doi : 10.17596/0002993

RV Natsushima Cruise Report

NT13-06

Hyper-Dolphin Dive Research

Off Hatsushima & Off Atami (Sagami Bay)

March, 24th-30th, 2013

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

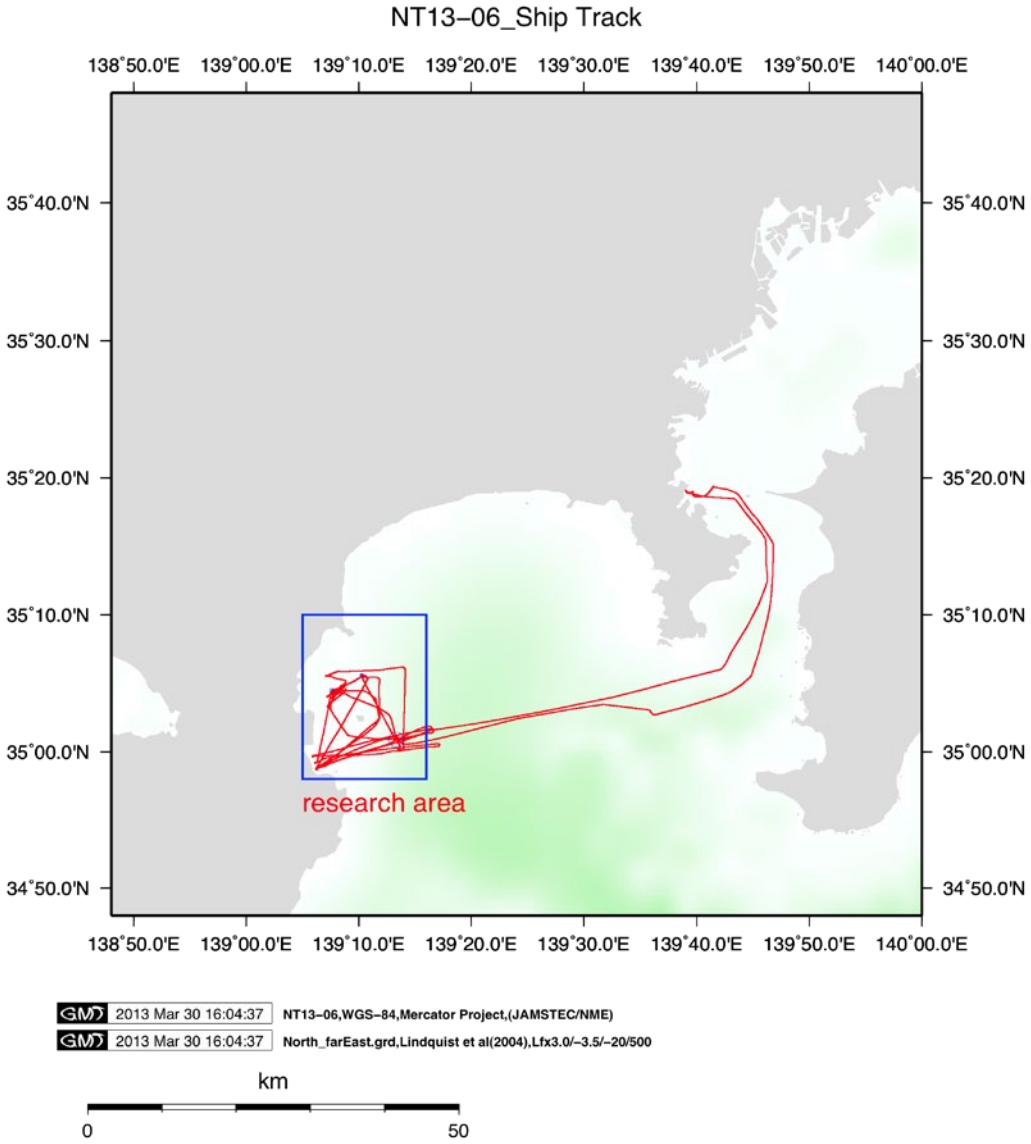
Contents

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. Yoshinari Ohno and “Hyper-Dolphin” operation team for their operations in sampling. We also thank Mr Masashi Ito, Nippon Marine Enterprises, Ltd., for his 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: NT13-06, R/V Natsushima
2) Title of the Cruise: “Hyper-Dolphin Research Dive, Deep-sea Research, FY2012
3) Title of Proposal:
   I) Transfer analysis of intracellular bacterial symbiont from *Calyptogena* clams to next generation
   II) Morphological observation of *Osedax* polychaetes using MRI
   III) Development of the technique to photograph the fluorescence that deep sea organisms emit on *in-situ*
   IV) Exploring protists associated with tubeworms.
   V) Diverse Glycoconjugates and their Receptors in the Deep Sea Invertebrates
4) Cruise Period: March 24, 2013 ~ March 30, 2013
5) Port Call: from JAMSTEC (March 24, 2013) to JAMSTEC (March 30, 2013)
6) Research Area: Off Hatsushima and Off Atami, Sagami Bay



Cruise track of R/V Natsushima (NT13-06)

7) Cruise Log (JST)

Date	Local Time	Note	Position/Weather/Wind/Sea condition
24.Mar.13		**Sail out and HPD1498_Off HATSUSHIMA**	3/24 12:00(UTC+9h)
	7:30-8:00	Scientists meeting.	35-01.0N 139-13.4E
	8:00	Let go all shore line,left YOKOSUKA.Then com'ced proceeding to research area(Off HATSUSHIMA).	Cloudy
	9:00-10:00	On board education for scientists.	NNE-4(Moderate breeze)
	11:20	Arrived at research area.	3(Sea slight)
	11:28	Released XBT.	1(Calm)
	13:23	HPD dove & started her operation#1498.	Visibly:8
	13:56	HPD landed on the sea bottom.(D=942m)	
	16:50	HPD left the sea bottom.(D=830m)	
	17:31	Recovered HPD & finished the operation.	
	18:20	Let go anchor at off ITO.	
25.Mar.13		**HPD1499_Off HATSUSHIMA**	3/25 12:00(UTC+9h)
	6:00	Heaving anchor,then com'ced proceeding to dive point.	35-01.0N 139-13.4E
	7:30	Arrived at dive point.	Rainy
	8:47	HPD dove & started her operation#1499.	NNE-4(Moderate breeze)
	9:21	HPD landed on the sea bottom.(D=911m)	3(Sea slight)
	15:59	HPD left the sea bottom.(D=808m)	1(Calm)
	16:39	Recovered HPD & finished the operation.	Visibly:6
	17:30	Let go anchor at off ITO.	
26.Mar.13		**HPD1500_Site A & HPD1501_Site B**	3/26 12:00(UTC+9h)
	6:00	Heaving anchor,then com'ced proceeding to dive point.	35-05.6N 139-10.3E
	7:15	Arrived at dive point.	Fine but cloudy
	7:17	Released XBT.	NE-3(Gentle breeze)
	8:26	HPD dove & started her operation#1500.	2(Sea smooth)
	8:50	HPD landed on the sea bottom.(D=490m)	1(Calm)
	11:03	HPD left the sea bottom.(D=491m)	Visibly:8
	11:35	Recovered HPD & finished the operation.	
	13:22	HPD dove & started her operation#1501.	
	13:52	HPD landed on the sea bottom.(D=406m)	
	16:26	HPD left the sea bottom.(D=402m)	
	16:56	Recovered HPD & finished the operation.	
	17:45	Let go anchor at off ITO.	
27.Mar.13		**HPD1502_Off HATSUSHIMA**	3/27 12:00(UTC+9h)
	6:00	Heaving anchor,then com'ced proceeding to dive point.	35-00.0N 139-13.5E
	7:15	Arrived at dive point.	Overcast
	8:21	HPD dove & started her operation#1502.	NNE-5(Fresh breeze)
	9:03	HPD landed on the sea bottom.(D=1178m)	3(Sea slight)
	15:47	HPD left the sea bottom.(D=1170m)	1(Calm)
	16:35	Recovered HPD & finished the operation.	Visibly:6
	17:30	Let go anchor at off ITO.	
28.Mar.13		**HPD1503_Off HATSUSHIMA & HPD1504_Site A**	3/28 12:00(UTC+9h)
	6:00	Heaving anchor,then com'ced proceeding to dive point.	35-03.8N 139-11.4E
	7:15	Arrived at dive point.	Fine but cloudy
	8:20	HPD dove & started her operation#1503.	NE-2(Light breeze)
	8:58	HPD landed on the sea bottom.(D=897m)	2(Sea smooth)
	10:48	HPD left the sea bottom.(D=857m)	1(Calm)
	11:30	Recovered HPD & finished the operation.	Visibly:5
	13:57	HPD dove & started her operation#1504.	
	14:18	HPD landed on the sea bottom.(D=489m)	
	16:17	HPD left the sea bottom.(D=492m)	
	16:53	Recovered HPD & finished the operation.	
	18:00	Let go anchor at off ITO.	
29.Mar.13		**HPD1505_Site B & HPD1506_Off HATSUSHIMA**	3/29 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.	Fine but cloudy
	8:17	HPD dove & started her operation#1505.	ENE-3(Gentle breeze)
	8:36	HPD landed on the sea bottom.(D=399m)	2(Sea smooth)
	10:33	HPD left the sea bottom.(D=400m)	1(Calm)
	11:01	Recovered HPD & finished the operation.	Visibly:4
	12:29	HPD dove & started her operation#1506.	
	13:00	HPD landed on the sea bottom.(D=898m)	
	16:25	HPD left the sea bottom.(D=858m)	
	17:02	Recovered HPD & finished the operation.	
30.Mar.13		**HPD1507_Off HATSUSHIMA & finished NT13-06**	3/30 12:00(UTC+9h)
	7:16	HPD dove & started her operation#1507.	35-05.2N 139-44.0E
	7:50	HPD landed on the sea bottom.(D=921m)	Overcast
	9:11	HPD left the sea bottom.(D=905m)	North-5(Fresh breeze)
	9:52	Recovered HPD & finished the operation.	3(Sea slight)
	10:00	Left research area,then com'ced proceeding to YOKOSUKA.	1(Calm)
	15:00	Sent out 1st shore line,then arrived at YOKOSUKA.	Visibly:6

2. Participants List

Scientists

Principal Investigator

Takao Yoshida
Marine Biodiversity Research Program
Institute of Biogeosciences
Japan Agency for Marine-Earth Science and Technology (JAMSTEC)

Onboard Scientists

Yoshihiro Fujiwara
Marine Biodiversity Research Program
Institute of Biogeosciences
Japan Agency for Marine-Earth Sciences and Technology (JAMSTEC)

Kiyotaka Takishita
Marine Biodiversity Research Program
Institute of Biogeosciences
Japan Agency for Marine-Earth Science and Technology (JAMSTEC)

Yasuo Furushima
Marine Biodiversity Research Program
Institute of Biogeosciences
Japan Agency for Marine-Earth Science and Technology (JAMSTEC)

Tesuro Ikuta
Marine Biodiversity Research Program
Institute of Biogeosciences
Japan Agency for Marine-Earth Science and Technology (JAMSTEC)

Yuki Hongo
Marine Biodiversity Research Program
Institute of Biogeosciences
Japan Agency for Marine-Earth Science and Technology (JAMSTEC)

Hidetaka Nomaki
Earth and Life History Research Program
Institute of Biogeosciences
Japan Agency for Marine-Earth Science and Technology (JAMSTEC)

Akinori Yabuki
Marine Biodiversity Research Program
Institute of Biogeosciences
Japan Agency for Marine-Earth Science and Technology (JAMSTEC)

Fumiya Noguchi
Marine Biodiversity Research Program
Institute of Biogeosciences
Japan Agency for Marine-Earth Science and Technology (JAMSTEC)

Ayaka Kasai
Marine Biodiversity Research Program
Institute of Biogeosciences
Japan Agency for Marine-Earth Science and Technology (JAMSTEC)

Yasuhiro Ozeki
Laboratory of Glycobiology and Marine Biochemistry
Department of Life and Environmental System Science
Graduate School of Yokohama City University (YCU)

Yasuhiro Koide
Laboratory of Glycobiology and Marine Biochemistry
Department of Life and Environmental System Science

Graduate School of Yokohama City University (YCU)

Misaki Fujio
Science Partnership Program, JST
Yokohama Science Frontier High School (YSFH)

Noriaki Kojima
Science Partnership Program, JST
Yokohama Science Frontier High School (YSFH)

Sadao Suzuki
Oceanographic Research Engineering

Masahiko Sasano
National Maritime Research Institute

Masayuki Miyazaki
Extremobiosphere Research Program
Institute of Biogeosciences
Japan Agency for Marine-Earth Sciences and Technology (JAMSTEC)

Shore base Scientists

Tadashi Maruyama
Marine Biodiversity Research Program
Institute of Biogeosciences
Japan Agency for Marine-Earth Science and Technology (JAMSTEC)

Kazaue Ohishi
Marine Biodiversity Research Program
Institute of Biogeosciences
Japan Agency for Marine-Earth Science and Technology (JAMSTEC)

Yoshihiro Takaki
Extremobiosphere Research Program
Institute of Biogeosciences
Japan Agency for Marine-Earth Sciences and Technology (JAMSTEC)

Genki Ozawa
Marine Biodiversity Research Program
Institute of Biogeosciences
Japan Agency for Marine-Earth Science and Technology (JAMSTEC)

Sumihiro Koyama
Marine Biodiversity Research Program
Institute of Biogeosciences
Japan Agency for Marine-Earth Science and Technology (JAMSTEC)

Mitsuru Jimbo
School of Marine Biosciences
Kitasato University

Kanae Igawa
Marine Biodiversity Research Program
Institute of Biogeosciences
Japan Agency for Marine-Earth Science and Technology (JAMSTEC)

Kaoru Kaihotsu
Marine Biodiversity Research Program
Institute of Biogeosciences
Japan Agency for Marine-Earth Science and Technology (JAMSTEC)

Takenori Sasaki
The University of Tokyo

Ryusaku Deguchi

Graduate Schools, Research division of advanced teacher training
Miyagi University of Education

Koji Inoue
Atmosphere and Ocean Research Institute (AORI),
The University of Tokyo

Akihiro Tame
Marine Works Japan Ltd.

