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Kairei Cruise Report

KR12-19

Ogasawara Trench

& Japan Trench

Nov, 30th – Dec 7th, 2012

Japan Agency for Marine-Earth Science and

Technology

(JAMSTEC)

1. Cruise Information

● Cruise ID KR12-19

● Name of vessel R/V Kairei

● Title of the cruise Geochemical and microbiological investigations for the trench biosphere

● Chief scientist [Affiliation] Takuro Nunoura [JAMSTEC]

● Representative of the Science Party [Affiliation] Takuro Nunoura [JAMSTEC]

●Title of proposal Expedition for Trench Biosphere - Microbiological and geochemical investigation for the trench environments

● Cruise period 30^{th} Nov – 7^{th} Dec, 2012

● Ports of call JAMSTEC (Yokosuka) - JAMSTEC (Yokosuka)

● Research area Ogasawara Trench and Japan Trench (Pacific Ocean)

2. Acknowledgements

We are grateful to Captain Mr. Ishiwata, Chief Officer Mr. Masujima and Chief Engineer Mr. Abe for their safe navigation and their skillful handling of “R/V Kairei”. Great thanks are due to Commander Mr. Miura and “Kaiko” operation team for their operations in sampling. We also thank Mr. Ito, Nippon Marine Enterprise, Ltd. and Mr. Hashimoto and Ms. Miyamoto, Marine Works Japan for their attentive supports. We thank all the JAMSTEC personnel who have supported us. Finally, we would like to appreciate all the person who supported directly or indirectly this cruise.

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4. List of participants

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Marine Technicians

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Yasushi Hashimoto

Marine Works Japan, LTD.

Naoko Miyamoto
Marine Works Japan, LTD.

RV Kairei Crew

Captain	Masayoshi Ishiwata
Chief Officer	Hiroaki Masujima
2nd Officer	Isao Maeda
3rd Officer	Hidehiko Konno
Jr3rdOfficer	Motoi Katsumata
Chief Engineer	Tadashi Abe
1st Engineer	Kazunori Noguchi
2nd Engineer	Ryuso Mikami
3rd Engineer	Shogo Yoshimura
Chief Electronic Operator	Tokinori Nasu
2nd Electronic Operator	Shunsuke Fukagawa
3rd Electronic Operator	Takatomo Shirozume
Boat Swain	Tadahiko Toguchi
Able Seaman	Takao Kubota
Able Seaman	Yoshiaki Matsuo
Able Seaman	Saikan Hirai
Sailor	Hirotaka Shigeta
Sailor	Yoshihiro Ogawa
Sailor	Yasunobu Kawabe
No.1 Oiler	Kazuaki Nakai
Oiler	Shinya Sugi
Oiler	Masanori Ueda
Assistant Oiler	Daiki Igarashi
Assistant Oiler	Aoi Takamiya
Chief Steward	Isao Matsumoto
Steward	Hideo Fukumura
Steward	Kazuhiro Hirayama
Steward	Kana Yuasa
Steward	Nakamichi Kanda

KAIKO Operation team (ABISMO operation during the cruise)

Submersible Operation Manager	Atsumori Miura
2nd Submersible Technical Officer	Kiyoshi Takishita
3rd Submersible Technical Officer	Ryu Asai
3rd Submersible Technical Officer	Shota Ihara
3rd Submersible Technical Officer	Takuma Goto

