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)
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   1) Transfer analysis of intracellular bacterial symbiont from Calyptogena clams to next generation
   2) Investigating habitat environments of Calyptogena spp., and sampling the bivalves for development of a rearing system for invertebrates in chemosynthetic ecosystem
   3) Development for the latest ocean bottom seismic and tilt measurements.
   4) Incorporation of algal organic matters into microbial biomass
   5) Long-term rearing of seep animals 36

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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 invertebrates in chemosynthetic ecosystem.
   III) Development for the latest ocean bottom seismic and tilt measurements.
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)
<table>
<thead>
<tr>
<th>Date</th>
<th>Local Time</th>
<th>Note</th>
<th>Position/Weather/Wind/Sea condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>2/Apr</td>
<td>06:00</td>
<td>Boarded</td>
<td>4/2 12:00(UTC+9)</td>
</tr>
<tr>
<td></td>
<td>10:00</td>
<td>Left all shores line, left YOKOSUKA.</td>
<td>Rain</td>
</tr>
<tr>
<td></td>
<td>10:35-12:30</td>
<td>Scientific meeting.</td>
<td>North-East (Moderate breeze)</td>
</tr>
<tr>
<td></td>
<td>11:30-13:00</td>
<td>On board education for scientists.</td>
<td>Low Cloud (880m)</td>
</tr>
<tr>
<td></td>
<td>13:40</td>
<td>Arrived at research area (off HATSUSHIMA)</td>
<td>Low Cloud (880m)</td>
</tr>
<tr>
<td></td>
<td>13:41</td>
<td>Released KIT.</td>
<td>Visibly 2</td>
</tr>
<tr>
<td></td>
<td>14:40</td>
<td>Got anchor off ITTO.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>16:42</td>
<td>Kooka Ceremony.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>18:30-19:40</td>
<td>Scientific meeting.</td>
<td></td>
</tr>
<tr>
<td>3/Apr</td>
<td>00:00</td>
<td>Heaving anchor, then continued proceeding to dive point.</td>
<td>55.00.9N, 135.16.4E</td>
</tr>
<tr>
<td></td>
<td>07:00</td>
<td>Arrived at dive point.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>08:22</td>
<td>HPD dove &amp; started her operation (1508).</td>
<td>NWW-5 F Fresh breeze</td>
</tr>
<tr>
<td></td>
<td>08:57</td>
<td>HPD landed on the sea bottom (D=876m)</td>
<td>(Sea depth)</td>
</tr>
<tr>
<td></td>
<td>16:13</td>
<td>HPD left the sea bottom (D=850m)</td>
<td>Low (Sea height)</td>
</tr>
<tr>
<td></td>
<td>16:56</td>
<td>Recovered HPD &amp; finished the operation.</td>
<td>Visibly 3</td>
</tr>
<tr>
<td></td>
<td>17:00</td>
<td>Got anchor off ITTO.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>18:30-19:40</td>
<td>Scientific meeting.</td>
<td></td>
</tr>
<tr>
<td>4/Apr</td>
<td>00:00</td>
<td>Heaving anchor, then continued proceeding to dive point.</td>
<td>34.01.9N, 135.16.3E</td>
</tr>
<tr>
<td></td>
<td>07:00</td>
<td>Arrived at dive point.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>08:23</td>
<td>HPD dove &amp; started her operation (1059)</td>
<td>SE-3 G F (Genlic breeze)</td>
</tr>
<tr>
<td></td>
<td>09:07</td>
<td>HPD landed on the sea bottom (D=890m)</td>
<td>2 Gen smooth</td>
</tr>
<tr>
<td></td>
<td>10:01</td>
<td>HPD left the sea bottom (D=900m)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>11:47</td>
<td>Recovered HPD &amp; finished the operation.</td>
<td>Visibly 3</td>
</tr>
<tr>
<td></td>
<td>17:00</td>
<td>Proceeded to research area (off BOSSO).</td>
<td></td>
</tr>
<tr>
<td></td>
<td>18:30-19:40</td>
<td>Scientific meeting.</td>
<td></td>
</tr>
<tr>
<td>5/Apr</td>
<td>00:00</td>
<td>Deployed BIT-SP (2000 class, 166487)</td>
<td>34.09.09N, 140.05.08E</td>
</tr>
<tr>
<td></td>
<td>01:00</td>
<td>Arrived at research area (off BOSSO).</td>
<td></td>
</tr>
<tr>
<td></td>
<td>01:06</td>
<td>Released KBIT.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>06:05-11:54</td>
<td>Scientific meeting.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12:01</td>
<td>HPD dove &amp; started her operation (1151)</td>
<td>4 Gen moderate</td>
</tr>
<tr>
<td></td>
<td>12:47</td>
<td>HPD landed on the sea bottom (D=1354m)</td>
<td>Visibly 5</td>
</tr>
<tr>
<td></td>
<td>13:46</td>
<td>Recovered HPD &amp; finished the operation.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>18:30-19:30</td>
<td>Scientific meeting.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>21:00</td>
<td>Arrived at above area</td>
<td></td>
</tr>
<tr>
<td></td>
<td>21:23</td>
<td>Deployed NUDORS. (           34.09.09N, 141.05.08E)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>21:27</td>
<td>Started to NUDORS calibration.</td>
<td></td>
</tr>
<tr>
<td>6/Apr</td>
<td>00:07</td>
<td>Completed NUDORS calibration (34.09.09N, 141.05.08E, D=1009m)</td>
<td>35.05.9N, 135.16.4E</td>
</tr>
<tr>
<td></td>
<td>08:28</td>
<td>Commed proceeding to YOKOSUKA due to rough sea condition.</td>
<td>SSE-6 Strong breeze</td>
</tr>
<tr>
<td></td>
<td>13:00</td>
<td>Got anchor at off YOKOSUKA.</td>
<td>Visibly 2</td>
</tr>
<tr>
<td></td>
<td>18:30-19:35</td>
<td>Scientific meeting.</td>
<td></td>
</tr>
<tr>
<td>7/Apr</td>
<td>00:00</td>
<td>Recovered HPD &amp; finished the operation.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>18:30-19:35</td>
<td>Scientific meeting.</td>
<td></td>
</tr>
<tr>
<td>8/Apr</td>
<td>00:00</td>
<td>HPD1511, OFF HATSUSHIMA.</td>
<td>4/5 12:00(UTC+9)</td>
</tr>
<tr>
<td></td>
<td>00:30</td>
<td>Released KIT.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>07:30</td>
<td>Arrived at research area (off HATSUSHIMA).</td>
<td></td>
</tr>
<tr>
<td></td>
<td>08:48</td>
<td>HPD dove &amp; started her operation (1511).</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10:00</td>
<td>HPD landed on the sea bottom (D=735m)</td>
<td>3 Low cloud (890m)</td>
</tr>
<tr>
<td></td>
<td>10:01</td>
<td>HPD left the sea bottom (D=832m)</td>
<td>Visibly 8</td>
</tr>
<tr>
<td></td>
<td>11:49</td>
<td>Recovered HPD &amp; finished the operation.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>18:30-19:35</td>
<td>Scientific meeting.</td>
<td></td>
</tr>
<tr>
<td>9/Apr</td>
<td>00:00</td>
<td>HPD1512, OFF HATSUSHIMA, and proceeding to YOKOSUKA.</td>
<td>4/9 12:00(UTC+9)</td>
</tr>
<tr>
<td></td>
<td>07:00</td>
<td>Arrived at dive point.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>08:29</td>
<td>HPD dove &amp; started her operation (1512).</td>
<td></td>
</tr>
<tr>
<td></td>
<td>09:00</td>
<td>HPD landed on the sea bottom (D=909m)</td>
<td>Low (Sea height)</td>
</tr>
<tr>
<td></td>
<td>13:49</td>
<td>HPD left the sea bottom (D=890m)</td>
<td>Visibly 7</td>
</tr>
<tr>
<td></td>
<td>17:00</td>
<td>Recovered HPD &amp; finished the operation.</td>
<td></td>
</tr>
<tr>
<td>10/Apr</td>
<td>00:00</td>
<td>Arrived at YOKOSUKA.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10:00</td>
<td>scientific (Research boat from) NUDORS.</td>
<td></td>
</tr>
</tbody>
</table>
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
Proposal 1:

Tetsuro 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)

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

Akihiro Tame
Marine Works Japan Ltd.

Makoto Sugimura
Enoshima Aquarium

Proposal 2:
Masaaki Konishi
Department of Biotechnology and Environmental Technology
Kitami Institute of Technology

Tatsuhiro Fukuba,
Marine Technology and Engineering Center
Japan Agency of Marine-Earth Science and Technology (JAMSTEC)

Katsunori Yanagawa,
Extremobiosphere Research Program
Institute of Biogeoscience,
Japan Agency of Marine-Earth Science and Technology (JAMSTEC)

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

Shinro Nishi, Biogeos, JAMSTEC
Marine Biodiversity Research Program
Institute of Biogeosciences
Japan Agency for Marine-Earth Science and Technology (JAMSTEC)

Hisako Hirayama
Extremobiosphere Research Program
Institute of Biogeoscience,
Japan Agency of Marine-Earth Science and Technology (JAMSTEC)

Kazuna Shitaka, Biogeos, JAMSTEC
Marine Biodiversity Research Program
Institute of Biogeosciences
Japan Agency for Marine-Earth Science and Technology (JAMSTEC)

Proposal 3:

Hajime Shiobara
Ocean Hemisphere Research Center, Earthquake Research Institute
University of Tokyo

Aki Ito
Institute for Research on Earth Evolution
Japan Agency of Marine-Earth Science and Technology (JAMSTEC)

Shore base Scientists

Proposal 1:

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)
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)

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

Naoki Ito
Tohoku University

Proposal 2
Taishi Tsubouchi
Marine Biodiversity Research Program
Institute of Biogeosciences
Japan Agency for Marine-Earth Science and Technology (JAMSTEC)

Masahiro Yamamoto,
Extremobiosphere Research Program
Institute of Biogeoscience,
Japan Agency of Marine-Earth Science and Technology (JAMSTEC)

Hiroko Makita
Extremobiosphere Research Program
Institute of Biogeoscience,
Japan Agency of Marine-Earth Science and Technology (JAMSTEC)

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

Jun-ichi Horiuchi
Department of Biotechnology and Environmental Technology
Kitami Institute of Technology

Proposal 3:
Masanao Shinohara
Center for Geophysical Observation and Instrumentation, Earthquake Research Institute
University of Tokyo
Takehi Isse  
Ocean Hemisphere Research Center, Earthquake Research Institute  
University of Tokyo  

Hiroko Sugioka  
Institute for Research on Earth Evolution  
Japan Agency of Marine-Earth Science and Technology (JAMSTEC)  

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  

11
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 invertebrates  
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 H₂S by D-POTE system  
The site located 35°00’937N, 139°13’379E, 903 m

‡Detecting H₂S by D-POTE system  
‡Sampling *Calyptogena* spp. by slurp gun  
The site located 35°00’935N, 139°13’376E, 901 m

‡Detecting H₂S by D-POTE system  
The site located 35°00’941N, 139°13’375E, 901 m

‡Detecting H₂S by D-POTE system  
‡Sampling sediment by MT core (Green)  
The site located 35° 00.957N, 139° 13.336E, 857 m
‡Detecting H₂S by D-POTE system
The site located 35° 00.953N, 139° 13.329E, 856 m

‡Detecting H₂S by D-POTE system
‡Sampling sediment by MT core (Red)
The site located 35° 00.960N, 139° 13.322E, 849 m

‡Detecting H₂S by D-POTE system
‡Sampling sediment by H core (Blue)
The site located 35° 00.942N, 139° 13.290E, 849 m

‡Detecting H₂S by D-POTE system
The site located 35° 00.940N, 139° 13.284E, 846 m

‡Detecting H₂S by D-POTE system
‡Sampling sediment by MT core (Blue and Yarrow)
The site located 35° 00.939N, 139° 13.253E, 829 m

‡Detecting H₂S by D-POTE system
The site located 35° 00.948N, 139° 13.222E, 803 m

‡Detecting H₂S 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 Mycinae gen. et sp.
‡Sampling Calyptogena spp. by slupe gun
The site located 35° 00.936N, 139° 13.280E, 909 m
Payload of dive HPD#1509
Dive track and event list of HPD#1509
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
Dive track and event list of HPD#1511

1. 11:09 時点 D=0m
   - 11:06 時点 D=0m
   - 11:03 時点 D=0m

2. 11:29 時点 D=0m
   - 11:27 時点 D=0m
   - 11:25 時点 D=0m

3. 12:16 時点 D=0m
   - 12:14 時点 D=0m
   - 12:12 時点 D=0m

4. 14:32 時点 D=0m
   - 14:30 時点 D=0m

5. 16:09 時点 D=0m
   - 16:07 時点 D=0m
   - 16:05 時点 D=0m

6. 17:08 時点 D=0m
   - 17:06 時点 D=0m
   - 17:04 時点 D=0m

Heard of the Dolphin
# 1511 DIVE
2013年04月08日
相模湾 初島沖
潮 gauge 1 / 3000

位 置 : GPS (UTC+9) 18'31.7378" N, 140'17.4537" E
装備 : WSC-64 DATEN (浪速県水産)
航 速 14.4 kn / s (D=1090 m)
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

1. 09:07 砂丘 D-97a
   (23:05, 50m, 10°-12.4°E)
2. 09:25 D-92b 砂丘 D-97a 予想地点 (23:05, 30°-12.4°E)
3. 09:35 25 30°-12.4°E
4. 09:35 砂丘 D-97a 予想地点 (23:05, 30°-12.4°E)
5. 09:35 砂丘 D-97a 予想地点 (23:05, 30°-12.4°E)
6. 09:35 砂丘 D-97a 予想地点 (23:05, 30°-12.4°E)
7. 09:35 砂丘 D-97a 予想地点 (23:05, 30°-12.4°E)
8. 09:35 砂丘 D-97a 予想地点 (23:05, 30°-12.4°E)
9. 09:35 砂丘 D-97a 予想地点 (23:05, 30°-12.4°E)
10. 09:35 砂丘 D-97a 予想地点 (23:05, 30°-12.4°E)
5. Preliminary research reports

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

Tetsuro Ikuta¹, Takao Yoshida¹, Yoshihiro Takaki¹, Tadashi Maruyama¹, Genki Ozawa¹, Kazue Ohishi¹, Yuki Hongo¹, Sumihiro Koyama¹, Hidetaka Nomaki¹, Kanae Igawa¹, Kaoru Kaihotsu¹, Ryusaku Deguchi², Akihiro Tame³ Hiroshi Miyake⁴, Mitsuru Jimbo⁴, Suguru Nemoto⁵, Takenori Sasaki⁶, Naoki Ito⁷

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 Tohoku 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 chemosynthetic 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 symbionts 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 4°C for developing, and the clams and bivalves were immediately dissected, and blood, serum, and other tissues were frozen in liquid nitrogen and stored at -80°C until used. Other samples were also stored at -80°C. 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 Konishi¹,², Tatsuhiro Fukuba², Katsunori Yanagawa², Hidetaka Nomaki², Kazuna Shitaka², Yuriko Nagano², Shinro Nishi², Masahiro Yamamoto², Hiroko Makita², Hisako Hirayama², Tetsuo Ikuta², Takao Yoshida²

¹Kitami Institute of Technology
²Japan 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 symbionts have been developed insufficient. Therefore, we are developing a tank system controlling H₂S 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 H₂S sensor (DEEP-POTE) (Fig. B) and was pointed just above *Calyptogena* and *Bathymodiolus* colonies using a manipulator of HPD for pH and H₂S 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.
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$_2$S profiles. The data will feedback to the setting experimental condition for cultivating clams and their symbionts.

The profiles of concentrations of H$_2$S, NH$_4$, and DO in core water was measured on board. The concentrations of H$_2$S 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.
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 Nomaki¹, Yoshinori Takano¹
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 HgCl₂ 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 *Calyptogena* 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