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# RV *Natsushima* Cruise Report

# NT14-05

## Hyper-Dolphin Dive Research

**Biological Research in Off Hatsushima**
**and Earthquake Research in Off Bousou**

**April, 2th-8th, 2014**

**Japan Agency for Marine-Earth Science and Technology (JAMSTEC)**

**Contents**

**1. Cruise Information**

● Cruise ID: NT14-05

● Name of vessel: R/V *Natsushima*

● Title of the cruise: Biological Research in Off Hatsushima and Earthquake Research in Off Bousou

● Title of proposal:

1) Transmission and distribution analysis of intracellular bacterial symbiont from *Calyptogena* clams

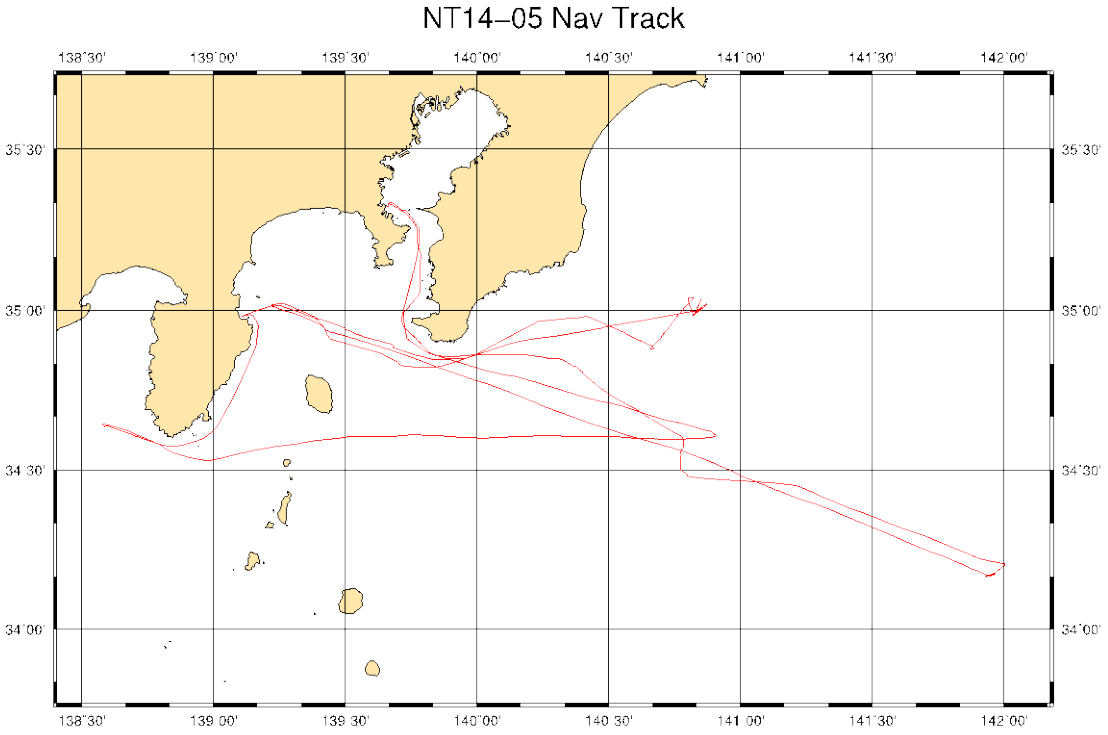
2) Development for the latest ocean bottom seismic and tilt measurements

● Cruise period: April 2, 2014 ~ April 8, 2014

● Ports of call: from Sumitomo Juko (April 2, 2014) to Sumitomo Juko (April 8, 2014)

● Research area: Off Hatsushima, Sagami Bay and Off Boso

● Research Map




Cruise track of R/V Natsushima (NT14-05)

**2. Researchers**

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Department of Marine Biodiversity Research

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## 3. Observation

### ● Overview of the cruise

In this cruise, two proposals participated. To investigate these objectives, we planned to two dive days at Sagami Bay and one dive and one operation day at Off Bouso (seven days in total). During the cruise, several biological samples, such as *Calyptogena* clams, *Bathymodiolus* mussels, tubeworms, etc., and segiment by H-type cores were collected. Detailed analyses of these samples will be performed after the cruise. We also retrieved ocean bottom seismometers for the latest broadband seismic and tilt measurements and for areas of the ultra deep sea.

### ● Cruise log

| 日付<br>Date | 時間<br>Local Time | 内容<br>Note | 数値表現<br>Description | 本船位置／気象／海象<br>Position/Weather/Wind/Sea condition |
|---|---|---|---|---|
| 02-Apr-14 | | Let go all shore lines & left YOKOSUKA for Research Area. | | 4/2 12:00(UTC+9h) |
| | 09:00 | Let go all shore lines & left YOKOSUKA for OFF BOUSOU. | | OFF SUZAKI |
| | 09:15-10:10 | Scientists meeting. | | 34-55.0N, 139-11.0E |
| | 10:30-11:00 | Carried out shipboard education & training for scientists. | | Cloudy |
| | 16:40-17:00 | Carried out KONPIRA pray. | | NNE-3(Gentle breeze) |
| | 19:00 | Change the destination to SURUGAWAN, due to rough sea. | | 3(sea slight) |
| | | | | 2(Low swell long) |
| | | | | Visibly:8 |
| 03-Apr-14 | | HPD umbilical cable free fall. | | 4/3 12:00(UTC+9h) |
| | 06:30 | Arrived at SURUGAWAN. | | SURUGAWAN |
| | 08:00-09:00 | Scientists meeting. | | 34-38.2N, 138-35.2E |
| | 08:29-11:02 | Carried out HPD umbilical cable free fall. | | Rain |
| | 13:35 | Left SURUGAWAN for OFF ITO. | | North-5(Fresh breeze) |
| | 18:00 | OFF ITO then stationed for anchoring. | | 3(sea slight) |
| | 18:00-18:50 | Scientists meeting. | | 2(Low swell long) |
| | | | | Visibly:6 |
| 04-Apr-14 | | HPD Operation #1641. | | 4/4 12:00(UTC+9h) |
| | 07:10 | Arrived at SRUGAWAN. | | SAGAMIWAN |
| | 07:23 | Released XBT. | 35-01.0237N, 139-13.7068E | 35-00.9N, 139-13.4E |
| | 09:51 | Hoisted up HPD. | | Fine but cloudy |
| | 09:55 | Launched HPD. | | WSW-6(Strong breeze) |
| | 10:07 | HPD dove & started her operation (#1641) | | 3(sea slight) |
| | 10:42 | HPD bounded on the sea bottom. | Depth=955m | 3(Moderate short) |
| | 17:13 | HPD left the sea bottom. | Depth=880m | Visibly:8 |
| | 17:21 | HPD floated. | | |
| | 17:59 | Recovered HPD & finished her operation. | | |
| | 18:15 | Proceeded to OFF BOUSOU. | | |
| 05-Apr-14 | | Recoverd OBDC & HPD Operation #1642. | | 4/5 12:00(UTC+9h) |
| | 05:00 | Arrived at BOUSOU. | | OFF BOUSOU |
| | 06:16 | Released XBT. | 34-59.9482N, 140-50.4223E | 34-59.8N, 140-50.2E |
| | 09:37 | Recovered OBDC. | | Fine but cloudy |
| | 10:21 | Hoisted up HPD. | | North-2(Light breeze) |
| | 10:29 | HPD dove & started her operation (#1642) | | 2(sea smooth) |
| | 12:29 | HPD bounded on the sea bottom. | | 3(Moderate short) |
| | 13:29 | HPD left the sea bottom. | Depth=1,361m | Visibly:8 |
| | 14:10 | HPD floated. | Depth=1,366m | |
| | 14:31 | Recovered HPD & finished her operation. | | |
| | 15:53-16:09 | Carried out calibration of OBSP#1. | | |
| | 16:00-18:30 | Scientist meeting. | | |
| | 18:47-18:50 | Carried out calibration of OBSP#2. | | |
| | 19:00 | Left research area (OFF BOUSOU) for SAGAMIWAN. | | |
| 06-Apr-14 | | HPD Operation #1643 & #1611. | | 4/6 12:00(UTC+9h) |
| | 04:00 | Arrived at SAGAMIWAN. | | SAGAMIWAN |
| | 07:12 | Hoisted up HPD. | | 35-13.6N, 139-13.6E |
| | 07:17 | Launched HPD. | | Fine but cloudy |
| | 07:30 | HPD dove & started her operation (#1643). | | NE-3(Gentle breeze) |
| | 08:07 | HPD bounded on the sea bottom. | Depth=940m | 3(sea slight) |
| | 10:10 | HPD left the sea bottom. | Depth=949m | 2(Low well long) |
| | 10:10 | HPD floated. | | Visibly:8 |
| | 10:58 | Recovered HPD & finished her operation. | | |
| | 12:07 | Hoisted up HPD. | | |
| | 12:11 | Launched HPD. | | |
| | 12:22 | HPD dove & started her operation (#1644). | | |
| | 12:56 | HPD bounded on the sea bottom. | Depth=841m | |
| | 15:07 | HPD left the sea bottom. | Depth=859m | |
| | 15:34 | HPD floated. | | |
| | 15:48 | Recovered HPD & finished her operation. | | |
| | 16:00 | Left research area (SAGAMIWAN) for OFF BOUSOU. | | |
| | 18:00-18:30 | Scientist meeting. | | |
| 07-Apr-14 | | Search for NUDOBS & HPD umbilical cable free fall. | | 4/7 12:00(UTC+9h) |
| | 05:00 | Arrived at OFF BOUSOU. | | OFF BOUSOU |
| | 05:18-10:00 | Carried out search for NUDOBS. | | 34-20.0N, 141-35.0E |
| | 10:00 | Left research area(OFF BOUSOU for HPD umbilical cable free fall area(OFF BOUSOU). | | Fine but cloudy |
| | | | | NW-2(Light breeze) |
| | | | | 2(sea sooth) |
| | | | | 3(Moderate short) |
| | | | | Visibly:8 |
| 08-Apr-14 | | Arrived at YOKOSUKA. | | 4/8 09:00(UTC+9h) |
| | 09:00 | Sent out 1st shore line, arrived at Yokosuka,completed NT14-05 | | YOKOSUKA |

