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RV Natsushima Cruise Report

NT13-07

Hyper-Dolphin Dive Research

Off Hatsushima, Sagami Bay and Off Boso

April, 2th-10th, 2013

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

Contents

Acknowledgements

We are grateful to Captain Mr. Eiko Ukekura, Chief Officer Mr. Akihisa Tsuji and Chief Engineer Mr. Hiroyasu Shibata for their safe navigation and their skillful handling of “R/V Natsushima”. Great thanks are due to Commander Mr. H. Wakamatsu and “Hyper-Dolphin” operation team for their operations in sampling. We also thank Ms Satomi Minamizawa, Nippon Marine Enterprises, Ltd., for her attentive supports. Finally, we would like to appreciate all the person who supported directly or indirectly this cruise.

1. Cruise Information

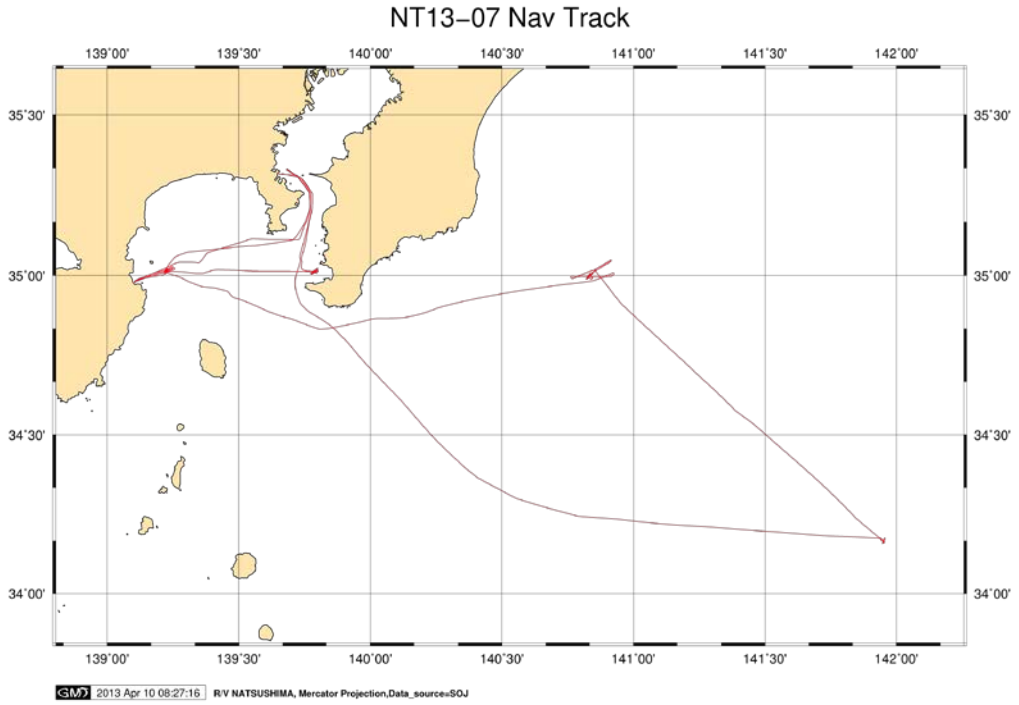
1) Cruise ID, Name of Vessel: NT13-07 R/V Natsushima
2) Title of the Cruise: “Hyper-Dolphin Research Dive, Deep-sea Research, FY2013
3) Title of Proposal:

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

II) Investigating habitat environments of *Calyptogena* spp., and sampling the bivalves for development of a rearing system for invertabrates in chemosynthetic ecosystem.

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

4) Cruise Period: April 2, 2013 ~ April 10, 2013
5) Port Call: from JAMSTEC (April 2, 2013) to JAMSTEC (April 10, 2013)
6) Research Area: Off Hatsushima, Sagami Bay and Off Boso



Cruise track of R/V Natsushima (NT13-07)

7) Cruise Log (JST)

Date	Local Time	Note	Position/Weather/Wind/Sea condition
2.Apr.13		**Sail out, proceeding to research area**	4/2 12:00(UTC+9h)
	9:00	Boarded.	35-06.6N,139-35.4E
	10:00	Let go all shore line,left YOKOSUKA.	Rain
	10:05-10:35	Scientific meeting.	North-4(Moderate breeze)
	13:00-14:00	On board education for scientists.	3(Sea slight)
	13:30	Arrived at research area (off HATSUSHIMA).	1(Low Swell short)
	13:41	Released XBT.	Visibly:2
	14:40	Let go anchor at off ITO.	
	16:40	Konpira ceremony.	
	18:30-18:40	Scientific meeting.	
3.Apr.13		**HPD1508_Off HATSUSHIMA**	4/3 12:00(UTC+9h)
	6:00	Heaving anchor,then com'ced proceeding to dive point.	35-00.9N,139-13.4E
	7:00	Arrived at dive point.	Rain
	8:22	HPD dove & started her operation#1508.	WNW-5(Fresh breeze)
	8:57	HPD landed on the sea bottom.(D=876m)	3(Sea slight)
	16:13	HPD left the sea bottom.(D=850m)	1(Low Swell short)
	16:56	Recovered HPD & finished the operation.	Visibly:3
	18:00	Let go anchor at off ITO.	
	18:30-18:35	Scientific meeting.	
4.Apr.13		**HPD1509_Off HATSUSHIMA**	4/4 12:00(UTC+9h)
	6:00	Heaving anchor,then com'ced proceeding to dive point.	35-01.0N,139-13.4E
	7:00	Arrived at dive point.	Fine but cloudy
	8:29	HPD dove & started her operation#1509.	SE-3(Gentle breeze)
	9:07	HPD landed on the sea bottom.(D=903m)	2(Sea smooth)
	16:04	HPD left the sea bottom.(D=909m)	1(Low Swell short)
	16:47	Recovered HPD & finished the operation.	Visibly:8
	17:00	Proceeded to research area (off BOSO).	
	18:30-18:40	Scientific meeting.	
5.Apr.13		**Deploy BBOBST-NX, HPD1510 dive, and deploy NUDOBS at OFFBOSO**	4/5 12:00(UTC+9h)
	0:10	Arrived at research area (off BOSO).	34-59.7N,140-50.0E
	0:16	Released XBT.	Rain
	5:59	Deployed BBOBST-NX. (@ 34-59.6392N, 140-49.8226E)	SE-4(Moderate breeze)
	6:16-6:41	Carried out BBOBST-NX calibration (34-59.7871N, 140-50.0164E, D=1372m).	4(Sea Moderate)
	7:42	HPD dove & started her operation#1510.	4(Moderate average)
	11:18	HPD landed on the sea bottom.(D=1364m)	Visibly:5
	12:37	HPD left the sea bottom.(D=1364m)	
	13:36	Recovered HPD & finished the operation.	
	13:45	Commenced proceeding to triple junction of off BOSO	
	18:30-18:35	Scientific meeting.	
	21:00	Arrived at above area	
	21:23	Deployed NUDOBS. (@ 34-09.9819N, 141-56.9767E)	
	23:23	Started to NUDOBS calibration.	
6.Apr.13		**Proceeding to research area**	4/6 12:00(UTC+9h)
	0:07	Finished NUDOBS calibration (34-10.2219N, 141-56.9360E, D=8920m).	35-03.0N,139-44.3E
	0:15	Commenced proceeding to SAGAMI WAN off HATSUSHIMA.	Cloudy
	8:00	Commenced proceeding to YOKOSUKA due to rough sea condition.	SSE-6(Strong breeze)
	14:00	Let go anchor at off YOKOSUKA	4(Sea Moderate)
	18:30-18:35	Scientific meeting.	1(Low Swell short)
			Visibly:6
7.Apr.13		**Proceeding to research area**	4/7 12:00(UTC+9h)
	18:30-18:35	Scientific meeting.	35-19.7N,139-41.1E
			Fine but cloudy
			SW-8(Gale)
			5(Sea Rough)
			1(Low Swell short)
			Visibly:6
8.Apr.13		**HPD1511_Off HATSUSHIMA**	4/8 12:00(UTC+9h)
	6:00	Heaving anchor,then commenced proceeding to research area.	35-00.9N,139-13.4E
	9:30	Arrived at research are(off HATSUSHIMA).	Fine but cloudy
	9:33	Released XBT.	SSE-4(Moderate breeze)
	10:22	HPD dove & started her operation#1511.	3(Sea slight)
	11:00	HPD landed on the sea bottom.(D=978m)	3(Moderate short)
	17:06	HPD left the sea bottom.(D=852m)	Visibly:8
	17:49	Recovered HPD & finished the operation.	
	18:30-18:35	Scientific meeting.	
	18:45	Let go anchor at off ITO.	
9.Apr.13		**HPD1512_Off HATSUSHIMA, and proceeding to YOKOSUKA**	4/9 12:00(UTC+9h)
	6:00	Heaving anchor,then com'ced proceeding to dive point.	35-00.9N,139-13.4E
	7:00	Arrived at dive point.	Cloudy
	8:28	HPD dove & started her operation#1512.	SW-6(Strong breeze)
	9:07	HPD landed on the sea bottom.(D=967m)	4(Sea moderate)
	16:31	HPD left the sea bottom.(D=809m)	3(Moderate short)
	17:10	Recovered HPD & finished the operation.	Visibly:7
	17:30	Left research area for YOKOSUKA.	
10.Apr.13		**Arrived at YOKOSUKA**	
	9:00	Arrived at YOKOSUKA.	
	10:00	Scientists disembark from NATSUSHIMA	
		Finished NT13-07 cruise	