5. Cruise Log (JST)

Date	Local Time	Note	Description	Position/Weather /Wind/Sea condition
01-Dec-16		**Sail out, proceeding to research area**		11/30 12:00 (UTC+9h)
	09:00	Let go all shore lines,left YOKOSUKA.Then com'ced proceeding to research area.		34-47.3N 139-49.4E
	9:30-10:15	Carried out shipboard education for scientists.		Overcast
	10:30-11:00	Carried out master station drill.		NNE-4(Moderate breeze)
				2(Sea smooth)
				2(Low swell long)
				Visibly: 6'
2.Dec.16		**Suspended ABISMO#18,due to rough sea**		12/01 12:00(UTC+9h)
	05:45	Arrived at research area.(Izu・Ogasawara trench)		34-55.7N 140-59.6E
	05:54	Released XBT at 34-17.7637N 141-56.6248E.		Cloudy
	07:25	Suspended ABISMO#18,due to rough sea.Proceeding to next dive p't(South of Japan Trench).		North-6(Strong breeze)
				4(Sea moderate)
				3(Moderate short)
				Visibly: 8'
3.Dec.16		**Deployed Free Fall camera system operation and carried out CTD water sampling**		12/02 12:00(UTC+9h)
	06:00	Arrived at research area.(South Japan trench)		36-04.0N 142-44.0E
	06:30	Released XBT at 36-09.2094N 142-43.9551E.		Cloudy
	08:50-09:13	Carried out MBES site surevey.		North-3(Gentle breeze)
	10:26	Deployed 11000m free fall camera.		3(Sea slight)
	13:05-16:12	Carried out CTD water sampling.		3(Moderate short)
	20:39	Com'ced MBES site survey.		Visibly:8'
4.Dec.16		**ABISMO#18**		12/03 12:00(UTC+9h)
	02:50	Finished MBES site survey.		36-05.0N 142-45.0E
	07:38	Recovered 11000m free fall camera.		Cloudy
	09:34	ABISMO dove & started her operation#18.		ESE-5(Fresh breeze)
	10:02	ABISMO refloted.		4(Sea moderate)
	10:19	Recovered ABISMO,then finished operation.		3(Moderate short)
	Noon	Com'ced proceeding to Sendai,due to rough sea.		Visibly:7'
5.Dec.16		**Ancoring at off Sendai**		12/04 12:00(UTC+9h)
	06:00	Let go anchor at off SENDAI.		38-12.0N 141-05.2E
				Rainy
				West-5(Fresh breeze)
				3(Sea slight)
				3(Moderate short)
				Visibly:3
6.Dec.16		**Carried out CTD water sampling**		12/05 12:00(UTC+9h)
	06:00	Heaving anchor,then com'ced proceeding to research area.(South Japan Trench)		36-53.0N 142-04.0E
	15:30	Arrived at research area.(South Japan Trench)		Fine but cloudy
	16:13-20:17	Carried out CTD water sampling.		WSW-4(Moderate breeze)
	21:00	Left research area,com'ced proceeding to YOKOSUKA.		3(Sea slight)
				2(Low swell long)
				Visibly:8'
7.Dec.16		**ABISMO test dive**		12/06 12:00(UTC+9h)
	13:30	Let go anchor at off YOKOSUKA.		35-07.5N 139-45.5E
	14:30-15:01	Carried out ABISMO test dive.		Fine but cloudy
				WSW-8(Gale)
				5(Sea rough)
				3(Moderate short)
				Visibly:8'
8.Dec.16		**Arrived at YOKOSUKA**		
	08:00	Heaving anchor then com'ced proceeding to YOKOSUKA.		
	08:45	Let go 1st shore line then arrived at YOKOSUKA.		