**● Dive report**

**1) Summary of the HPD Dive #1641**

Date: April. 04, 2014

Site: Off Hatsushima, Sagami Bay

Landing: 35°00.924'N, 139°13.432'E, 955 m (10:42)

Leaving: 35°00.948'N, 139°13.310'E, 860 m (17:13)

Purpose:

Collection of *Calyptogena* clams, *Bathymodiolus* mussels, tubeworms, and sediments

Induction of spawning of *Calyptogena* clams

Sampling of seawater

Payload Equipment:

Incubation box x1

Egg incubation sampler x1

Suction sampler（multiple canister & single canister）

Sample box x1

H-type core x2

Scoop sampler x1

CCD camera

Animal trap

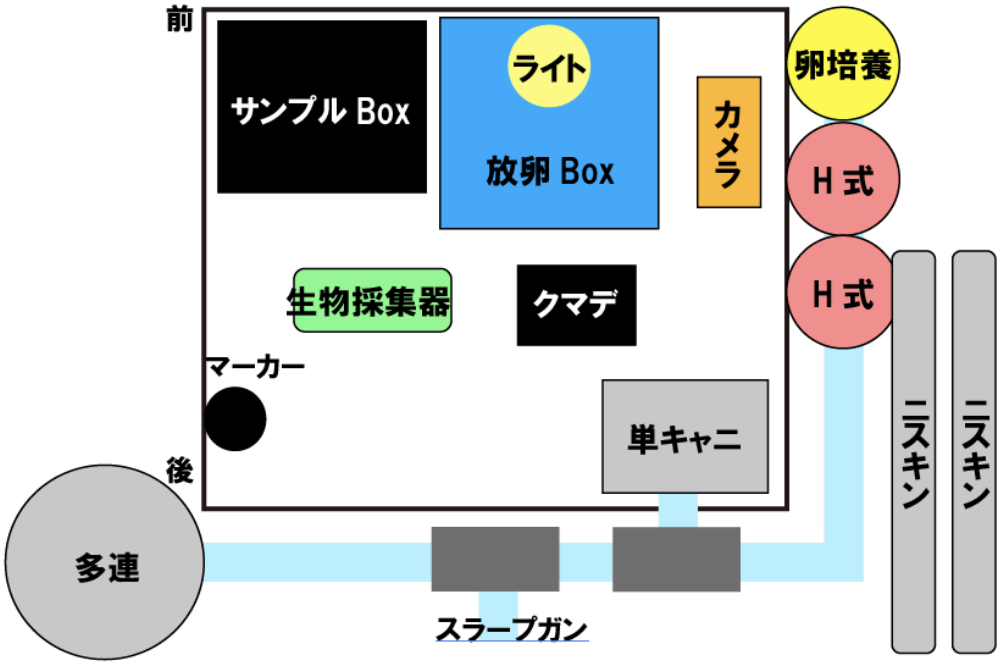
Niskin water sampler

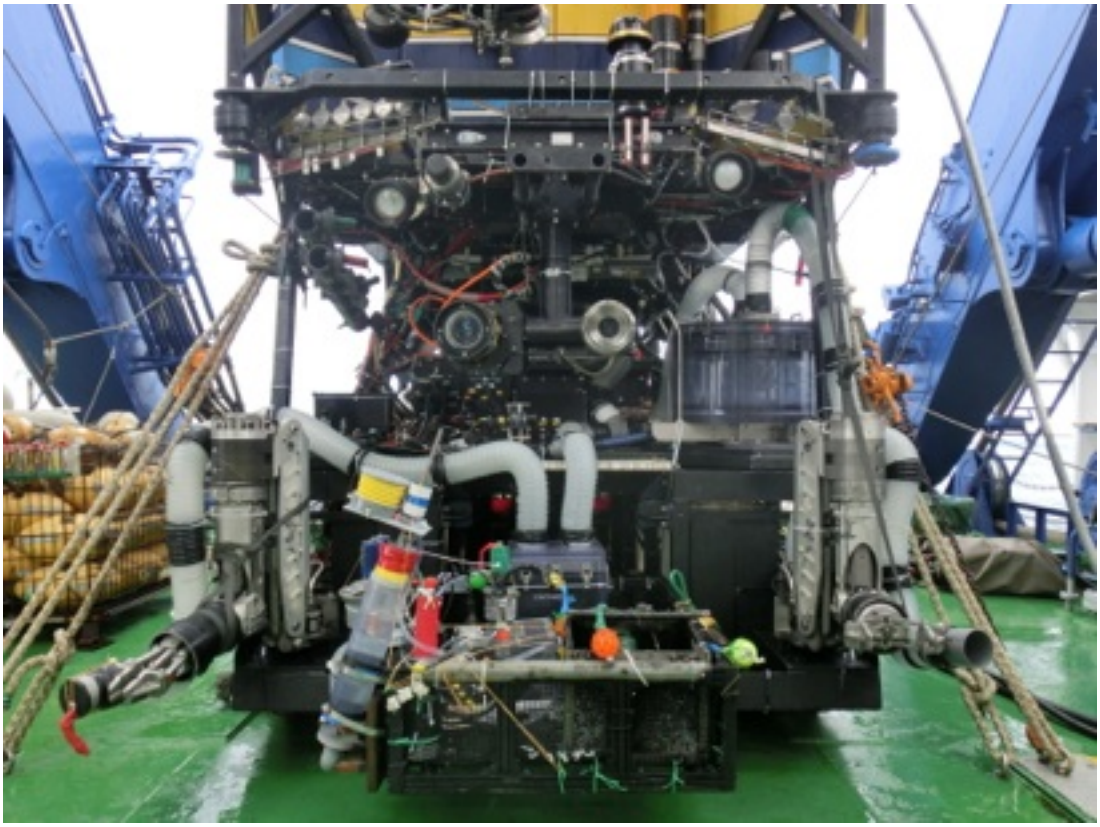
Kumade sampler

Dive Summary

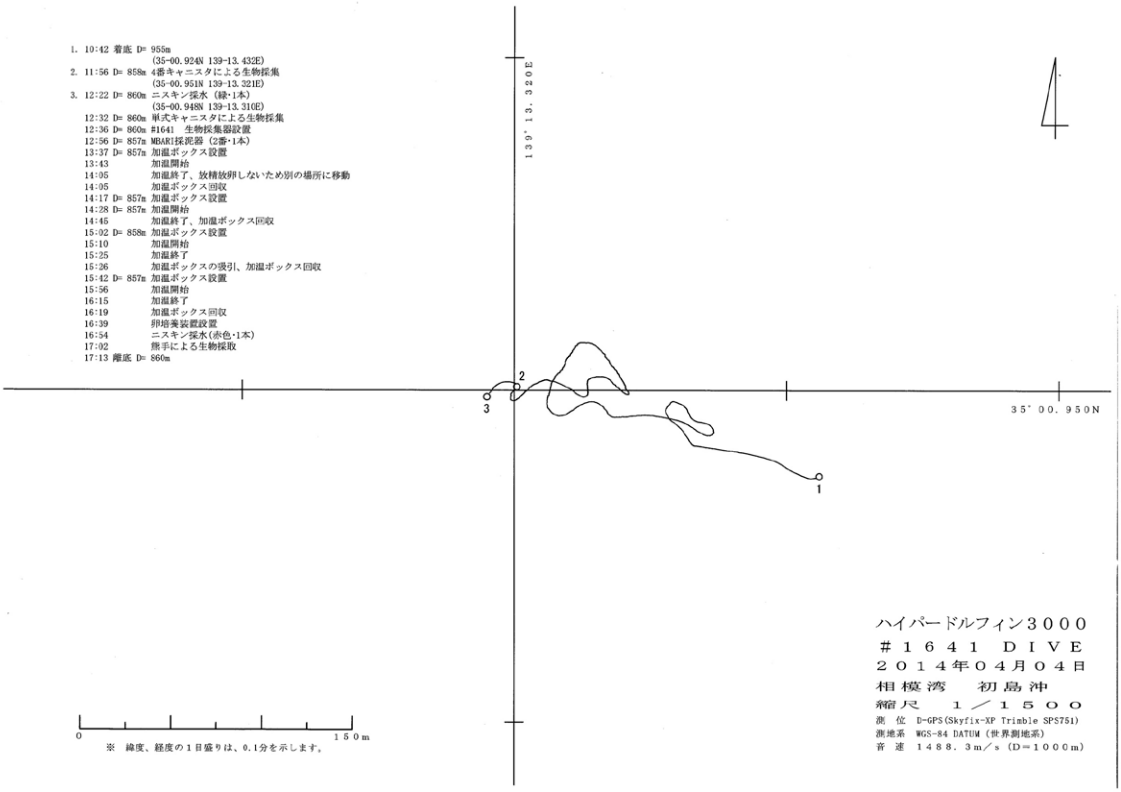
‡Sampling of *Calyptogena* clams and *Bathymodiolus* mussels with the suction samplers. Setting of the animal trap. Sampling of sediments with the H-type corers. Incubation of *Calyptogena* colonies