Naoki Ito
Tohoku University

Hiroshi Miyake
School of Marine Biosciences
Kitasato University

Imtiaj Hasan
MEXT Ph.D Program Fellow
Laboratory of Glycobiology and Marine Biochemistry
Department of Life and Environmental System Science
Graduate School of Yokohama City University (YCU)
Department of Biochemistry and Molecular Biology
Rajshahi University, Bangladesh

Masaru Kawato
Marine Biodiversity Research Program
Institute of Biogeosciences
Japan Agency for Marine-Earth Science and Technology (JAMSTEC)

Norio Miyamoto
Marine Biodiversity Research Program
Institute of Biogeosciences

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

Yoshiteru Seo
Graduate School of Medicine
Dokkyo Medical University

Suguru Nemoto
Enoshima Aquarium

Marine Technician

M. Ito	Nippon Marine Enterprises, LTD.

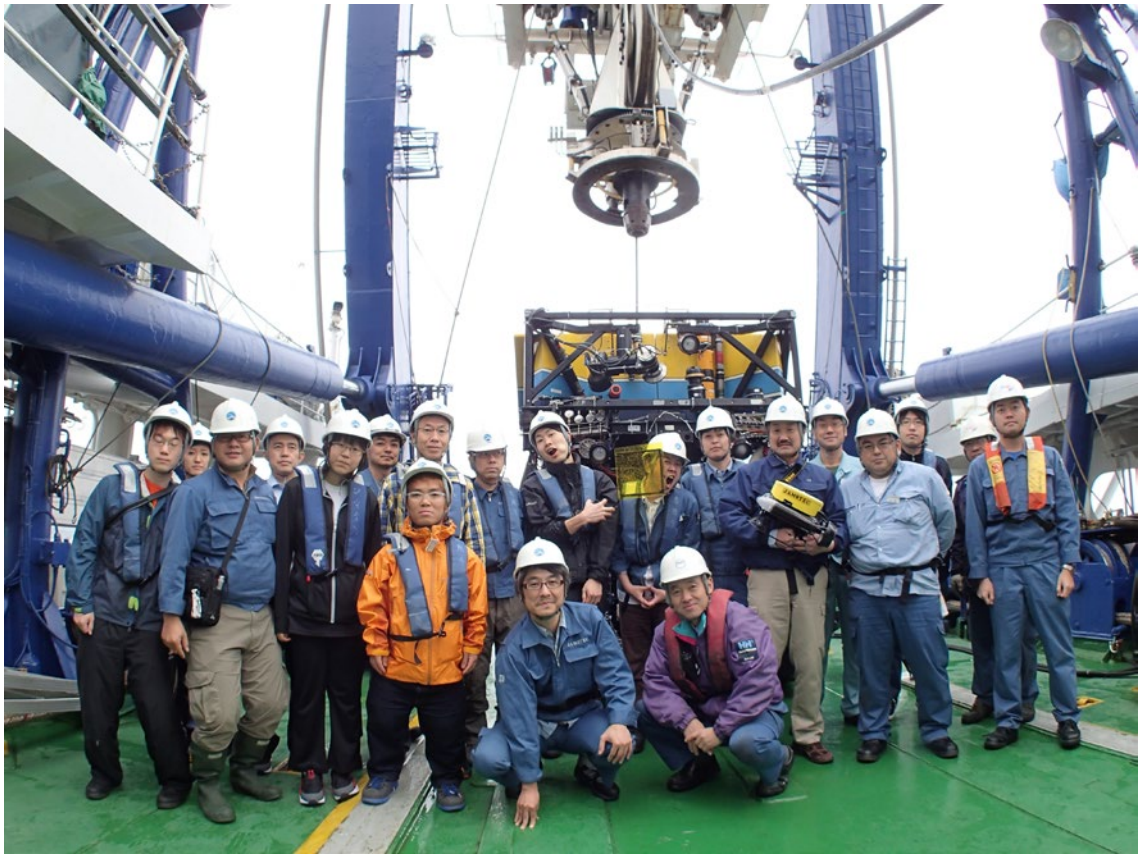
Hyper-dolphin operation team

Y. Ono	Operation Manager
K. Chiba	2nd ROV Operator
Y. Chida	2nd ROV Operator
A. Takenouchi	2nd ROV Operator
Y. Sakakibara	2nd ROV Operator
R. Saigo	2nd ROV Operator

R/V YOKOSUKA Officers and Crew

E. Ukekura	Captain
A. Tsuji	Chief Officer
I. Maeda	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
T.Onoue	Steward
Y. Chikuba	Steward
A. Saito	Steward
K. Kawase	Steward



Member of onboard science party

3. Overview of the cruise

In this cruise, five 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 Atami, Sagami Bay. The research subjects of the five groups were physiological, biochemical, parasitic single-cell eukaryotic or molecular-biological studies of cold seep-specific invertebrates or whale carcasses (see Section 5), and thus we spent most time to collect live samples, e.g., deep-sea mussels, vesicomyid clams, vestimentiferan tubeworm, and so on. In addition, time laps camera was used for observation of whale carcasses, and *in situ* fluorescent observation was examined. 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 HPD Dive #1498

Date: March. 24, 2013
Site: Off Hatsushima, Sagami Bay
Landing: 35°00.915'N, 139°13.403'E, 942 m (13:56)
Leaving: 35°00.943'N, 139°13.267'E, 830 m (16:50)

Purpose:
Collection of *Bathymodiolus* bivalves, *Calyptogena* clams, tubeworms, and sediments
Fluorescent observation of biological samples

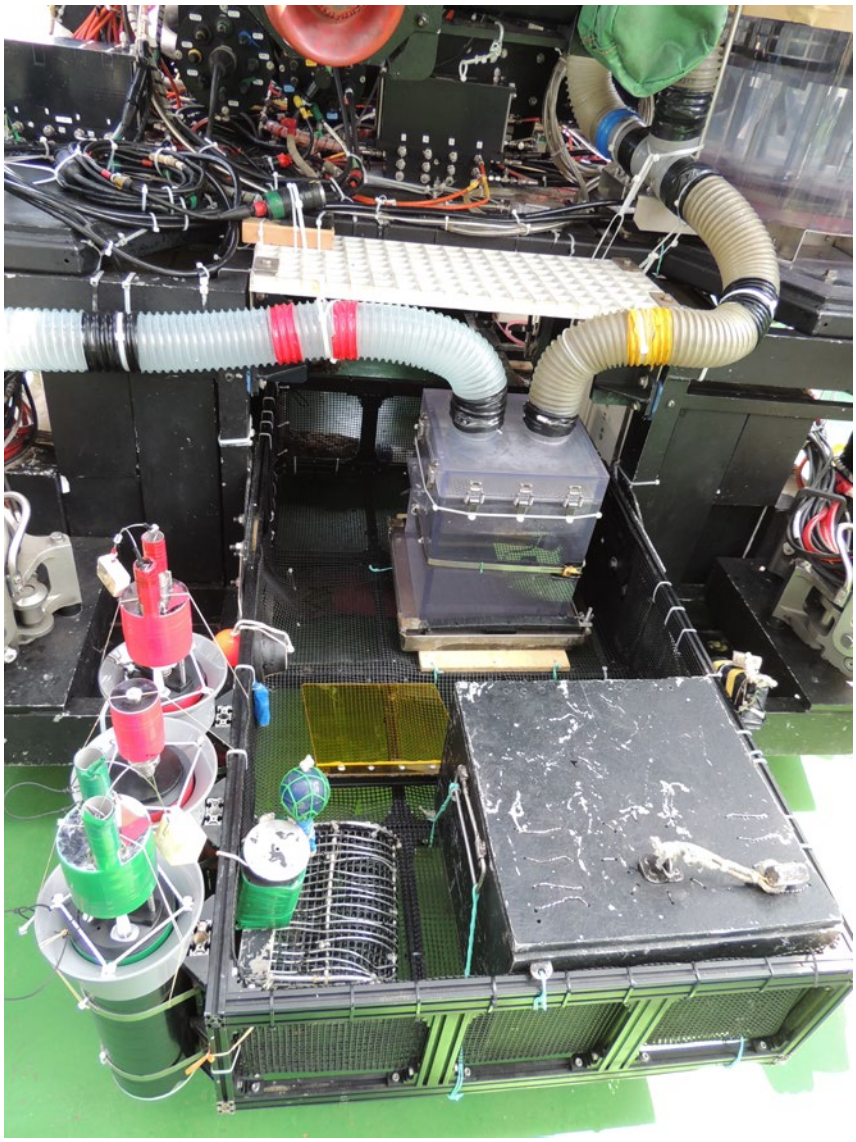
Payload Equipment:
Suction sampler（multiple canister & single canister）
Scoop sampler X2
Sample box X1
Nomaki-type core X3
Furushima-type fluorescent filter X1

Dive Summary
‡ Fluorescent observation and sampling of *Bathymodiolus* bivalves with suction samplers.
The site location 35°00.939'N, 139°13.385'E, 909 m

‡ Chemical fixation and collection of sediment with Nomaki-type core (green) and sampling of *Calyptogena* clams with coop sampler into sample box.
The sampling site location 35°00.957'N, 139°13.331'E, 857 m

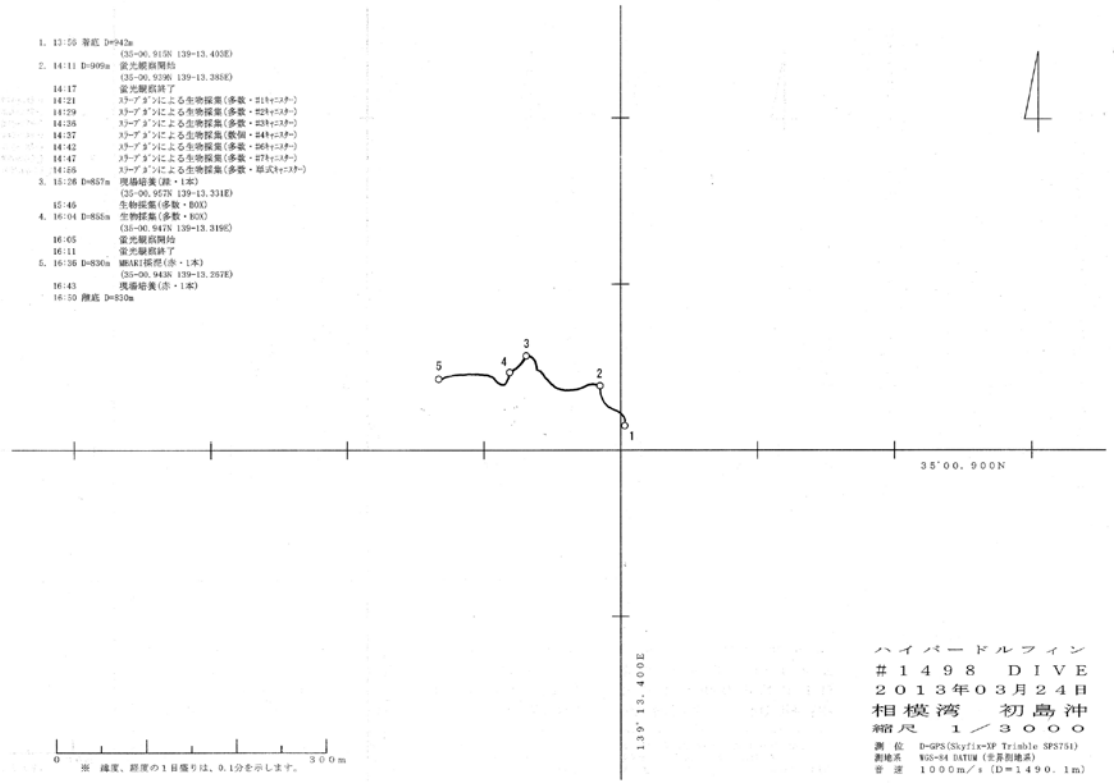
‡ Sampling of tubeworms into sample box and fluorescent observation.
The site location 35°00.947'N, 139°13.319'E, 855 m

‡Chemical fixation and collection of sediment with Nomaki-type core (red and red).
The sampling site location 35°00.943'N, 139°13.267'E, 830 m



Payload of HPD#1498

Dive track and event list of HPD#1498



2) Summary of the HPD Dive #1499

Date: March. 25, 2013
Site: Off Hatsushima, Sagami Bay
Landing: 35°00.939'N, 139°13.391'E, 911m (9:21)
Leaving: 35°00.940'N, 139°13.237'E, 830 m (15:59)

Purpose:
Collection of tubeworms and *Calyptogena* clam's eggs
Fluorescent observation of biological samples
in situ ^{13}C incubation of sediment and *Calyptogena* clams