Date	Local Time	Note	Description	Position/Weather /Wind/Sea condition
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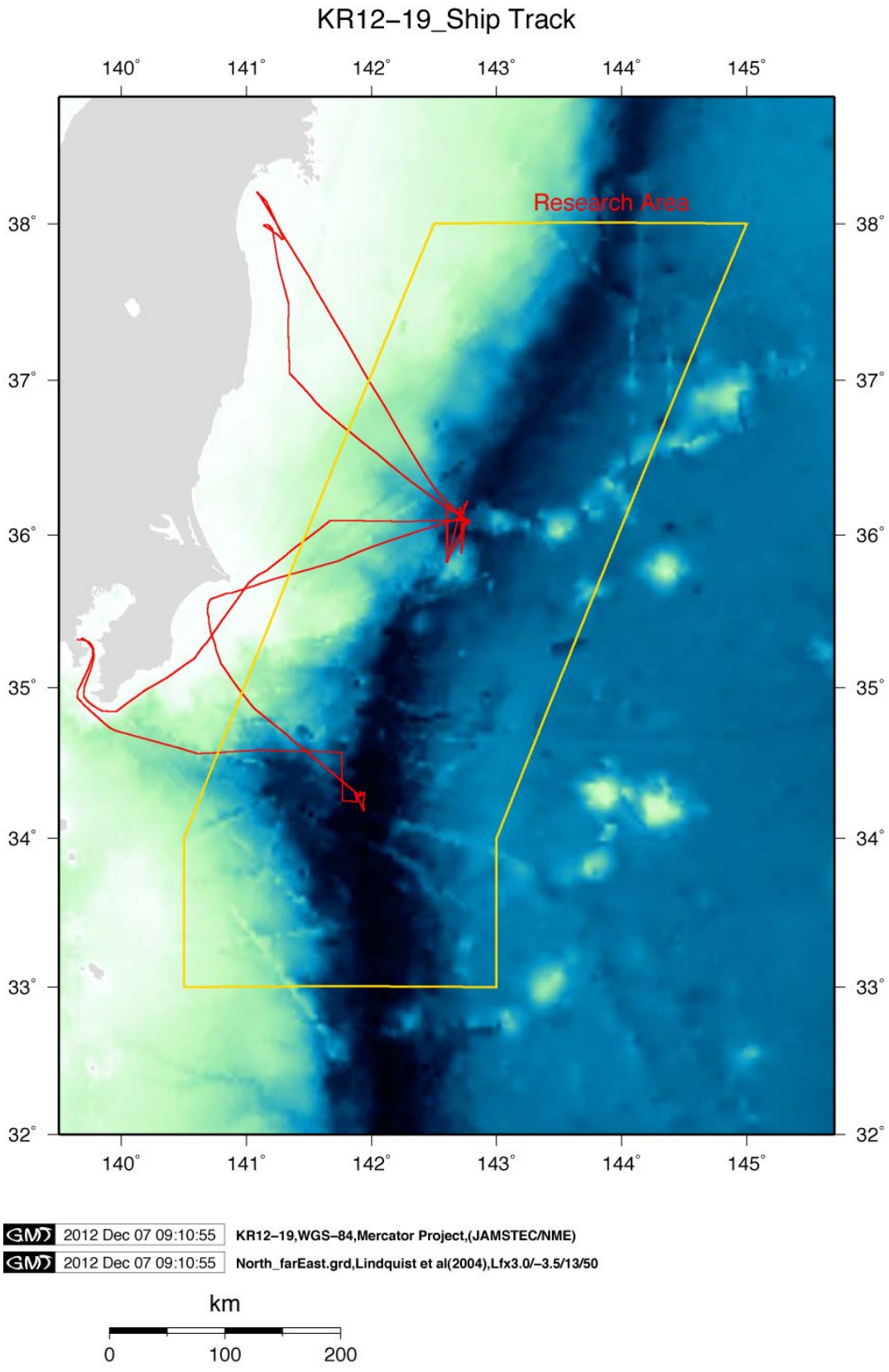
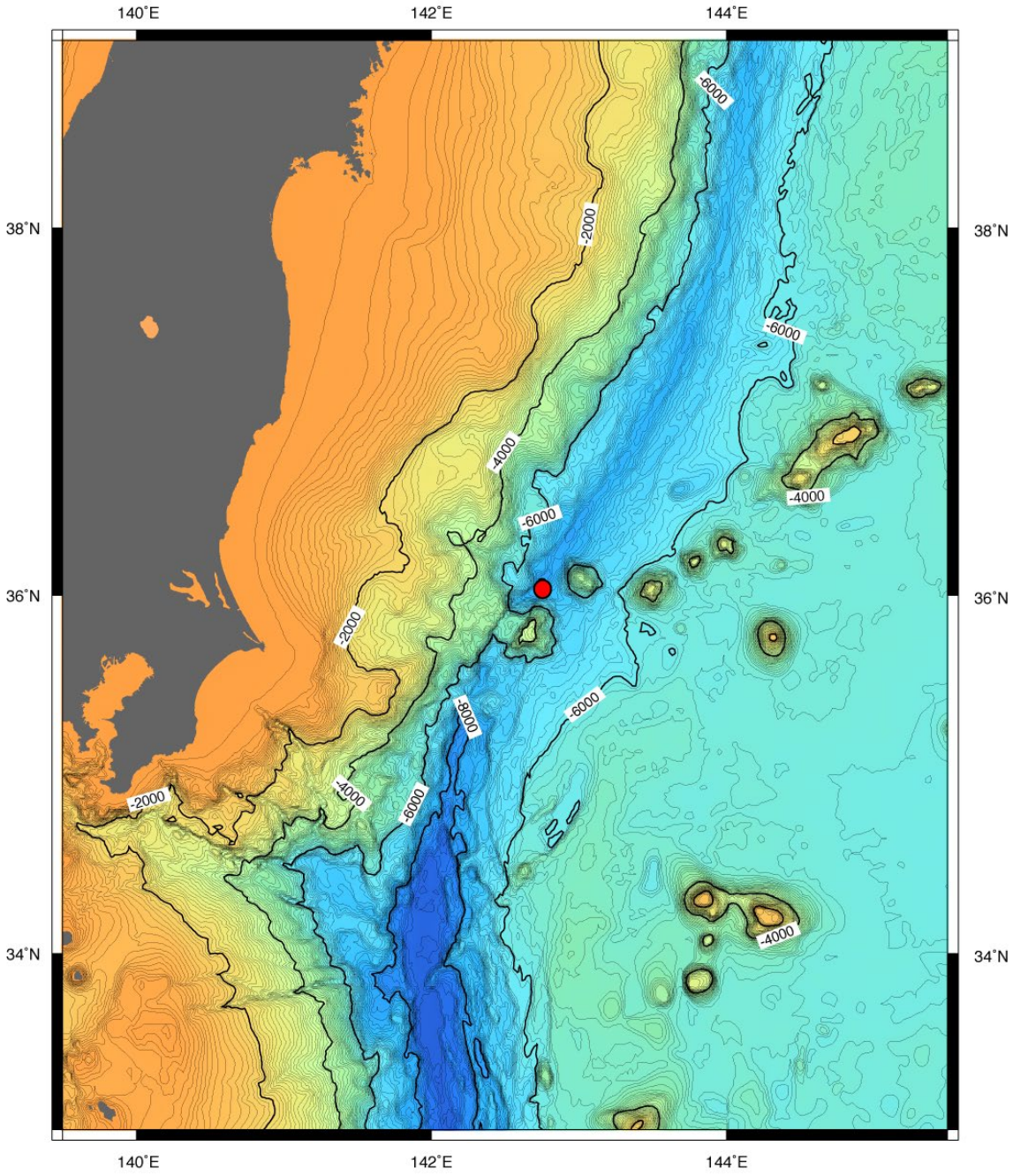