with the incubation box, and collection of their eggs into the egg incubation sampler. Sampling of *Calyptogena* clams with the Kumade sampler. Sampling of seawater with the niskin samplers.

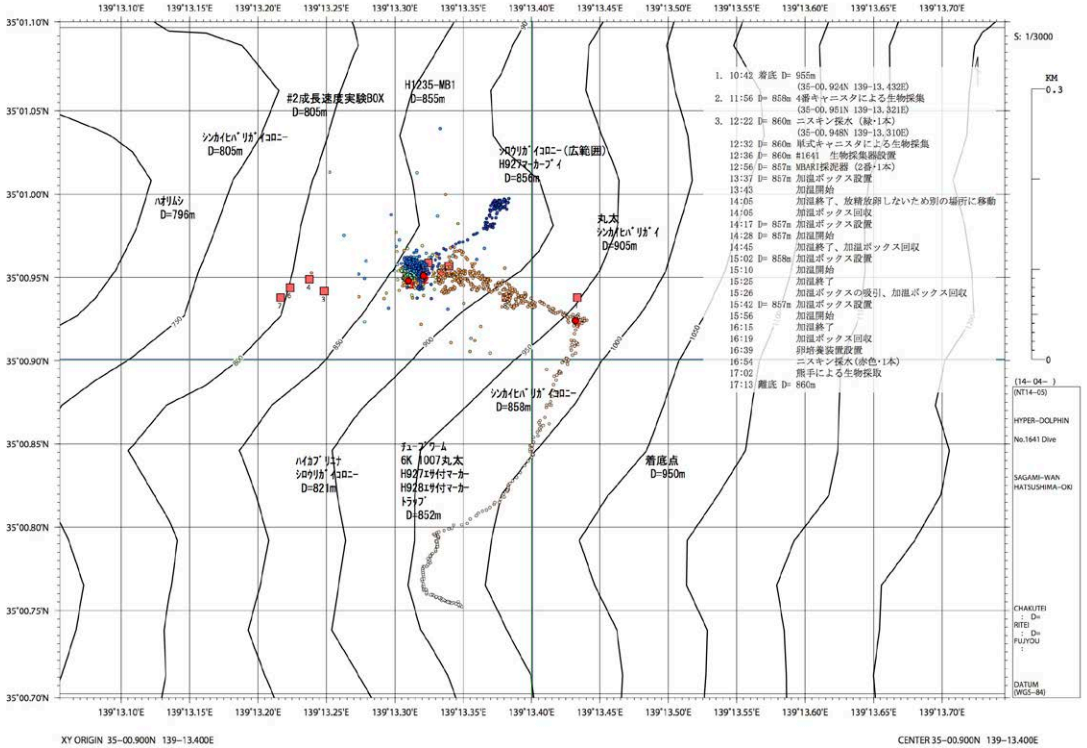
The site location: 35°00.948'N, 139°13.310'E, 860m



Payload of HPD#1641




Dive track and event list of HPD#1641

**2) Summary of the HPD Dive #1642**

Date: April 05, 2014
Site: Eastern Off Bousou Peninsula
Target: 34°59.79'N, 140°50.02'E, 1364m

Purpose:
Recovery of the BBOBST-NX for long-term tilt measurement

Payload Equipment:
Remote commander for the SI2 acoustic transponder

Dive Summary

All events were at the target position of the BBOBST-NX was deployed in 2013.
Time is in JST.

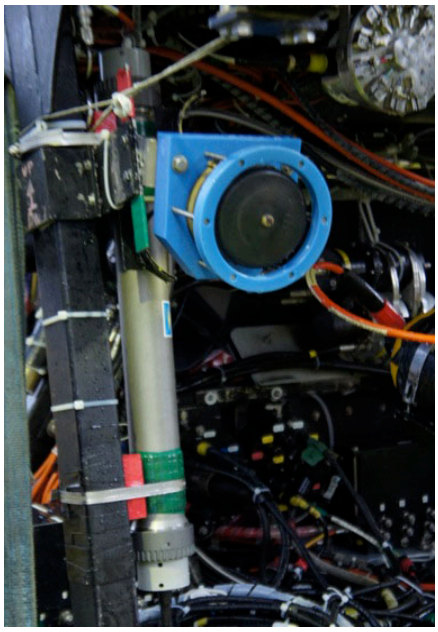
‡ Stopped the recording of the BBOBST-NX during approaching by the remote commander (11:46).

‡ Found the BBOBST-NX (12:36), and landed for the operation (12:40).

