirmed the chimney-clasping. The ISCS into the black smoker vent was buried in to the chimney. We recovered the ISCS. After recovering, the strong black smoker was gushed.

We finished the work around the Sagrada Familia chimney, we moved to Himeji-jo chimney (#111) on the north of Archaean site. We planed the course in which we went to Himeji-jo chimney via 6k#17 site. But we could not find #17 site. But we found 6k#111 so that we directly moved to the chimney. At 13:40, we arrived at the chimney site. In seafloor in front of the chimney, we observed the shimmering from everywhere. For a while, we observed the chimney and moved to one of the clear smoker chimney. We sampled fluid by WHATS and the max fluid temperature was 165°C. Next, we sampled clear smoker chimney by Bamboo rake sampler. Next, we next looked for chimneys erupting black smoker. We found black-smoker vent in the top of the chimney but we could not sample the fluid because of the height. So that we sampled pseudo-black smoker near main chimney vent by WHATS. The max fluid temperature was 175°C. After sampling fluid, we looked for *Alviniconcha* and *Buccinidae* but we could not found them. So that we went to 6k#17 site. On the way to 6k#17, we found a Buccinidae but we did not sampled. We arrived at shimmering site located at the top of Archaean site, but we could not find *Alviniconcha* and *Buccinidae* colony. We gave up sampling animals and headed to west. We left the bottom at 15:53.

Payloads:

1) Suction sampler
2) WHATS with 4 bottles
3) Sample box with lid (3 separated box (left basket))
4) 2 Cases for in situ colonaization system (Old type)
5) Bamboo rake sampler (in the right sample box)

Location of Events:

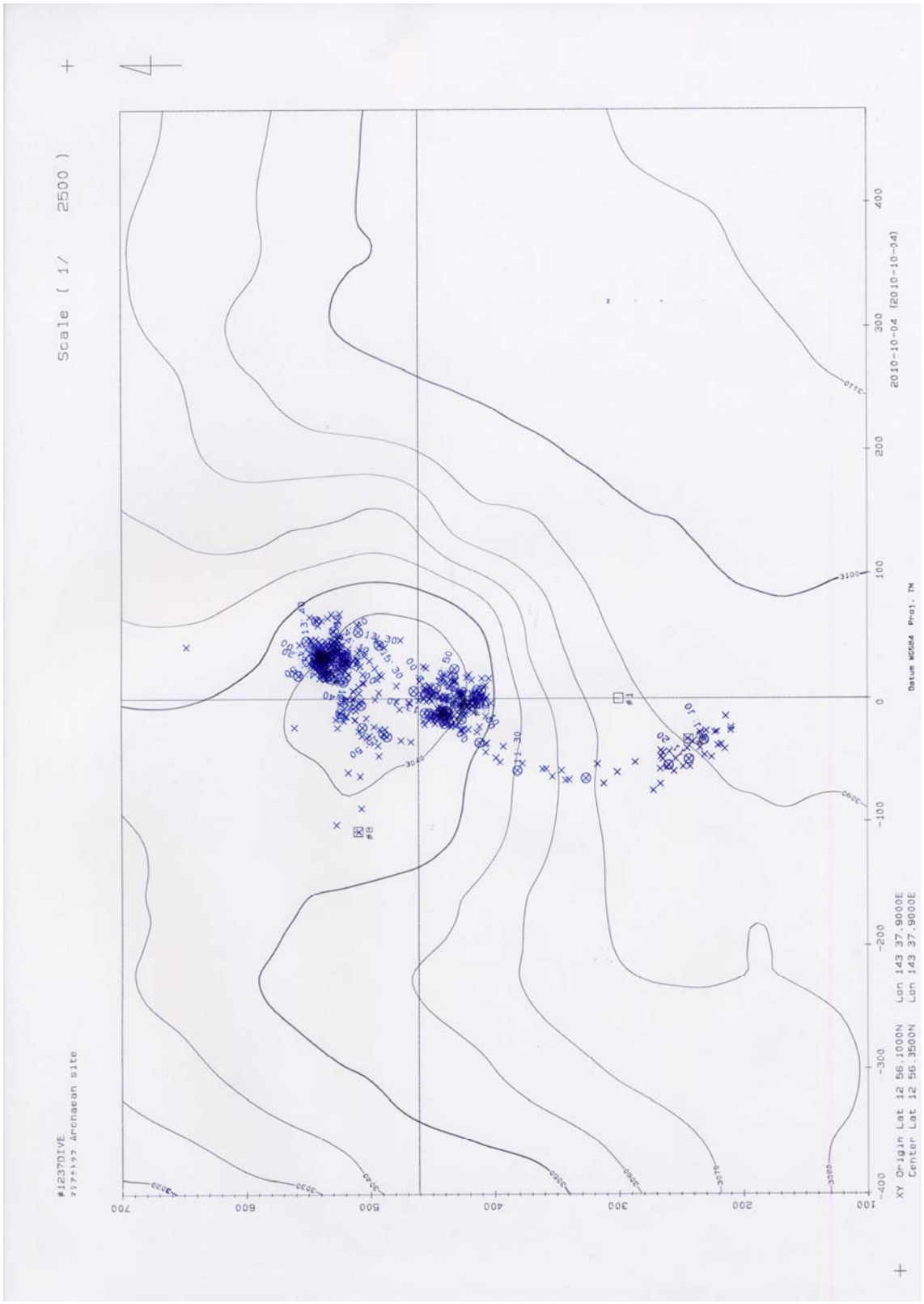
As in the section, “Event List”.