KR12−19_Ship Track
Research Area
140°
141°
142°
143°
144°
145°
38°
37°
36°
35°
34°
33°
32°
2012 Dec 07 09:10:55 KR12−19,WGS−84,Mercator Project,(JAMSTEC/NME)
2012 Dec 07 09:10:55 North_farEast.grd,Lindquist et al(2004),Lfx3.0/−3.5/13/50
km
0
100
200

Sampling station in the KR12-19 cruise



6. Instruments

1) Automatic Bottom Inspection and Sampling Mobile "ABISMO"

ABISMO has been developed to reach the deepest sea bottom, observe the area with a camera, and sample the bottom layer. Its maximum operating depth is 11,000 m. Figure 1 shows ABISMO just about to dive, and Fig. 2 gives an overview of the system used to operate ABISMO. Table 1 shows the dimensions of ABISMO. The underwater system of ABISMO consists of a launcher and vehicle. The vehicle is docked with the launcher at the first step of diving, and it remains docked until ABISMO arrives at the target depth, while the primary cable is being reeled out. After making observations with a camera and sampling the bottom layer, the vehicle approaches and touches down on the bottom as the secondary cable is reeled out, if this is required. The vehicle inspects the bottom surface with a camera by moving around with the crawler. In addition, we have thought about the operational flexibility of the ABISMO system. Therefore ABISMO is designed so that it is lightweight and has a small volume, and it will be possible to change its mother ship in future.