Payload Equipment:
Suction sampler（multiple canister）
Scoop sampler X1
Sample box X1
Nomaki-type core X4
Furushima-type fluorescent filter X1
Incubation box X1
Egg collection sampler with hand pomp

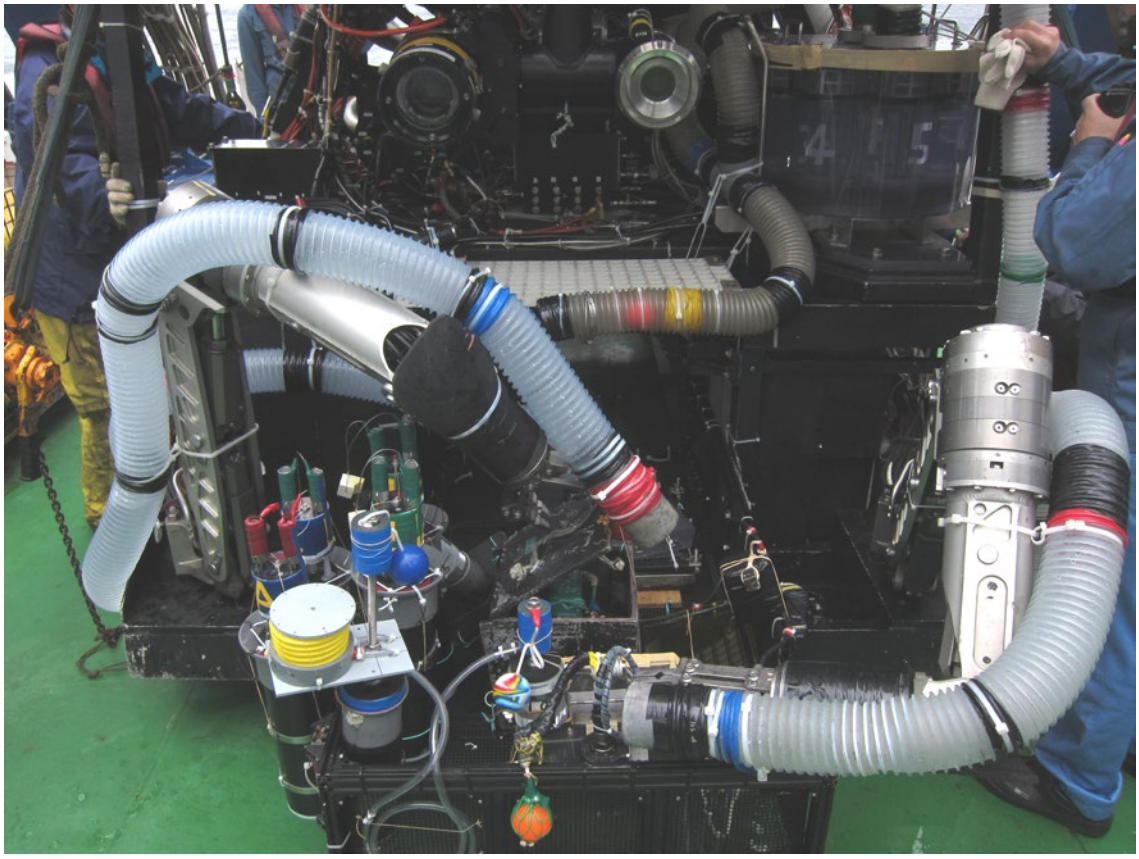
Dive Summary
‡ *in situ* ^{13}C incubation of sediment by Nomaki-type core (#12Green, #13Green, ROV homer ID16)
The site location 35°00.963'N, 139°13.323'E, 852 m

‡ *in situ* ^{13}C incubation of *Calyptogena* clams by Nomaki-type core (A_blue, A_blue)
The sampling site location 35°00.961'N, 139°13.333'E, 856 m

‡ Try of sampling of *Calyptogena* eggs and fluorescent observation of biological samples
The site location 35°00.943'N, 139°13.238'E, 810 m

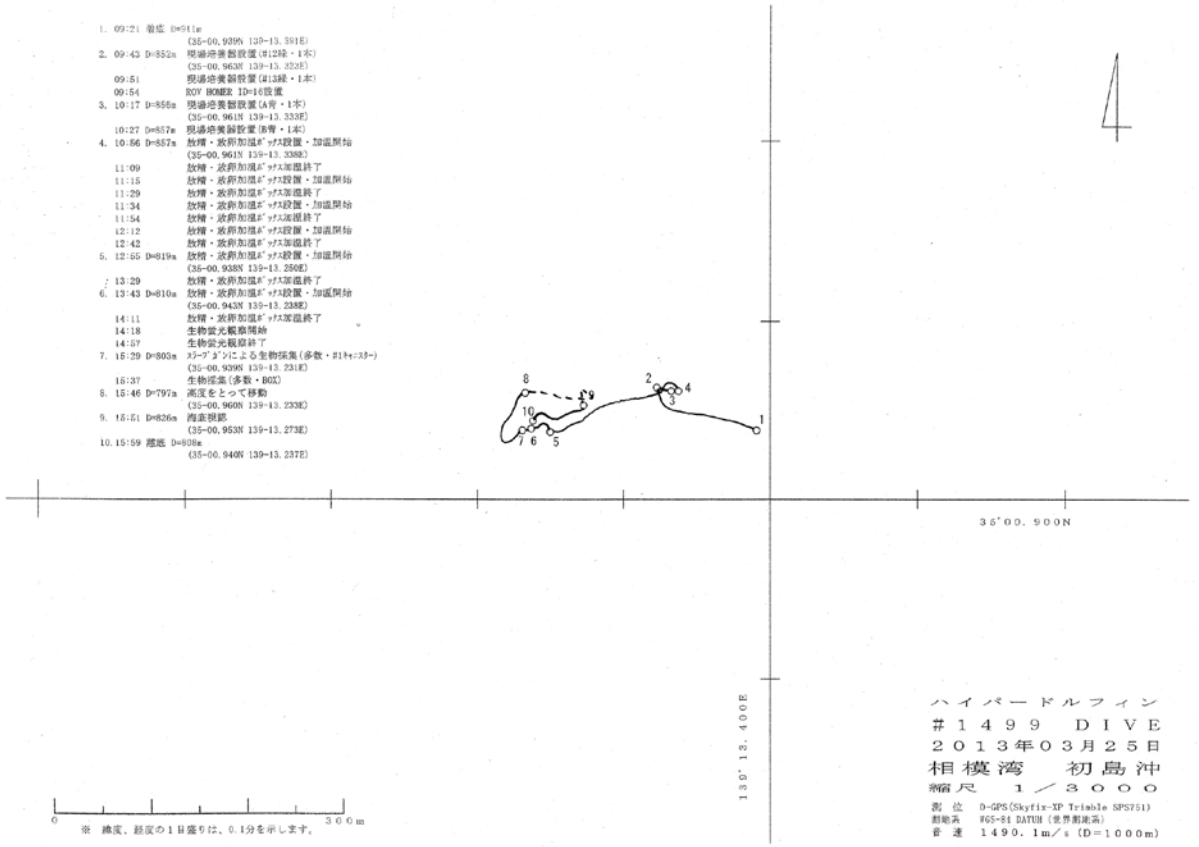
‡Sampling of tubeworms and shrimp

The sampling site location 35°00.939’N, 139°13.231’E, 803 m



Payload of HPD#1499

Dive track and event list of HPD#1499



3) Summary of the HPD Dive #1500

Date: March. 26, 2013
Site: Off Atami, Sagami Bay
Landing: 35°05.523'N, 139°10.259'E, 490 m (08:50)
Leaving: 35°05.575'N, 139°10.268'E, 491 m (11:03)

Purpose:

- Deployment of a time-lapse video camera and cow bones
- Observation and biological sampling at "SAITO" whale

Payload Equipment:

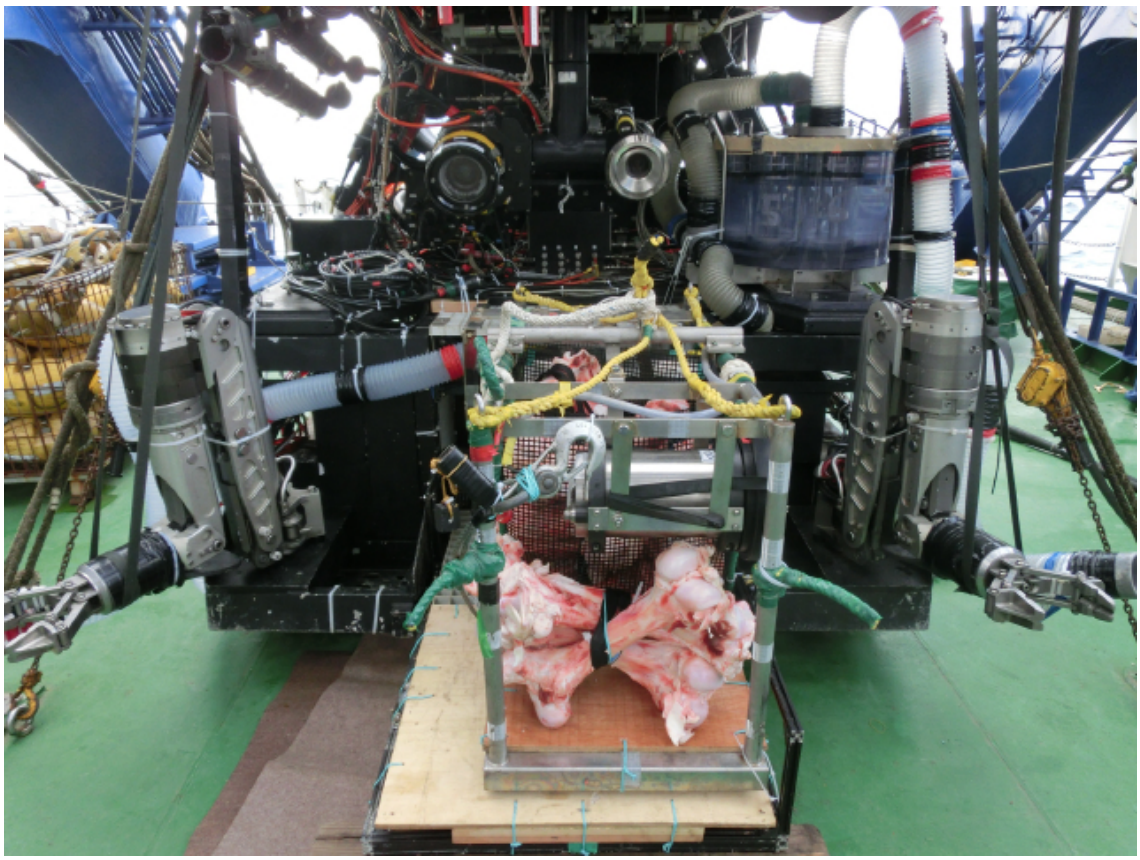
- Suction sampler（multiple canister）x1 set
- Time-lapse video camera
- Niskin bottle x2

Dive Summary

‡ Deployment of a time-lapse video camera and cow bones
The site location 35°05.575'N, 139°10.268'E, 491 m

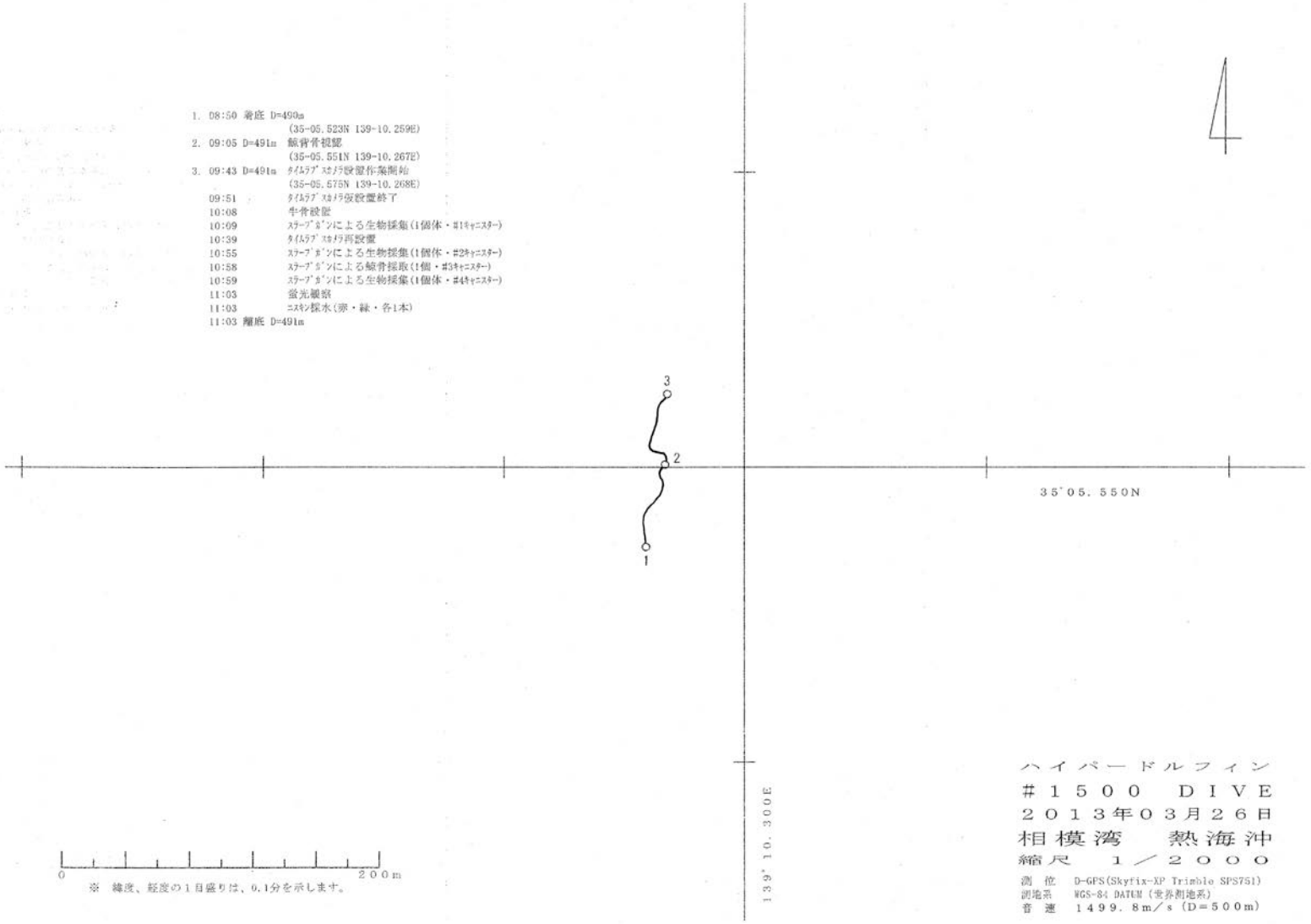
‡ Suction sampling of benthic fauna and whale bones
The site location 35°05.575'N, 139°10.268'E, 491 m

‡ Fluorescence observation around whale bones
The site location 35°05.575'N, 139°10.268'E, 491 m



Payload of HPD#1500

Dive track and event list of HPD#1500



4) Summary of the HPD Dive #1501

Date: March. 26, 2013
Site: Off Atami, Sagami Bay
Landing: 35°04.472'N, 139°07.604'E, 401 m (13:52)
Leaving: 35°04.480'N, 139°07.572'E, 402 m (16:26)

Purpose:
Collection of bone of *Megaptera novaeangliae*, water, and sediments.
Fluorescent observation of biological samples

Payload Equipment:
Suction sampler（multiple canister & single canister）
Sample box X1
Niskin weter sampler X2
MBARI core sampler X3
Furushima-type fluorescent filter X1

Dive Summary
‡ The water sample as control seawater with Niskin sampler (red).
The site location 35°04.460'N, 139°07.676'E, 405 m

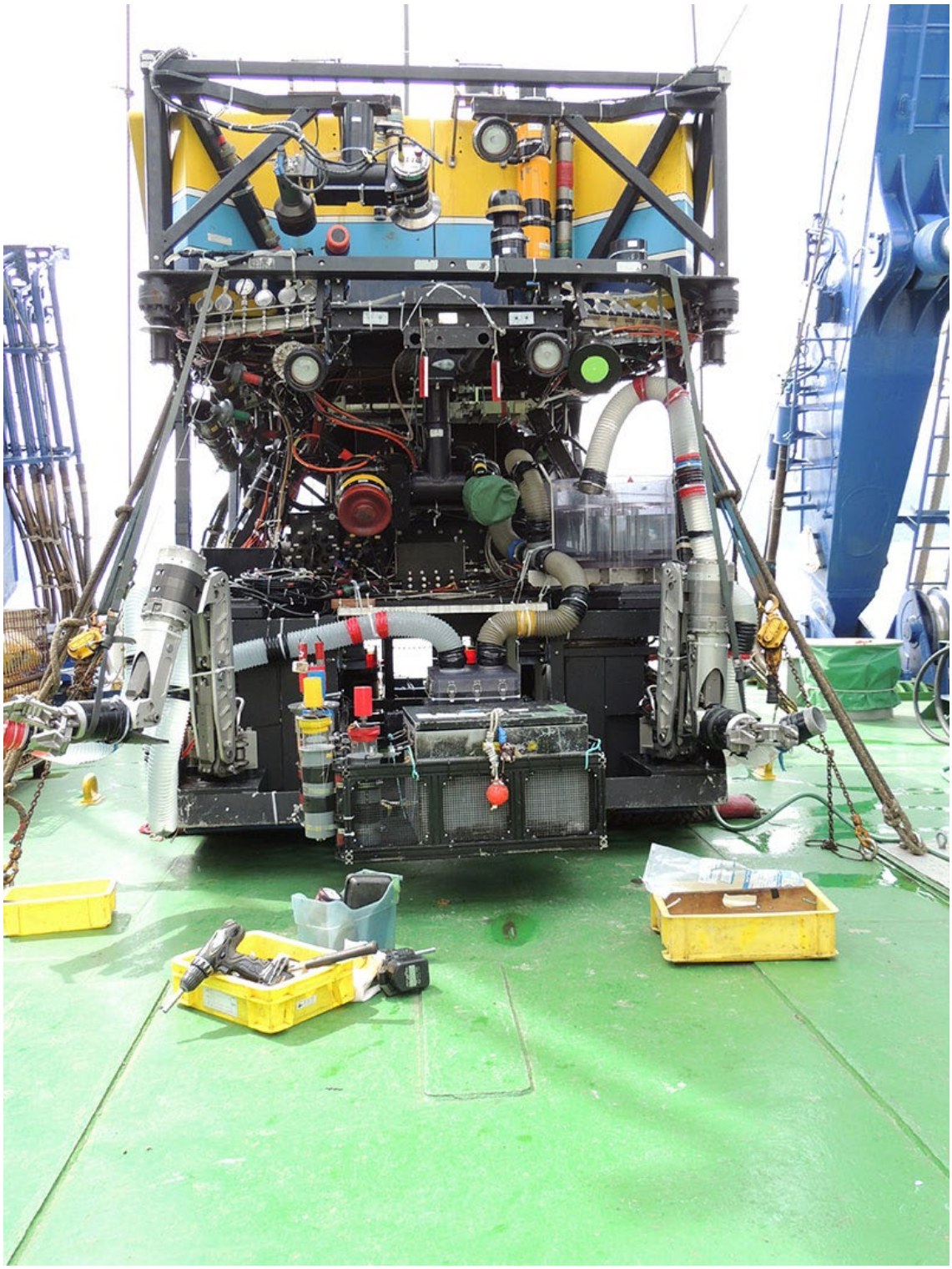
‡Make an observation about long bamboo.
The site location 35°04.472'N, 139°07.604'E, 400 m

‡ Sampling of sea water with niskin water Niskin sampler (green) and observation of *Megaptera novaeangliae*
The site location 35°04.480'N, 139°07.572'E, 400 m

‡Moving at head's left, sampling bone of finger in sample box and sediment with MBARI core sampler (red & blue)
The sampling site location 35°04.480'N, 139°07.572'E, 401 m

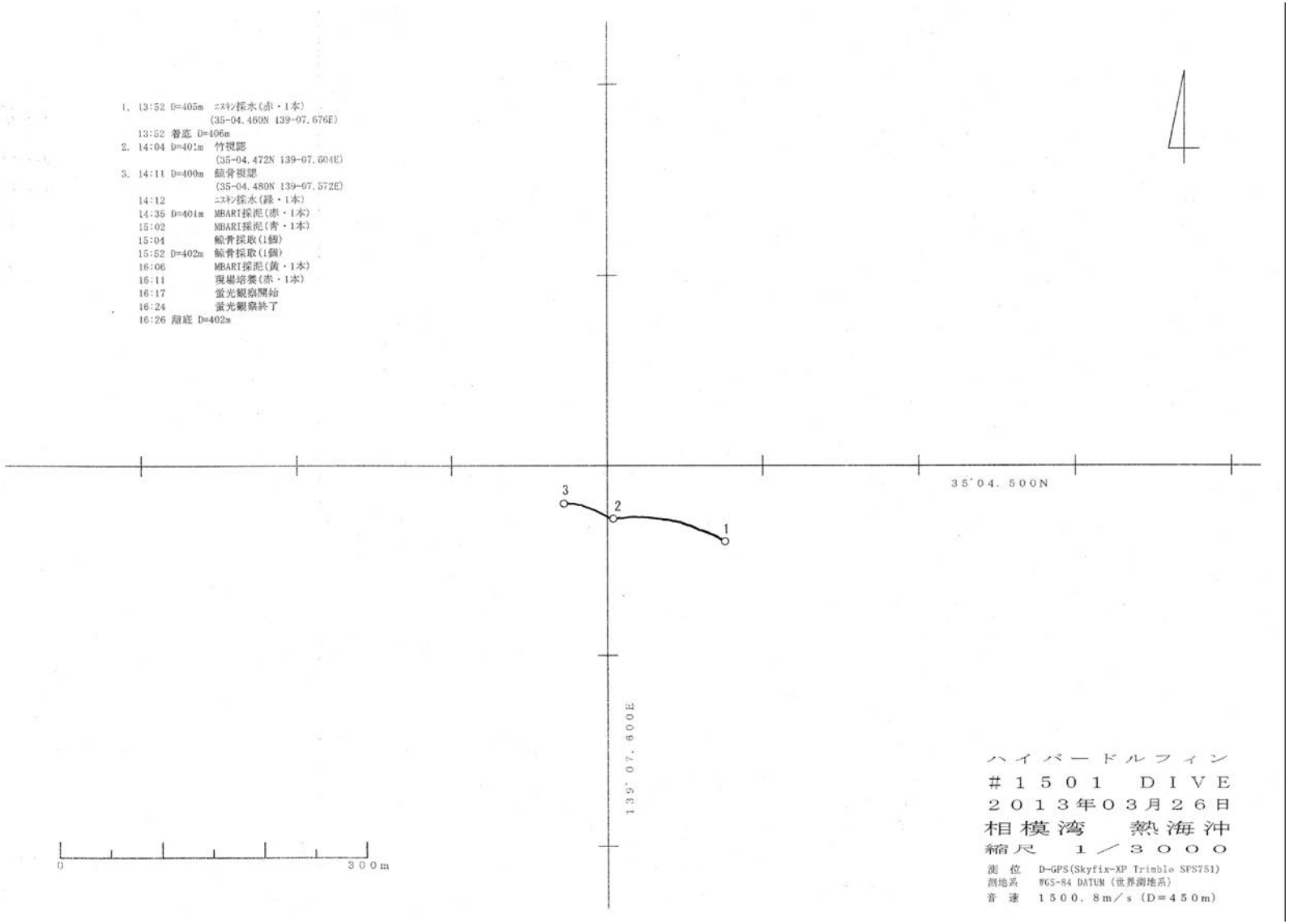
‡Moving at head's right, sampling bone of finger in sample box, chemical fixation and collection of sediment with Nomaki-type (red) and sediment with MBARI core sampler yellow)

The sampling site location 35°04.480'N, 139°07.572'E, 402 m



Payload of HPD#1501

Dive track and event list of HPD#1501



5) Summary of the HPD Dive #1502

Date: March. 27, 2013
Site: Off Hatsushima, Sagami Bay
Landing: 35°00.051'N, 139°13.520'E, 1178 m (9:03)
Leaving: 35°00.086'N, 139°13.485'E, 1170 m (15:47)

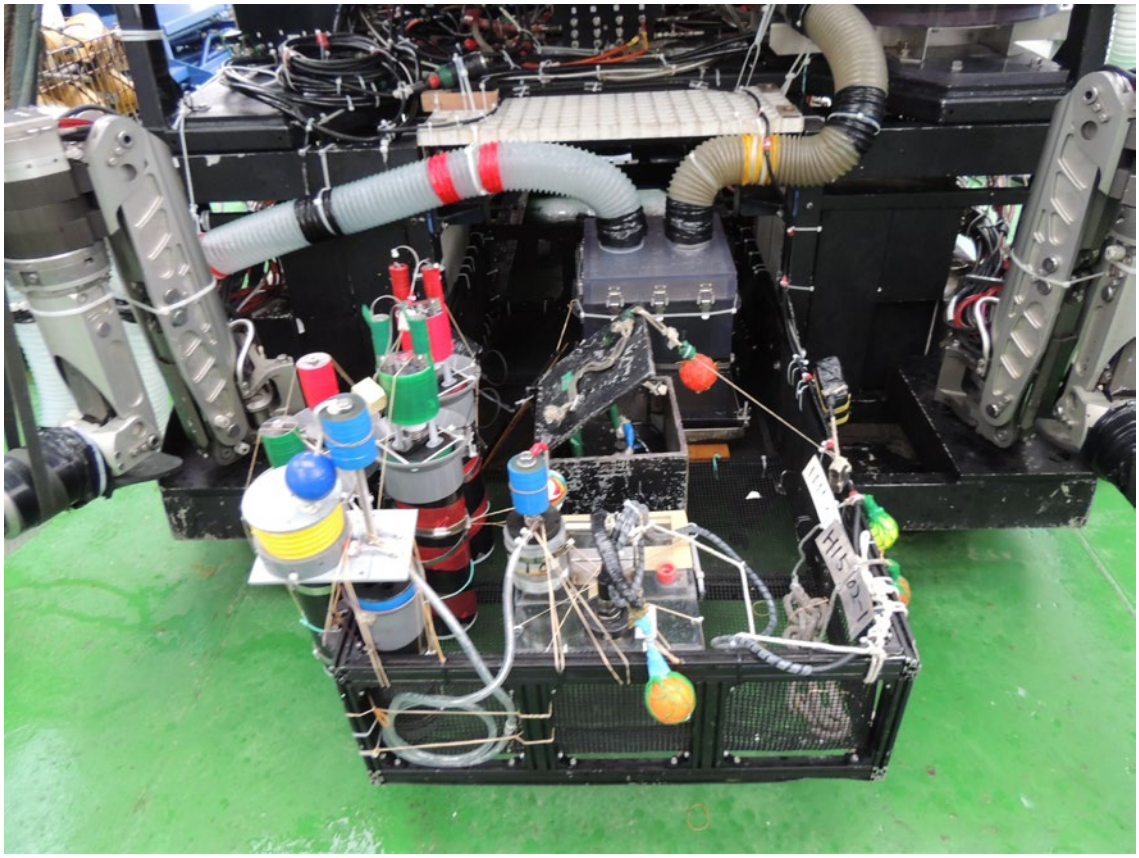
Purpose:
Collection of *Calyptogena* clams and their eggs
Collection of microbial mats
Sampling of seawater
Fluorescent observation of biological and sediment samples

Payload Equipment:
Incubation box X1
Suction sampler（multiple canister & single canister）
Sample box X1
Nomaki-type core X4
Furushima-type fluorescent filter X1

Dive Summary
‡ Observation of fluorescence of *Calyptogena* colonies and sampling of seawater with a niskin sampler.
The site location 35°00.063'N, 139°13.529'E, 1177 m

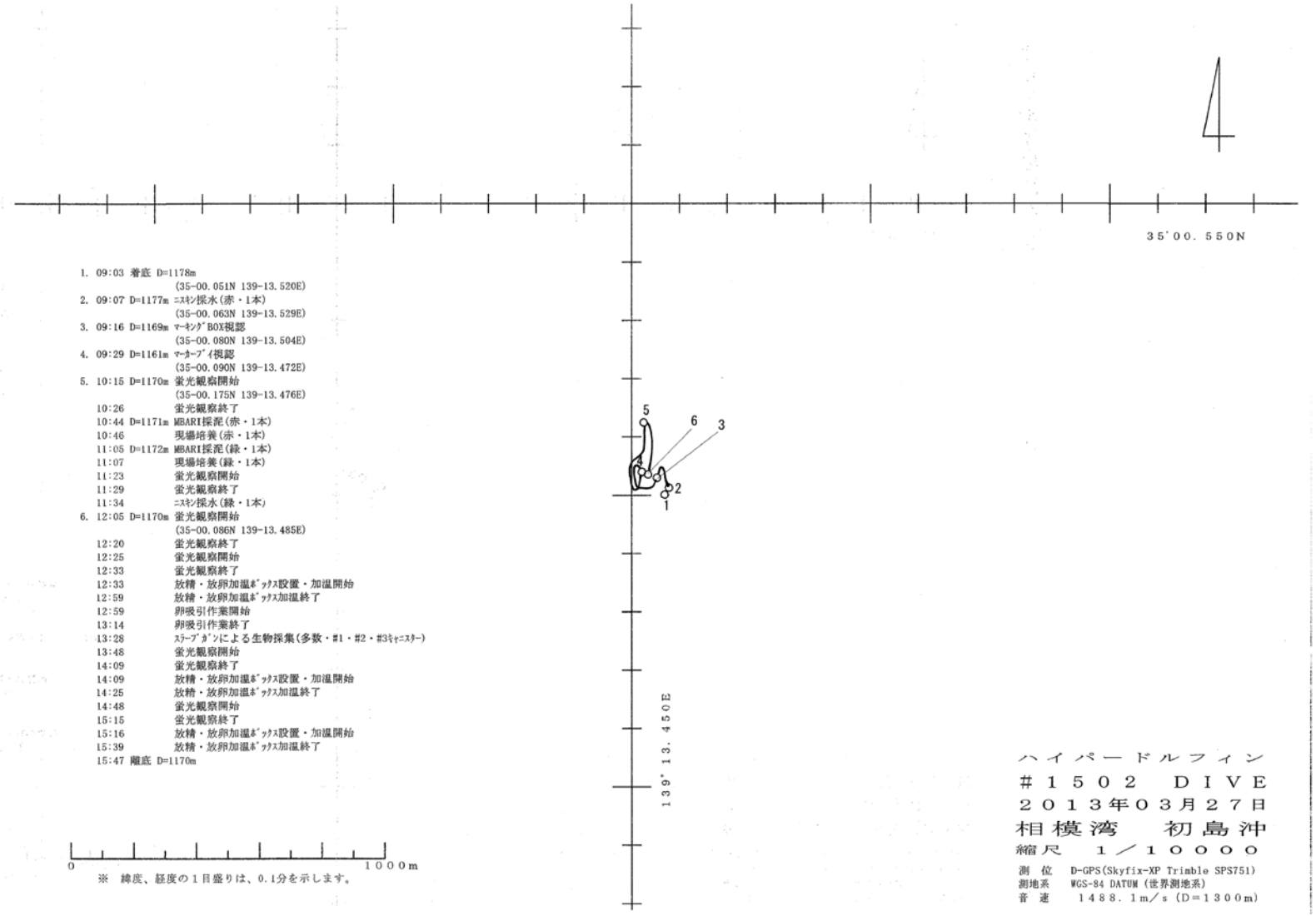
‡ Chemical fixation and collection of sediments with Nomaki-type cores (green and red), sampling of seawater with a niskin sampler, and observation of fluorescence of microbial mats.
The site location 35°00.175'N, 139°13.476'E, 1170 m

‡ Incubation of *Calyptogena* colonies with an incubation box to collect their eggs and observation of fluorescence of *Calyptogena* colonies.
The site location 35°00.086'N, 139°13.485'E, 1170 m



Payload of HPD#1502

Dive track and event list of HPD#1502



1. 09:03 着底 D=1178m
(35-00.051N 139-13.520E)
2. 09:07 D=1177m ﾆｽｷﾝ採水(赤・1本)
(35-00.063N 139-13.529E)
3. 09:16 D=1169m ﾏｰｷﾝｸﾞBOX視認
(35-00.080N 139-13.504E)
4. 09:29 D=1161m ﾏｰｶｰﾌﾞｲ視認
(35-00.090N 139-13.472E)
5. 10:15 D=1170m 蛍光観察開始
(35-00.175N 139-13.476E)
10:26 蛍光観察終了
10:44 D=1171m MBARI採泥(赤・1本)
10:46 現場培養(赤・1本)
11:05 D=1172m MBARI採泥(緑・1本)
11:07 現場培養(緑・1本)
11:23 蛍光観察開始
11:29 蛍光観察終了
11:34 ﾆｽｷﾝ採水(緑・1本)
6. 12:05 D=1170m 蛍光観察開始
(35-00.086N 139-13.485E)
12:20 蛍光観察終了
12:25 蛍光観察開始
12:33 蛍光観察終了
12:33 放精・放卵加温ﾎﾞｯｸｽ設置・加温開始
12:59 放精・放卵加温ﾎﾞｯｸｽ加温終了
12:59 卵吸引作業開始
13:14 卵吸引作業終了
13:28 ｽﾃｰﾌﾞｶﾞﾝによる生物採集(多数・#1・#2・#3ｷｬﾆｽﾀｰ)
13:48 蛍光観察開始
14:09 蛍光観察終了
14:09 放精・放卵加温ﾎﾞｯｸｽ設置・加温開始
14:25 放精・放卵加温ﾎﾞｯｸｽ加温終了
14:48 蛍光観察開始
15:15 蛍光観察終了
15:16 放精・放卵加温ﾎﾞｯｸｽ設置・加温開始
15:39 放精・放卵加温ﾎﾞｯｸｽ加温終了
15:47 離底 D=1170m

※ 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
＃1502 DIVE
2013年03月27日
相模湾 初島沖
縮尺 1／10000
測 位 D-GPS(Skyfix-XP Trimble SPS751)
測地系 WGS-84 DATUM (世界測地系)
音 速 1488.1m／s (D=1300m)

6) Summary of the HPD Dive #1503

Date: March. 28, 2013
Site: Off Hatsushima, Sagami Bay
Landing: 35°00.951'N, 139°13.384'E, 897m (8:58)
Leaving: 35°00.965'N, 139°13.325'E, 857 m (10:48)

Purpose:
in situ ^{13}C incubation of *Calyptogena* clams
Fluorescent observation of biological and sediment samples

Payload Equipment:
Incubation box X1
Suction sampler（multiple canister）
Nomaki-type core X4
Furushima-type fluorescent filter X1

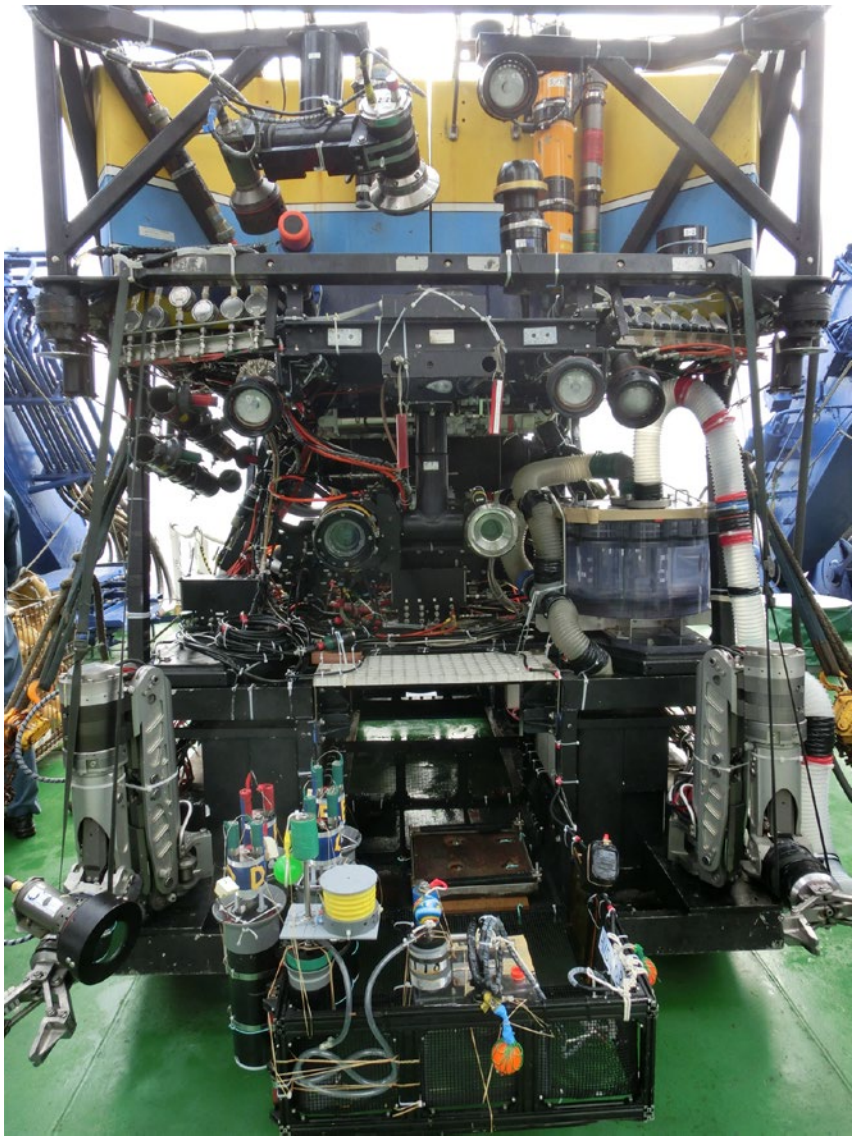
Dive Summary

‡ Sampling of seawater with a niskin sampler.
The site location 35°00.965'N, 139°13.325'E, 857 m

‡ *in situ* ^{13}C incubation of *Calyptogena* clams by Nomaki-type core (C, D, E, and F) and marker H1503
The sampling site location 35°00.965'N, 139°13.325'E, 857 m

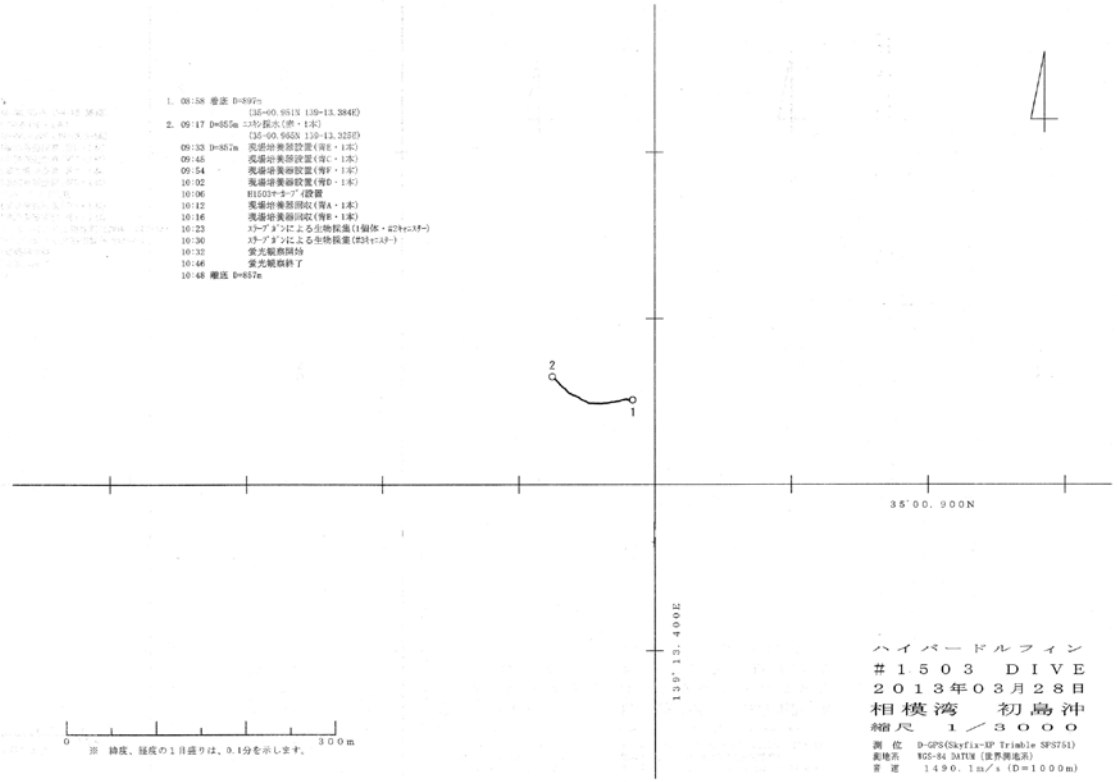
‡Retrieved two Nomai-type core (HPD#1499) in *Calyptogena* colony
The sampling site location 35°00.965'N, 139°13.325'E, 857 m

‡ Observation of fluorescence of *Calyptogena* colonies
The site location 35°00.965'N, 139°13.325'E, 857 m



Payload of HPD#1503

Dive track and event list of HPD#1503



7) Summary of the HPD Dive #1504

Date: March. 28, 2013
Site: Off Atami, Sagami Bay
Landing: 35°05.528'N, 139°10.261'E, 489 m (14:18)
Leaving: 35°05.576'N, 139°10.271'E, 492 m (16:17)

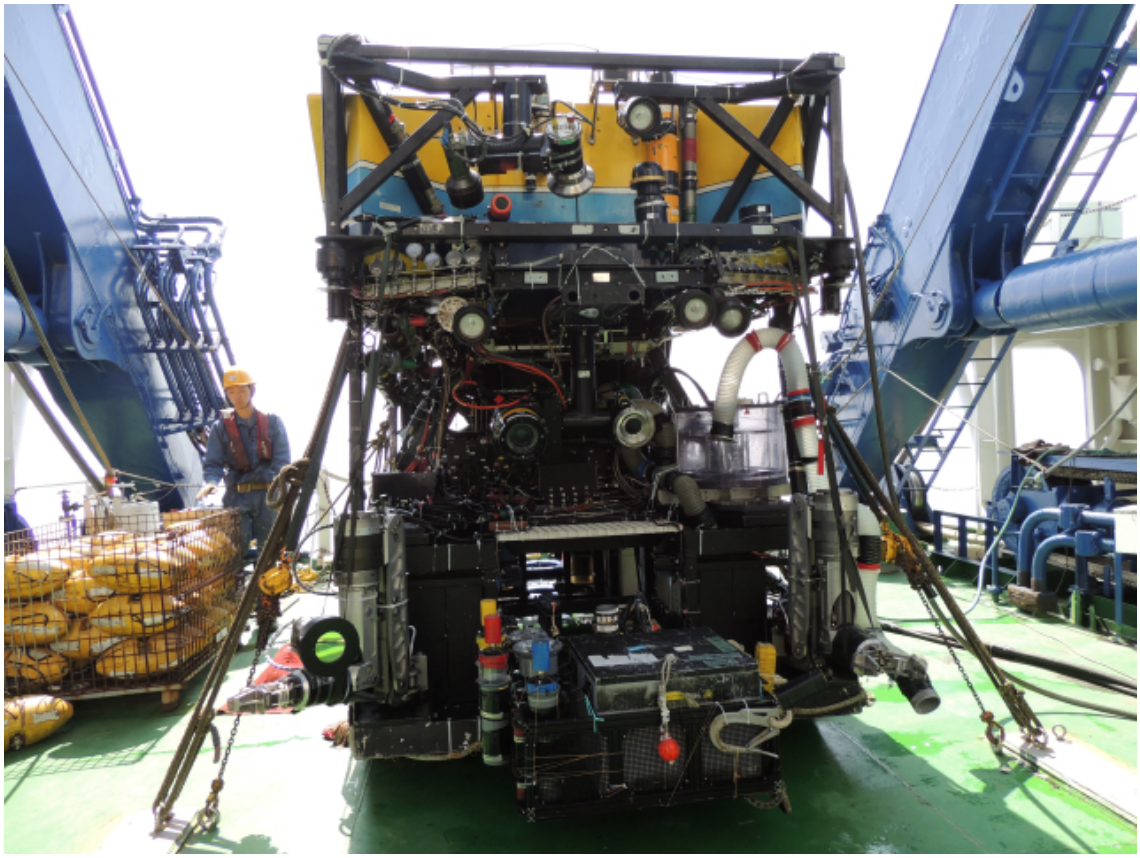
Purpose:

- Retrieval of a time-lapse video camera
- Observation and biological sampling at "SAITO" whale

Payload Equipment:

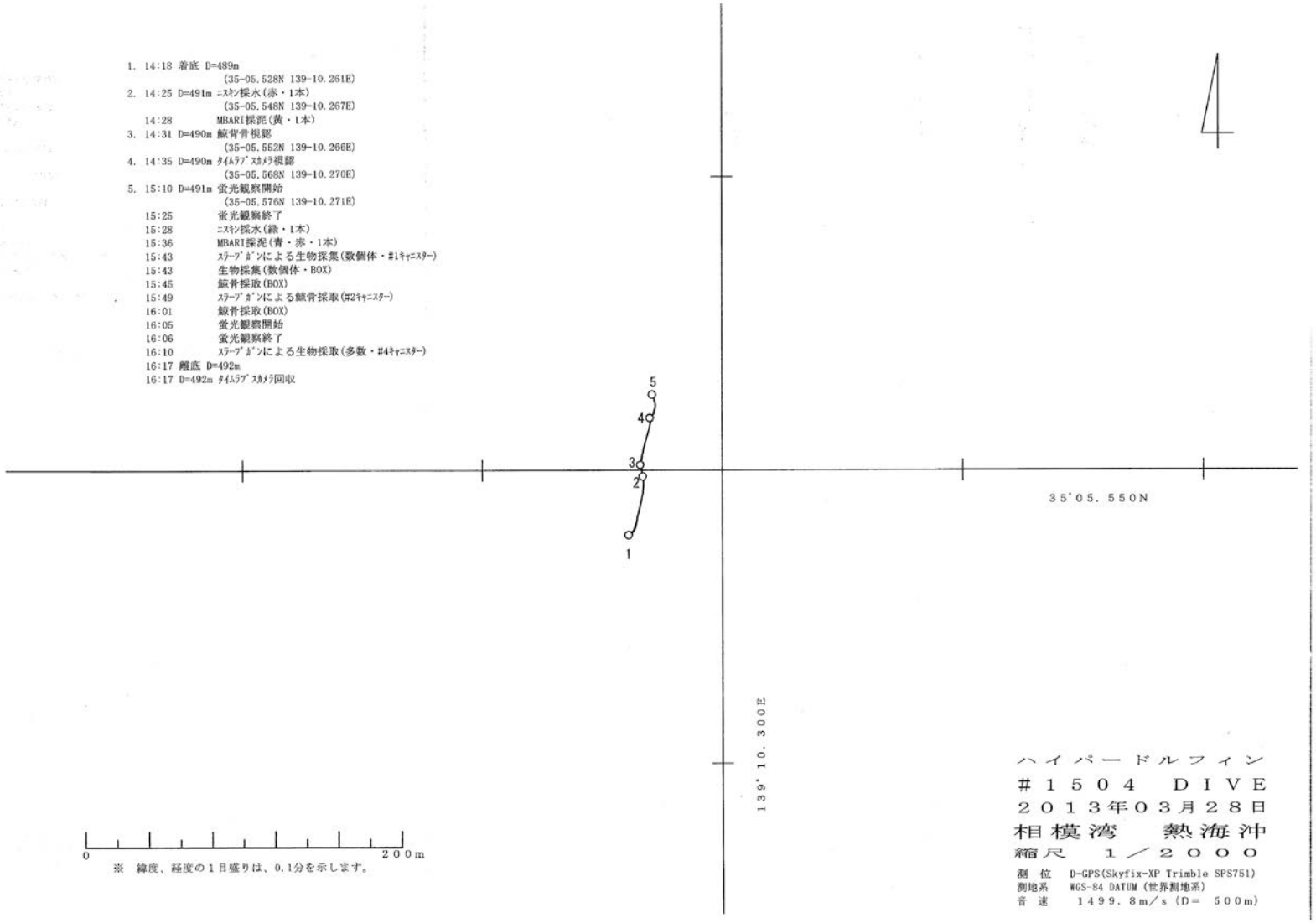
- Suction sampler（multiple canister）x1 set
- Sampling box (large) x1
- MBARI corer x3
- Niskin bottle x2
- Blue light installed on a right manipulator

Dive Summary
‡ Biological, core and water sampling, fluorescence observation, retrieval of a time-lapse video camera
The site location 35°05.576'N, 139°10.271'E, 492 m



Payload of HPD#1504

Dive track and event list of HPD#1504



8) Summary of the HPD Dive #1505

Date: March. 29, 2013
Site: Off Atami, Sagami Bay
Landing: 35°04.465'N, 139°07.618'E, 401 m (8:36)
Leaving: 35°04.490'N, 139°07.571'E, 402 m (10:33)

Purpose:
Explore of Sperm Whale

Payload Equipment:
Suction sampler（multiple canister & single canister）
Sample box X1
Niskin weter sampler X2
MBARI core sampler X3

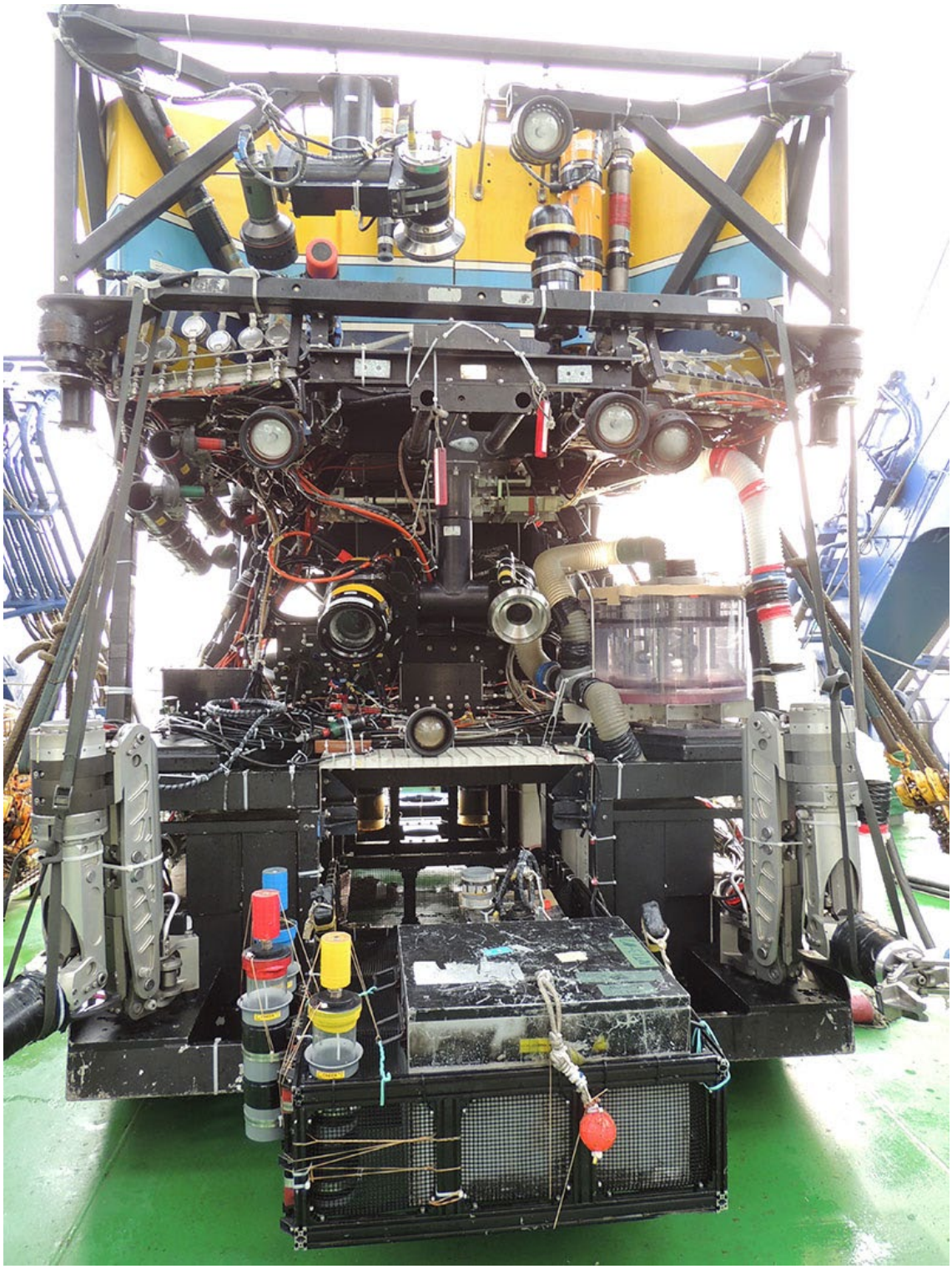
Dive Summary
‡ The water sample as control seawater with Niskin sampler (red) and MBARI core sampler (blue).
The site location 35°04.477'N, 139°07.583'E, 400 m

‡Make an observation about long bamboo.
The site location 35°04.472'N, 139°07.604'E, 400 m

‡Detection of *Megaptera novaeangliae*.
The site location 35°04.486'N, 139°07.572'E, 400 m

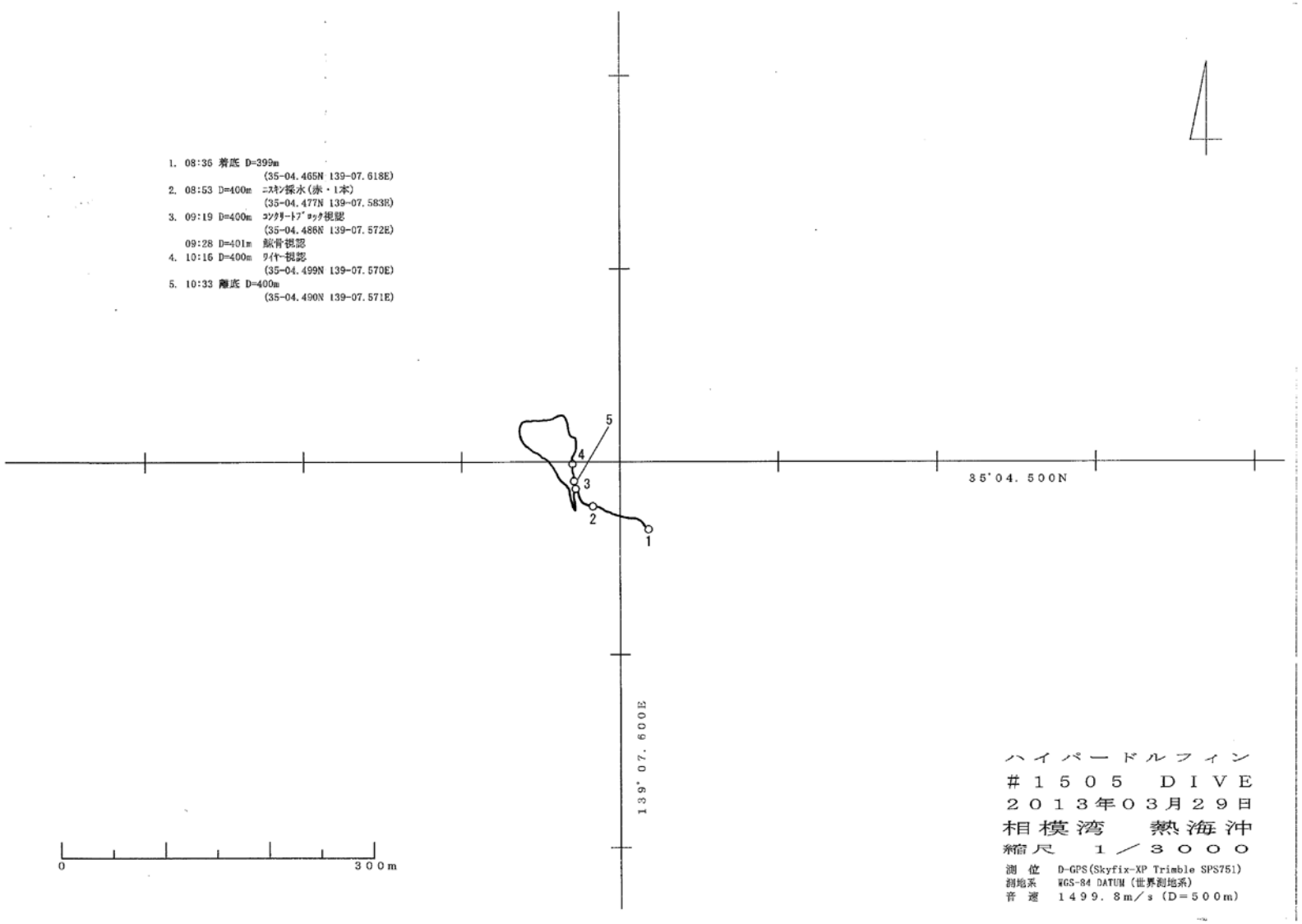
‡Explore of Sperm Whale

‡Sampling of acorn barnacle with suction samplers.
The site location 35°04.490'N, 139°07.571'E, 400 m



Payload of HPD#1505

Dive track and event list of HPD#1505



9) Summary of the HPD Dive #1506

Date: March. 29, 2013
Site: Off Hatsushima, Sagami Bay
Landing: 35°00.939'N, 139°13.408'E, 898m (13:00)
Leaving: 35°00.950'N, 139°13.331'E, 857 m (16:25)

Purpose:
Collection of *Calyptogena* clam's eggs

Payload Equipment:
Incubation box X1
Egg collection sampler with hand pomp
Suction sampler（multiple canister-single use）
Scoop sampler X1
Sample box X1

Dive Summary

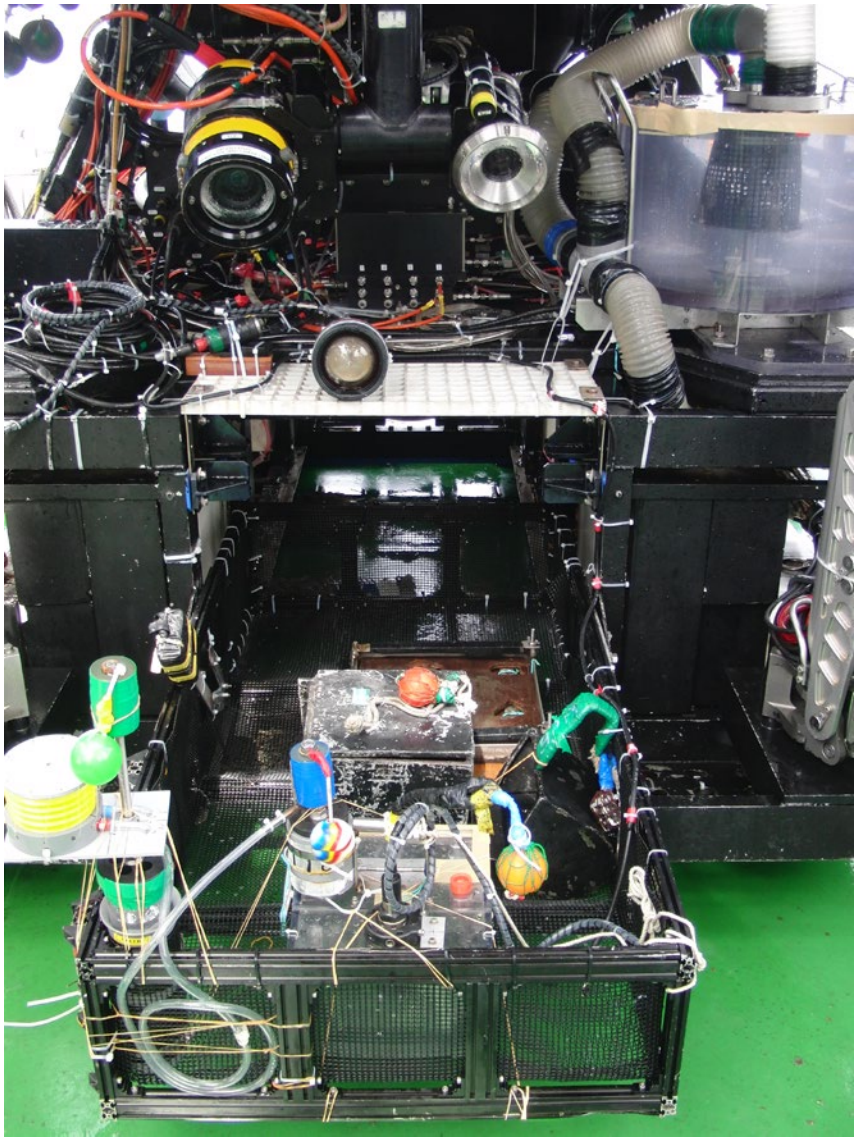
‡ Sampling of *Bathymodiolus* bivalves and *Calyptogena* clams with suction samplers.
The site location 35°00.933'N, 139°13.390'E, 910 m

‡ Sampling of tubeworms into sample box.
The site location 35°00.939'N, 139°13.386'E, 902 m

‡ Incubation of *Calyptogena* colonies with an incubation box.
The site location 35°00.946'N, 139°13.330'E, 860 m

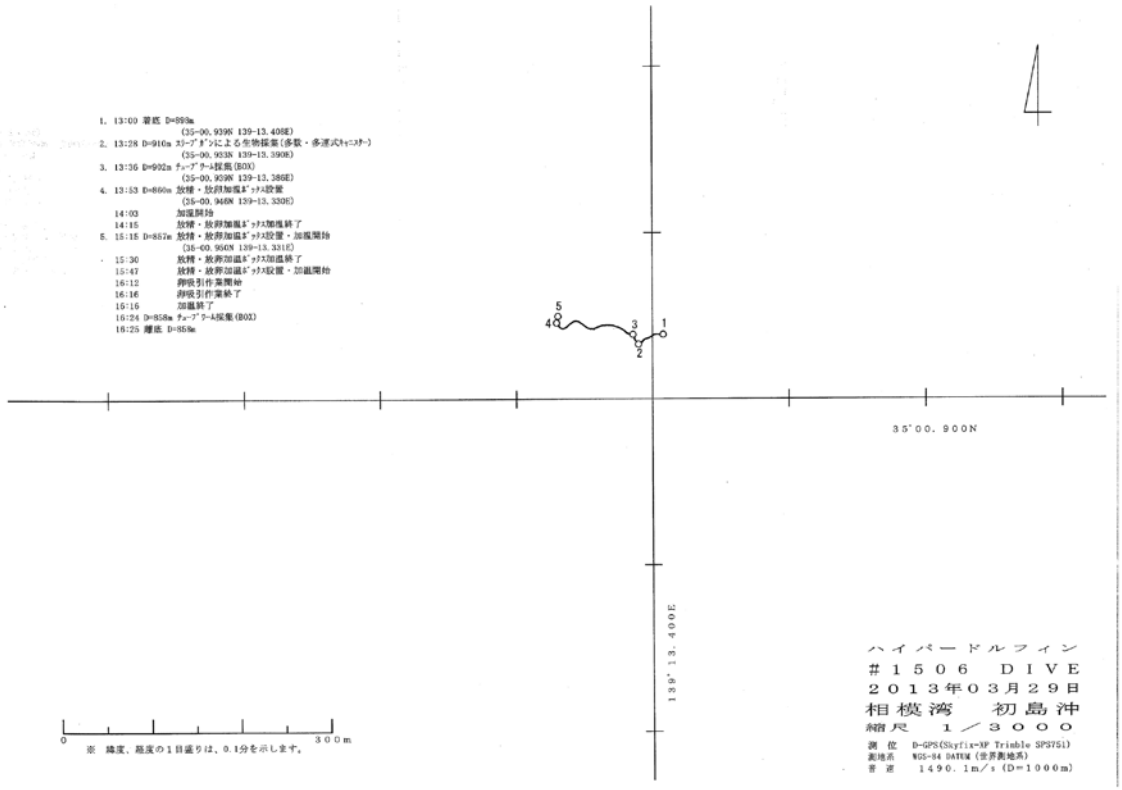
‡ Incubation of *Calyptogena* colonies with an incubation box.
The site location 35°00.950'N, 139°13.331'E, 857m

‡ Sampling of tubeworms into sample box.
The site location 35°00.950'N, 139°13.331'E, 858m



Payload of HPD#1506

Dive track and event list of HPD#1506



10) Summary of the HPD Dive #1507

Date: March. 30, 2013
Site: Off Hatsushima, Sagami Bay
Landing: 35°00.936'N, 139°13.399'E, 921m (7:50)
Leaving: 35°00.942'N, 139°13.395'E, 905 m (9:11)

Purpose:
Fluorescent imaging observation and biological sampling

Payload Equipment:
Niskin bottle X2
Suction sampler（multiple canister）
Furushima-type fluorescent filter X2