2. Participants List

Scientists

Principal Investigator

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Onboard Scientists

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Marine Technician

S. Minamizawa	Nippon Marine Enterprises, LTD.

Hyper-dolphin operation team

H. Wakamatsu	Operation Manager
T. Kondo	1st ROV Operator
K. Chiba	2nd ROV Operator
Y. Chida	2nd ROV Operator
S. Kikuya	2nd ROV Operator
R. Saigo	2nd ROV Operator
R. Asai	2nd ROV Operator

R/V YOKOSUKA Officers and Crew

E. Ukekura	Captain
A. Tsuji	Chief Officer
I. Maeda	2nd Officer
T. Shishikura	Jr. 2nd Officer
H. Omae	3rd Officer
H. Shibata	Chief Engineer
N. Tadooka	1st Engineer
M. Murakami	2nd Engineer
N. Uemura	3rd Engineer
M. Takahashi	Chief Radio Operator
H. Ishiwata	2nd Radio Operator
T. Takakuwa	3rd Radio Operator

Y. Kyuki	Boat Swain
Y. Fujii	Able Seaman
K. Ogasawara	Able Seaman
T. Chimoto	Able Seaman
N. Ishizuka	Able Seaman
Y. Ogawa	Sailor
K. Kanda	Sailor
J. Mori	No.1 Oiler
T. Chino	Oiler
M. Tanaka	Oiler
K. Aizawa	Oiler
K. Taniguchi	Oiler
S. Sasaki	Chief Steward
Y. Chikuba	Steward
H. Ohba	Steward
A. Saito	Steward
K. Kawase	Steward

Member of onboard science party

3. Overview of the cruise

In this cruise, three research groups participated to this cruise. As the total number of scientists who wanted to join the cruise was more than the number of the bed of R/V Natsushima, we divided the cruise into two research area; first one is to Off Hatsushima, Sagami Bay and the other one to Off Bousou. The research subjects of the three groups were environmental, physiological, biochemical, or molecular-biological studies of cold seep-specific invertebrates and development of new type seismometers (see Section 5), and thus we spent most time to collect samples, e.g., deep-sea mussels, vesicomyid clams, sediments, and so on. We deployed two new type seismometers. Some samples are also used for short-term rearing experiments. Other samples were kept alive and brought back to JAMSTEC and Enoshima Aquarium for rearing experiments. Detailed analyses of genes, amino acids, proteins and enzymes will be performed after the cruise. The preliminary reports of each group are in the section 5.

4. Dive report

1) Summary of the Hyper-dolphin Dive #1508

Date: April. 3, 2013
Site: Off Hatsushima, Sagami Bay
Landing: 35°00.956'N, 139°13.357'E, 876m (8:57)
Leaving: 35°00.954'N, 139°13.284'E, 850m (16:13)

Purpose:
Collection of *Calyptogena* clam's eggs

Payload Equipment:
Incubation box x1
Egg collection sampler with hand pomp
Suction sampler (multiple canister & single canister)
Scoop sampler x1
Sample box x1
Cases for Nomai-type core x4

Dive Summary

‡ Retrieved four Nomai-type cores (NT13-06, HPD#1503) in *Calyptogena* colony
The sampling site location 35°00.965'N, 139°13.324'E, 857m

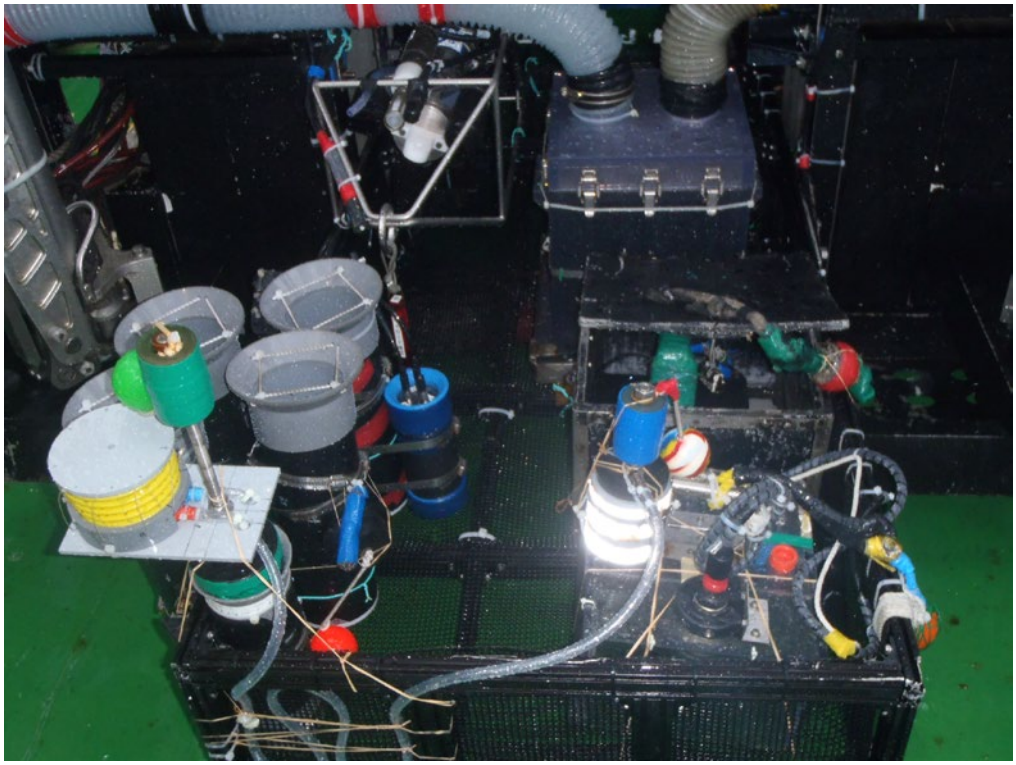
‡ Sampling of *Calyptogena* clams with a scoop sampler.
The sampling site location 35°00.965'N, 139°13.324'E, 857m

‡ Sampling of *Bathymodiolus* bivalves with suction samplers.
The sampling site location 35°00.965'N, 139°13.324'E, 857m

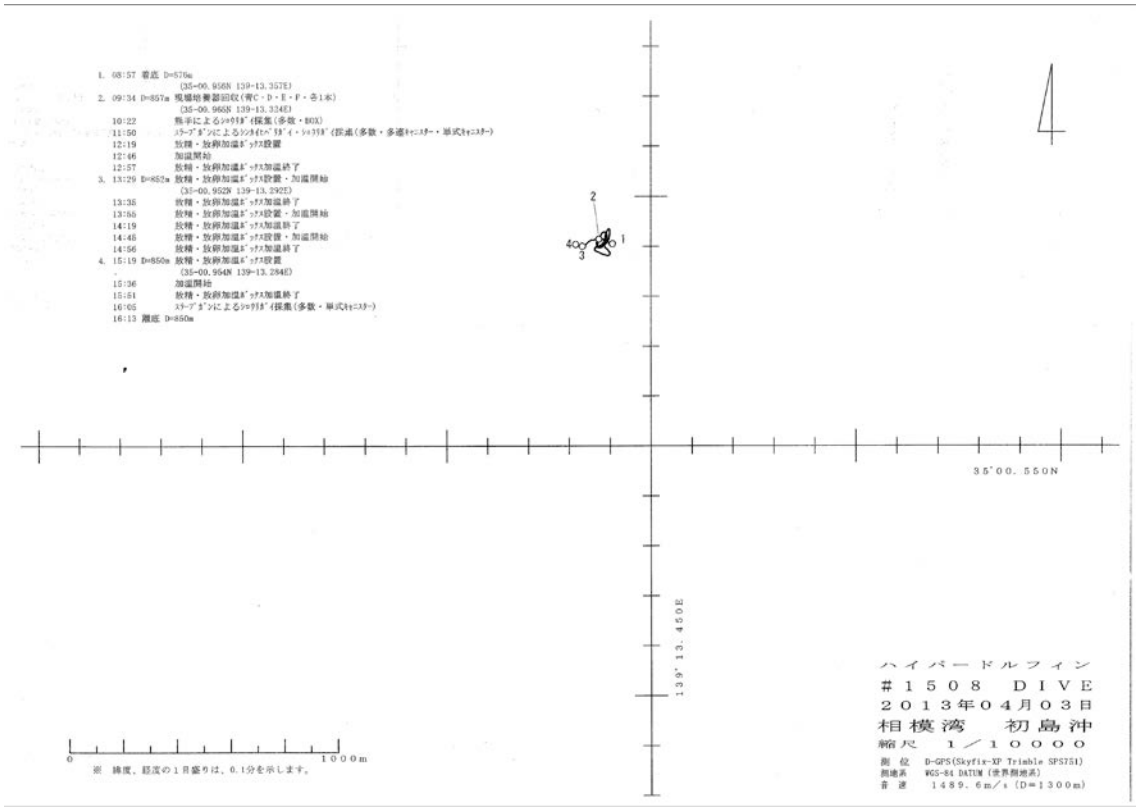
‡ Incubation of *Calyptogena* colonies with an incubation box.
The site location 35°00.952'N, 139°13.292'E, 852m