The launcher of ABISMO has a 160-m secondary cable drum system, a vehicle docking device, two kinds of mud samplers, a sampler winch, twelve water samplers, two 1,000-W thrusters, two NTSC surveillance video cameras, an HD observation camera, a 500-W halogen light, a gyro, an altimeter, a depth gauge, a responder, and an SSBL receiver array for vehicle tracking. Figure 3 shows an overview of the launcher and (docked) vehicle. The launcher reels the vehicle in and out with the cable drum systems. The vehicle is docked with the launcher by using a docking device, and released at the target depth. The cable drum system reels the released vehicle in and out, and the vehicle is fully reeled in and docked again after it has made its observations. The launcher can choose from a two- or three-meter-long gravity corer and a Smith-McIntyre mud sampler that is 220 mm in width and length. The gravity corer is dropped from the vehicle, free falls, and penetrates the seabed's bottom layers. Some material from the bottom layer is captured and retrieved by reeling in the corer with the winch drum. The launcher has a responder at its tail so it can be tracked, and an SBL receiver array at the bottom to track the vehicle.

The vehicle of ABISMO has buoyancies, four 400-W thrusters, crawlers for moving on the sea bottom, an NTSC observation camera, a 500-W halogen light, an LED array, a

gyro and a depth gauge. The vehicle can move on the sea bottom and then the crawlers can approach the targets closely and observe them with the NTSC camera in a stable environment. The thrusters help the vehicle to move along the soft sea bottom where the crawler is buried. The transponder, named an “ultra deepwater transponder”, can respond to acoustic pings from the mother ship, so we can directly navigate the vehicle at a depth of 11,000 m. In addition, the transponder can receive acoustic commands and active a release switch. If the secondary cable is cut accidentally and the crawler is not able to dock with the vehicle, the transponder can be given a command to drop a weight to enable the vehicle to surface. (But no drop weights are loaded on the vehicle at the moment.)

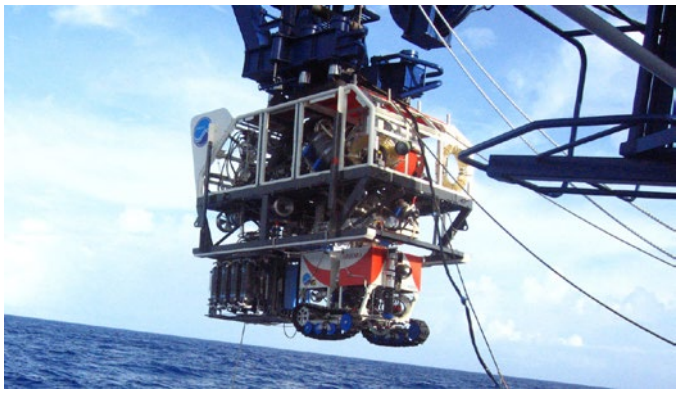


Fig. 1 The automatic bottom inspection and sampling mobile ABISMO.

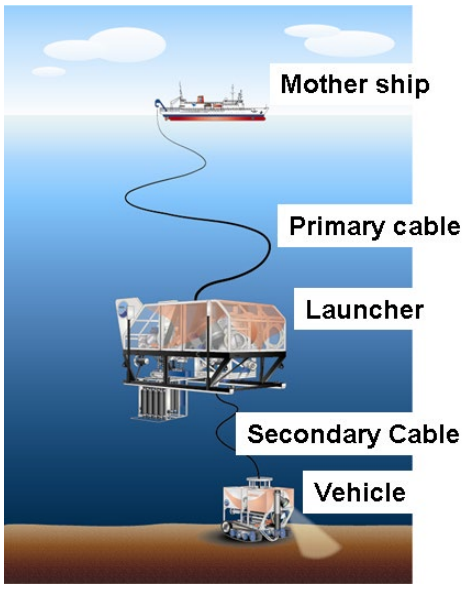


Fig. 2 Overview of ABISMO system.