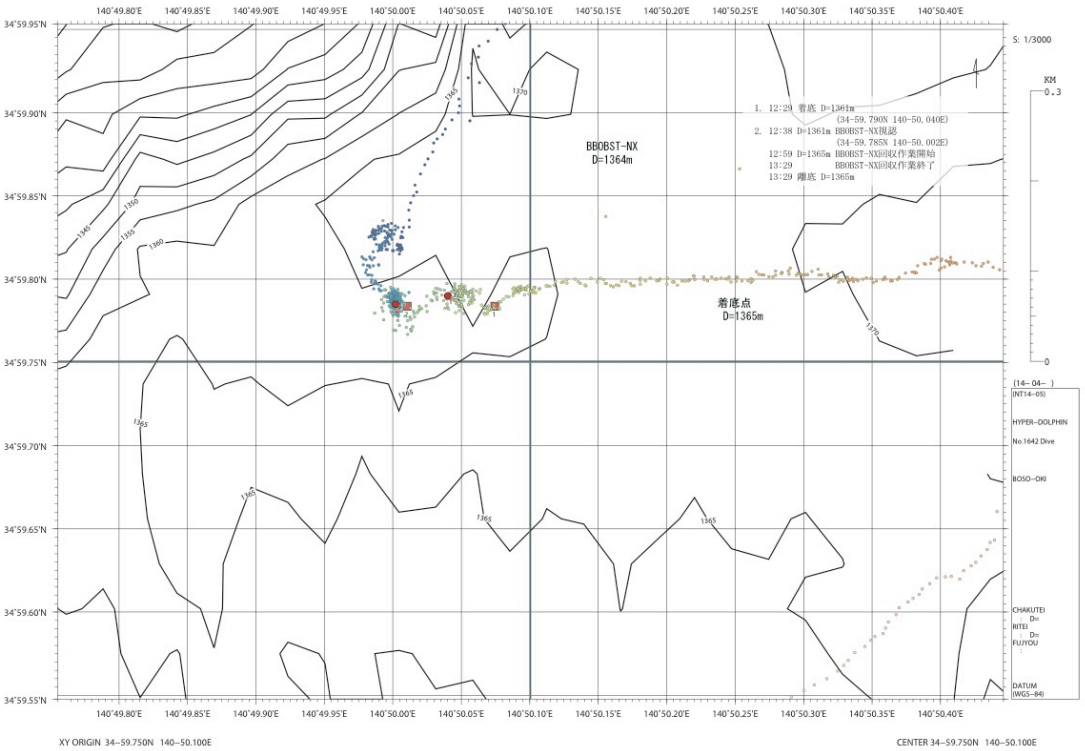
‡ Extracted the sensor unit from the sediment (12:50).

‡ Started to connect the recovery rope (12:59).

‡ Finished the connection and left the sea floor (13:29).



Payloads of HPD#1642 (remote commander for the transponder)




Dive track and event list of HPD#1642

**3) Summary of the HPD Dive #1643**

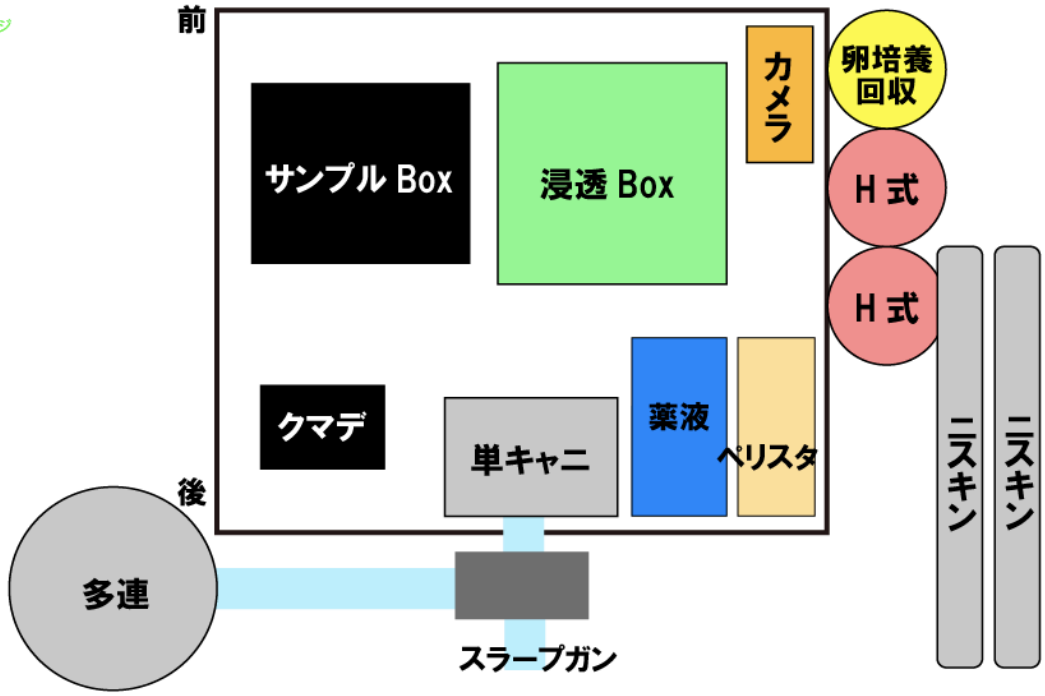
Date: April. 06, 2014
Site: Off Hatsushima, Sagami Bay
Landing: 35°00.931'N, 139°13.445'E, 955 m (08:07)
Leaving: 35°00.924'N, 139°13.426'E, 949 m (10:10)

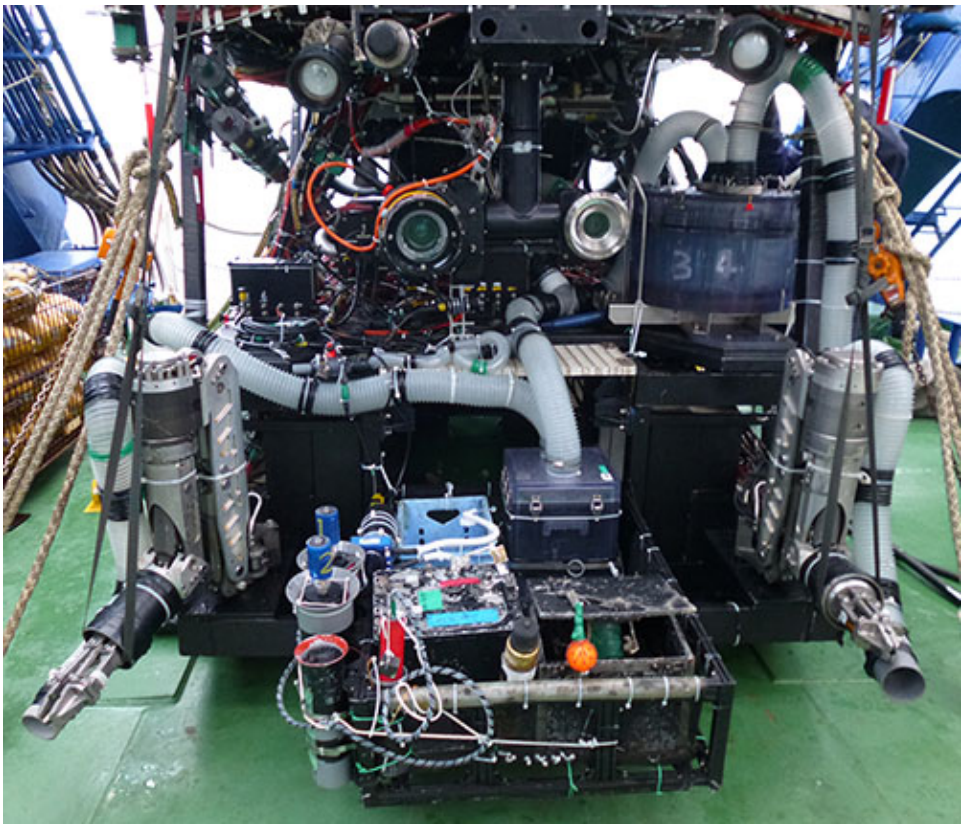
Purpose:
Collection of *Calyptogena* clams, *Bathymodiolus* mussels and tubeworms.
Sampling of seawater

Payload Equipment:
*in situ* sample treatment system x1
Suction sampler（multiple canister & single canister）
Sample box x1
H-type core x2
Scoop sampler x1
CCD camera
Niskin water sampler
Kumade sampler