Event List:

1) 10:00, 12°56.2627'N, 143°37.900'E, Landing Target
2) 11:16, 12°56.2322'N, 143°37.8816'E, Landing, D=3083 m

3) 12:07, 12°56.3294'N, 143°37.8956'E, Recovering Watsuji’s hair ISCS and Miyazaki11’s ISCS, D=3001 m
4) 12:17, 12°56.3374'N, 143°37.8930'E, Recovering OSL dosimeter, D=2998m
5) 13:01, 12°56.3336'N, 143°37.8991'E, Recovering ISCS (True Black Smoker), D=3001 m
7) 14:36, 12°56.3925'N, 143°37.8991'E, Sampling WHATS & Chimney (Clear Smoker, Max Temp=153°C, 165°C), D=3000 m
8) 15:21, 12°56.3904'N, 143°37.9098'E, Sampling WHATS (Pseudo Black Smoker, Max Temp=175°C, 155°C)
9) 15:53, 12°56.3763'N, 143°37.8403'E, Left Bottom, D=2982 m (A=40m)

Dive Track:


Scale (1/ 2500)
#1237DIVE
2010-10-04 (2010-10-04)
Datum WGS84 Proj: TM
XY Origin Lat 12 56.1000N Lon 143 37.9000E
Center Lat 12 56.3500N Lon 143 37.9000E

Dive Report: Shinkai 6500 Dive#1238

Date: October 5, 2010
Site: Snail site
Landing: 11:18; 12°57.2224'N, 143°37.1424'E, 2854 m
Leaving: 15:57; 12°57.1295'N, 143°37.1564’E, 2827 m
Observer: Sanae Sakai (JAMSTEC)
Pilot: K. Chiba **Co-Pilot:** A. Ishikawa

Objectives:

Primary objectives of this dive are recovering 3 *in situ* colonization systems (ISCS, Gali8 and Gali9) and finding ANKOU IWA site found in Leg 10-10, the dive #1215. Other objectives are collecting seawater and animals, and deploy in situ colonization systems.