‡ Incubation of *Calyptogena* colonies with an incubation box.
The site location 35°00.954’N, 139°13.284’E, 850m

‡Sampling of *Calyptogena* clams with a suction sampler.
The sampling site location 35°00.954’N, 139°13.284’E, 850m



Payload of HPD#1508



Dive track and event list of HPD#1508

2) Summary of the Hyper Dolphin Dive #1509

Date: Apr. 4, 2013

Site: Off Hatsushima Island, Suruga Bay

Purpose: Investigating habitat condition of *Calyptogena* spp. and sampling deep-sea invertabrates

Landing: 9:07 35°00.934N, 139°13.418E, 930 m

Leaving: 16:04 35°00.936N, 139°13.380E, 909 m

Payload Equipment:

Suction sampler (multi canister):	1
Single canister	1
Scoop sampler:	1
Sample box:	1
MT core:	3
H core:	2
D-POTE system:	1
ISFEET-pH sensor system:	2
CTD Sea-bird 19 plus V2:	1

Dive Summary

‡Sampling Bathymodiolus spp. clams by slurp gun

‡Detecting H_2S by D-POTE system

The site located 35°00’937N, 139°13’379E, 903 m

‡Detecting H_2S by D-POTE system

‡Sampling *Calyptogena* spp. by slurp gun

The site located 35°00’935N, 139°13’376E, 901 m

‡Detecting H_2S by D-POTE system

The site located 35°00’941N, 139°13’375E, 901 m

‡Detecting H_2S by D-POTE system

‡Sampling sediment by MT core (Green)

The site located 35° 00.957N, 139° 13.336E, 857 m

‡Detecting H_2S by D-POTE system
The site located 35° 00.953N, 139° 13.329E, 856 m

‡Detecting H_2S by D-POTE system
‡Sampling sediment by MT core (Red)
The site located 35° 00.960N, 139° 13.322E, 849 m

‡Detecting H_2S by D-POTE system
‡Sampling sediment by H core (Blue)
The site located 35° 00.942N, 139° 13.290E, 849 m

‡Detecting H_2S by D-POTE system
The site located 35° 00.940N, 139° 13.284E, 846 m

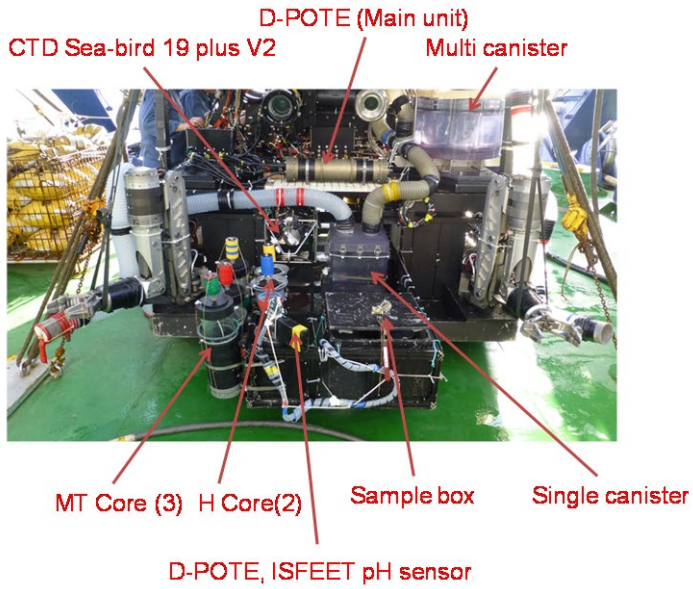
‡Detecting H_2S by D-POTE system
‡Sampling sediment by MT core (Blue and Yarrow)
The site located 35° 00.939N, 139° 13.253E, 829 m

‡Detecting H_2S by D-POTE system
The site located 35° 00.948N, 139° 13.222E, 803 m

‡Detecting H_2S by D-POTE system
‡Sampling sediment by H core (Yarrow)
The site located 35° 00.963N, 139° 13.237E, 806 m

‡Sampling Bathymodiolus spp. clams by slurp gun
‡Sampling a rock
‡Sampling tube worm
‡Sampling *Myciniae* gen. et sp.
‡Sampling *Calyptogena* spp. by slupe gun
The site located 35° 00.936N, 139° 13.280E , 909 m