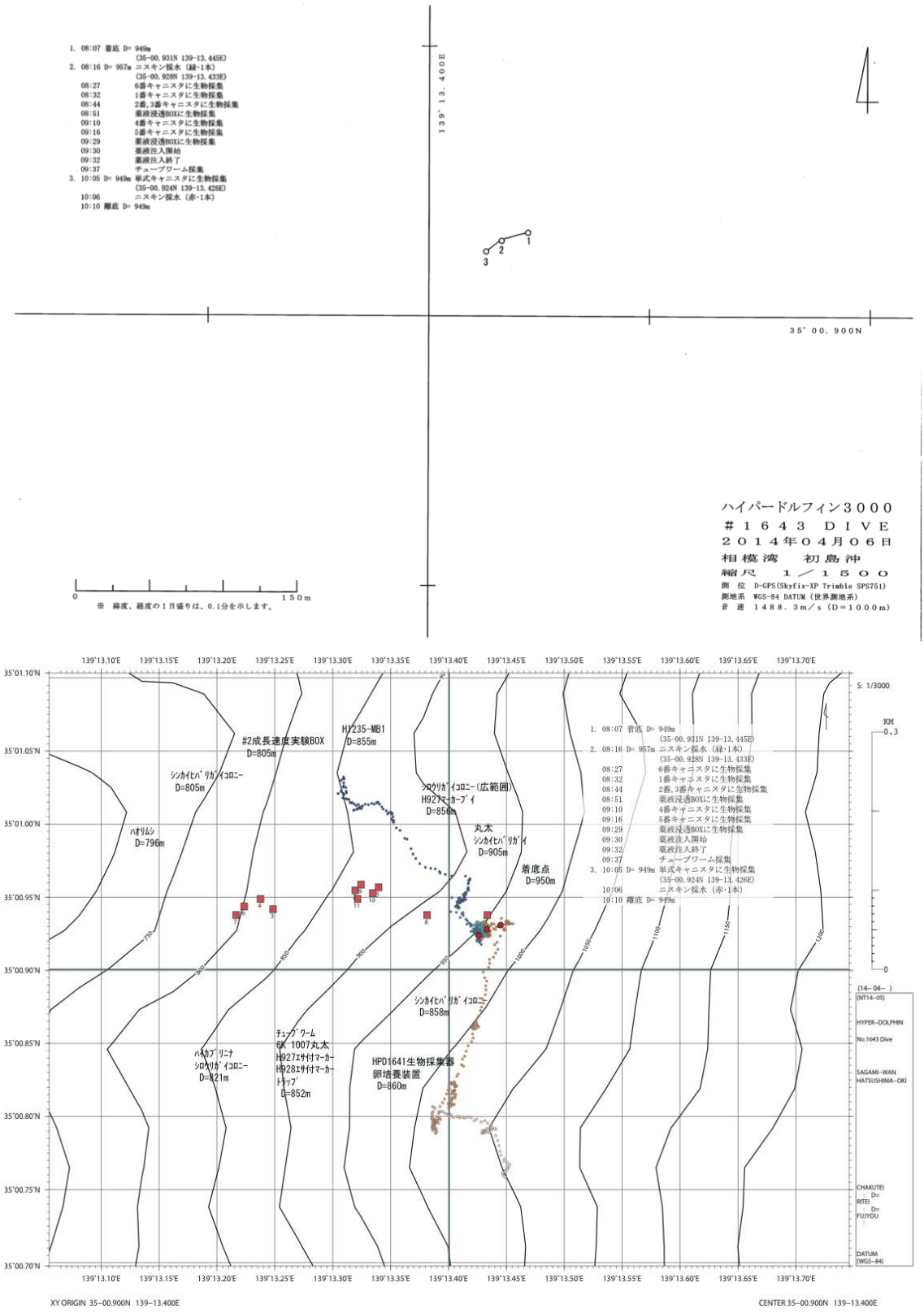
Dive Summary
‡ Sampling of seawater with the niskin sampler. Sampling of *Calyptogena* clams and *Bathymodiolus* mussels with the suction samplers. Sampling of tubeworms into the sample box. Sampling of *Calyptogena* clams and *Bathymodiolus* mussels with the *in situ* sample treatment system.
The site location: 35°00.928'N, 139°13.433'E, 957m

‡ Sampling of *Calyptogena* clams *Bathymodiolus* mussels with suction sampler. Sampling of seawater with the niskin sampler.
The site location: 35°00.924'N, 139°13.426'E, 949m



Payload of HPD#1643




Dive track and event list of HPD#1643

**4) Summary of the HPD Dive #1644**

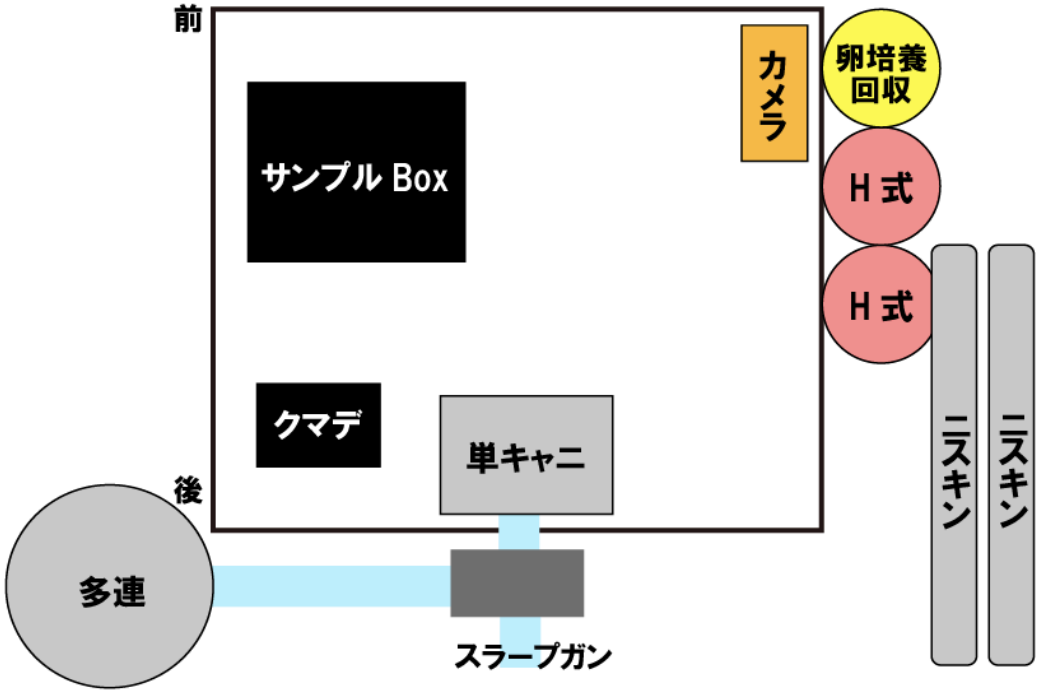
Date: April. 06, 2014
Site: Off Hatsushima, Sagami Bay
Landing: 35°00.954'N, 139°13.384'E, 884 m (12:56)
Leaving: 35°00.966'N' 139°13.329'E, 860 m (15:07)

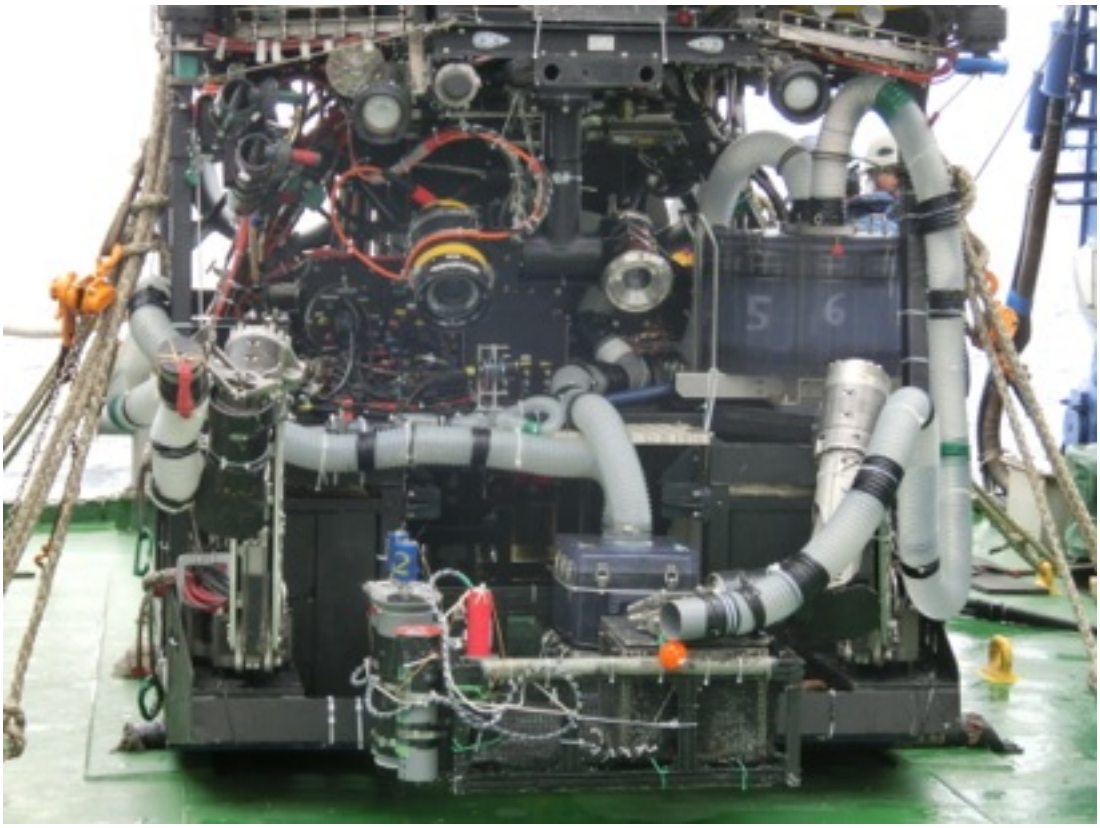
Purpose:
Collection of *Calyptogena* clams, *Bathymodiolus* mussels, tubeworms, and sediments.
Sampling of seawater

