

RV Kaimei Cruise Report

KM16-04

Operation training of the ROV Hyper-Dolphin and ROV KM-ROV

Off Hatsushima, Izu-Ogasawara, and Suruga Bay

June, 30th-July, 5th, 2016

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

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

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

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

1. Cruise Information

- 1) Cruise ID, Name of Vessel: KM16-04 R/V Kaimei
- Title of the Cruise: "Operation training of ROV Hyper-Dolphin and ROV KM-ROV"
- 3) Cruise Period: June 30, 2016 ~ July 5, 2016
- 4) Port Call: from JAMSTEC (June 30, 2016) to JAMSTEC (July 5, 2016)
- 5) Research Area: Off Hatsushima, Izu-Ogasawara, and Suruga Bay



Cruise track of R/V Kaimei (KM16-04)

6) Cruise Log (JST)

日付 Date	時間 Local Time	内容 Note	特記事項 Description	本船位置/気象/ 海象 Position/Weather/ Wind/Sea condition
30-Jun-16		Sail out & started KM16-04 and HPD dive #1991.		6/30 12:00 (JST)
	09:00	Let go all shore line & left YOKOSUKA for research area.	SAGAMI BAY	OFF East HATSUSHIMA
	10:00-10:45	Carried out education and training for scientist.		35-02.2'N、 139-20.0'E
	12:10	Arrived at research area.	OFF HATSUSHIMA	Overcast
	12:13	Released XBT.		SSW-2(Light breeze)
	14:04	Hoisted up HPD.		1(Calm)
	14:27	Launched HPD then it dove and com'ced her operation.	Dive No.1992	1(Low swell sea)
	15:01	HPD landed on the sea bottom.	Depth = 907m	Visibly: 6'
	16:17	HPD left the sea bottom.	Depth = 857m	
	16:53	Hoisted up HPD		
	16:59	Recovered HPD and finished avobe operation.		
	17:20-17:40	Carried out KONPIRA prey.		
	17:30	Com'ced proceeded to next dive point.	IZU OGASAWARA	
	18:30-18:45	Scientist meeting.		
01-Jul-16		HPD dive #1992		7/1 12:00 (JST)
	05:50	Arrived at research area.	IZU OGASAWARA	OFF East MIKURAJIMA
	05:54	Released XBT.		33-57.4'N、 141-04.8'E
	06:19-07:27	Carried out MBES mapping survey.		Mist
	09:40	Hoisted up HPD.		WSW-3(Gentle Breeze)

	10:04	Launched HPD then it dove and com'ced her operation.	Dive No.1993	2(Sea smooth)
	12:21	HPD landed on the sea bottom.	Depth = 4,407m	1(Low swell sea)
	14:17	HPD left the sea bottom.	Depth = 4,412m	Visibly: 2'
	16:36	Hoisted up HPD.		
	16:41	Recovered HPD and finished avobe operation.		
	17:00	Com'ced proceeded to next dive point.	OFF HATSUSHIMA	
	18:30-18:45	Scientist meeting.		
02-Jul-16		KM-ROV dive #15		7/2 12:00 (JST)
	06:50	Arrived at research area.	OFF SURUGA BAY	OFF South OMAEZAKI
	06:55	Released XBT.		34-14.0'N、 138-25.0'E
	09:24	Hoisted up KM-ROV.		Fine but cloudy
	09:38	Launched KM-ROV then it dove and com'ced her operation.	Dive No.15	WSW-4(Moderate breeze)
	11:27	KM-ROV landed on the sea bottom.	Depth = 2,992m	3(Sea slight)
	13:22	KM-ROV left the sea bottom.	Depth = 2,989m	1(Low swell sea)
	15:29	Hoisted up KM-ROV.		Visibly: 5'
	15:40	Recovered KM-ROV and finished avobe operation.		
	16:30-22:40	Carried out proceeded to next dive point.	OFF HATSUSHIMA	
	18:30-18:45	Scientist meeting.		
03-Jul-16		KM-ROV dive #16 & #17		7/3 12:00 (JST)
	08:24 Hoisted up KM-ROV.	Listed on VM DOV		OFF South-East
			HATSUSHIMA	
	08:42	Launched KM-ROV then it dove and com'ced her operation.	Dive No.16	35-01.0'N、 139-13.3'E
	09:23	KM-ROV landed on the sea bottom.	Depth = 880m	Fine but cloudy
	11:19	KM-ROV left the sea bottom.	Depth = 863m	SW-6(Strong breeze)
	11:54	Hoisted up KM-ROV.		4(Sea moderate)
	12:04	Recovered KM-ROV and finished avobe operation.		1(Low swell sea)

	13:32	Hoisted up KM-ROV.		Visibly: 7'
	13:44	Launched KM-ROV then it dove and com'ced her operation.	Dive No.17	
	14:20	KM-ROV landed on the sea bottom.	Depth = 868m	
	15:45	KM-ROV left the sea bottom.	Depth = 862m	
	16:14	Hoisted up KM-ROV.		
	16:21	Recovered KM-ROV and finished avobe operation.		
	17:19	Com'ced SBP survey.		
	19:00-19:15	Scientist meeting.		
04-Jul-16		KM-ROV dive #18		7/4 12:00 (JST)
	04:50	Einished CDD summer		OFF South-East
	04.59	Finished SBP survey.		HATSUSHIMA
	08.15	Hoisted up KM-ROV.		35-01.0'N
	08.15			139-13.3'E
	08:25	Launched KM-ROV then it dove and com'ced her operation.		Fine but cloudy
09:	00.05	KM-ROV landed on the sea bottom.	Dive No.18	South-2(light
	03.02			breeze)
	10:50	KM-ROV left the sea bottom.	Depth = 876m	1(Calm)
	11:22	Hoisted up KM-ROV.	Depth = 864m	1(Low swell sea)
	11:30	Recovered KM-ROV and finished avobe operation.		Visibly: 7'
	14:15-15:48	Carried out SBP survey.		
	16:00	Left research area for YOKOSUKA.		
	16:30-16:45	Scientist meeting.		
05-Jul-16		Disembarkation scientist group & completed KM16-04.		
	09:00	Arrived at the JAMSTEC. Then completed KM16-04.		

2. Participants List

Principal Investigator

Kazuki Iijima Marine Technology and Engineering Center Japan Agency for Marine-Earth Science and Technology (JAMSTEC)

Onboard Researchers

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Toshimasa Nasu Nippon Marine Enterprises, LTD.

Kouichi Inagaki Nippon Marine Enterprises, LTD.

ROV HYPER DOLPHIN and KM ROV Team

Operation Manager	KONDO TOMOE
Operation Manager	SAKURAI TOSHIAKI
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1st ROV Operator	ONO YOSHINARI
1st ROV Operator	MIURA ATSUMORI
2nd ROV Operator	KIDO TEPPEI
2nd ROV Operator	KIKUYA SHIGERU
2nd ROV Operator	TAKENOUCHI ATSUSHI
2nd ROV Operator	KATAGIRI MASAYA
3rd ROV Operator	KUMAGAI SHINOSUKE

R/V Kaimei Crew Member

Captain	SUSAMI SATOSHI
Chief Officer	ADACHI TATSUO
2nd Officer	MIYAKE KAZUKI
3rd Officer	SUZUKI AKIRA
Chief Engineer	KANEDA KAZUHIKO
1st Engineer	KATO KENZO
2nd Engineer	MORI TAKAHIRO
3rd Engineer	IKEGUCHI KENTA
Chief Radio Operator	INOUE YOICHI
2nd Radio Operator	ISHIWATA HIROKI
3rd Radio Operator	SHIROZUME TAKATOMO
Boat Swain	OHATA MASANORI
Able Seaman	YOSHINO YUKI
Able Seaman	HIRAI SAIKAN
Able Seaman	ABE SHUN
Able Seaman	NAKANISHI TORU
Sailor	SUZUKI SHO
Sailor	NASU KENTA
No.1 Oiler	IKEDA TOSHIKAZU
Oiler	FUNAWATARI KEITA
Oiler	MISAGO SOTA
Oiler	KOZAKI MAKOTO
Chief Steward	TAKEMURA RYUEI
Steward	TANAKA SHINSUKE

Steward Steward ABE TAKAHIRO SONODA KAZUMA

3. Overview of the cruise

Operation training and performance check of ROV Hyper-Dolphin and ROV KM-ROV in R/V Kaimei were main propose of this cruise. We had two dives of ROV Hyper-Dolphin and four dives of ROV KM-ROV at Off Hatsushima in Sagami Bay, Izu-Ogasawara area, and Off Suruga Bay. In this cruise, several scientists participated to this cruise. The research subjects of scientists were environmental, physiological, biochemical, chemical, and molecular-biological studies of cold seep-specific organisms, and sediments, and topographic servey of sea floor. During dives, we collect samples, e.g., deep-sea mussels, vesicomyid clams, sediments, and so on. Bivelves were kept alive and brought back to JAMSTEC. Detailed analyses of samples will be performed after the cruise.

4. Dive report

1) Summary of the Hyper-dolphin Dive #1992

Date: June 30, 2016 Site: Off Hatsushima, Sagami Bay Landing: 35°00.940'N, 139°13.384'E, 907m (15:01) Leaving: 35°00.953'N, 139°13.324'E, 857m (16:17)

Purpose:

Operation training of ROV Hyper-Dolphin on R/V Kaimei and sample collection

Payload Equipment: Suction sampler (single canister) Scoop sampler x1 Sample box x1 MBARI core x1 Sterilizing mud sampler X2

Dive Summary

[‡] Sampling of Bathymodiolus mussels with a suction sampler. The sampling site location 35°00.937'N, 139°13.375'E, 904m

‡ Sampling of sediments with MBARI core and sterilizing mud sampler. The sampling site location 35°00.957'N, 139°13.328'E, 855m

‡ Sampling of *Calyptogena* clams with a scoop sampler.The sampling site location 35°00.953'N, 139°13.324'E, 857m



XY ORIGIN 35-01.000N 139-13.400E

CENTER 35-00.950N 139-13.350E

2) Summary of the Hyper-dolphin Dive #1993

Date: July 1, 2016 Site: Izu-Ogasawara Landing: 33°57.463'N, 141°07.820'E, 4407m (12:21) Leaving: 33°57.411'N, 141°07.818'E, 4412m (14:17)

Purpose:

Operation training of ROV Hyper-Dolphin on R/V Kaimei and sample collection

Payload Equipment: MBARI core x1 Sterilizing mud sampler X2

Dive Summary

‡ Test of acoustic positioning system in ROVSite location 33°57.463'N, 141°07.820'E, 4407m

‡ Sampling of sediments with sterilizing mud sampler.The sampling site location 33°57.463'N, 141°07.820'E, 4407m



XY ORIGIN 33-56.900N 141-08.700E

CENTER 33-57,496N 141-07.856E

3) Summary of the KM-ROV Dive #15

Date: July 2, 2016 Site: Suruga Bay Landing: 34°13.947'N, 138°24.968'E, 2992m (11:27) Leaving: 34°13.947'N, 138°24.968'E, 2989m (13:22)

Purpose: Operation training of ROV KM-ROV on R/V Kaimei and sample collection

Payload Equipment: Sterilizing mud sampler X2

Dive Summary

‡ Sampling of sediments with sterilizing mud sampler.The sampling site location 34°13.947'N, 138°24.968'E, 2992m

‡ Training of separation and connection of ROV via TMS



CENTER 34-14.000N 138-25.000E

4) Summary of the KM-ROV Dive #16

Date: July 3, 2016 Site: Off Hatsushima, Sagami Bay Landing: 35°00.952'N, 139°13.371'E, 880m (9:23) Leaving: 35°00.948'N, 139°13.336'E, 863m (11:19)

Purpose: Operation training of ROV KM-ROV on R/V Kaimei and sample collection

Payload Equipment: Scoop sampler x1 Sample box x1 Monnma mud sampler X1 Sterilizing mud sampler X2

Dive Summary

‡ Sampling of *Calyptogena* clams with a scoop sampler.The sampling site location 35°00.953'N, 139°13.326'E, 859m

‡ Sampling of sediments with sterilizing mud sampler.The sampling site location 35°00.959'N, 139°13.324'E, 854m

‡ Sampling test of sediments with Monnma mud sampler.The sampling site location 35°00.948'N, 139°13.336'E, 863m



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5) Summary of the KM-ROV Dive #17

Date: July 3, 2016 Site: Off Hatsushima, Sagami Bay Landing: 35°00.971'N, 139°13.347'E, 868m (14:20) Leaving: 35°00.948'N, 139°13.336'E, 862m (15:45)

Purpose: Operation training of ROV KM-ROV on R/V Kaimei and sample collection

Payload Equipment: Scoop sampler x1 Sample box x1 Monnma mud sampler X1 Sterilizing mud sampler X2

Dive Summary

‡ Sampling of sediments with sterilizing mud sampler.The sampling site location 35°00.971'N, 139°13.347'E, 868m

‡ Sampling of *Calyptogena* clams with a scoop sampler.The sampling site location 35°00.952'N, 139°13.324'E, 858m

‡ Sampling test of sediments with Monnma mud sampler.The sampling site location 35°00.948'N, 139°13.336'E, 863m



6) Summary of the KM-ROV Dive #18

Date: July 4, 2016 Site: Off Hatsushima, Sagami Bay Landing: 35°00.951'N, 139°13.369'E, 876m (9:02) Leaving: 35°00.955'N, 139°13.375'E, 864m (10:50)

Purpose: Operation training of ROV KM-ROV on R/V Kaimei and sample collection

Payload Equipment: Scoop sampler x1 Sample box x1 Monnma mud sampler X1 Sterilizing mud sampler X2

Dive Summary

‡ Sampling of sediments with sterilizing mud sampler.The sampling site location 35°00.953'N, 139°13.362'E, 870m

\$\product Sampling of Calyptogena clams with a scoop sampler.
The sampling site location 35°00.954'N, 139°13.327'E, 857m

‡ Sampling test of sediments with Monnma mud sampler.The sampling site location 35°00.955'N, 139°13.375'E, 877m



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5. Preliminary research reports

1) The analysis of symbiosis between deep-sea bivalves and intracelluer symbiont.

Takao Yoshida (JAMSTEC), Kiyotaka Takishita (JAMSTEC), Akihiro Tame (JAMSTEC/Marine Works Japan, Ltd), and Tesuro Ikuta (JAMTEC)

Deep-sea bivalves, including *Calyptogena* spp. and *Bathymodiolus* spp, form dense communities on the deep sea floor near hydrothermal vents and seeps. These bivalves have vestigial digestive tracts and are nutritionally dependent on chemoautotrophic symbiotic bacteria, which are harbored within their gill epithelial cells. However, detailed symbiotic mechanisms are still unknown. To elucidate the symbiotic relationship between deep-sea bivalves and intracellular bacterial symbiont, we collected the *Calyptogena* clams and *Bathymodiolus* mussels.

Bathymodiolus mussels were collected at approximately 900 m site in Off Hatsushima. After collecting, we moved from 900m to 850m. At 850m site, *Calyptogena* clams were collected. After dive, the *Calyptogena* clams, and *Bathymodiolus* mussels were immediately dissected, and blood, serum, and other tissues were frozen and stored at -80°C until used. Other samples were also stored at -80°C. Gill of these bivalves were fixed in 4% paraformaldehyde in PBS, and stored at 4°C or -20°C. Other samples were keep in aquarium at 4°C.

Future plan

*Analysis of expression of several genes in bivalves

*Analysis of metabolite of bivalves

Detailed analyses of these samples will be performed after the cruise.

2) Sterilizing mud sampler

Yuichi Nogi (JAMSTEC)

When searching for microorganism lives to a specific place in the deep-sea, it is necessary to prevent the microorganism being mixed other than the field setting. It is necessary to use the sterilized apparatus for that. It is necessary to prevent the microorganism from the outside being mixed during the round trip to the point. The device developed for that is a sterilizing mud sampler. This sampler uses the centrifuging tube of 50ml on the market. The part where the centrifuging tube was installed is sealed up, the pressure adjustment with the outside is done through the filter of $0.22\mu m$, it is possible to gather it without mixing of the microorganism of other points.

ROV *Hyper-Dolphin* Dive #1992, two sediment sample were obtained at Area A in the deep methane seep site off Hatsushima, Sagami Bay (depth 855 m). These sample were taken on the rim of the *Calyptogena okutanii* clam colony, as close as possible to the active seepage area.

ROV *Hyper-Dolphin* Dive #1993, two sediment sample were obtained at Area C in the Izu-Ogasawara Trench (depth 4407 m). These samples were taken on the normal bottom.

KM-ROV Dive #15, two sediment sample were obtained at Area B in the Nankai Trough northern marginal part (depth 2992 m). These samples were taken on the sediment deposited on seabed.

KM-ROV Dive #16, two sediment sample were obtained at Area A in the deep methane seep site off Hatsushima, Sagami Bay (depth 854 m). These samples were taken on around the neighborhood of the *Calyptogena okutanii* clam colony.

KM-ROV Dive #17, two sediment sample were obtained at Area A in the deep methane seep site off Hatsushima, Sagami Bay (depth 868 m). These samples were taken on the sediment deposited on seabed.

KM-ROV Dive #18, two sediment sample were obtained at Area A in the deep methane seep site off Hatsushima, Sagami Bay. The samples were taken on the sediment deposited on seabed (depth 870 m) and in the *Calyptogena okutanii* clam colony (depth 857 m).

The samples were repackage into smaller sizes and stored at 4°C and container of

liquid nitrogen. After the cruise, useful bacteria isolated and genomic DNA will be extracted from the samples.

3) Cruise report in KM16-04

Yoshihiro Takaki (JAMSTEC)

Objective:

The bacterial epigenome is dynamic feature that changes during growth in response to external stimuli, and thereby facilitating adjustment to varying environmental conditions that controls gene exchange, transcription, and genome stability on a broad scale. Recently, several model proteobacteria, *Escherichia ecoli* and *Caulobacter crescentus*, is well studied in the control of the timing of the cell cycle and differentiation. However, less is known about the epigenetic regulation in the symbiosis. Thus the present study is an attempt at exploring symbiotic epigenomics using the next-generation sequencing technology.

Material and Methods

Two species of *Bathymodiolus* clam, *B. japonicus* and *B. platifrons*, was sampled from the 850 m deep methane seep site off Hatsushima, Sagami Bay during ROV *HyperDolphin* Dive #1992. Gill tissues from three individuals of each species were homogenized in Tris-HCl bufer (pH7.5) with xx mM EDTA. Symbiont cells in the suspension were collected by centrifuge and treated with DNaseI. The cells were stored at -80°C. After the cruise, genomic DNA of these samples will be extracted and used in the methylome analysis.

Future plan:

We have a plan to perform the single molecule real time (SMRT) DNA sequencing of symbiont genome. SMRT sequencing enables to identify the DNA modification along with methylation over genome. Furthermore, we study the biological significance of these modifications in the correlation between host and symbiont.

4) Megafauna Sampling

Chong Chen (JAMSTEC), and Yoko Chiba (JAMSTEC)

During the KM16-04 cruise, megafauna samples were obtained from a methane seep site (850 m deep) off Hatsushima, Sagami Bay on multiple ROV dives. During ROV *Hyper-Dolphin* dive #1992, specimens were collected using both slurp gun (with single canister) and kumade scoop. A kumade scoop was used for collections by *KAIMEI ROV* during dives #0016, #0017, and #0018. When kumade scoop was used, the samples were emptied into a watertight biobox with the lid tightly closed afterwards.

After recovery of the ROV on to the ship, the canister / sample box was immediately removed from ROV and brought to the wet lab (Lab. 3). There, the contents were washed on a 1 mm mesh and sorted to the lowest taxonomic level possible. The sorted specimens were preserved in 99% ethanol, 10% buffered formalin, or frozen in -80°C freezer for shore-based taxonomy and genetic analyses. Specimens of *Bathyacmaea nipponica* Okutani, Tsuchida & Fujikura, 1992 successfully recovered in living state were immediately transferred to cold condition (4 °C) for long-term rearing. They will be used for live rearing experiments in JAMSTEC after the cruise. All megafauna samples obtained during this cruise are listed on the biology metadatasheet.

5) MBARI Push-core

Yoko Chiba (JAMSTEC), and Chong Chen (JAMSTEC)

A push-core sample was taken from the 850 m deep methane seep site off Hatsushima, Sagami Bay during ROV *HyperDolphin* Dive #1992. The core sample was taken on the rim of the *Calyptogena okutanii* clam colony, as close as possible to the active seepage area. A subset of the core was taken at every 5 cm intervals (i.e., at 0 cm, 5 cm, 10 cm, and so on) and placed into sterile glass vials. The samples were fixed and stored at room temperature. After the cruise, these samples will be used for geochemical analyses by Dr. Shinsuke Kawagucci (D-SUGAR, JAMSTEC).

During ROV *Hyper-Dolphin* Dive #1993, a sediment core sample was obtained using MBARI push-core at Area C in the Izu-Ogasawara Trench (depth 4407 m). Immediately

after recovery of the ROV, the core sample was cut into 5 cm thick sections from top to bottom. Sections were placed into ZipLoc bags and stored at -80°C. The total length of the core sample was 28 cm. After the cruise, genomic DNA will be extracted from the samples to detect several marker protein genes of prokaryotic fermentation metabolism.

The push-core samples obtained during this cruise are listed on the metadatasheet for 'other samples'.

6) Temperature Probe

Chong Chen (JAMSTEC), and Yoko Chiba (JAMSTEC)

A temperature probe built to measure the temperature of hydrothermal fluid (owned by D-SUGAR, JAMSTEC) was tested on both ROV *Hyper-Dolphin* and *KAIMEI ROV* for the purpose of signal transmission testing. This probe has been used many times with ROV *Hyper-Dolphin* on-board other JAMSTEC research vessels, but this is the first time it is used with R/V *KAIMEI*. In addition, it has never been tested on *KAIMEI ROV*. Although probe is usually mounted on a pistol-shaped mount and held by manipulator to acquire measurements from specific vent orifices, this was not required for the purpose of this cruise and thus the probe was simply attached to the vehicle body using cable-ties.

The test on ROV *Hyper-Dolphin* was carried out during dive #1993 to a depth of over 4400 m, and was completed successfully without any error. Temperature recordings showed generally expected values and were stable. Therefore the probe is ready to be used with the *Hyper-Dolphin* / R/V *KAIMEI* pair for research purposes in the future.

The probe was tested on *KAIMEI ROV* dives #0016 and #0017 to a depth of approximately 850 m off Hatsushima, Sagami Bay. These were two consecutive dives carried out on the same day. On dive #0016, a small issue occurred due to the stop button being accidentally pressed, but otherwise the communication between probe and computer programme was fine. The test on dive #0017 was completed without any error, and the temperature recordings showed generally expected values and were stable. The probe appears to be in good shape for use with *KAIMEI ROV* in the future for scientific purposes.