Dive Summary:

We sampled reference seawater (NISKIN) before landing. Then we landed on lava at 11:18 (depth 2854m). We headed to the south to find the starting point #20 (TAIGA10M-BMS01). However before we found #20, we found #18 (18ODP maker). Therefore, we first retrieved *in situ* colonization system Gali (#8) at #18 (depth 2853 m). After that, we headed to the north to find #20. A half-hour later we found the casing pipe located in #20 (depth 2850 m). At #20, we retrieved the ISCS and took 2 WHATS bottles of water (No.1 and No.2, the temperature of fluids was approximately 7-8 °C). Moreover we collected 2 rocks lying around the casing pipe. Subsequently we headed to the southeast to go to #19. About 20 minutes later, we found No.108 marker indicating #19. Soon after, we found Gali (#9) and retrieved it (depth 2854 m). Then we began to search ANKOU IWA site, where hydrothermal fluids were observed in previous dive (dive #1215, YK10-10). About 10 minutes of search, we found ANKOU IWA site at 12˚57.1750N, 143˚37.1629E. At the site, the huge rocks, which partially covered with white bio-film like mat, were presented. The hydrothermal fluids sprang out surround the rocks. At first, we planed to take 2 bottles of WHATS water from the hydrothermal fluids of this site. However, it is difficult to find the main source of the fluids. We measured temperature of the fluids at several points, and the average temperature of the fluid was around 15 ˚C. After we measured temperature, we judged that the fluid was inappropriate for sample and canceled this sampling. Thus we just wrenched a rock

piece off as a rock sample at this site. After that, we left this site for #16 (*Alviniconcha* site). After 20 minutes of ride, we got to *Alviniconcha* site and landed near *Alviniconcha* sp. colony. First, we collected *Alviniconcha* sp. individuals by using slurp gun. Then, we collected two WHATS bottles of the fluid sample (No.3 and No.4) from the point where the young chimney was constructed (although we broke this young chimney before sampling). Because of the trouble of thermometer, we could not measure the temperature of collected sample. However the maximum temperature recorded before sampling was 134 ˚C. Moreover, we wrenched a rock piece off as a rock sample from *Alviniconcha* site. In the last, we moved to #17 (APM01). A little fluid was observed when we found the casing pipe. The pipe was seemed like that it was filled with sediments. So we poked the pipe with the end of water sampling pipe to make the flow of fluid better. Then we started fluid sampling by using the bag sampler. However, there was a trouble with the sampler and it didn't work well. So we were just able to collect 6L of the fluid although we set up a 20L sample bag. During the fluid sampling, we collected 2 rocks surrounding the casing pipe. Finally, we deployed an *in situ* colonization system in the casing pipe, although we could not put it deeply because the pipe still chokes with sediments. We left the bottom at 15:57.

Payloads:

1) WHATS with 4 bottles
2) Niskin bottle
3) Sample box with lid (3-separated box (left basket) and 1 box (right basket))
4) 2 x in situ colonization system
5) 1case for in situ colonization system
6) Suction sampler (single canister)
7) Shovel (in the right sample box)

Location of Events:

As in the section, "Event List".

Event List:

1) 10:00, 12˚57.2434N, 143˚37.1498E, Landing Target
2) 11:18, 12˚57.2224N, 143˚37.1424E, Landing and sampling reference water using NISKIN, D=2854m
3) 11:40, 12˚57.1763N, 143˚37.1548E, Retrieve Gali(#8), D=2853m

4) 12:31, 12˚57.1976N, 143˚37.1605E, BMS01, Retrieve ISCS and sampling WHATS (1, 2) (BMS01 Casing pipe, Temp. = 8 °C, 7 °C), D=2850m
5) 12:37, 12˚57.1976N, 143˚37.1604E, BMS01, Set ISCS(#1), Sampling Rocks(2), D=2850m
6) 12:55, 12˚57.1763N, 143˚37.1712E, Retrieve Gali(#9), D=2854m
7) 13:21, 12˚57.1750N, 143˚37.1629E, Sampling Rock(1), D=2855m
8) 14:21, 12˚57.1866N, 143˚37.1701E, Alviniconcha, Sampling Animals, WHATS(3, 4) (Alviniconcha site, Temp.= 134 ˚C before sampling) and Rocks(1) , D=2849m
9) 14:41, 12˚57.1541N, 143˚37.1697E, Finding #114 Marker, D=2849m
10) 15:45, 12˚57.1653N, 143˚37.1448E, AMP01, Sampling Water (Bag) and Rocks(2), Set ISCS(#2), D=2854m
11) 15:57, 12˚57.1295N, 143˚37.1564E, Left Bottom, D=2827m

Dive Track: