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NATSUSHIMA Cruise Report NT10-08

Myojin Knoll (Izu-Ogasawara Area)
and Off Hatsushima (Sagami Bay)

May 11-18, 2010

Principal Investigator
Koji INOUE
Atmosphere and Ocean Research Institute,
The University of Tokyo

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

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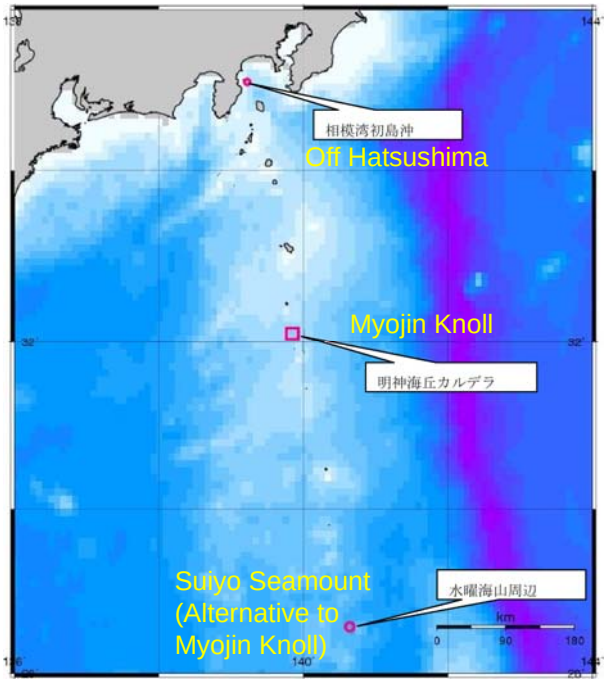
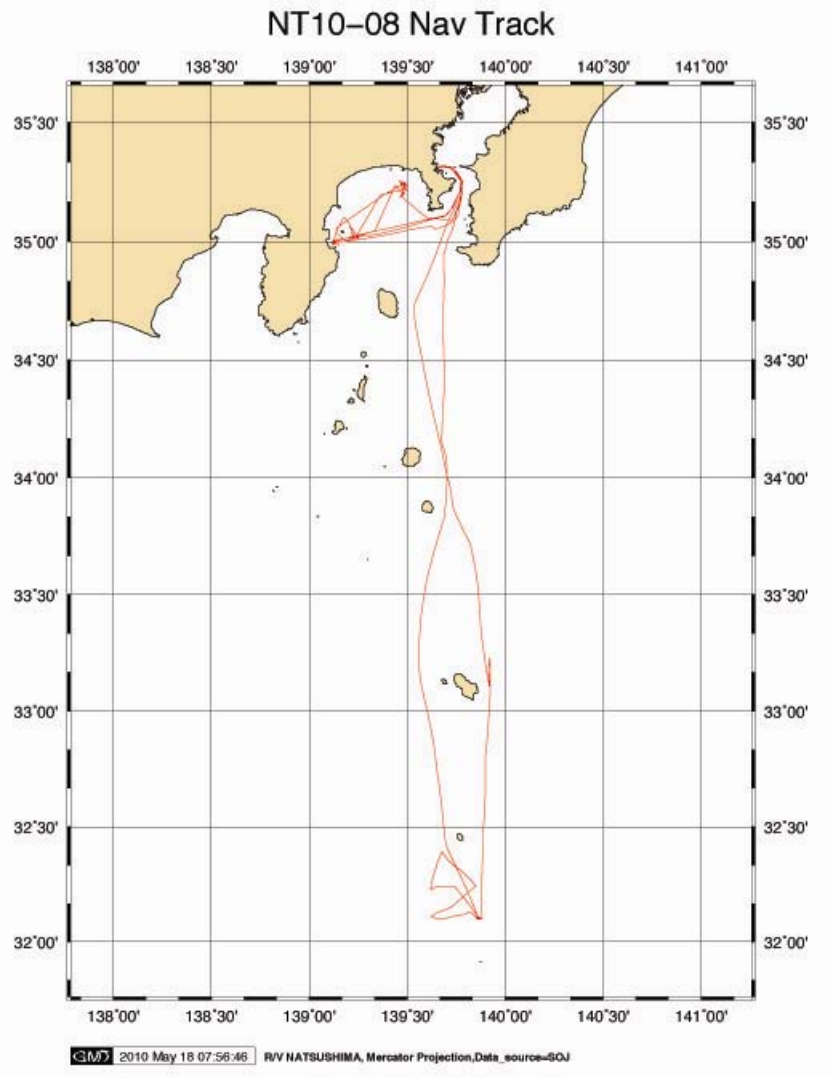
We express sincere thanks to the crew of R/V Natsushima, the operation team of ROV Hyper-Dolphin, and the stuff of JAMSTEC for their support of this cruise.

1. CRUISE INFORMATION

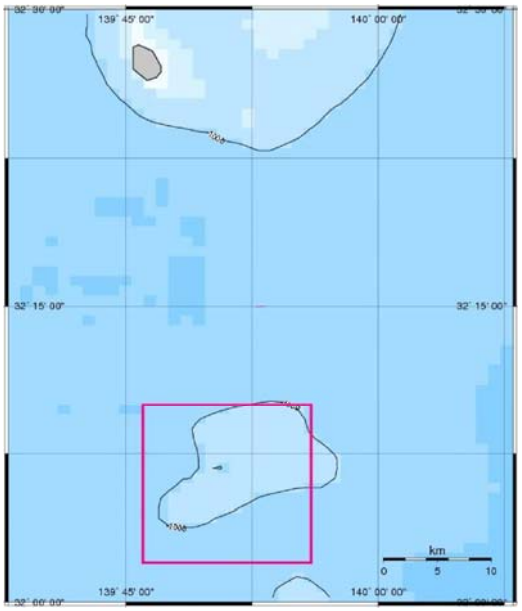
1) Cruise ID/Name of Vessel
NT10-08/RV Natsushima and ROV Hyper-Dolphin
2) Title of cruise
"Hyper-Dolphin" Research Dive, Deep-sea Research, FY2010.
3) Title of the proposals (Representative of the proposals)
Elucidation of the mechanism and evolutionary history of the adaptation system to toxic sulfides (Koji INOUE, AORI, The University of Tokyo)
The carbonic anhydrase is important factor for symbiosis (Takao YOSHIDA, JAMSTEC)
Phylogeny and ultrastructure of the deep-sea microorganisms with m itochondria and incomplete nuclear envelope (Masashi YAMAGUCHI, Chiba University)
4) Period of cruise
From May 11, 2010 to May 18, 2010
5) Ports of calls
From Yokosuka (JAMSTEC) to Yokosuka (JAMSTEC)
(Called at JAMSTEC, Yokosuka on May 13 and May 16)
6) Research area
Izu-Ogasawara Area (Myojin Knoll).
Sagami Bay (Off Hatsushima)



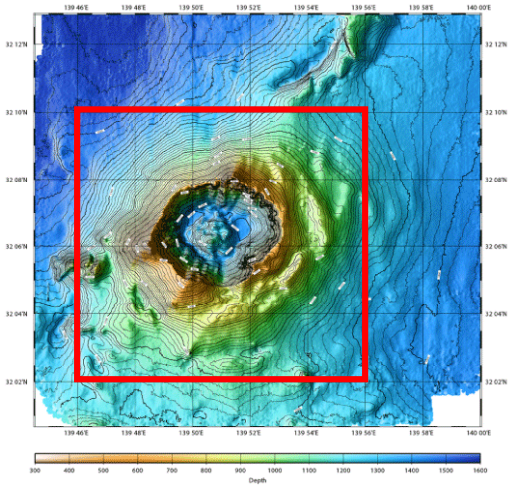
Research Area



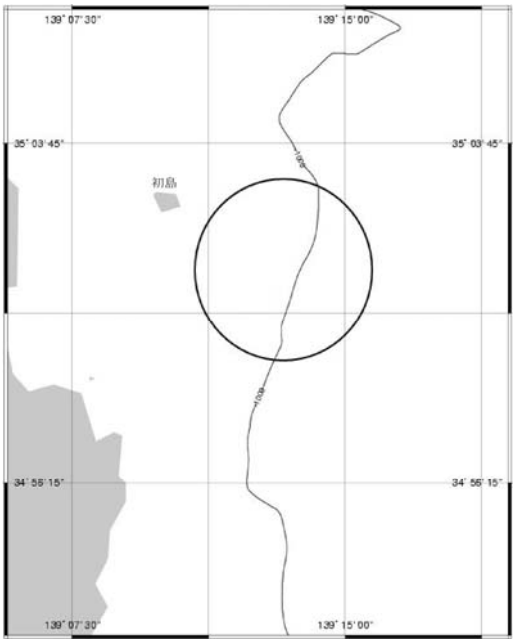
Cruise track of R/V Natsushima during NT10-08



The position of Myojin Knoll.
The red square indicates the area
proposed in the cruise plan.



Topographic map of Myojin Knoll.
The red square indicates the area
proposed in the cruise plan.



Map of Off Hatsushima.
The circle indicates the area
proposed in the cruise plan.

2. RESEARCHERS

1) Chief Scientist

Koji INOUE
Associate Professor, Atmosphere and Ocean Research Institute, The University of Tokyo.
5-1-5 Kashiwanoha, Kashiwa 277-8564, Japan.
e-mail, inouek(at)aori.u-tokyo.ac.jp
Phone +81-4-7136-6212

2) Representatives of Science Parties

Koji INOUE (shown above)
Takao YOSHIDA, Japan Agency for Marine-Earth Science and Technology (JAMSTEC), 2-15 Natsushima, Yokosuka Kanagawa 237-0061, Japan.
Masashi YAMAGUCHI, Medical Mycology Research Center , Chiba University, 1-8-1 Inohana, Chuo, Chiba 260-8673, Japan.

3) Science Party (except for the representatives of science parties)

Misumi AOKI (Nippon Marine Enterprises, Ltd.)
Yuki HONGO (JAMSTEC)
Mitsuru JIMBO (Kitasato University)
Masaru KATO (The University of Tokyo)
Azusa KINJO (The University of Tokyo)
Madoka KITAJIMA (Enoshima Aquarium)
Tomoko KOITO (Nihon University)
Yoshimichi KOZUKA (Tokyo Medical University)
Hiroshi MIYAKE (Kitasato University)
Toshihiro NAGASAKI (The University of Tokyo)
Yoshimitsu NAKAMURA (JAMSTEC)
Yuichi NAMIKI (Chiba University)
Suguru NEMOTO (Enoshima Aquarium)
Hitoshi OKADA (Chiba University)
Motoki OZAWA (JAMSTEC)
Takuho SHUTO (The University of Tokyo)
Shino SUZUKI (JAMSTEC)
Akihiro TAME (Marine Works Japan, Ltd.)
Haruhiko TOYOHARA (Kyoto University)
Katsuyuki UEMATSU (Marine Works Japan, Ltd.)
Kyohei YAMADA (Kyoto University)

4) Collaborators (in JAMSTEC)

Katsunori FUJIKURA, Hisako HIRAYAMA, Tadashi MARUYAMA, Yukiko NAGANO, Kazue OHISHI, Daisuke SEKINE, Eriko SEO, Yoshimi TAKAHASHI, Hiromi WATANABE

Members of the science party of NT10-08

3. OBSERVATION

1) Overview of the cruise

We had planned two days of dive researches (two dives a day) at Myojin Knoll first, followed by two days (also two dives a day) at Off Hatsushima after changing some members. However, we could not but change the schedule because of the stormy weather. We departed JAMSTEC (Yokosuka) on the afternoon of May 11th, and went toward Off Hatsushima first because we had information that the weather around Myojin Knoll was very stormy. However, among two dives expected on May 12th, the morning dive (#1124) was discontinued just after the start of diving because of oil spill accident, and thus only the afternoon dive (#1125) was successful. On May 13th, after replacement of some members at JAMSTEC, we sailed toward Myojin Knoll. We arrived there in the early morning of May 14th and waited for the weather to improve. However, stormy condition continued until the night and we could perform dive researches only on May 15th. We returned to JAMSTEC on May 16th, and replaced some researchers again, and performed two dives at Off Hatsushima on May 17th. Finally, we returned to JAMSTEC on the morning of May 18th.

During the cruise, mussels, clams, and polychaetes were collected around the hydrothermal vents at Myojin Knoll, and cold seeps at Off Hatsushima. Fixation and biochemical analyses were performed on some of the samples immediately after collection. Other samples were kept alive and brought back to AORI, JAMSTEC, and Enoshima Aquarium for rearing experiments. Detailed analyses of genes, amino acids, enzymes, and ultrastructure will be performed after the cruise.

Although we could have only one day for the research at Myojin Knoll and one dive at Of Hatsushima (#1124) was not successful, we could obtain most of the samples requested by the scientists, by giving top priority to collection of samples at well-known sites. However, we regret that we could not have enough time for in situ observation of at the vent area and for wide survey of unknown area.

2) Cruise Log (By Ms. Aoki)

NT10-08 Cruise Log (Time=UTC+9:00)
From Yokosuka to Yokosuka

05/11 departure delayed
10:30-11:30 science meeting
13:00 departure from JAMSTEC
14:00-15:00 science meeting

05/12 @noon: weather: rain / wind direction: East / wind speed index: 2 / wave: 2m / swell: 2m / visibility: 6 nautical mile
06:30 XBT
HPD#1124 / Off Hatsushima Island
dive interrupted because of oil leakage
HPD#1125 / Off Hatsushima Island
13:52 on bottom(D=857m)
16:45 off bottom(D=860m)
18:45-19:15 science meeting

05/13 @noon: weather: fine but cloudy / wind direction: NE / wind speed index: 2 / wave: 2m / swell: 2m / visibility: 8 nautical mile
08:30 2 scientists disembark at JAMSTEC
08:45 2 scientists onboard, transit to Myoujin Knoll
18:00- science meeting
05/14 @noon: weather: fine but cloudy / wind direction: WNW / wind speed index: 6 / wave: 5m / swell: 3m / visibility: 9 nautical mile
06:30 XBT
dive canceled because of bad sea condition
05/15 @noon: weather: fine but cloudy / wind direction: NE / wind speed index: 4/ wave: 3m / swell: 2m / visibility: 9 nautical mile
HPD#1126 / Myojin Knoll
09:01 on bottom(D=1,240m)
11:41 off bottom(D=1,226m)
HPD#1127 / Myojin Knoll
14:22 on bottom(D=1,237m)
16:31 off bottom(D=1,248m)
transit to JAMSTEC
05/16
11:20 5 scientists disembark at JAMSTEC
15:00 4 scientists onboard, transit to Off Hatsushima
05/17 @noon: weather: fine but cloudy / wind direction: South / wind speed index: 2 / wave: 1m / swell: 1m / visibility: 8 nautical mile
HPD#1128 / Off Hatsushima Island
08:55 on bottom(D=910m)
11:05 off bottom(D=899m)
HPD#1129 / Off Hatsushima Island
13:55 on bottom(D=858m)
16:20 off bottom(D=816m)
19:10-19:45 science meeting
05/18 08:00 arrive at JAMSTEC, scientists disembark

wind speed index= 0 = 0 - 0.2 m/sec., 1 = 0.3 - 1.5m/sec., 2 = 1.6 - 3.3m/sec., 3 = 3.4 - 5.4m/sec.,4 = 5.5 - 7.9m/sec., 5 = 8.0 - 10.7m/sec., 6 = 10.8 - 13.8m/sec., 7 = 13.9 - 17.1m/sec., 8 = 17.2 - 20.7m/sec., 9 = 20.8 - 24.4m/sec., 10 = 24.5 - 28.4m/sec., 11 = 28.5 - 32.6m/sec., 12 = more than 32.7 m/sec.

3) Dive list

Dive #	Dive points	Keywords
1124	Methane Seep at Off Hatsushima	Operation discontinued during the descending dive.
1125	Methane Seep at Off Hatsushima	Collection and observation of seep-specific organisms, water sampling
1126	Hydrothermal Vents at Myojin Knoll	Collection and observation of vent-specific organisms, water sampling
1127	Hydrothermal Vents at Myojin Knoll	Collection and observation of vent-specific organisms, water sampling
1128	Methane Seep at Off Hatsushima	Collection and observation of seep-specific organisms, water sampling
1129	Methane Seep at Off Hatsushima	Collection and observation of seep-specific organisms, water sampling

4) Major equipments loaded to Hyper-Dolphin

a) Slurp Gun (Suction sampler)

It was used to collect benthos and fish. The nozzle attached to the left hand of the manipulator was connected to a rotary canister containing 6 bottles, which en able to keep samples from different points separated. Bottles were removed when necessary. In this cruise, another nozzle was set on the right hand, which was connected to another canister, which is cubic shape and contained no bottle.

The left nozzle connected to the cubic canister

The right nozzle, which is connected to the rotary canister.

The cubic canister

The rotary canister with canister bottles

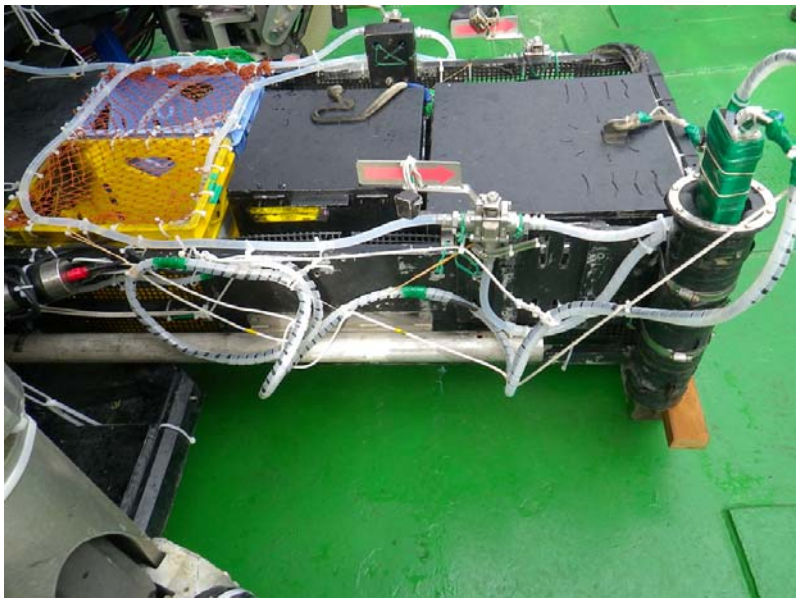
b) Sample boxes

Two sample boxes were loaded in front of the vehicle.

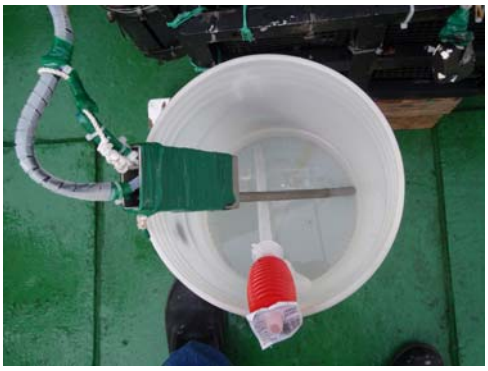
Sample boxes

c) Bag-type water sampler and RMT thermometer

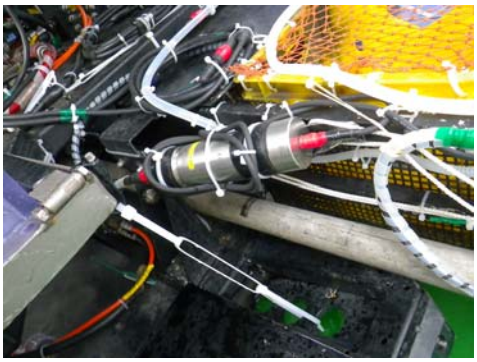
RMT ther mometer was attached to the nozzle of the water sampler. The nozzle was connected to two or three plastic bags through a three direction connector with a selector lever. Water was evacuated using a perista pump.



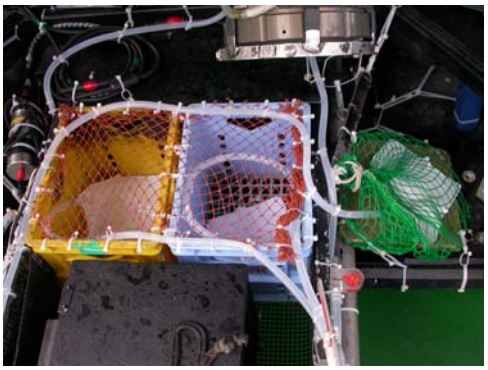
Arrangement of water sampler



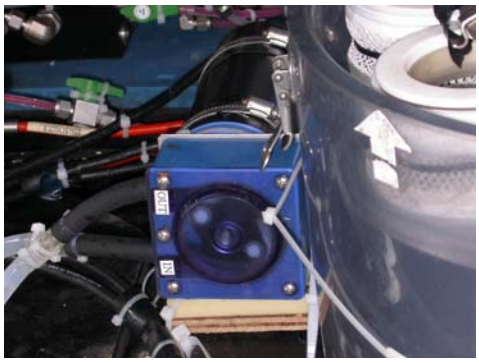
The nozzle of the water sampler



Data logger of the RMT thermometer



Water bags set on the front bay



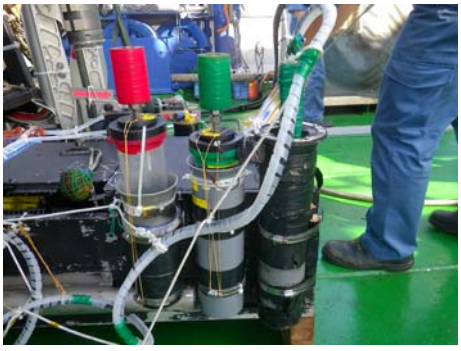
Perista pump

d) Niskin water sampler and MBARI corer

Non-vent and non-seep seawater was sampled using this type of water sampler.

The sediment around the seep was collected using MBARI corers.

Niskin water sampler



MBARI corers

5) Summary of dives

a) Dive #1124 (May 12, 2010; Off Hatsushima)

Objective：Collection of seep-specific animals, sampling of water just above colonies

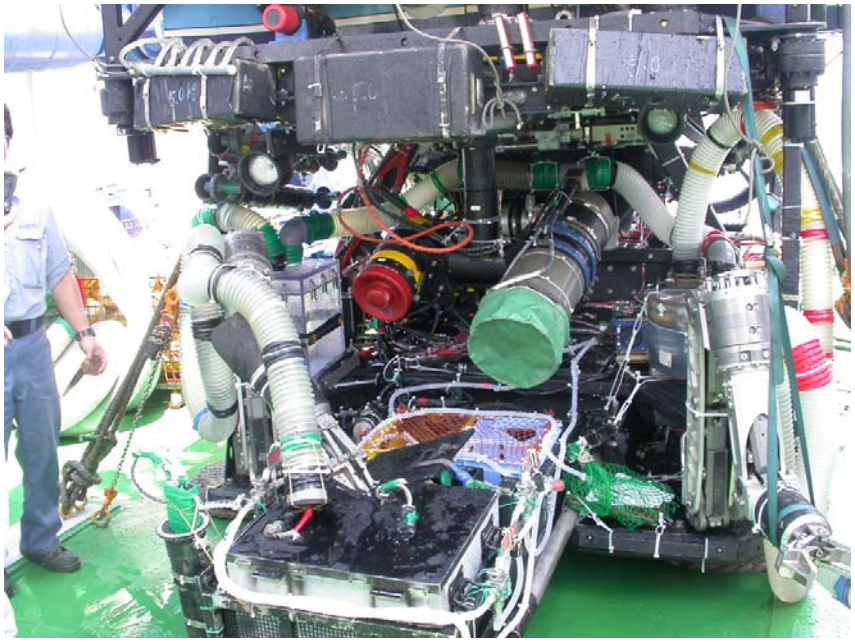
Equipments loaded: Suction sampler (Slurp gun) with a rotary canister containing 6-bottles, and that with a cubic canister. Bag-type water sampler and RMT thermometer, Niskin water sampler, Sample Boxes, Scoop.

Summary: Started diving at 8:17 but it was stopped at a depth of 270 m due to oil spill from the system of the vehicle.

b) Dive #1125 May 12, 2010; Off Hatsushima; Reporter, T. Yoshida)

Objective：Collection of seep-specific animals, sampling of water just above colonies

Equipments loaded: Suction sampler (Slurp gun) with a rotary canister containing 6-bottles, and that with a cubic canister. Bag-type water sampler and RMT thermometer, Niskin water sampler, Sample Boxes, Scoop.

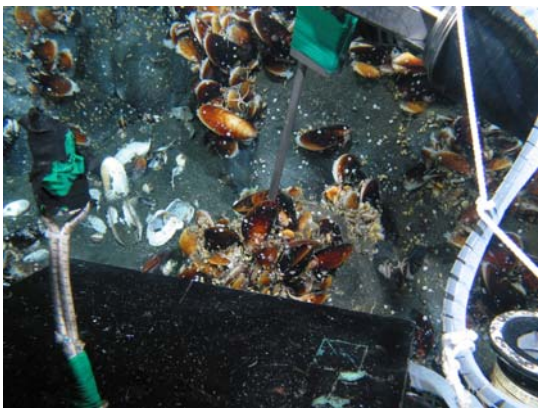


Arrangement of research equipments at Dive #1125

Summary: To investigate the symbiosis of *Calyptogena* clams, and environmental adaptation of *Bathymodiolus* mussles, we dived the 850m site of seep community in Off Hatsushima, Sagami Bay. HPD ROV moved to the *Bathymodiolus* mussel colony in the rock at 861m site (35-00.948N, 139-13.329E). At this colony, we corrected the *Bathymodiolus* and environmental

water at thr ee points. After that, ROV was moved to *Calyptogena* colony in 859m site (35-00.952N, 139-13 .320E). *Calyptogena* clams were collected by scoop in the two boxes. After that, we moved to the *Alaysia* colony in 856 m (35-00.945N, 139-13.304). We deployed the bait trap in front of the *Alaysia* colony for correcting fishes. Three trap was picked up in the box, and *Alaysia* was corrected in the box. We moved to the *Lamellibrachia* colony, and corrected the *Lamellibrachia*.

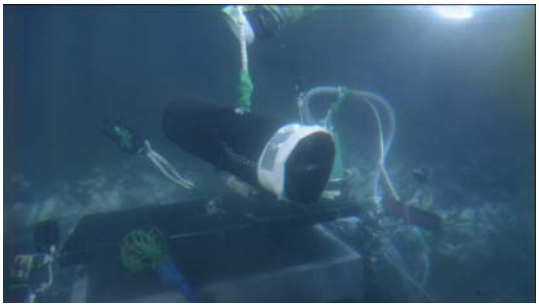
Mussel and *Lamellibrachia* colony



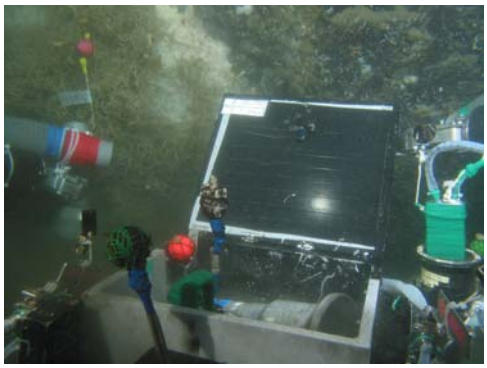
Water sampling

Sampling of *Bathymodiolus*

Sampling of *Calyptgena*

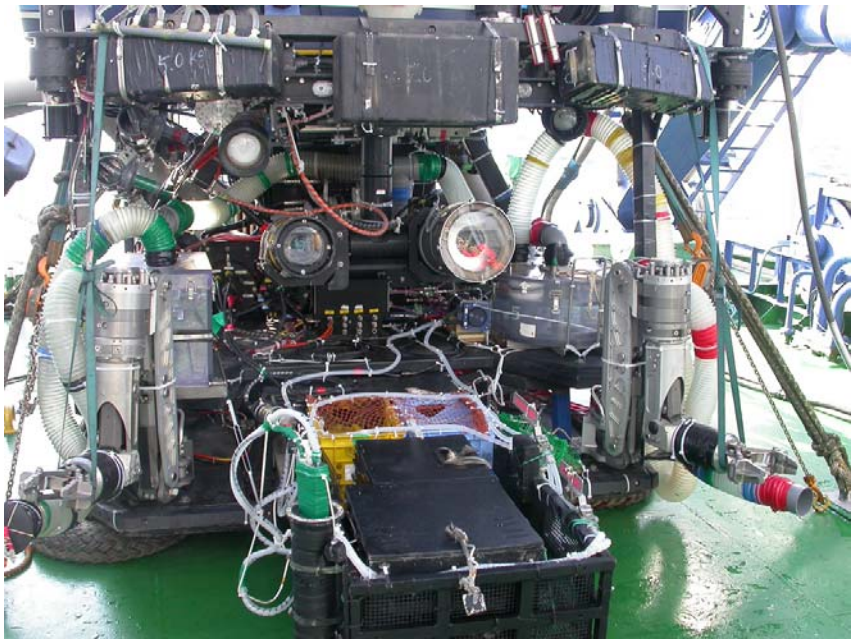


Setting of a bait trap



Recovery of the trap for larvae
(set at #796)

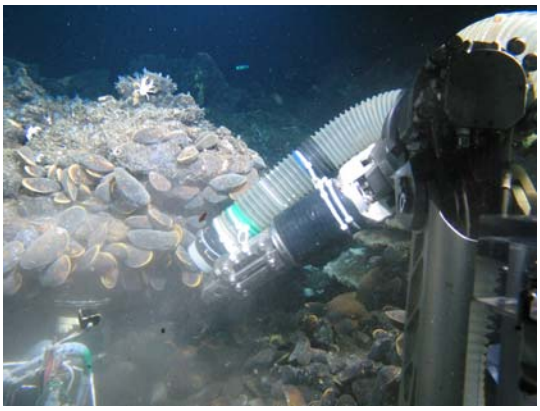
c) Dive #1126 (May 15, 2010; Myojin Knoll; Reporter, K. Inoue)
Objective：Collection of vent-specific animals, Sampling of water above mussel colonies
Equipments loaded: Suction sampler (Slurp gun) with a rotary canister containing 6-bottles, and that with a cubic canister. Bag-type water sampler and RMT thermometer, Niskin water sampler, Sample Boxes, Scoop.



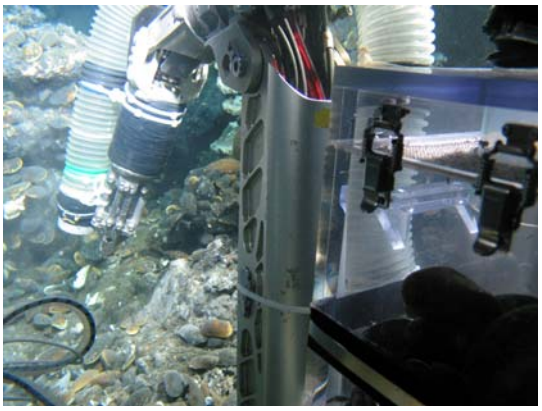
Arrangement of research equipments at Dive #1126

Summary: This is the first dive at Myojin Knoll in this cruise after waiting for the weather to improve for one day. The vehicle dived toward the points 1 and 13 (see appendix). After reached to the bottom, the main cable appeared in the vision of the camera, which means slack of the cable. After winding up the loosen cable, the vehicle moved along the bottom to find mussel colonies. At the first large colony, seawater was sampled into the bag #1, and then mussels in the colony were collected into the cubic canister with the suction sampler. Subsequently, we tried to catch crabs into the rotary canister but the canister stacked soon. Thus, we could not continue the sampling using the suction sampler. We collected two chimney heads and left the bottom.

Sampling of the water just above the mussel colony

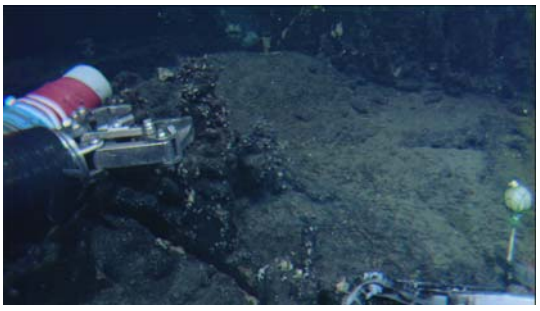


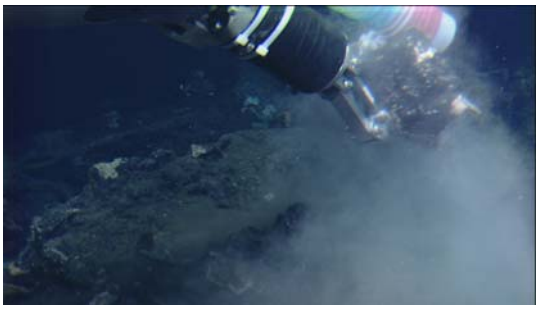
Sampling of the mussels



Mussels are sucked into the canister

Sampling of crabs



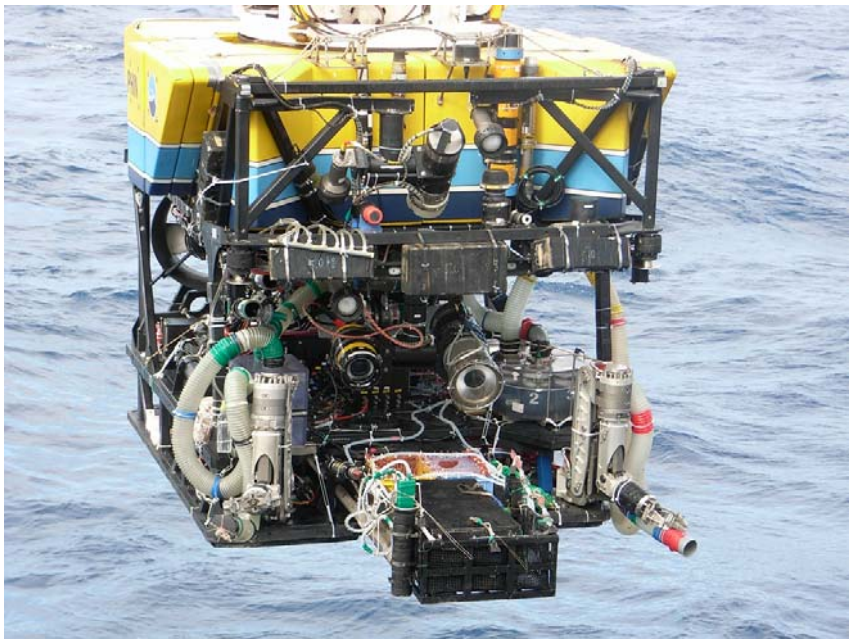


Sampling of a piece of chimney

d)Dive #1127 (May 15, 2010; Myojin Knoll; Reporter K. Inoue)

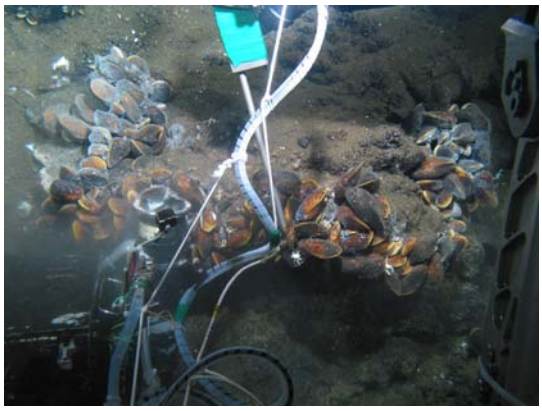
Objective：Collection of vent-specific animals, Sampling of water above mussel colonies

Equipments loaded: Suction sampler (Slurp gun) with a rotary canister containing 6-bottles, and that with a cubic canister. Bag-type water sampler and RMT thermometer, Niskin water sampler, Sample Boxes, Scoop.

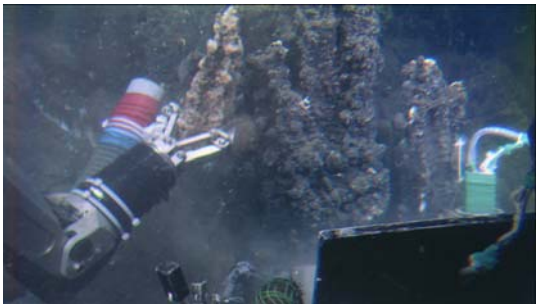


Arrangement of research equipments at Dive #1127

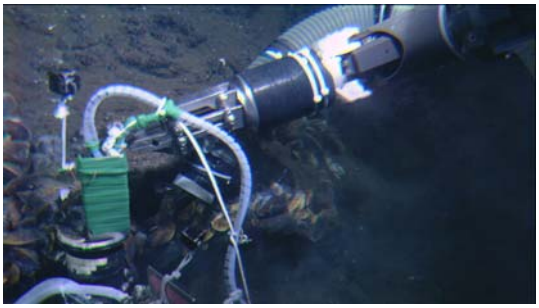
Summary: The vehicle dived toward the sampling point of the morning dive. First, we collected some pieces of chimneys to obtain polychaetes and barnacles. Then, we found another mussel colony and performed water sampling (bag #1) followed by mussel collection (into cubic canister) as did in the morning dive. Vent-specific animals were collected into the canister # 1 using the suction sampler. At the next mussel colony, water sampling into the bag #2 and mussel sampling into the canister #2 were carried out. Additional pieces of chimneys were sampled. The vehicle moved toward the giant chimney ("Dai-Myojin") and we took pictures of the mussel colony, which has been continuously observed for 8 years. After collection of polynoid-like polychaetes, the vehicle left the bottom.



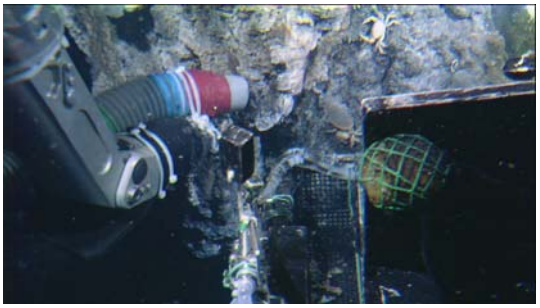
Sampling of the water from a mussel colony



Sampling of a piece of chimney



Sampling of mussels

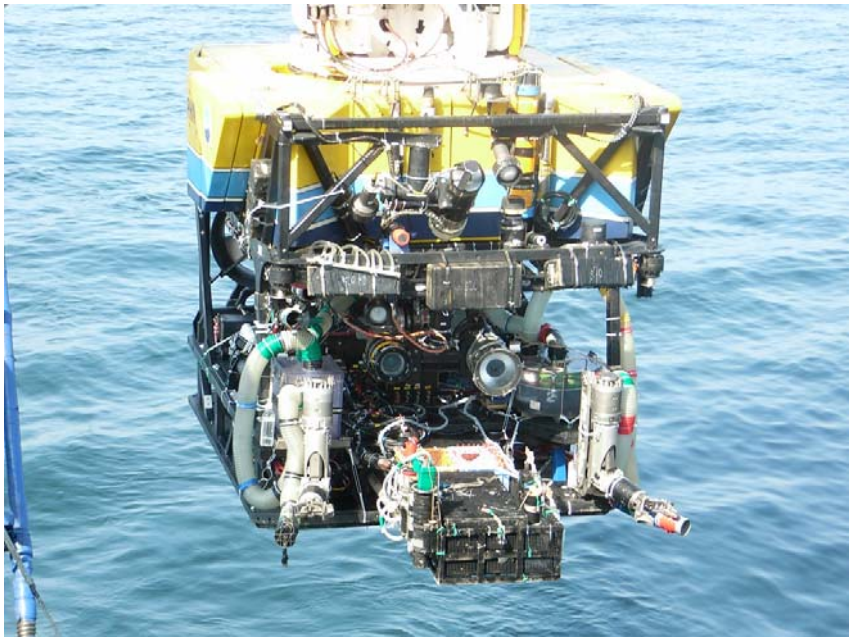


Sampling of polychaetes

e)Dive #1128 (Date :May 17, 2010; Off Hatsushima; Reporter, M. Jimbo)

Objective：Collection of seep-specific animals, sampling of water just above colonies

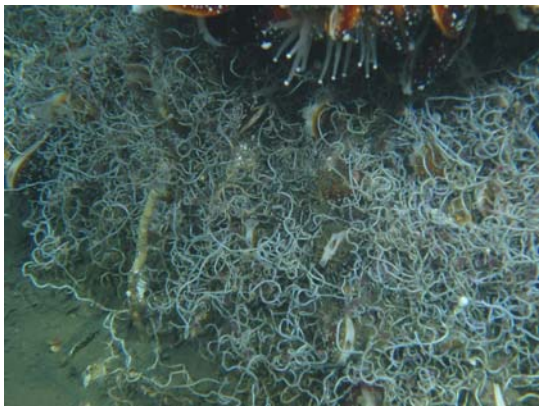
Equipments loaded: Suction sampler (Slurp gun) with a rotary canister containing 6-bottles, and that with a cubic canister. Bag-type water sampler and RMT thermometer, Niskin water sampler, Sample Boxes, Scoop, MBARI corers.



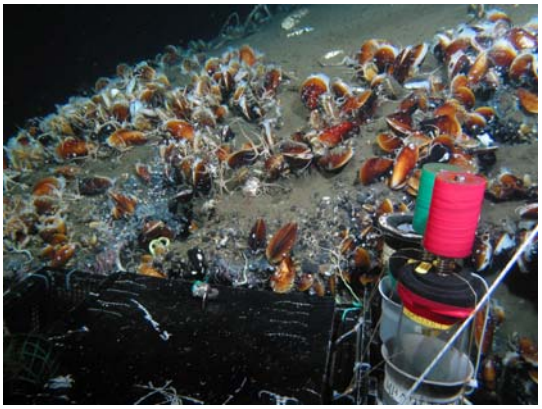
Arrangement of research equipments at Dive #1128

Summary: The vehicle dived toward the point #8, and arrived at the bottom slightly east to the target point. On the way to the target point, we found a mussel coloney, where we sampled water and mussels, using the bag-type sampler and the suction sampler with rotary canister, respectively. At the point #8, we observed a piece of log. Subsequently, we found an *Alaysia* coloney, where some tubeworms were collected. We also found another mussel colony, where water and mussels were sampled. At that time, as the power of the suction sampler decreased

due to accumulation of samples in the tube system, mussels were collected by pealing and pushing them, using the manipulator, into the sample box. We could obtain enough number of mussels here because we collected them from several large colonies around the point. We set a marker buoy here. We also attempted to collect *Calyptogena* clams. However, it was not successful because of insufficient power of the suction sampler. We collected some individuals of *Lamellibrachia* sp. and left the bottom.

An *Alaysia* colony




Mussel colonies

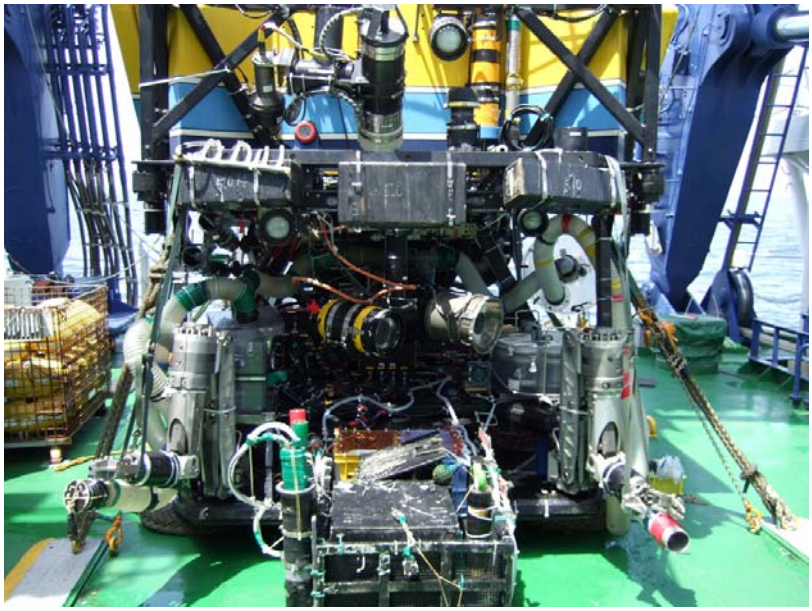

Calyptogena colony

Sampling of tubeworms

f) Dive #1129 (May 17, 2010; Off Hatsushima; Reporter, T. Yoshida)

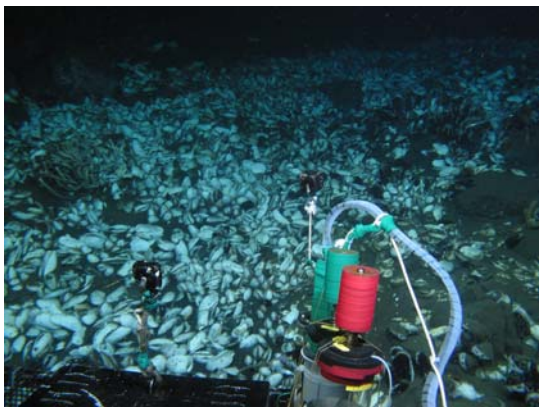
Objective：Collection of seep-specific animals, sampling of water just above colonies

Equipments loaded: Suction sampler (Slurp gun) with a rotary canister containing 6-bottles, and that with a cubic canister. Bag-type water sampler and RMT thermometer, Niskin water sampler, Sample Boxes, Scoop, MBARI corers.



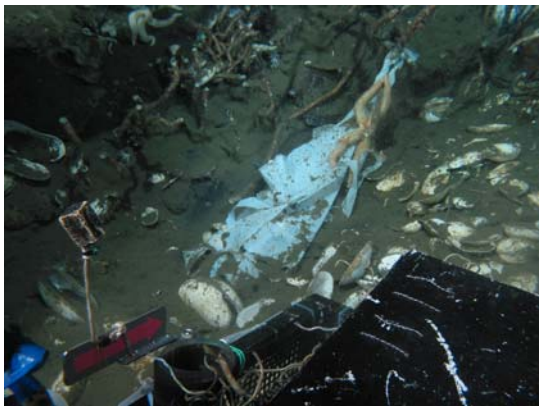
Arrangement of research equipments at Dive #1129

Summary: We reached on the bottom at a depth of 858m at 13:55 and found the colony of *Calyptogena* clam and *Bathymodiolus* mussel soon. Sampling using suction sampler was conducted to sample small size *Calyptogena* buried in mud. We moved to another colony and sampled seawater just above the mussel colony and then collected some *Bathymodiolus* mussels. We also did the same sampling on *Calyptogena* clam colony and deployed a marker buoy. After that, ROV went to the site where a bait trap was deployed at HD Dive #1125. A species of conger eels which was triggered by the bait was sampled and Bait trap was retrieved. We moved to the *Alaysia* tube worm colony and sample some shrimps and a squat robster in the *Alaysia* colony and many *Alaysia*.

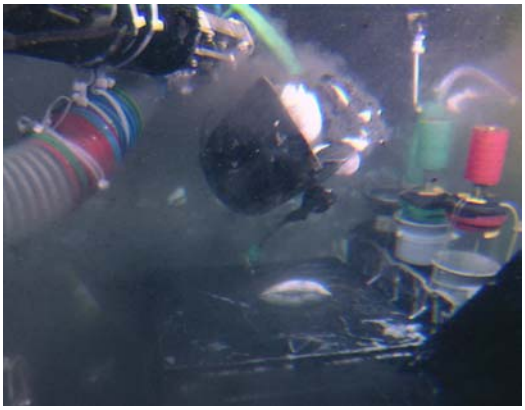


Colony of *Calyptogena* sp.

A bottle was found on the deep-sea floor

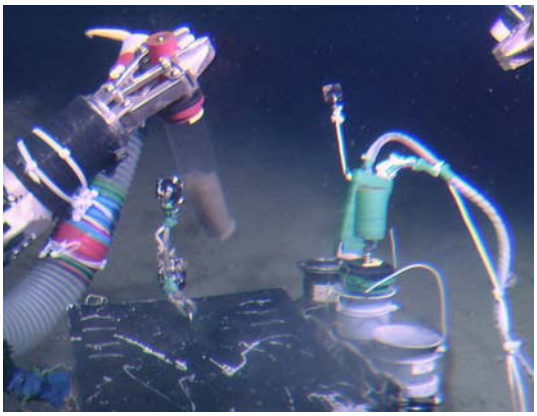


Plastic waste found on the deep-sea floor



Sampling of *Calyptogena*

Bait trap



Sediment sampling

4. RESEARCH PEPORTS

1) Detoxification of hydrogen sulfides using amino acids: mechanisms and evolution

Koji INOUE[1], Tomoko KOITO[2], Takuho SHUTO[1], Azusa KINJO[1], Masaru KATO[1], Toshihiro MAGASAKI[1], Kyohei YAMADA[3] and Haruhiko TOYOHARA[3]
[1]*Ocean Research Institute, The University of Tokyo;* [2]*College of Bioresource Sciences, Nihon University;* [3]*Graduate School of Agriculture, Kyoto University*

Objective

Many invertebrates inhabiting hydrothermal vents contain thiotrophic bacteria in their tissues. However, the mechanisms to deliver toxic sulfides to the endosymbionts remain unknown. Recently, it has been suggested that thiotaurine is involved in sulfide detoxification. We are trying to understand the detoxification process by characterizing the taurine transporter (TAUT), which transport taurine and related amino acids across the cell membrane. We have already cloned the TAUT cDNA from the hydrothermal vent mussel *Bathymodiolus septemdierum* and the cold-seep mussel *B. platifrons*, and have analyzed its functions (Inoue et al. FEBS letters 582, 1542-1546, 2008; Koito et al. Fish. Sci. 76, 382-388, 2010; Koito et al. Cah. Biol. Mar. 51, 429-433, 2010). We also established a real-time PCR system to quantify the level of mRNA and also a method for detection of thiotaurine and hypotaurine using HPLC. In the present cruise, we tried to compare TAUT mRNA level and thiotaurine level between mussels collected at the high sulfide environment and those from low sulfide environment. We also performed aquarium experiments to expose the mussels to sulfide and also to osmotic stress, because taurine is know as an osmolyte.

Achievement in this cruise

We collected live *B. septemdierum* specimens from a colony facing to an active vent and those from a colony that is not exposed directly to vent water. We also collected live *B. platifrons* from several colonies. The specimens were dissected and frozen for mRNA analyses and also fixed for histological analyses. Some mussels are reared for 2 days in sulfide-containing seawater or in high or low salinity seawater, and dissected and frozen during the cruise. We also brought back some live specimens for laboratory experiments.

Future studies

- Rearing of the mussels in aquarium under the different condition (long-term).
- Quantification of TAUT mRNA level by real-time PCR.
- Analysis of free amino acids.
- Histological analyses.
- Phylogenetic analyses on TAUT sequences of various marine organisms.

2) The carbonic anhydrase is important factor for chemosynthetic symbiosis

Takao YOS HIDA[1], Y uki HONGO [1], Y oshimitsu NAKAM URA[1], Genki OZA WA[1], Shino SUZUKI[1], Akihiro TAME[2], and Katsuyuki UEMATSU[2]
[1]*Japan Agency for Marine-Earth Science and Technology (JAMSTEC)*
[2]*Marine Works Japan, Ltd.*

Objective

Intracellular symbioses between chem oautotrophic bacteria and marine invertebrates dominate the fauna at deep-sea hydrothermal vents and seeps. Intracellular chemoautotrophic bacteria synthesize all necessary organic compounds from carbon dioxide. The host invertebrates are nutritionally dependent on their symbiont. The hosts have to take up carbon dioxide and deliver to the symbiont to obtain nutrition. The carbonic anhydrase may facilitate uptake of inorganic carbon by catalyzing the reversible dehydration of bicarbonate to carbon dioxide. This enzyme may also facilitate intracel lular conversion of bicarbonate to carbon dioxide, and enhance the inorganic carbon fixation by symbiont. To understand the mechanism of carbonic anhydrase in chemosynthetic symbiosis, we analyze the function of carbonic anhy drase in the marine invertebrates.

Achievement in this cruise

Calyptogena spps, *Bathymodiolus* spps were collected from the Off Hatsushima Island site, Sagami Bay, and the Myojin Knoll. The samples were immediately dissected, gill and other tissues were frozen in liquid nitrogen and stored at -80ºC. These samples were also fixed in 4% paraformaldehyde.

Future studies

- Expression and activity of carbonic anhydrase will be measured..
- Localization of carbonic anhydrase will be observed.
- Cultivation of gill tissue will be tested.

3) Ultrastructure and evolution of deep-sea microorganisms having mitochondria and incomplete nuclear envelope

Masashi YAMAGUCHI[1], Katsuyuki UEMATSU[2], Hitoshi OKADA [1,3], Yuichi NAMIKI [1] and Yoshimichi KOZUKA[4]

[1]*Medical Mycology Research Center, Chiba University;* [2]*Marine Works Japan, Ltd.;* [3] *Integrated Imaging Research Support;* [4]*Tokyo Medical University, Japan*

Objective

There are two kinds of organisms on earth, eukaryotes having nucleus with nuclear envelope and prokaryotes having no nucleus with nuclear envelope. Eukaryotes are considered to have evolved from prokaryotes. If this hypothesis is correct, then, there must have been organisms that were in the process of evolving from prokaryotes to eukaryotes. We consider there might be such organisms in the deep sea which is isolated from other environments. One of us (Kozuka) found, in a previous study , deep-sea microorganisms having mitochondria and incomplete nuclear envelope that might be such organisms. In the present study, we will further look for such microorganisms using electron microscopy and try to get direct evidence that shows evolutionary process from prokaryotes to eukaryotes.

Achievement in this cruise

In the caldera of Myojin Knoll, we collected live small organisms including species of Aphroditoidea, *Alvinella*, Polychaeta, Bathymodiolinae, spiral shell and sea urchin. The specimens were fixed with 2.5 % glutaraldehyde and dissected into small pieces with razor blades for electron microscopic examination. A part of the specimens were frozen for DNA analyses without fixation. About 100 specimens were processed and embedded for electron microscopy in the laboratory.

Future studies

- Making ultrathin sections and taking many micrographs of microorganisms associated with deep-sea small organisms.
- Analyzing DNA for species identification.
- Rapid freezing of remaining glutaraldehyde-fixed tissues and embedding to obtain better images of associated microorganisms.

4) Purification and Characterization of lectins from deep sea mollusks

Mitsuru JIMBO
School of Marine Biosciences, Kitasato University

Objective

A lectin is a generic name of sugar binding proteins, and is reported to involve to self-defense, biomineralisation, and so on. We found that the lectins from the haemolymph of *Lamellibrachia satsuma* and *Calyptogena okutanii*, agglutinated their symbiotic bacteria. These suggests that the lectin from deep sea involved in symbiosis, like zooxanthell a-coral and legume-rhizobia symbiosis. Since the struc ture and function of lectins from deep sea organisms is not known thus far, the lectin is purified and characterized.

Achievement of this cruise

1. *Calyptogena okutanii* lectin

The lectin in haemolymph usually bind to nonself organisms and start defensive reaction. *C. okutanii* lectin COL may also have same function, since it exsits in haemolymph. In this research, Yeast was injected to *C. okutanii*, and before injection and after 24h of injection, the haemolymph was obtained. They were stored at -70°C.

This lectin seems to relate maintenance of symbiotic bacteria . The ovary of *C. okutanii* contains symbiotic bacteria, the tissue was fixed to detect the distribution of the lectin.

2. *Bathymodiolus* lectin

Recently, we detect haemagglutination activity from the haemolymph of *B. septemdierum*. Thus, we obtained the hamolymph of them to purify lectin. We also obtained the haemolymph of *B. japonicus* and *B. platifrons*.

Future plan

I am going to examine the lectin quantity of haemolymph from yeast-injected *C. okutanii* by ELISA, and to examine the distribution of *C, okutanii* lectin in ovary.
The lectin from *Bathymodiolus* is going to be purified.

5) Study of Immune defense system in the deep-sea mussel with symbiotic bacteria

Kazue OHISHI[1], Daisuke SEKINE [1,2], Yoshimitsu NAKAMURA [1], Akihiro TAME[3], Takao YOSHIDA[1], Hiroshi MIYAKE[2], Tadashi MARUYAMA[2]
[1]*Japan Agency for Marine-Earth Science and Technology;* [2]*Kitasato University;* [3]*Marine Works Japan Co.*

Objective

In deep-sea environments near hydrothermal vents and seeps, various invertebrates including mussels harbouring symbiotic bacteria are found to make dense colonies. However, it is not clear how the mussels recognize and keep the symbionts, although they have an immune defense system for eliminating the exogenous bacteria. To understand the stable association between the mussels and symbiotic bacteria, the immune system of the deep-sea mussels must be studied. We study about the blood cells from the mussels, which are generally thought to play a central role in the immune defense system as the first step of the research.

Achievement in this cruise

We sampled *Bathymodiolus* spp. at a seep environment off Hatsushima island in Sagami-bay. The blood cells were collected.

Future studies

- Classification and characterization of the blood cells will be conducted.
- The distribution of the blood cells in the mussel body will be examined using the tissue samples.

6) Studies on reproductive ecology of deep-sea bivalves, *Calyptogena* spp. and *Bathymodiolus* spp.

Katsunori FUJIKURA, Eriko SEO, Yoshimi TAKAHASHI, Hiromi WATANABE,
and Takao YOSHIDA
BioGeos, Japan Agency for Marine-Earth Science and Technology (JAMSTEC)

Objective

Reproduction is the most important factor in the life-history of organisms to maintain population. In the deep-sea chemosynthesis-based ecosystems, *Calyptogena*, *Bathymodiolus* and Thyasirid bivalves are dominant animals. To understand reproductive characteristics, sex ratio and developing sizes will be estimated. Additionally, these bivalves have parasite animals including polychaeta and copepoda. We will estimate the relationship between these bivalves and parasite animals.

Achievement in this cruise

Calyptogena okutanii, C. soyoae, Bathymodiolus platifrons, B, japonicus and Thyasiridae gen. sp. from the Off Hatsushima Island site, Sagami Bay, and *B. septemdierum* from the Myojin Knoll were shared. These specimens were fixed ethanol, 10 % form alin and freeze. After dissection, gonad tissues will be observed to observe mature stage.

Future studies

- Gonad tissues observation for mature stage.
- Taxonomy of parasite animals.
- Analysis of food web using stable isotopic ratio.

7) Studies on fungi in deep-sea environments

Yuriko NAGANO
Marine Bioresource Exploration Research Team, Marine Biodiversity Research Program, Institute of Biogeosciences, JAMSTEC

Objective

Fungi are one of the most important components in ecosystems. They occupy a wide variety of environments by virtue of their highly versatile physiology function. Although the presence of fungi in deep-sea environments has started to be recognized, its distribution and diversity are still largely unknown. In order to increase our knowledge of fungal communities in deep-sea ecosystems, we are trying to obtain culturable fungi and fungal DNA from deep-sea supply materials, such as sediments and also deep-sea animals. We also intend to explore deep-sea fungi for application use.

Achievement in this cruise

In the caldera of Myojin Knoll, we collected *Munidobsis myojneasis*, *Aphroditoidoa gen*. sp., *Paraluinella hessleri* and other species for investigating the fungal diversity in deep-sea ecosystems. The specimens were kept at 4 degrees until used. Homogenized samples were cultured on plates and also used for DNA extraction.

Future studies

- Isolation of culturable fungi from deep-sea animals
- Investigating the fungal diversity in deep-sea animals by molecular methods.
- Phylogenetic analyses on fungal sequences from deep-sea environments
- Exploration of antimicrobiotic agents and other useful agents in deep-sea fungal culture collections.

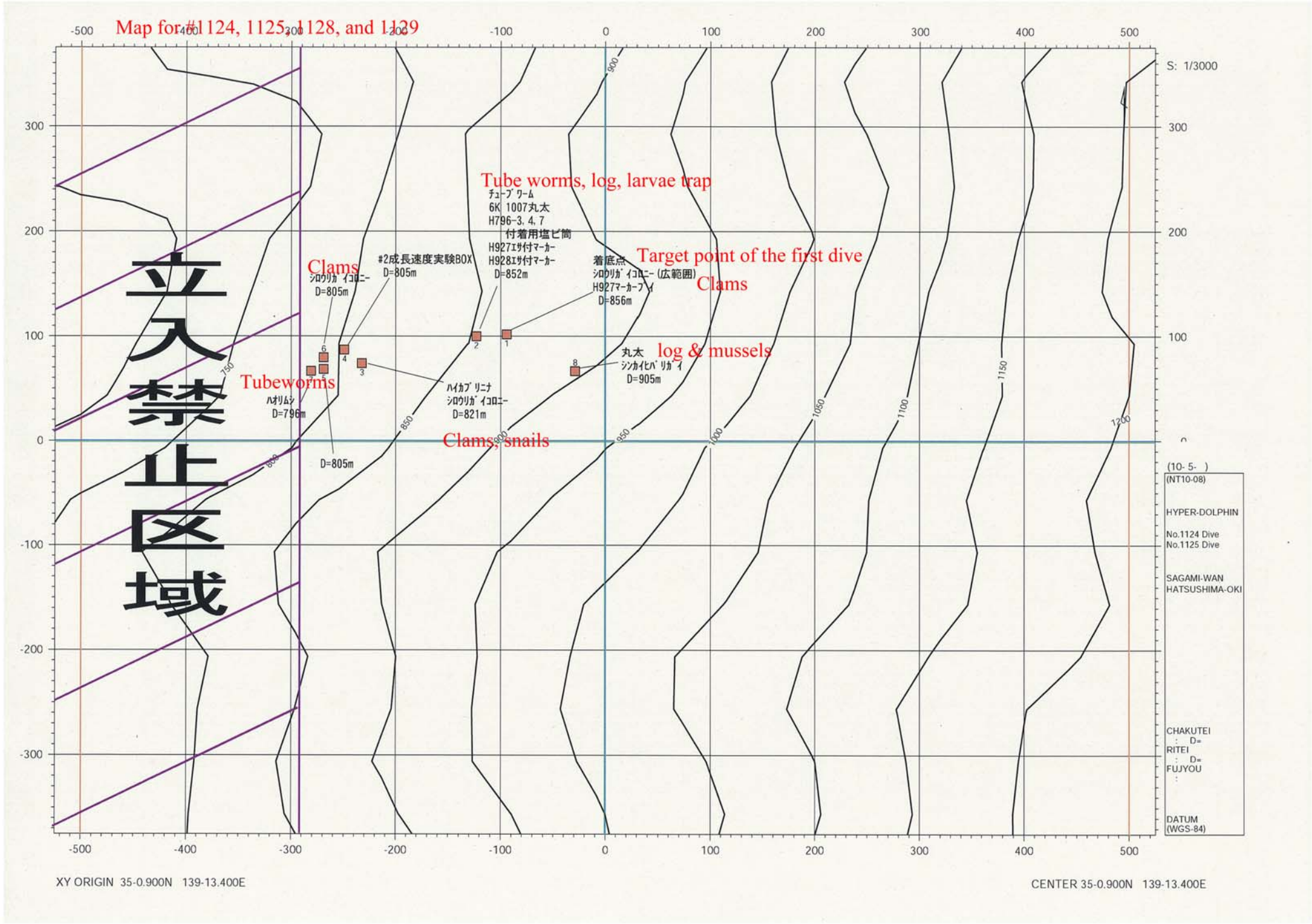
8) Culture study of methanotrophic symbionts of *Bathymodiolus* mussels

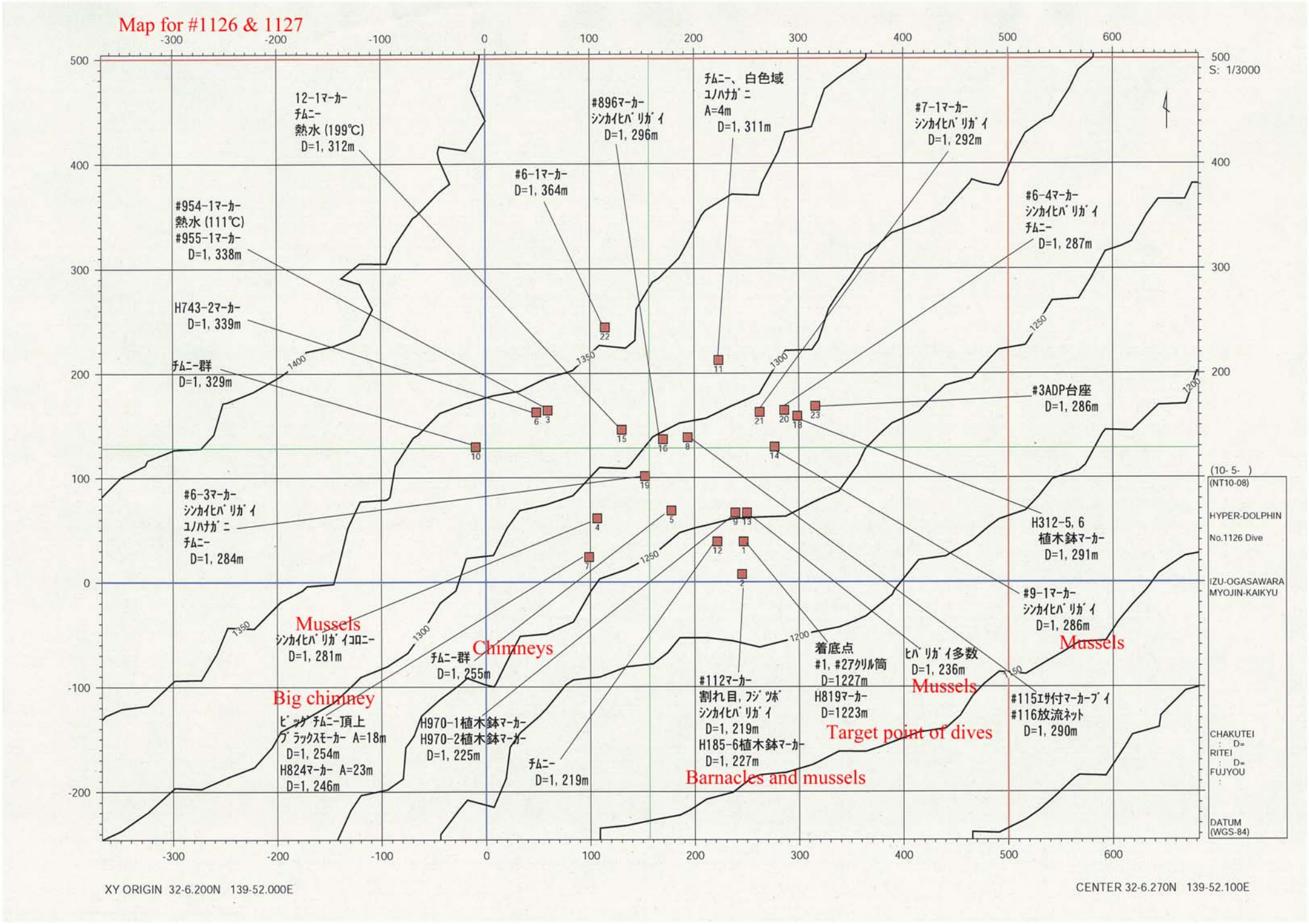
Hisako HIRAYAMA
Japan Agency for Marine-Earth Science and Technology (JAMSTEC)

Methanotrophic symbionts of *Bathymodiolus* mussels have never been cultured independently in the laboratory. This situation doesn't allow us to know the detailed physiology of methanotrophic symbionts and also the optimum conditions of rearing tanks for the mussels. In this study I try to culture methanotrophs from the inocula of fresh gill tissues of *B. japonicus* and *B. platifrons* collected in Sagami Bay cold seeps. In addition, the effect of methane-supply on the maintenance of methanotrophic symbionts within the mussels for a certain period of time will be examined by rearing several individuals with and without methane-supply into the experimental tank.

Appendix

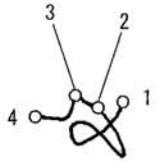
(Point maps and track charts)


Map for #1124, 1125, 1128, and 1129
立入禁止区域
Tube worms, log, larvae trap
チューブワーム
6K 1007丸太
H796-3, 4, 7
付着用塩ビ筒
H927エサ付マーカー
H928エサ付マーカー
D=852m
Target point of the first dive
着底点
シロウリガイコロニー(広範囲)
H927マーカーブイ
D=856m
Clams
Clams
シロウリガイコロニー
D=805m
#2成長速度実験BOX
D=805m
log & mussels
丸太
シンカイヒバリガイ
D=905m
Tubeworms
ハオリムシ
D=796m
ハイカブリニナ
シロウリガイコロニー
D=821m
Clams, snails
D=805m
S: 1/3000
(10- 5-)
(NT10-08)
HYPER-DOLPHIN
No.1124 Dive
No.1125 Dive
SAGAMI-WAN
HATSUSHIMA-OKI
CHAKUTEI
: D=
RITEI
: D=
FUJYOU
:
DATUM
(WGS-84)
XY ORIGIN 35-0.900N 139-13.400E
CENTER 35-0.900N 139-13.400E


Map for #1126 & 1127
12-1マーカー
チムニー
熱水 (199℃)
D=1, 312m
#954-1マーカー
熱水 (111℃)
#955-1マーカー
D=1, 338m
H743-2マーカー
D=1, 339m
チムニー群
D=1, 329m
#6-3マーカー
シンカイヒバリガイ
ユノハナガニ
チムニー
D=1, 284m
#896マーカー
シンカイヒバリガイ
D=1, 296m
#6-1マーカー
D=1, 364m
チムニー、白色域
ユノハナガニ
A=4m
D=1, 311m
#7-1マーカー
シンカイヒバリガイ
D=1, 292m
#6-4マーカー
シンカイヒバリガイ
チムニー
D=1, 287m
#3ADP台座
D=1, 286m
H312-5, 6
植木鉢マーカー
D=1, 291m
#9-1マーカー
シンカイヒバリガイ
D=1, 286m
Mussels
シンカイヒバリガイコロニー
D=1, 281m
Big chimney
ビッグチムニー頂上
ブラックスモーカー A=18m
D=1, 254m
H824マーカー A=23m
D=1, 246m
Chimneys
チムニー群
D=1, 255m
H970-1植木鉢マーカー
H970-2植木鉢マーカー
D=1, 225m
チムニー
D=1, 219m
#112マーカー
割れ目, フジツボ
シンカイヒバリガイ
D=1, 219m
H185-6植木鉢マーカー
D=1, 227m
着底点
#1, #2アクリル筒
D=1227m
H819マーカー
D=1223m
Target point of dives
ヒバリガイ多数
D=1, 236m
Mussels
Barnacles and mussels
#115エサ付マーカーブイ
#116放流ネット
D=1, 290m
Mussels
S: 1/3000
(10- 5-)
(NT10-08)
HYPER-DOLPHIN
No.1126 Dive
IZU-OGASAWARA
MYOJIN-KAIKYU
CHAKUTEI
: D=
RITEI
: D=
FUJYOU
:
DATUM
(WGS-84)
XY ORIGIN 32-6.200N 139-52.000E
CENTER 32-6.270N 139-52.100E

Track chart of #1125

1. 13:52 着底 D=857m — Arrival at the bottom
   (35-00.950N 139-13.339E)
   13:54 ﾆｽｷﾝ採水(#1,#2)
2. 14:17 D=861m ﾊﾞｯｸﾞ採水開始(#1) — Water sampling (Niskin)
   (35-00.948N 139-13.329E)
   14:22 ﾊﾞｯｸﾞ採水終了(#1) — Water sampling (Bag)
   14:32 ｼﾝｶｲﾋﾊﾞﾘｶﾞｲ採集(多数)(#1ｷｬﾆｽﾀｰ) — Mussel sampling
   14:40 ﾊﾞｯｸﾞ採水開始(#2)
   14:44 ﾊﾞｯｸﾞ採水終了(#2) — Water sampling
   14:52 ｼﾝｶｲﾋﾊﾞﾘｶﾞｲ採集(多数)(#2ｷｬﾆｽﾀｰ) — Mussel sampling
   15:07 ﾊﾞｯｸﾞ採水開始(#3)
   15:12 ﾊﾞｯｸﾞ採水終了(#3) — Water sampling
   15:41 ｼﾝｶｲﾋﾊﾞﾘｶﾞｲ採集(多数)(単式ｷｬﾆｽﾀｰ) — Mussel sampling
3. 16:04 D=859m 熊手による採泥及びｼｵｳﾘｶﾞｲ採集(多数) — Clam sampling
   (35-00.952N 139-13.320E)
4. 16:28 D=856m ﾄﾗｯﾌﾟ設置 — Trap set & recovery
   (35-00.945N 139-13.304E)
   16:36 H796-3,4,7付着用塩ﾋﾞ筒回収
   16:40 ﾍｵﾘﾑｼ採集(多数) — Tubeworm sampling
3. 16:45 D=860m ﾍｵﾘﾑｼ採集(多数)
   16:45 離底 D=860m — Left the bottom



35° 00. 90N

139° 13. 40E

0 300m

※ 緯度、経度の1目盛りは、0.1分を示します。

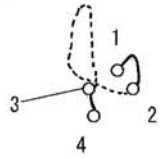
ハイパードルフィン
＃１１２５ ＤＩＶＥ
２０１０年０５月１２日
相模湾 初島沖
縮尺 １／３０００

測 位 D-GPS(Skyfix-XP Trimble SPS751)
測地系 WGS-84 DATUM (世界測地系)
音 速 １４８９．８ｍ／ｓ (D＝９００ｍ)

Track chart of #1126

1. 09:01 着底 D=1240m Arrival at the bottom
   (32-06.248N 139-52.160E)
2. 09:17 D=1244m ケーブル巻取り Cable winding
   (32-06.242N 139-52.166E)
3. 09:34 D=1240m ﾆｽｷﾝ採水(#1) Water sampling (Niskin)
   (32-06.242N 139-52.149E)
4. 09:48 D=1228m ﾊﾞｯｸﾞ採水開始(#1) Water sampling (Bag)
   (32-06.233N 139-52.151E)
   09:54 ﾊﾞｯｸﾞ採水終了(#1)
   10:23 ｼﾝｶｲﾋﾊﾞﾘｶﾞｲ採集(多数)(単式ｷｬﾆｽﾀｰ) Mussel sampling
   10:31 ﾕﾉﾊﾅｶﾞﾆ採集(多数)(#1ｷｬﾆｽﾀｰ) Crab sampling
   10:59 D=1227m 生物付ﾁﾑﾆｰ片採取(2個) Chimney sampling
   10:59 ﾏｰｶｰﾌﾞｲ(番号なし)設置 Set of buoy
   11:05 離底 D=1226m

Left the bottom



32° 06.20N

139° 52.00E

0 300m

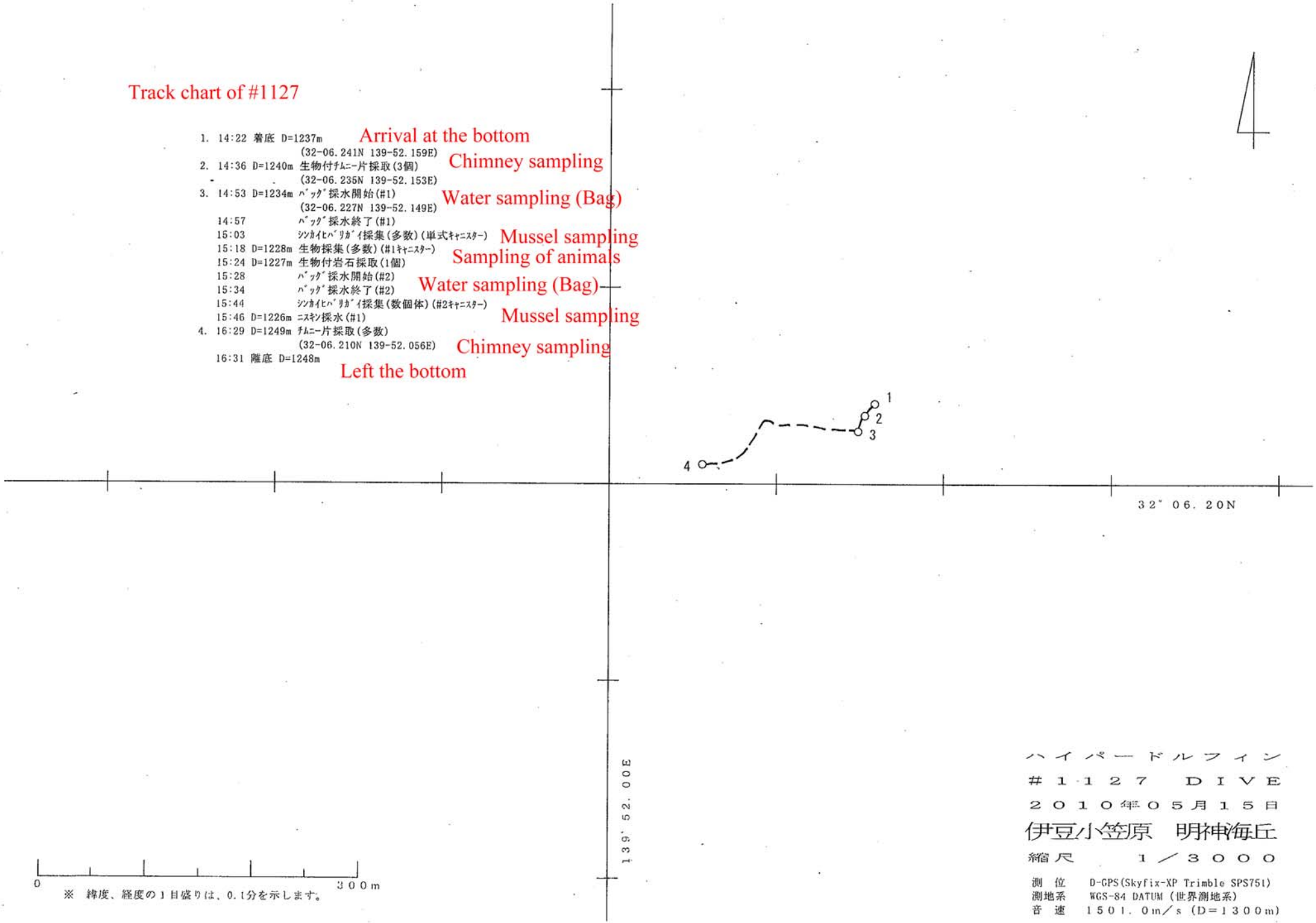
※ 緯度、経度の1目盛りは、0.1分を示します。

ハイパードルフィン
＃1126 DIVE
2010年05月15日
伊豆小笠原 明神海丘
縮尺 1／3000

測 位 D-GPS(Skyfix-XP Trimble SPS751)
測地系 WGS-84 DATUM (世界測地系)
音 速 1501.0m／s (D=1300m)

Track chart of #1127

1. 14:22 着底 D=1237m — Arrival at the bottom
   (32-06.241N 139-52.159E)
2. 14:36 D=1240m 生物付ﾁﾑﾆｰ片採取(3個) — Chimney sampling
   (32-06.235N 139-52.153E)
3. 14:53 D=1234m ﾊﾞｯｸﾞ採水開始(#1) — Water sampling (Bag)
   (32-06.227N 139-52.149E)
   14:57 ﾊﾞｯｸﾞ採水終了(#1)
   15:03 ｼﾝｶｲﾋﾊﾞﾘｶﾞｲ採集(多数)(単式ｷｬﾆｽﾀｰ) — Mussel sampling
   15:18 D=1228m 生物採集(多数)(#1ｷｬﾆｽﾀｰ) — Sampling of animals
   15:24 D=1227m 生物付岩石採取(1個)
   15:28 ﾊﾞｯｸﾞ採水開始(#2) — Water sampling (Bag)
   15:34 ﾊﾞｯｸﾞ採水終了(#2)
   15:44 ｼﾝｶｲﾋﾊﾞﾘｶﾞｲ採集(数個体)(#2ｷｬﾆｽﾀｰ) — Mussel sampling
   15:46 D=1226m ﾆｽｷﾝ採水(#1)
4. 16:29 D=1249m ﾁﾑﾆｰ片採取(多数)
   (32-06.210N 139-52.056E) — Chimney sampling
   16:31 離底 D=1248m — Left the bottom



0 300m

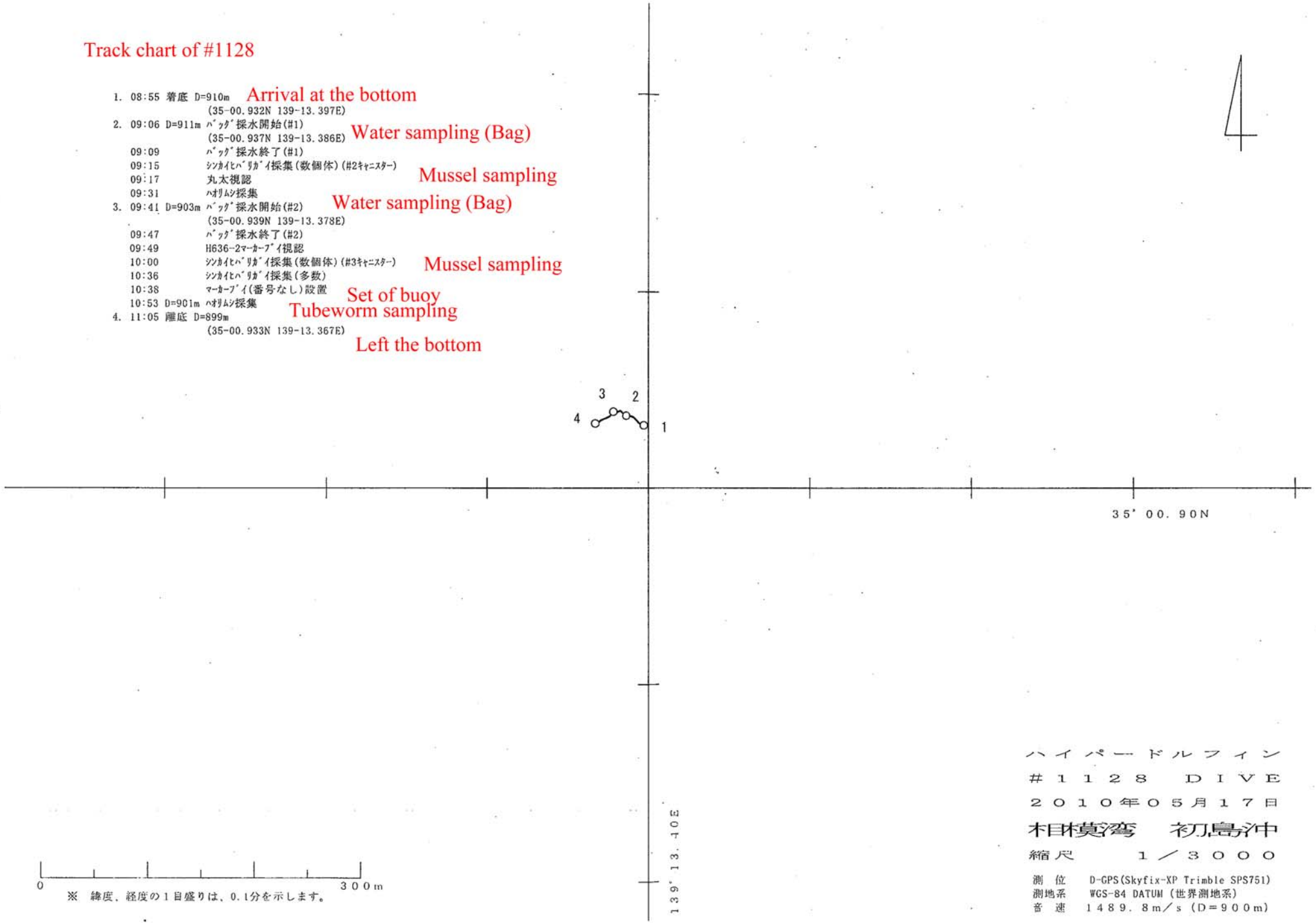
※ 緯度、経度の1目盛りは、0.1分を示します。

ハイパードルフィン
#1127 DIVE
2010年05月15日
伊豆小笠原 明神海丘
縮尺 1／3000

測 位 D-GPS(Skyfix-XP Trimble SPS751)
測地系 WGS-84 DATUM (世界測地系)
音 速 1501.0m／s (D=1300m)

Track chart of #1128

1. 08:55 着底 D=910m — Arrival at the bottom
   (35-00.932N 139-13.397E)
2. 09:06 D=911m ﾊﾞｯｸﾞ採水開始(#1) — Water sampling (Bag)
   (35-00.937N 139-13.386E)
   09:09 ﾊﾞｯｸﾞ採水終了(#1)
   09:15 ｼﾝｶｲﾋﾊﾞﾘｶﾞｲ採集(数個体)(#2ｷｬﾆｽﾀｰ) — Mussel sampling
   09:17 丸太視認
   09:31 ﾊｵﾘﾑｼ採集
3. 09:41 D=903m ﾊﾞｯｸﾞ採水開始(#2) — Water sampling (Bag)
   (35-00.939N 139-13.378E)
   09:47 ﾊﾞｯｸﾞ採水終了(#2)
   09:49 H636-2ﾏｰｶｰﾌﾞｲ視認
   10:00 ｼﾝｶｲﾋﾊﾞﾘｶﾞｲ採集(数個体)(#3ｷｬﾆｽﾀｰ) — Mussel sampling
   10:36 ｼﾝｶｲﾋﾊﾞﾘｶﾞｲ採集(多数)
   10:38 ﾏｰｶｰﾌﾞｲ(番号なし)設置 — Set of buoy
   10:53 D=901m ﾊｵﾘﾑｼ採集 — Tubeworm sampling
4. 11:05 離底 D=899m — Left the bottom
   (35-00.933N 139-13.367E)

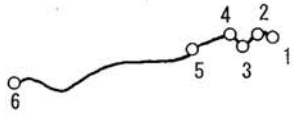


※ 緯度、経度の1目盛りは、0.1分を示します。

ハイパードルフィン
#1128 DIVE
2010年05月17日
相模湾 初島沖
縮尺 1／3000

測 位 D-GPS(Skyfix-XP Trimble SPS751)
測地系 WGS-84 DATUM (世界測地系)
音 速 1489.8m／s (D=900m)

Track chart of #1129



1. 13:55 着底 D=858m — Arrival at the bottom
   (35-00.951N 139-13.337E)
2. 13:57 D=858m H927ﾏｰｶｰﾌﾞｲ視認
   (35-00.952N 139-13.331E)
3. 14:22 D=861m 生物採集(多数)(単式ｷｬﾆｽﾀｰ) — Animal sampling
   (35-00.948N 139-13.325E)
4. 14:31 D=858m ﾊﾞｯｸﾞ採水開始(#1)
   (35-00.952N 139-13.320E) — Water sampling (Bag)
   14:35 ﾊﾞｯｸﾞ採水終了(#1)
   14:41 ｼﾝｶｲﾋﾊﾞﾘｶﾞｲ採集(数個体)(#2ｷｬﾆｽﾀｰ) — Mussel sampling
   14:48 ﾊﾞｯｸﾞ採水開始(#2)
   14:52 ﾊﾞｯｸﾞ採水終了(#2) — Water sampling (Bag)
   15:10 ｼｮｳﾘｶﾞｲ採集(多数)
   15:15 ﾏｰｶｰﾌﾞｲ(番号なし)設置 — Clam sampling
5. 15:23 D=856m ｹﾞﾝｹﾞ採集(1個体)(#3ｷｬﾆｽﾀｰ) — Fish sampling
   (35-00.947N 139-13.305E)
   15:26 ﾄﾗｯﾌﾟ回収
   15:31 生物採集(多数)(#4ｷｬﾆｽﾀｰ)
   15:39 ﾍｵﾘﾑｼ採集(多数) — Tubeworm sampling
6. 16:12 D=816m MBARI採泥(緑・1本)
   (35-00.936N 139-13.234E)
   16:12 ｺﾞﾐ袋採取(#5ｷｬﾆｽﾀｰ) — MBARI core sampling
   16:19 MBARI採泥(赤・1本)
   16:20 離底 D=816m — Left the bottom

35° 00.90N

139° 13.40E

0 — 300m

※ 緯度、経度の1目盛りは、0.1分を示します。

ハイパードルフィン
#1129 DIVE
2010年05月17日
相模湾 初島沖
縮尺 1／3000

測 位 D-GPS(Skyfix-XP Trimble SPS751)
測地系 WGS-84 DATUM (世界測地系)
音 速 1489.8m／s (D=900m)