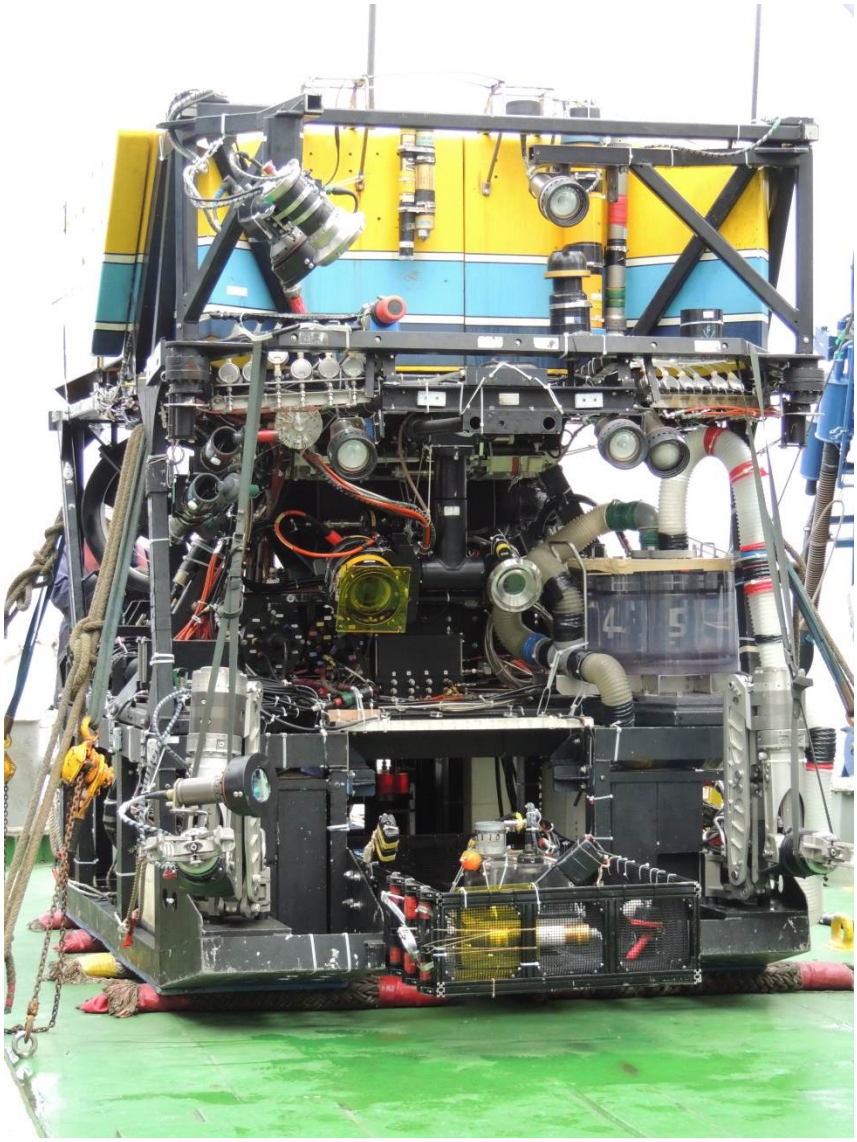
Dive Summary

‡Fluorescence photographing test with the fluorescence excitation filter from 250m depth to 700m

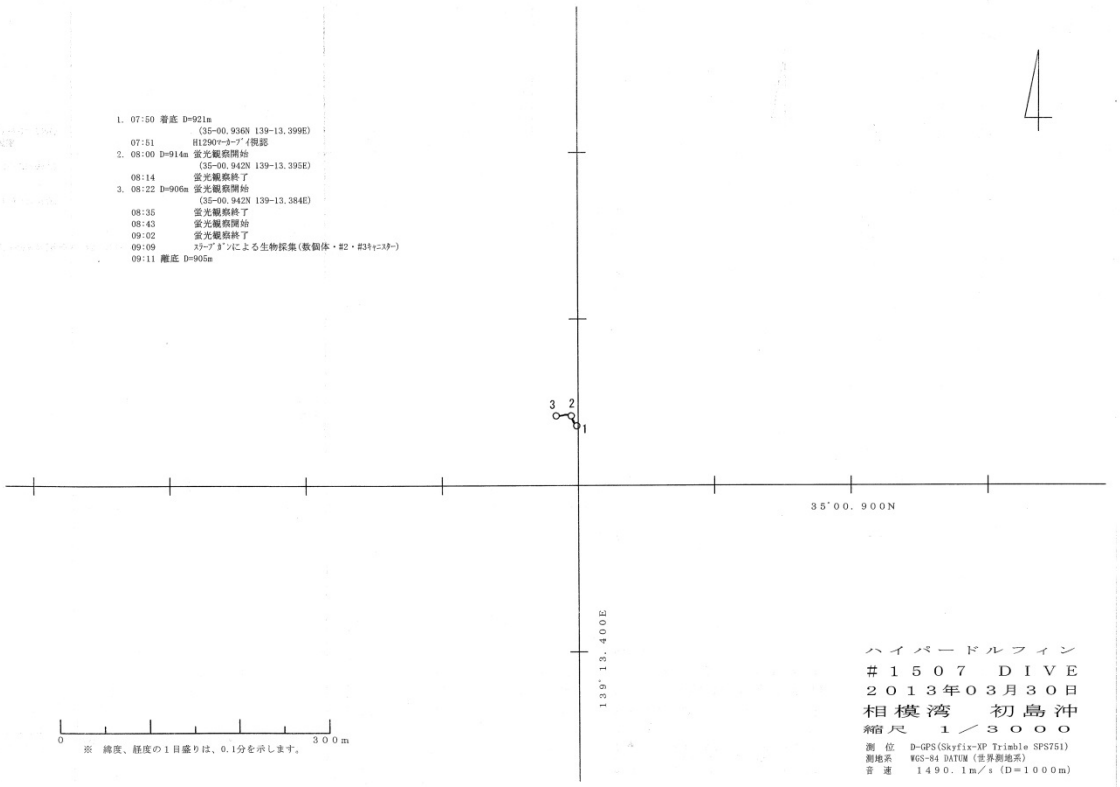
‡ Observation of fluorescence of *Bathymodiolus* colonies
The site location 35°00.942'N, 139°13.395'E, 914 m

‡Observation of fluorescence of *Calyptogena* colonies
The site location 35°00.942'N, 139°13.384'E, 906 m

‡Observation of fluorescence *and Calyptogena* and *Bathymodiolus* several were sampled.
The site location 35°00.942'N, 139°13.384'E, 906 m



Payload of HPD#1507



Dive track and event list of HPD#1507

5. Preliminary research reports

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

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. . 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) Morphological observation of *Osedax* polychaetes using MRI

Yoshihiro FUJIWARA (JAMSTEC)

Objective and achievement in this cruise

Osedax polychaetes inhabit whale bones and acquire nutrition from the bones through a characteristic organ named "root". As plant roots, morphology of *Osedax* roots is protean. Some shows a "potato" like structure and the other are fibrous root like. The roots locate in hard whale bones, which are very difficult to be excavated. Therefore, the precise morphology of the root system is still unknown.

The genus *Osedax* is a member of the class Polychaeta that is a segmented worm. However, no segmentation has been reported from the adult female *Osedax* worms. Vestimentiferan tubeworms are also one of the polychaetes and shows specialized body structure. Most of the body parts have no segment except the most posterior region named opisthosome. *Osedax* and vestimentiferans are closely related group phylogenetically. Therefore, *Osedax* may have a segmented part at the very end of the root tissue. For better visualization of the *Osedax* roots, we will conduct a nondestructive imaging analysis using magnetic resonance imaging (MRI) system.

During the NT13-06 cruise, we collected some pieces of a skull of a baby sperm whale that were deployed at a depth of 489 m off Atami in Sagami Bay on June 8, 2012. We also collected two pieces of finger bones of a humpback whale that were deployed at a depth of 400 m off Atami in Sagami Bay on December 7, 2011. Many *Osedax* polychaetes inhabited all the bones collected and were reared under laboratory condition onboard at their ambient temperature in the natural habitats.

We also deployed and retrieved a time-lapse video camera system and a 50-kg cow bone parcel at a depth of 490 m off Atami in Sagami Bay for understanding of faunal composition of the necropagous stage of "animal carcass" community. The video images were taken every 12 minutes for one minute from 10 a.m. on March 26, 2013 until retrieval. Many pugnose eels and large isopods were the most dominant at this stage in this region.

3) Development of the technique to photograph the fluorescence that deep sea organisms emit in *in-situ*

Yasuo Furushiam[1], Shinji Tsuchida[1], Shuichi Shigeno[1], Tadashi Maruyama[1], Sadao Suzuki[2], Masahiko Sasano[3]

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

[2] *Oceanographic Research Engineering (O.R.E.)*

[3] *National Maritime Research Institute (NMRI)*

Objective and achievement in this cruise

In this research cruise, we carry out taking photographs of the fluorescence which deep sea organisms emit using a camera system of the Hyper Dolphin. An exciting light filter (fluorescence excitation filter: BE1, Naightsea LLC) is attached to a lighting (1 light or 2 lights) of the Hyper Dolphin to get image and video of the fluorescence in *in-situ*. Through the cut filter (blue-blocking filter: Naightsea LLC)), the fluorescence taking photographs of deep sea organisms are carried out with a camera system of the Hyper Dolphin. Fluorescence image targets are *Calyptogena*, *Bathymodiolus*, tubeworms and crabs etc. inhabiting the deep-sea bottom off Hatsushima in Sagami Bay. And the identification of species emitting fluorescence, fluorescent color and the light emission part of various organisms, characteristics of two-dimensional distribution of organism are clarified. Furthermore, based on consequence of the provided image and video, we aim at the technical development to monitor the distribution of the deep sea organism using the fluorescence photography. As for the ecological knowledge about significance and the role of the fluorescence that deep sea organisms emit, there is much unexplained point. In this research cruise, as for obtaining fluorescence image and video in in-situ, it will be in basis to obtain the ecological (or physiological) knowledge of deep sea organisms.

Future studies

* Extraction of problems in this photographing research and improvement of the camerawork
* The analysis of wave length and the color of the fluorescence that deep sea organisms emit
* Establishment of the technique to estimate two-dimensional distribution and

population from a fluorescence image

* The tool development with a new photoenvironment to monitoring behavior and distribution of the deep sea organisms.

4) Exploring protists associated with tubeworms.

Kiyotaka Takishita[1], Fumiya Noguchi[2], Akinori Yabuki[1]

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

[2]*Graduate School of Marine Science and Technology, Tokyo University of Marine Science and Technology*

Objective and achievement in this cruise

It has recently been unveiled that a wide variety of microbial eukaryotes (protists) occur in chemosynthetic ecosystems, such as hydrothermal vents and methane seeps. However, there is little knowledge regarding protists associated with endemic animals inhabiting these environments. To address this issue, in the present study, the occurrence of protists in the tubeworms *Lamellibrachia* sp. and *Alaysia* sp. from methane seeps in Sagami Bay will be investigated with PCR and whole-mount *in situ* hybridization. Sediment samples obtained from this seep site will be also analyzed with PCR, microscopy and cultivation to understand whether the protists associated with the tubeworms have a free-living life stage. The tissues of *Lamellibrachia* sp. and *Alaysia* sp. as well as sediment samples were stored in liquid nitrogen for the extractions of DNA and RNA. The tissues of two tubeworm species were also fixed with 4% paraformaldehyde in filtered artificial seawater followed by substitution with 70% ethanol in filtered artificial seawater for whole-mount *in situ* hybridization. The sediment samples were fixed with glutaraldehyde for microscopic observation using an *in situ* incubation core. The sediment samples were also inoculated to several types of media for cultivation of protists.

Future studies

*PCR detection of protists associated with *Lamellibrachia* sp. and *Alaysia* sp. with non-metazoan primers of 18S rRNA gene

*Whole-mount *in situ* hybridization of the tubeworm tissues with a specific probe based on the 18S rRNA gene sequence detected with non-metazoan PCR

*Microscopic observation of fixed sediment samples and cultivation/isolation of protists with “raw” (non-fixed) sediment samples

5) Diverse Glycoconjugates and their Receptors in the Deep Sea Invertebrates

Yasuhiro Ozeki[1], Yasuhiro Koide[1], Imtiaj Hasan[1,2], Misaki Fujio[3], Noriaki Kojima[3]

[1]*Graduate School of NanoBio Sciences, Yokohama City University (YCU)*

[2]*Dept. Biochemistry and Molecular Biology, Rajshahi University, Bangladesh*

[3]*Yokohama Science Frontier High School (YSFH)*

Objective and achievement in this cruise

Oligosaccharides of glycoconjugates on cells essentially work for glycan-mediated signaling through the recognition with their receptors as carbohydrate-binding proteins (lectins). Proposers originally determined the two novel primary structure of α-D-galactose-binding lectin of SUEL (P22031) and MytiLec (B3EWR1) from the echinoderm and the mussel, respectively (Biochemistry 30, 2391-94 1991; JBC. 287, 44772-83. 2012). They curiously bound with the globotriaosylsphingolipids on cells down-regulated the cell proliferation and gene expression of a transporter (Protein J. 31, 15-26. 2012).

These scientific backgrounds indicate that oligosaccharides with α-D-galactoside and lectins to recognize the structure regulate different cell functions, however, the perspective studies on the significance of this subject is still not clear yet. Project No. 5 in cruse NT13-06 focus to find the presence of D-galactose containing glycoconjugates and lectins to decipher the sugar from the deep sea invertebrates. The investigation of them will lead us to know the information on various essential or valuable glyco-products and glyco-genes which have been apparently maintained during evolution and are still present in the deep sea invertebrates.

The research cruse provided that 1) The phyla mollusk and annelid lived in the deep sea contained glycoconjugates with D-galactoside in addition to the lectins which binds to α-galactoside. 2) Different from the D-galactose-binding lectins in sea shore invertebrates (Comp. Biochem. Physiol. 152B, 382-389. 2009), some lectins from the deep sea animals specifically distinguished anomer structures.

Future studies

1) Completion of purification on each glycoconjugate and lectin. 2) Glycan- and lectin-array analysis to find the details on both glycan-binding and oligosaccharide structure profiles. 3) Assay for cell regulation activities of them.

6) 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 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. Water depth, temperature, and dissolved oxygen concentration at the experimental site was 852 m, 4.1 dC, and 1.1 ml L^{-1}, respectively. ROV homer was also deployed next to the incubation cores to find the cores at the retrieve dive.

Future studies

The *in situ* incubation cores will be retrieved 10 to 14 days after the deployment, during the *Natsushima* cruise NT13-07. Environmental conditions in the cores after incubation will be measured onboard. The recovered cores will be sliced off into some different layers and kept frozen until further analyses in a laboratory. Geochemical analyses will be carried out to estimate microbial incorporation of algal organic matters.