Table 1 ABISMO's dimensions

Item	Launcher	Vehicle
Operating depth	11,000 m	11,000 m
Dimensions (L × B × D)	3.3 × 1.9 × 2.7 m	1.2 × 1.3 × 1.25 m (with crawler)
Weight in air	About 2,700 kgf	327 kgf
Weight in water	About 2,100 kgf	97 kgf

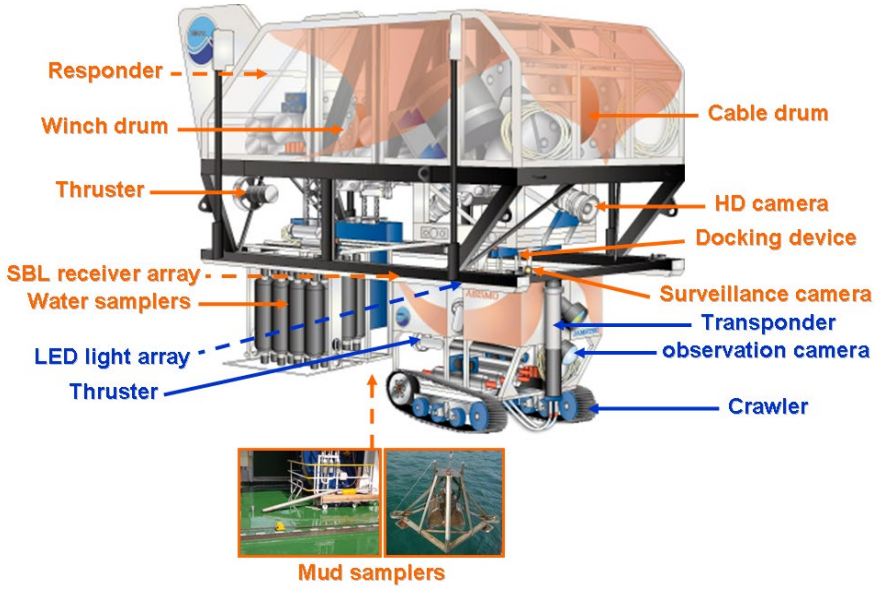


Fig. 3 Overview of the launcher and docked vehicle.

2) 11K Free Fall Camera System

A free fall camera system was used for the sampling of surface sediments and incubation experiments of sediments and water *in situ*. The free fall camera system was made by MARITEC, JAMSTEC, and consisted of CTD, a transponder, release weights, a battery, a deep-sea camera, two lights, and three sediment samplers attached to tripod aluminum frame.

For this cruise, we newly designed two *in situ* incubation systems. The first incubation system is set inside of sediment sampler tube which has 7.4 cm inner diameter. After landing on the seafloor, triggers are released and stable isotope tracer is injected onto sediment surface or into the sediments in the tubes. The second incubation system is a water sampler which includes chemicals and/or stable isotope labeled substrates. After certain time, trigger is released and surrounding bottom water is introduced into the

sampler.