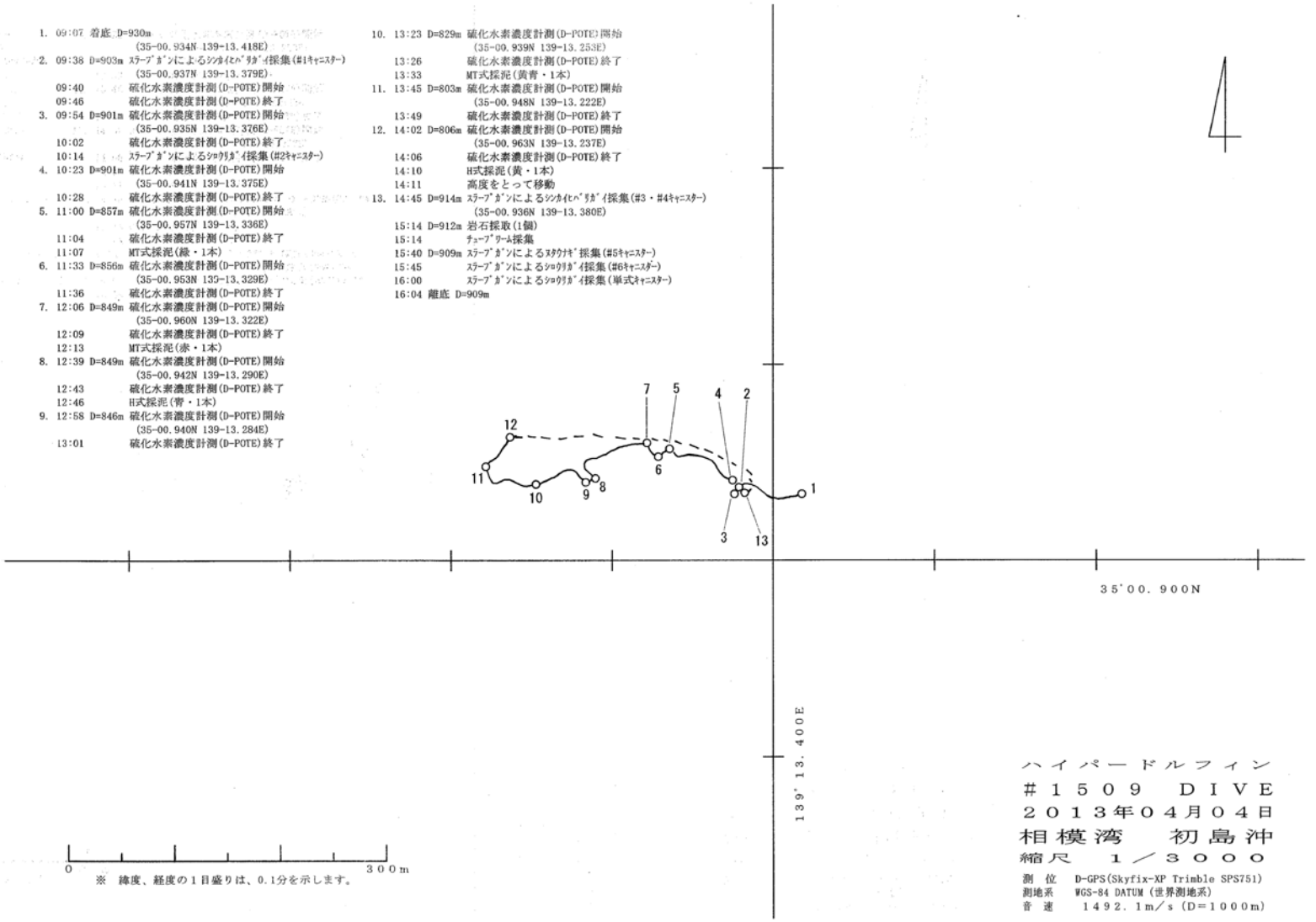
Payload HPD#1509



Payload of dive HPD#1509

Dive track and event list of HPD#1509

1. 09:07 着底 D=930m
(35-00.934N 139-13.418E)
2. 09:38 D=903m ｽﾃｰﾌﾞｶﾞﾝによるｼﾝｶｲﾋﾍﾞﾘｶﾞｲ採集(#1ｷｬﾆｽﾀｰ)
(35-00.937N 139-13.379E)
09:40 硫化水素濃度計測(D-POTE)開始
09:46 硫化水素濃度計測(D-POTE)終了
3. 09:54 D=901m 硫化水素濃度計測(D-POTE)開始
(35-00.935N 139-13.376E)
10:02 硫化水素濃度計測(D-POTE)終了
10:14 ｽﾃｰﾌﾞｶﾞﾝによるｼｮｳｸﾘｶﾞｲ採集(#2ｷｬﾆｽﾀｰ)
4. 10:23 D=901m 硫化水素濃度計測(D-POTE)開始
(35-00.941N 139-13.375E)
10:28 硫化水素濃度計測(D-POTE)終了
5. 11:00 D=857m 硫化水素濃度計測(D-POTE)開始
(35-00.957N 139-13.336E)
11:04 硫化水素濃度計測(D-POTE)終了
11:07 MT式採泥(緑・1本)
6. 11:33 D=856m 硫化水素濃度計測(D-POTE)開始
(35-00.953N 139-13.329E)
11:36 硫化水素濃度計測(D-POTE)終了
7. 12:06 D=849m 硫化水素濃度計測(D-POTE)開始
(35-00.960N 139-13.322E)
12:09 硫化水素濃度計測(D-POTE)終了
12:13 MT式採泥(赤・1本)
8. 12:39 D=849m 硫化水素濃度計測(D-POTE)開始
(35-00.942N 139-13.290E)
12:43 硫化水素濃度計測(D-POTE)終了
12:46 H式採泥(青・1本)
9. 12:58 D=846m 硫化水素濃度計測(D-POTE)開始
(35-00.940N 139-13.284E)
13:01 硫化水素濃度計測(D-POTE)終了
10. 13:23 D=829m 硫化水素濃度計測(D-POTE)開始
(35-00.939N 139-13.253E)
13:26 硫化水素濃度計測(D-POTE)終了
13:33 MT式採泥(黄青・1本)
11. 13:45 D=803m 硫化水素濃度計測(D-POTE)開始
(35-00.948N 139-13.222E)
13:49 硫化水素濃度計測(D-POTE)終了
12. 14:02 D=806m 硫化水素濃度計測(D-POTE)開始
(35-00.963N 139-13.237E)
14:06 硫化水素濃度計測(D-POTE)終了
14:10 H式採泥(黄・1本)
14:11 高度をとって移動
13. 14:45 D=914m ｽﾃｰﾌﾞｶﾞﾝによるｼﾝｶｲﾋﾍﾞﾘｶﾞｲ採集(#3・#4ｷｬﾆｽﾀｰ)
(35-00.936N 139-13.380E)
15:14 D=912m 岩石採取(1個)
15:14 ﾁｭｰﾌﾞﾜｰﾑ採集
15:40 D=909m ｽﾃｰﾌﾞｶﾞﾝによるﾇﾀｸﾅｷﾞ採集(#5ｷｬﾆｽﾀｰ)
15:45 ｽﾃｰﾌﾞｶﾞﾝによるｼｮｳｸﾘｶﾞｲ採集(#6ｷｬﾆｽﾀｰ)
16:00 ｽﾃｰﾌﾞｶﾞﾝによるｼｮｳｸﾘｶﾞｲ採集(単式ｷｬﾆｽﾀｰ)
16:04 離底 D=909m



3) Summary of the Hyper Dolphin Dive #1510

Date: April 05, 2013
Site: Eastern Off Bousou Peninsula
Landing: 34°59.832'N, 140°50.013'E, 1366m (11:18)
Leaving: 34°59.783'N, 140°50.010'E, 1364m (12:37)

Purpose:
Deployment of the BBOBST-NX and the OBDC for long-term tilt measurement

Payload Equipment:
The OBDC (ocean bottom Doppler current profiler)
Remote commander for the SI2 acoustic transponder

Dive Summary

All events were at the position of the BBOBST-NX landed;
34° 59.7872' N, 140° 50.0164' E, 1364 m.

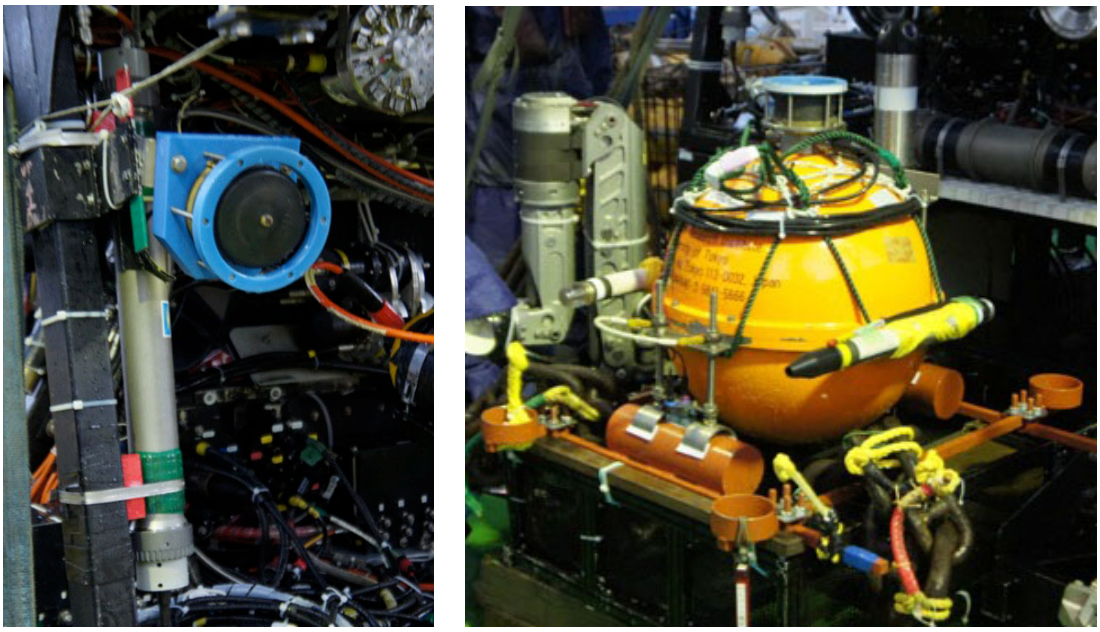
‡ Unloaded the OBDC at the SW side, 9 m from the BBOBST-NX.

‡ Untied the recording unit with the sensor unit, then moved it about 3 m away, NE side.