Payload Equipment:
Suction sampler（multiple canister & single canister）
Sample box x1
H-type core x2
Scoop sampler x1
CCD camera
Niskin water sampler
Kumade sampler

Dive Summary
‡ Retrieved the animal trap. Retrieved the egg incubation sampler. Sampling of *Calyptogena* clams and *Bathymodiolus* mussels with the suction samplers. Sampling of sediments with the H-type corers. Sampling of *Calyptogena* clams and tubeworms into the sample box. Sampling of seawater with the niskin sampler.
The site location: 35°00.966'N, 139°13.329'E, 860m

‡ Sampling of seawater with the niskin sampler.
The site location: 35°00.940'N, 139°13.416'E, 500m



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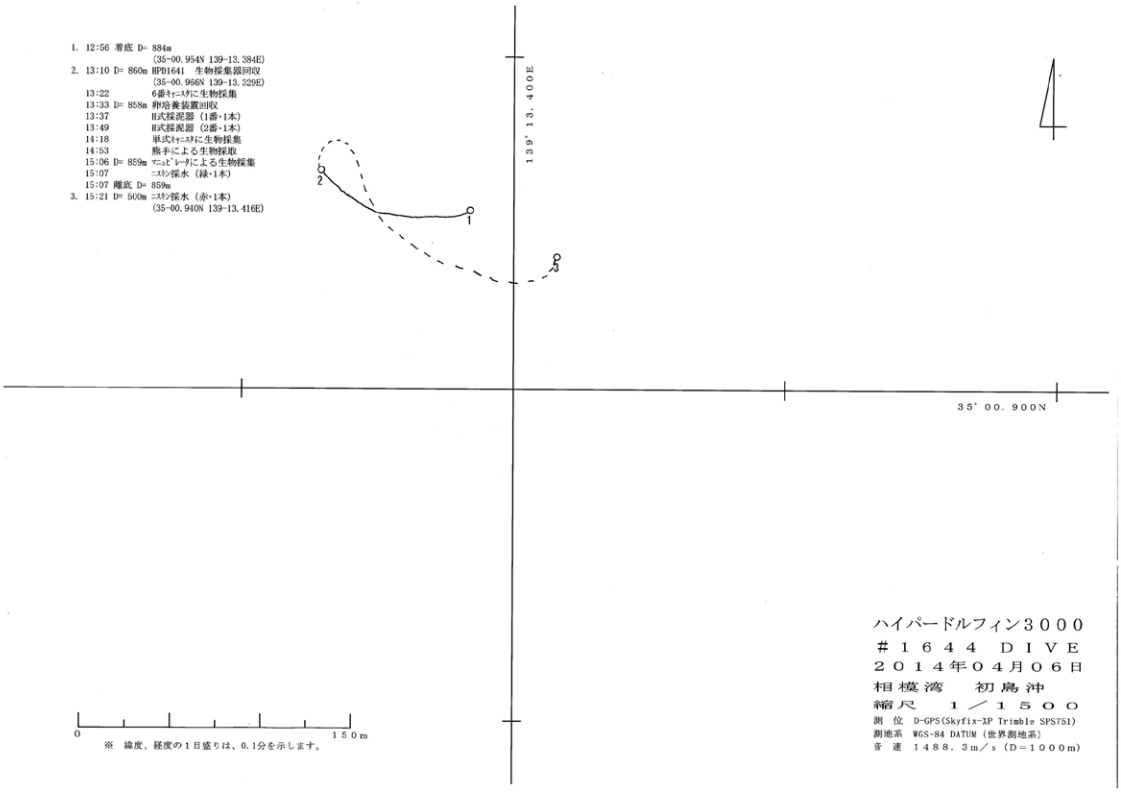


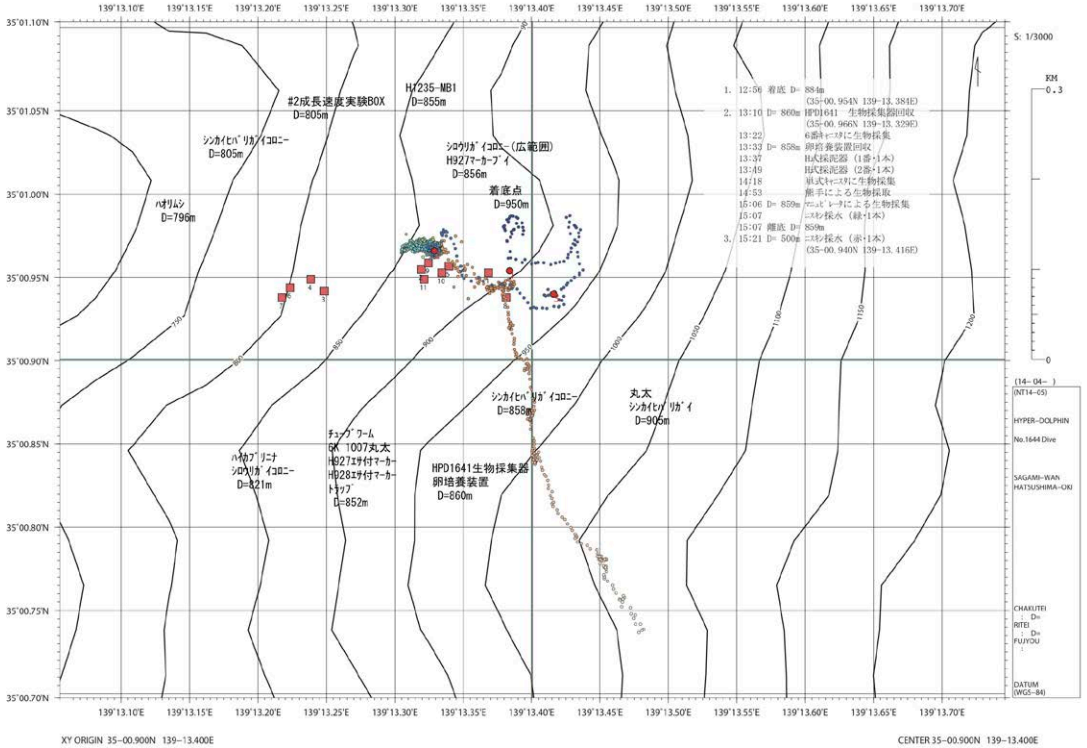


Dive track and event list of HPD#1644

**● Preliminary research reports**

**1) Transfer analysis of intracellular bacterial symbiont from *Calyptogena* clams to next generation**

Tetsuro Ikuta[1], Takao Yoshida[1], Yoshihiro Takaki[1], Tadashi Maruyama[1], Shigeru Shimamura[1], Genki Ozawa[1], Kazue Ohishi[1], Yuki Hongo[1], Kanae Igawa[1], Kaoru Kaihotsu[1], Yui Aoki[1], Ryusaku Deguchi[2], Akihiro Tame[3], Saki Tominaga[4], Makoto Sugimura[4]

[1] *Japan Agency for Marine-Earth Science and Technology (JAMSTEC)*

[2] *Miyagi university of Education*

[3] *Marine Works Japan, Ltd.*

[4] *Shin-Enoshima Aquarium*

**Objective and achievement in this cruise**

Vesicomyid clams, including *Calyptogena* spp., form dense communities on the deep sea floor near hydrothermal vents and seeps. These clams have vestigial digestive tracts and are nutritionally dependent on chemoautotrophic sulfur-oxidizing symbiotic bacteria, which are harbored within their gill epithelial cells. *Calyptogena* symbionts are vertically transmitted via eggs, and are transferred to the gill epithelial cells during development. However, detailed mechanisms of vertically transmission of symbonts and development of *Calyptogena* eggs are still unknown. In this cruise, we planned to collect the *Calyptogena* clam's eggs and clams at Off Hatsushima, Sagami Bay. During the cruise, *Calyptogena* clams, eggs, and other *Bathymodiolus* bivalves were collected at several colonies. Additionally, on the ship, we chemically induced spawning of *Calyptogena* eggs and tried to culture them. After dive, eggs were fixed or incubated at 4ºC for developing, and the clams and bivalves were immediately dissected, and blood, serum, and other tissues were frozen in liquid nitrogen and stored at -80ºC until used. Other samples were also stored at -80ºC. Detailed analyses of these samples will be performed after the cruise.

**Future studies**

*Analysis of early developmental pattern of *Calyptogena* clam

*Analysis of symbiont localization in *Calyptogena* eggs and embryos

*Analysis of expression pattern of symbiont genes in *Calyptogena* eggs and embryos

*Analysis of gene expression patterns of the host *Calyptogena* clam

**2) Long-term rearing of the seep animals**

Makoto Sugimura[1], Saki Tominaga[1], Yukiko Nagai[2], Takashi Toyofuku[2], Kazue Oishi[2], Tadashi Maruyama[2], Takao Yoshida[2] and Tetsuro Ikuta[2]

[1] *Shin-Enoshima Aquarium*

[2] *Japan Agency for Marine-Earth Science and Technology (JAMSTEC)*

**Objective and achievement in this cruise**

Long-term rearing of deep-sea animals at New Enoshima Aquarium aims to establish a stable rearing system. So far, we have successfully reared *Calyptogena* spp. for 153 days. However, for establishing a stable rearing system, a lot of problems remain to be solved and more detailed information to evaluate the water tank environment of the long-term rearing system is required. During this cruise, *Calyptogena* clams and the other biological samples are collected, and brought back to New Enoshima Aquarium. After the cruise, they will be reared in the long-term rearing system. The clams will be dissected and fixed at regular intervals, and used for various investigations for evaluation of the rearing system. Also, some biological samples sorted on board were photographed alive, and fixed.

**Outreach activity**

We will post a "logbook" in our WEB page to report the process of the cruise, and to enable a lot of people to enjoy and learn about the research and research activities by watching it.

**Future studies**

* Improvement of the long-term rearing system
* Behavioral observation study of deep-sea animals
* Public relations activities on the research

### 3) Laboratory observations of *Calyptrogena* spp.

Yukiko Nagai[1], Masaaki Konishi[2], Makoto Sugimura[3], Takami Nobuhara[4], Tetsuro Ikuta[1], Takao Yoshida[1], Sachiko Kawata[1] and Takashi Toyofuku[1],

[1] *Japan agency for Marine-Earth Science and Technology (JAMSTEC)*
[2] *Kitami Institute of Technlogy*
[3] *Shin-Enoshima Aquarium*
[4] *Shizuoka University*

It is good opportunity to investigate detailed behavior of deep-sea symbiotic bivalve *Calyptogena* spp in laboratory when we have freshly collected low damaged living individuals by ROV/HOV. Even the knowledge accumulation has been enhanced by recent efforts, still many ecological information are necessary to realize longer laboratory culture and well-designed experiments. For the purposes, we confirm recolonization activity, respiration rates and Male/Female rate during NT14-05 cruise and afterwards.

The animal behaviors of recolonization have been recorded visually by time lapse imaging of

*GoPro* HERO3 camera. Glass beakers are used as observation vessel. The specimen put on the artificial sediment. The black smelly deep-sea sediment taken from original *Capyptogena* colony was put on bottom of the beaker. Artificial glass beads (~3mm) are put on black sediment to see the motion of foot activity. In some case, the crashed and whole silica-gel beads (~5mm) are also put on the glass beads. These silica-gel have excellent transparency than glass beads. The character is suitable for the purpose. The whole vessel with sediment and animal are dunk into 60-litter water bath with other captured animals to keep nice oxygen concentration. The camera system observations were carried out from side of water bath. The fluorescent light was lit during observation. The observation will be continued on-land laboratory. The individuals will be put into our chemostat water bath system for hydrogen sulfide to expect the expanding of *Calyptogenas'* survival time.

The respiration rate are monitored on-board and on-land laboratory. We examine the amount of consumed oxygen in a well-closed bottle with specimen for ~2 hours. Oxygen concentrations were measured by PreSens optode system FiBox 3. The water temperature was kept at ~0 °C during measurement by ice.

The cultivated seawater of respiration rate monitoring is kept for Male/Female test. We expect *Calyptogena'* s steroids would be leaked into ambient sweater. We will be able to decide the sex of the specimen by leaked steroid if the sexual hormone is included in seawater. It might be great advantage to design the experiment because the sex could not identified by outer morphology on this species.

**4) Foraminifera (fossilizing benthic meiofauna) in cold seeps**

Takashi Toyofuku[1], Yukiko Nagai[1] and Christophe Fontanier[2]

[1] *Japan agency for Marine-Earth Science and Technology (JAMSTEC)*

[2] *Laboratoire Environnements Sédimentaires, IFREMER, Centre de Brest, Technopôle de Brest-Iroise, BP 70, F-29280 Plouzané, FRANCE*

Cold-seep activity is important to understand symbiosis in the seep animals and establish the stable rearing system. Live (Rose-Bengal stained) and dead benthic Foraminifera (Eukaryota, Rhizaria) from recent deep-sea cold seeps have been investigated in numerous cold-seep areas. An exhaustive synthesis of the major ecological and biogeochemical observations concerning recent foraminiferal assemblages is proposed by some previous studies (e.g. Akimoto et al., 1994; Sen Gupta and Aharon, 1994; Kitazato, 1996; Sen Gupta et al., 1997; Rathburn et al, 2000) To summarize, it seems that foraminiferal faunas are able to thrive in cold-seep areas and can tolerate adverse geochemical conditions prevailing there (sulfidic conditions, methane seepages). Both metabolic and habitat adaptations were proposed to explain foraminiferal distribution in cold seepage sediment.

In this project, we would like to study benthic foraminifera collected in a cold-seep area of off

Hatsushima. Live (Rose-Bengal stained) and dead faunas are considered. The main objective of our investigation is to precise whether Foraminifera can be used as reliable and relevant proxies of cold-seep activity. We work at the community level and, also, we study the shell geochemistry of both living and dead foraminifera with the environmental properties.

Surface undisturbed core samples are planned to use for foraminiferal faunal assemblage research. Syringe subcores are inserted into each core. Each subcore will be sliced every 0.5 cm intervals from core top to 3 cm and every 1 cm intervals for 3 to 5 cm. Sliced sediments are fixed by 50% seawater Et-OH 0.5% rose Bengal solution. Just before sorting, we plan to wash fractions with 63 and 150 μm opening mesh. Then, every stained individual will be sorted at species level under binocular stereo-microscope.

**5) Screening for bacteria expressing β-glucosidase from seawater and sediments**

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**Objective and achievement in this cruise**

Enzymes are now widely used in a wide range of industrial processes. Most of the enzymes are of bacterial origin. Therefore, screening for novel bacterial enzymes of industrial importance is a key step in the development of industrial processes. Furthermore, detecting bacterial catalytic activities at the habitat is useful to understand the symbiosis. To activate these processes, we have developed a screening method for bacteria expressing enzymes with a desired catalytic activity based on single-cell analysis. In this cruise, we planned screening for bacteria expressing β-glucosidase in seawater and sediments at Off Hatsushima, Sagami Bay. During the cruise, seawater and sediments were collected at several spots. Collected samples were also stored at -4ºC. Screening will be performed after the cruise.

**Future studies**

Bacteria expressing β-glucosidase will be detected, and isolated at the single-cell level. Single isolated cells will be subjected to Phi29 polymerase-mediated whole genome amplification (WGA). Then, it will be attempted to clone the genes encoding β-glucosidase from WGA products.

**6) Development for the latest ocean bottom seismic and tilt measurements.**

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**Objective and achievement in this cruise**

Generally, we have been trying to widen observation ranges in multi dimensions, such as term of observation, period, area (depth) and strength of signal, in ERI. In this research cruise, we started long-term observation of the next generation broadband ocean bottom seismometer with tilt-meter function (BBOBST-NX) and of the new design ultra deep ocean bottom seismometer (NUDOBS) too. Our final aim is to realize the spatially dense tilt observation on the sea floor and the reliable instrument in the ultra deep water area. The research area is shown in Fig. 3-1. The position of the BBOBST-NX (P1a) was chosen at the center of large displacement by the Boso slow slip event repeatedly occurred. We performed the continuous observation of about one year long by deploying the BBOBST-NX and the ocean bottom Doppler current profiler (OBDC) to get the environmental condition during the NT13-07 cruise in April 2013 (Fig. 3-2). As for the NUDOBS, it is still a prototype, but deployed in the deepest part (P2, about 9200 m depth) of the triple junction off Boso to check the real performance of it. The NUDOBS equips omni-directional geophones (15 Hz) and the MEMS accelerometer.

Recovery of the OBDC and the BBOBST-NX was performed on 5th April. In the early morning, the OBDC was called and released the anchor well. With about the 55m/min. ascending speed, it was reached the sea surface and recovered. The bottom current data for one year is obtained. The #1642 HPD dive was started soon after the recovery. The BBOBST-NX was easily found mainly by using the acoustic transponder ranging. There was a wooden peace at the EW component of the sensor unit. The extraction of the sensor unit from the sediment and the connection of the recovery rope system were finished within one hour. The data of the BBOBST-NX was expected to be recorded well from the log data. Additionally, positioning for three units of the ocean bottom seismometer with pressure gauge (OBSP) at P1a and P1b sites were tried. But, the first OBSP deployed in 2012 at the P1a site never answered. The second OBSP deployed in 2012 at the P1b site answered, but it was impossible to perform the ranging due to bad sea condition. We stopped to continue the ranging and left the site.

The recovery of the NUDOBS at the P2 site was tried on 7th April from the early morning. We started to call but never replied from the NUDOBS. So that, we sent the anchor releasing command and waited for four hours that seemed enough time to come up to the sea surface, but it never appeared.

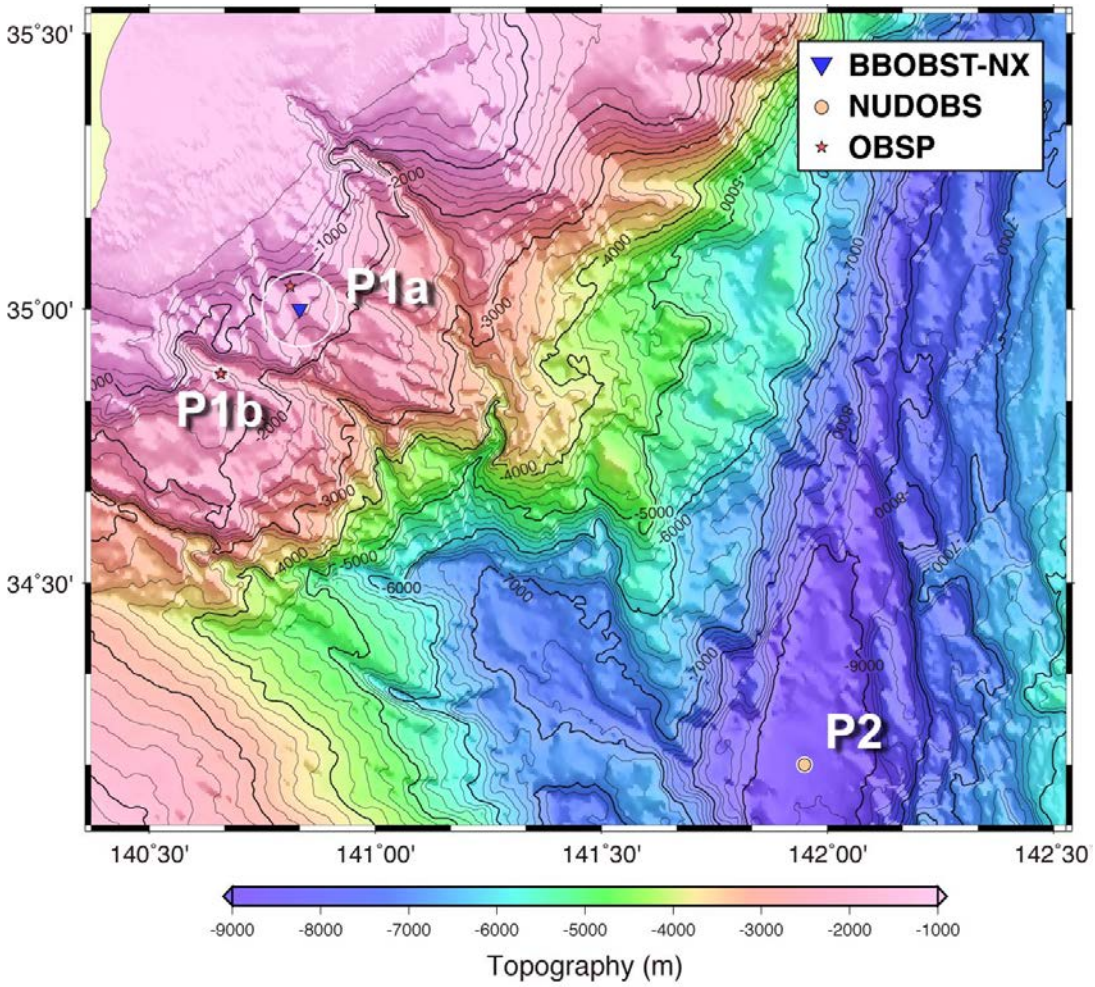


Fig. 3-1. Location map.

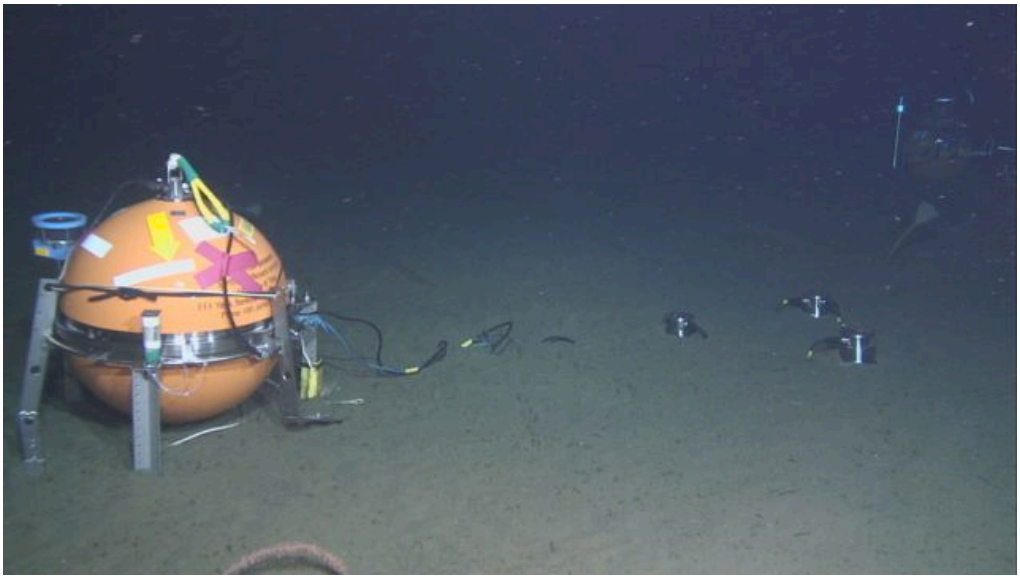

Fig. 3-2. The BBOBST-NX and the OBDC at the P1a site in the NT13-07 cruise.

**4. Notice on Using**

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

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

Users of data or results on this cruise report are requested to submit their results to the Data Management Group of JAMSTEC.