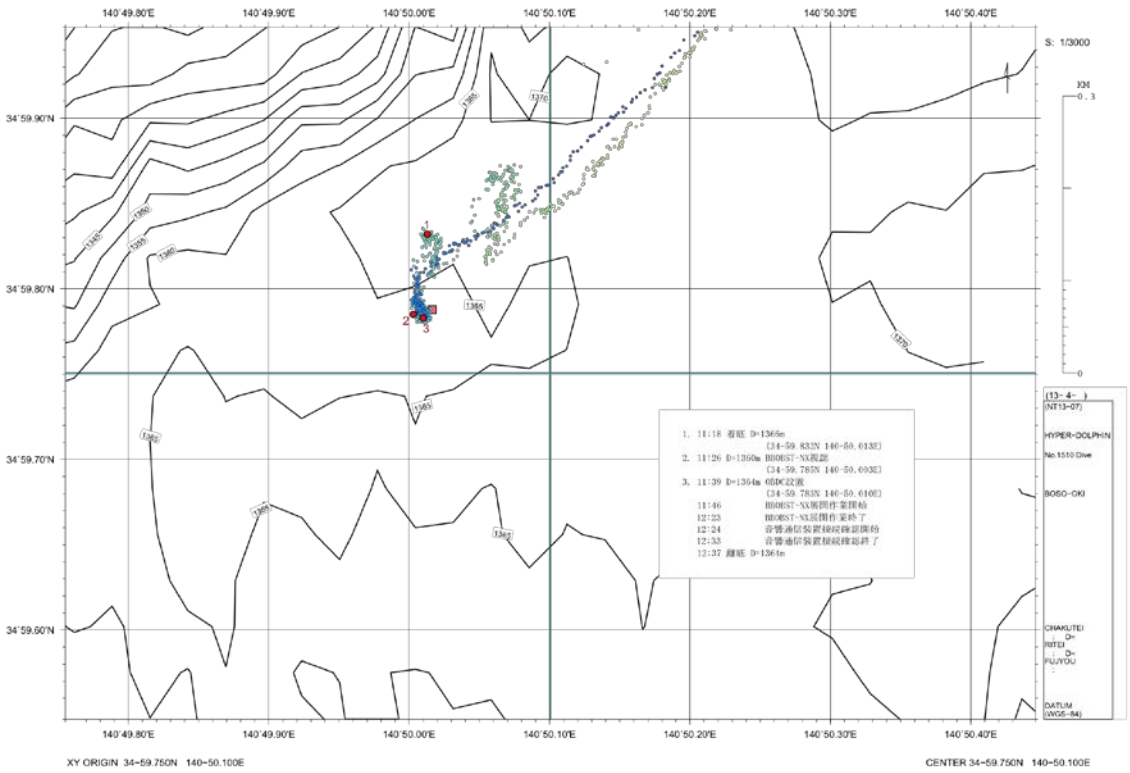
‡ Recovered three additional weights from the top of each component of the sensor unit.

‡ Checked tilt of the sensor and started the observation by using the acoustic transponder commander attached on the HPD.

‡ Took photographs of all instruments deployed.



Payloads of HPD#1510 (left: transponder controller, right: OBDC)



Dive track and event list of HPD#1510

4) Summary of the Hyper-dolphin Dive #1511

Date: April. 8, 2013
Site: Off Hatsushima, Sagami Bay

Landing: 35°00.913'N, 139°13.463'E, 978m (11:00)
Leaving: 35°00.939'N, 139°13.299'E, 852m (17:06)

Purpose:
Collection of *Calyptogena* clam's eggs

Payload Equipment:
Incubation box x1
Egg collection sampler with hand pomp
Suction sampler (multiple canister & single canister)
Scoop sampler x1
Sample box x1
Cases for Nomai-type core x2
Nomai-type core x1
D-port sensor

Dive Summary

‡ Sampling of fishes, shrimps and *Bathymodiolus* bivalves with suction samplers.
The sampling site location 35°00.939'N, 139°13.299'E, 852m

‡ Measuring hydrogen sulfide concentration by D-port sensor.
The site location 35°00.939'N, 139°13.299'E, 852m

‡ Retrieved two Nomai-type cores (NT13-06, HPD#1499)
The sampling site location 35°00.951'N, 139°13.313'E, 852m

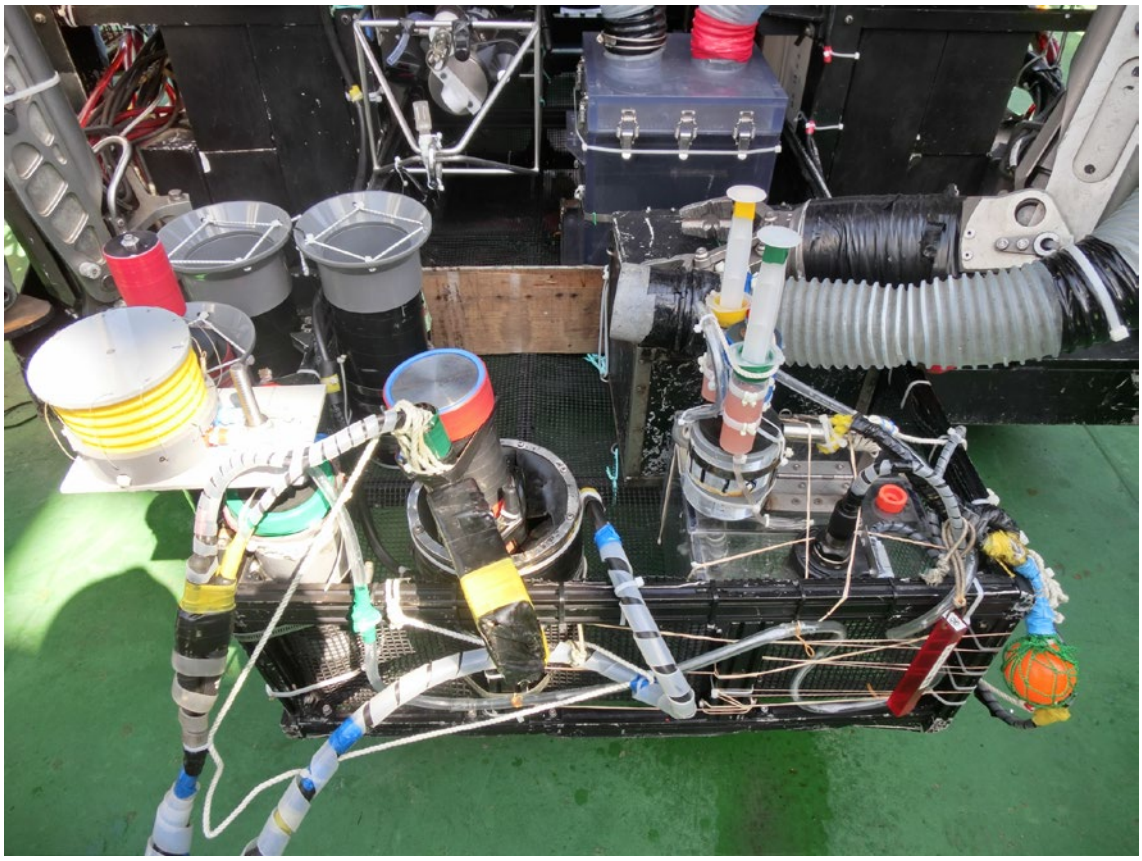
‡ Sampling of *Calyptogena* clams with a scoop sampler.

The sampling site location 35°00.947’N, 139°13.324’E, 857m

‡ Measuring hydrogen sulfide concentration by D-port sensor.
The site location 35°00.939’N, 139°13.299’E, 852m

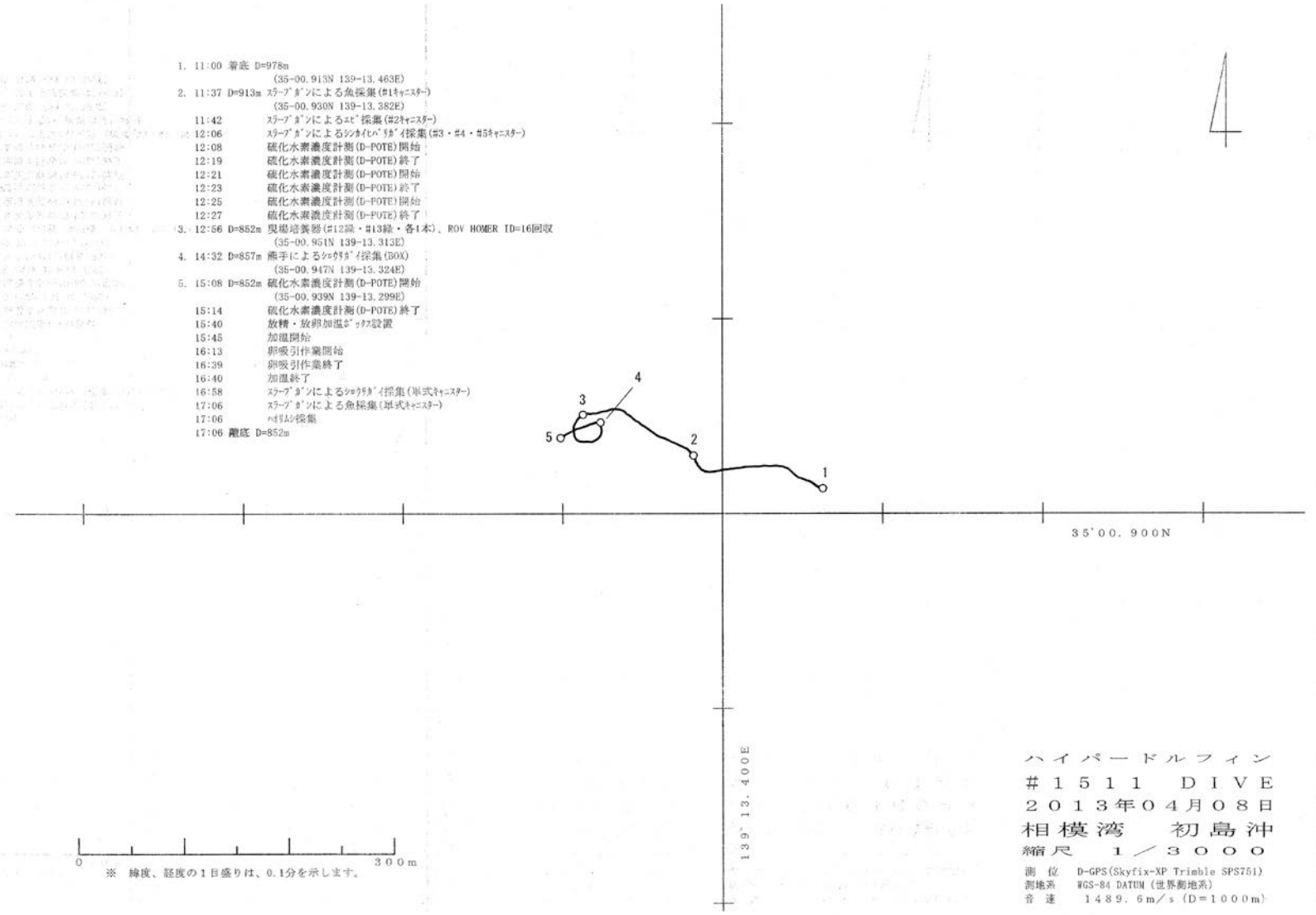
‡ Incubation of *Calyptogena* colonies with an incubation box.
The site location 35°00.939’N, 139°13.299’E, 852m

‡Sampling of *Calyptogena* clams, fishes and tube worms with a suction sampler.
The sampling site location 35°00.954’N, 139°13.284’E, 850m



Payload of HPD#1511

Dive track and event list of HPD#1511



5) Summary of the HPD Dive #1512

Date: April. 9, 2013
Site: Off Hatsushima, Sagami Bay

Landing: 35°00.907'N, 139°13.412'E, 967m (11:00)
Leaving: 35°00.938'N, 139°13.227'E, 809m (16:31)

Purpose:
Collection of *Calyptogena* clam's eggs

Payload Equipment:
Incubation box x1
Egg collection sampler with hand pomp
Suction sampler (multiple canister & single canister)
Scoop sampler x1
Sample box x1
MT cores x2
Nomai-type core x1
D-port sensor

Dive Summary

‡ Sampling of bacterial mat and shrimps with suction samplers.
The sampling site location 35°00.919'N, 139°13.403'E, 950m

‡ Measuring hydrogen sulfide concentration by D-port sensor.
The site location 35°00.919'N, 139°13.403'E, 950m

‡ Sampling of *Calyptogena* clams with suction samplers.
The sampling site location 35°00.927'N, 139°13.380'E, 924m

‡ Sampling of *Calyptogena* clams with a suction sampler.

The sampling site location 35°00.934’N, 139°13.375’E, 912m

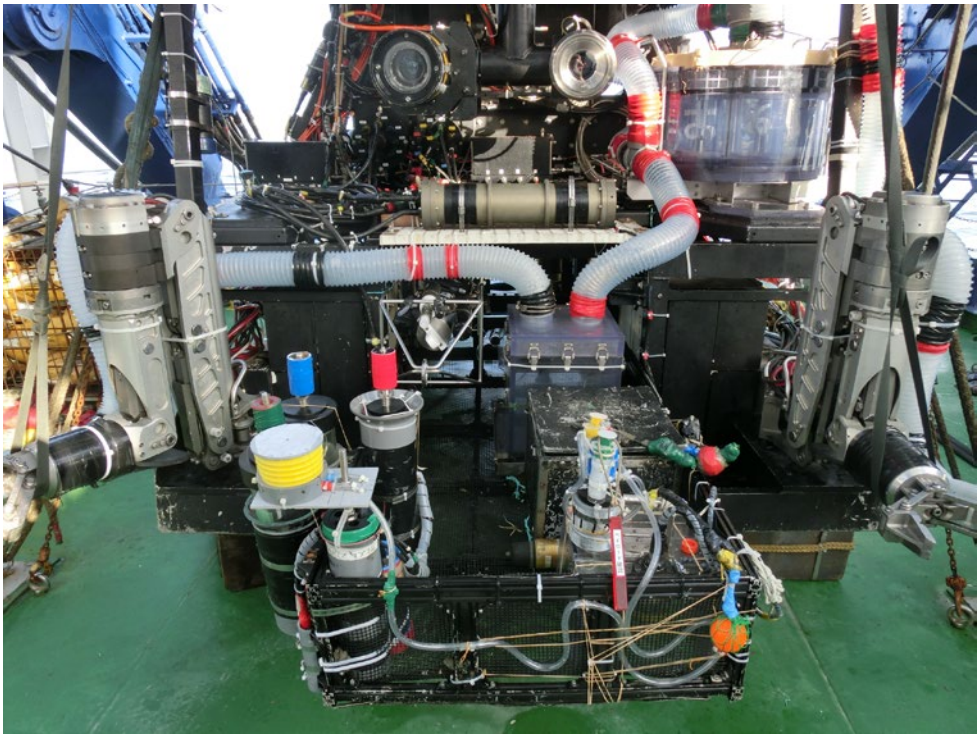
‡ Incubation of *Calyptogena* colonies with an incubation box.
The site location 35°00.936’N, 139°13.368’E, 907m

‡ Soil sampling by two MT cores.
The sampling site location 35°00.954’N, 139°13.338’E, 62m

‡ Sampling of *Calyptogena* clams with soil by Nomaki-type core.
The sampling site location 35°00.952’N, 139°13.321’E, 857m

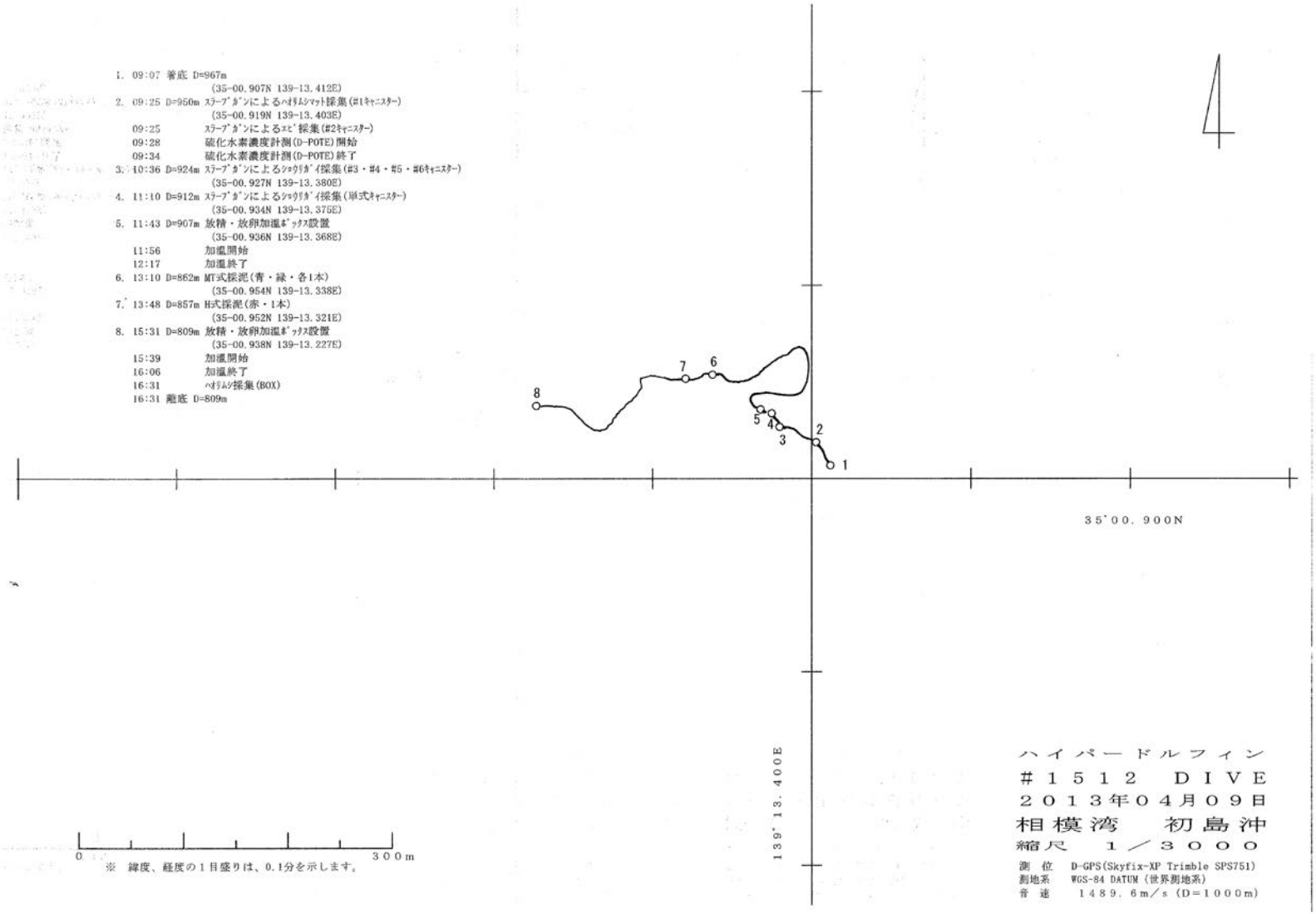
‡ Incubation of *Calyptogena* colonies with an incubation box.
The site location 35°00.938’N, 139°13.227’E, 809m

‡Sampling of tube worms into sample box.
The sampling site location 35°00.938’N, 139°13.227’E, 809m



Payload of HPD#1512

Dive track and event list of HPD#1512



5. 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], Genki Ozawa[1], Kazue Ohishi[1], Yuki Hongo[1], Sumihiro Koyama[1], Hidetaka Nomaki[1], Kanae Igawa[1], Kaoru Kaihotsu[1], Ryusaku Deguchi[2], Akihiro Tame[3] Hiroshi Miyake[4], Mitsuru Jimbo[4], Suguru Nemoto[5], Takenori Sasaki[6], Naoki Ito[7]

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

[2] *Miyagi university of Education*

[3] *Marine Works Japan, Ltd.*

[4] *School of Marine Biosciences, Kitasato University*

[5] *Enoshima aquarium*

[6] *Tokyo University*

[7] *Touhoku University*

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. To investigate these, 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 tried to chemically induce maturation and spawning of *Calyptogena* eggs. 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 symbiont localization and population in *Calyptogena* eggs

* Analysis of expression of several symbiont genes in *Calyptogena* eggs

*Analysis of blood cells of *Calyptogena* clam

*Analysis of expression of several genes in *Calyptogena* clam

2) Investigating habitat environments of *Calyptogena* spp., and sampling the bivalves for development of a rearing system for invertabrates in chemosynthetic ecosystem.

Masaaki Konishi[1,2], Tatsuhiro Fukuba[2], Katsunori Yanagawa[2], Hidetaka Nomaki[2], Kazuna Shitaka[2], Yuriko Nagano[2], Shinro Nishi[2], Masahiro Yamamoto[2], Hiroko Makita[2], Hisako Hirayama[2] , Tetsuo Ikuta[2], Takao Yoshida[2]

[1]Kitami Institute of Technology

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

Objectives and achievements in this cruise

Cultivation and rearing of deep sea invertabrates and their symbionts habitating in the deep-sea chemosynthetic ecosystem are one of significant technologies in embryology, biochemistry, and molecular biology. However, cultivating technology for deep sea invertabrates and their chemoautotrophic symtionts have been developed insufficient. Therefore, we are developing a tank system controlling H_2S feeding for rearing the deep sea invertabrates, at Yokosuka headquarter of JAMSTEC. However, there is not enough information of optimal habitat conditions for the deep sea invertabrates. In this cruise, we attempted to examine habitat conditions of *Calyptogena* spp. by *in situ* sensors, and to collect *Calyptogena* spp. for using cultivating experiments. Furthermore, we attempted to examine microbial diversities near the colony of *Calyptogena* spp.

A CTD profiler (SBE19 plus V2) with a pH, ORP (Oxidation-Reduction Potential), DO (Dissolved Oxygen) (Sea-Bird Electronics Inc.), turbidity, chlorophyll (Seapoint Inc.), and PAR (Photosynthetic Active Radiation: Biospherical Instrument Inc.) sensor was mounted on HPD (Fig. A) for environmental parameter measurements at the off-Hatsushima deep-sea habitats. A power for the CTD was supplied from internal batteries and the acquired data was stored in the CTD. The CTD was operated during dive HPD#1508, 1509, 1511, and 1512. The data was uploaded to a PC and was processed after each dive. (1 sec.-averaged data from a raw data recorded every 0.5 sec.).

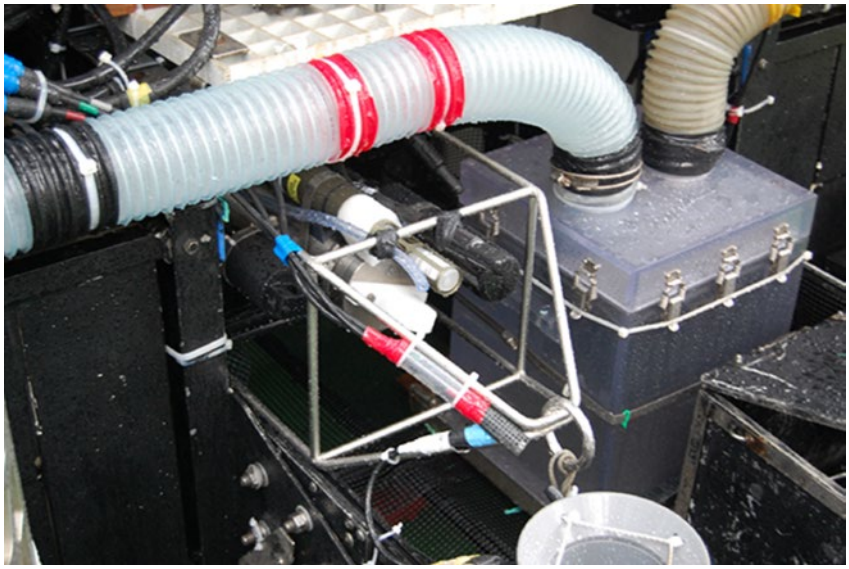


Fig. A CTD profiler mounted on HPD

Tow ISFET (Ion Sensitive Field Effect Transistor) based pH sensors were operated to measure seawater pH at sediment-seawater interface on bivalve's habitats. One of the ISFET-pH sensors was bundled with a H_2S sensor (DEEP-POTE) (Fig. B) and was pointed just above *Calyptogena* and *Bathymodiolus* colonies using a manipulator of HPD for pH and H_2S measurements during the dives. Another ISFET-pH sensor was mounted at a payload-bay of HPD and the sensor head was fixed near the pH sensor on the CTD for data comparison. Before and after each deployment, ISFET-pH sensors were calibrated using pH standard solutions (AMP, TRIS) onboard under a known temperature condition. ISFET pH sensors were operated during dive HPD#1508, 1509, 1511, and 1512.

Fig. B ISFET-pH sensor bundled with DEEP-POTE H_2S sensor head

In the dive of HPD#1509, habitat conditions of *Calyptogena* spp. were investigated at 849 m (35'00.942N, 139'13.248E) sites off Hatsushima Island. Core sediments with and without *Calyptogena* clams were obtained by using MT and H-type push cores. Dissolved oxygen (DO) and pH profiles of core sediment were observed by pH and DO sensors on board. Sediment cores were sliced into several layers at different depths from the surface, namely 0-4, 4-8, 8-12, 12-16, 16-20, and 20-24 cm, and stored at 4 to -80°C for microbial diversity, chemical and molecular biology. The stored sediments will be analyzed in details after the cruise. Core water was obtained by centrifugation. Hydrogen sulfide and ammonium concentrations in the water were analyzed by colorimetric analyses. *Calyptogena* clams (24 individuals), *Bathymodioulus* spp. (12 individuals) were stored in rearing tank on board before transferred to experimental tank system on shore.

Preliminary result

The CTD profiler was successfully collected the environmental data for the dive #1508, 1509, 1511, and 1512. Vertical and horizontal profiles of conductivity, temperature, pH, ORP, DO, turbidity, chlorophyll, and PAR can be utilized for a

detailed study about properties of the off-Hatsushima deep-sea habitat.

ISFET-pH sensors worked properly for during the dive HPD#1508, 1509, 1511, and 1512. The data will be used for analysis of local environmental properties of specific habitats of bivalves depending on a chemosynthetic symbiosis and primary production.

DEEP-POTE sensor was malfunctioned during the dive HPD#1508, 1509, and 1511. After sensor cable was repaired, DEEP-POTE described H_2S profiles. The data will feedback to the setting experimental condition for cultivating clams and their symbionts.

The profiles of concentrations of H_2S, NH_4, and DO in core water was measured on board. The concentrations of H_2S and NH_4 in the core water obtained from cores with *Calyptogena* colony were larger than those without colony. The result indicate that the habitat condition of *Calyptogena* spp. was reduced condition compared with conditions without *Calyptogena* spp.

Future studies

Analyses of microbial diversity of sediments

Cell count in the sediment

Cultivation of collected *Calyptogena* clams in an experimental tank.

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

Hajime Shiobara[1], Aki Ito[2], Hiroko Sugioka[2], Takehi Isse[1], Masanao Shinohara[1]

[1] *Earthquake Research Institute (ERI), University of Tokyo*

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

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 is chosen at the center of large displacement by the Bousou slow slip event repeatedly occurred. We perform 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. As for the NUDOBS, it is still a prototype, but deployed in the deepest part (about 9200 m depth) of the triple junction off Bousou to check the real performance of it. The NUDOBS equips omni-directional geophones (15 Hz) and the MEMS accelerometer.

Deployments of all instruments were performed well within 5th April, due to severely bad weather was expected on 7th. In the early morning, the BBOBST-NX with the ROV homer was dropped and landed at the position expected. The OBDC was loaded on the HPD, and the #1510 dive was started. The BBOBST-NX was easily found and the OBDC was placed about 9 m distance from it. Unlocking and moving of the recording unit from the sensor unit were finished within one hour. The tilt of the sensor unit was small enough to operate, which did not require any correction by the HPD. Finally, the view of all instruments was checked as in Fig. 3-2. The system of the OBDC has the small mooring buoy using 6 glass sphere floats for the deep water. The deployment was performed to release these floats, rope and the main unit of the OBDC (Fig. 3-3) at last. The descending speed seemed changed from 80 m to 66 m per minute at some depth deeper than 5000 m. The acoustic transponder worked well in this depth of 8920 m by 1500 m/s, and the status of the change into the observation mode was recognized after

the first stage command was sent. The positioning measurement was also finished with good condition with the maximum slant range of 8990 m. These instruments will be recovered in the next year.

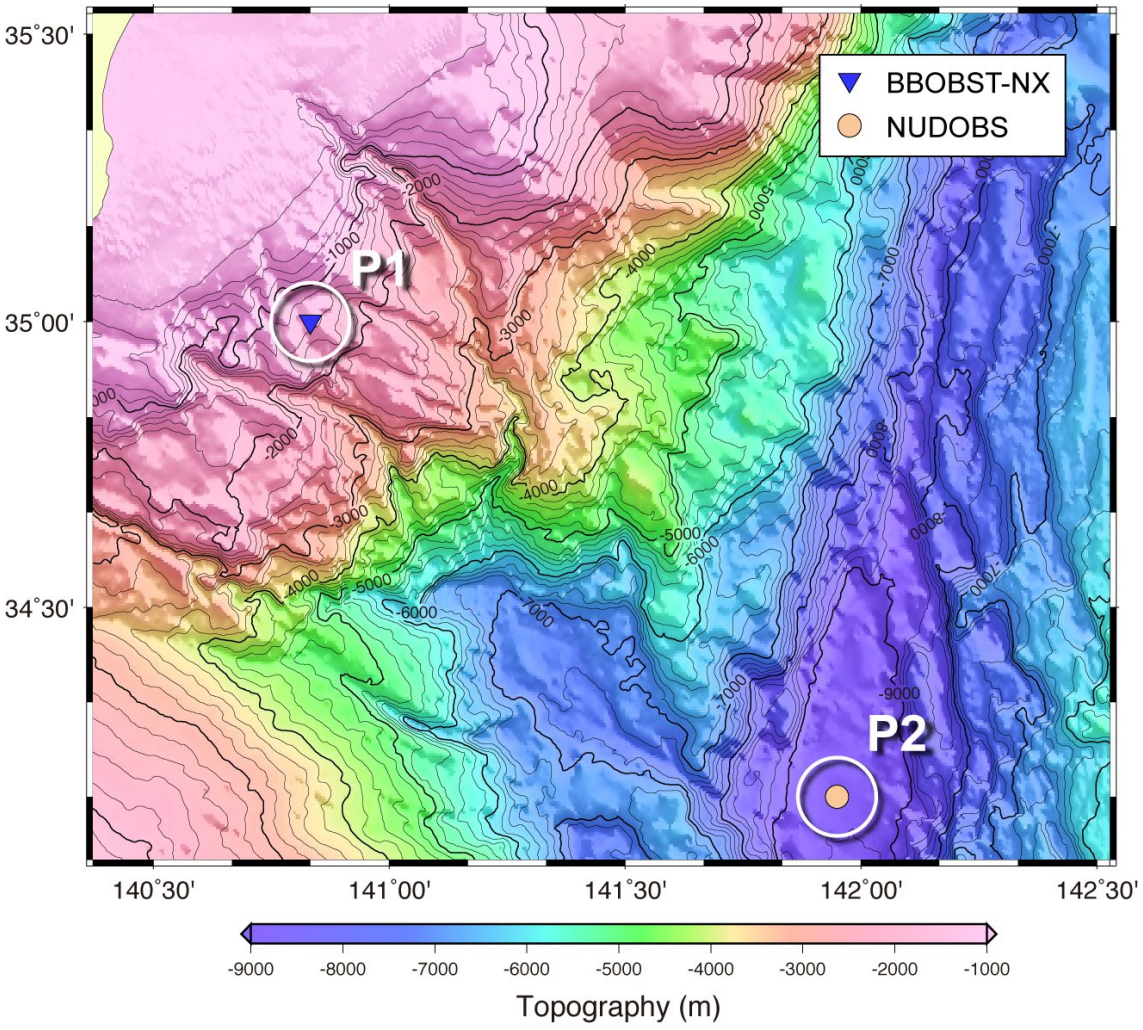


Fig. 3-1. Location map.

Fig. 3-2. The BBOBST-NX and the OBDC at the P1 site.

Fig. 3-3. The NUDOBS to be released soon.

4) Incorporation of algal organic matters into microbial biomass.

Hidetaka Nomaki[1], Yoshinori Takano[1]

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

Introduction

Organic matters produced by photoautotrophs are major energy sources for benthic communities. Since sinking organic matters diminished exponentially with increasing water depths, deep-sea benthic heterotrophs thrive in a limited energy condition. Here we carried out an *in situ* incubation experiment to investigate metabolic adaptation of microbes to such an energy limited environment.

Materials and Methods

During the HPD dive#1499 of *Natsushima* NT13-06 cruise on 25th March 2013, we deployed two *in situ* incubation cores containing algal organic matters at the normal seafloor 25 m apart from *Calyptogena* colony. ROV homer was also deployed next to the incubation cores to find the cores at the retrieve dive. During the HPD dive #1511 on 8th April, i.e., 14 days after the deployment, we retrieved the two *in situ* incubation cores and ROV homer. Water depth, temperature, and dissolved oxygen concentration at the experimental site was 852 m, 3.4 dC, and 1.0 ml L^{-1}, respectively.

Future studies

On board, chemical profiles through water-sediment interface were obtained using microelectrodes. Overlying water samples of the cores were collected into glass bottles and poisoned with $HgCl_2$ to stop microbial activity. Sediments were sliced off into different layers and kept frozen at -80 dC. Geochemical analyses will be carried out in a laboratory of JAMSTEC to estimate microbial incorporation of algal organic matters.

5) Long-term rearing of seep animals.

Makoto Sugimura (New Enoshima aquarium)

Objective and achievement in this cruise

Long-term rearing of deep sea animals that live in the seep sites, at Enoshima Aquarium is aiming to establish a rearing system.

As a result, for *Calyptogen*a spp. has lead to the rearing of 153 days.

During this cruise, we collect the seep animals in biological systems sampler. We have identified a biological sample. And we saved. Thereafter, the analysis of biodiversity of seep Sagami Bay Off Hatsushima Island.

In addition, the study sample is saved as a long-term rearing seep animals.

＊For sample collection

Calyptogena spp., *Lamellibrachia* sp., *Alaysia* sp., *Alvinocaris* sp., *Zoarcidae* gen.et sp. is performed for the purpose of rearing in storage on board as a sample for the study of long-term rearing .

After you have brought back to the aquarium, housed in a tank that reproduces the deep seep sites.

Observations are made of mud and behavior measurement sulfide, *Calyptogena* spp.and *Lamellibrachia* sp. explores the rearing conditions. *Alvinocaris* sp., *Zoarcidae* gen.et sp. explores the reproductive behavior in a water bath.

Research of biodiversity in seep Sagami Bay Off Hatsushima Island.

Biological samples sorted on board, and photographed alive.

After taking the picture, make a 70% alcohol fixation.

＊Outreach activity

We will post a "logbook" by WEB page to open to the public how the voyage.

We work so that it can be a lot of people, fun and learn about the research and research activities by watching a "logbook".

Future studies

＊Technological development of deep-sea animals continued long-term rearing

* Behavioral observation study of deep-sea animals
* Study of rearing in the aquarium of deep-sea animals
* Spread to the general public and research activities