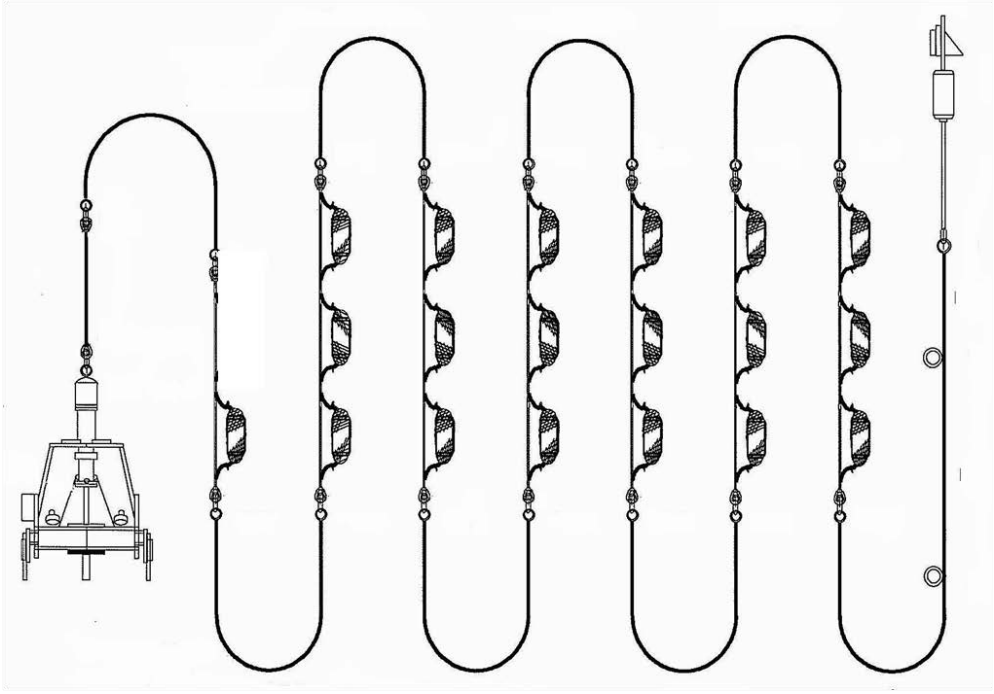


Figure. A schematic figure of the free fall camera system.

3) CTD (Conductivity-Temperature-Depth profiler) in the ABISMO and the Lander system

SBE49, Sea-Bird Electronics

Conductivity:

Range: 0-9

Accuracy: 0.0003

Resolution: 0.00005 (oceanic waters; resolves 0.4 ppm in salinity)

Temperature:

Range: -5 – 35˚C

Accuracy: 0.002

Resolution: 0.0001

Pressure:

Range: 10000m

Accuracy: 0.1% of full scale range
Resolution: 0.002% of full scale range

4) CTD (Conductivity-Temperature-Depth profiler) carousel system

(a) Deck Unit SBE11Plus (Seial No. 11P54451-0872), Sea-Bird Electronics, Inc.

(b) Carousel Water Sampler SBE32 (Serial No. 3227443-0389), Sea-Bird Electronics,Inc.

(c) Niskin-X Water Sampler 12-litter Bottle Model 1010X, General Oceanics, Inc.

(d) Thermometer SBE03Plus (Serial No. 03P5329), Sea-Bird Electronics, Inc.
Range: -5.0 to +35 ˚C
Accuracy: 0.001 ˚C
Resolution: 0.0002 ˚C

(e) Salinometer SBE04C (Serial No. 043889), Sea-Bird Electronics, Inc.
Range: 0.0 to 7 S/m
Accuracy: 0.0003 S/m
Resolution: 0.00004 S/m

(f) Manometer SBE9plus (Serial No. 09P27443-0677), Sea-Bird Electronics, Inc.
Range: up to 10500 m
Accuracy: 0.015 %F.S.
Resolution: 0.001 %F.S.

(g) Altimeter PSA-916T (Serial No. 52396), Teledyne Benthos, Inc.
Range: 0-100 m

(h) DO sensor SBE43 (Serial No.432036) , Sea-Bird Electronics, Inc.
Measurement range:120% of saturation on all natural waters, fresh and salt.
Initial accuracy : 2% of saturation
Typical stability : 0.5% per 1000hours (clean membrane)

(i) Software Seasave-Win32 (ver 7.21f), Sea-Bird Electronics, Inc.
SBEDataProcessing-Win32（ver 7.22）, Sea-Bird Electronics, Inc.

7. CTD Water Sampling Reports

CTD cast ID: 36NTS01

Date: Nov 2, 2012 (JST)

Site: Japan Trench (36N)

Start of Logging: Nov 2, 4:08 UTC

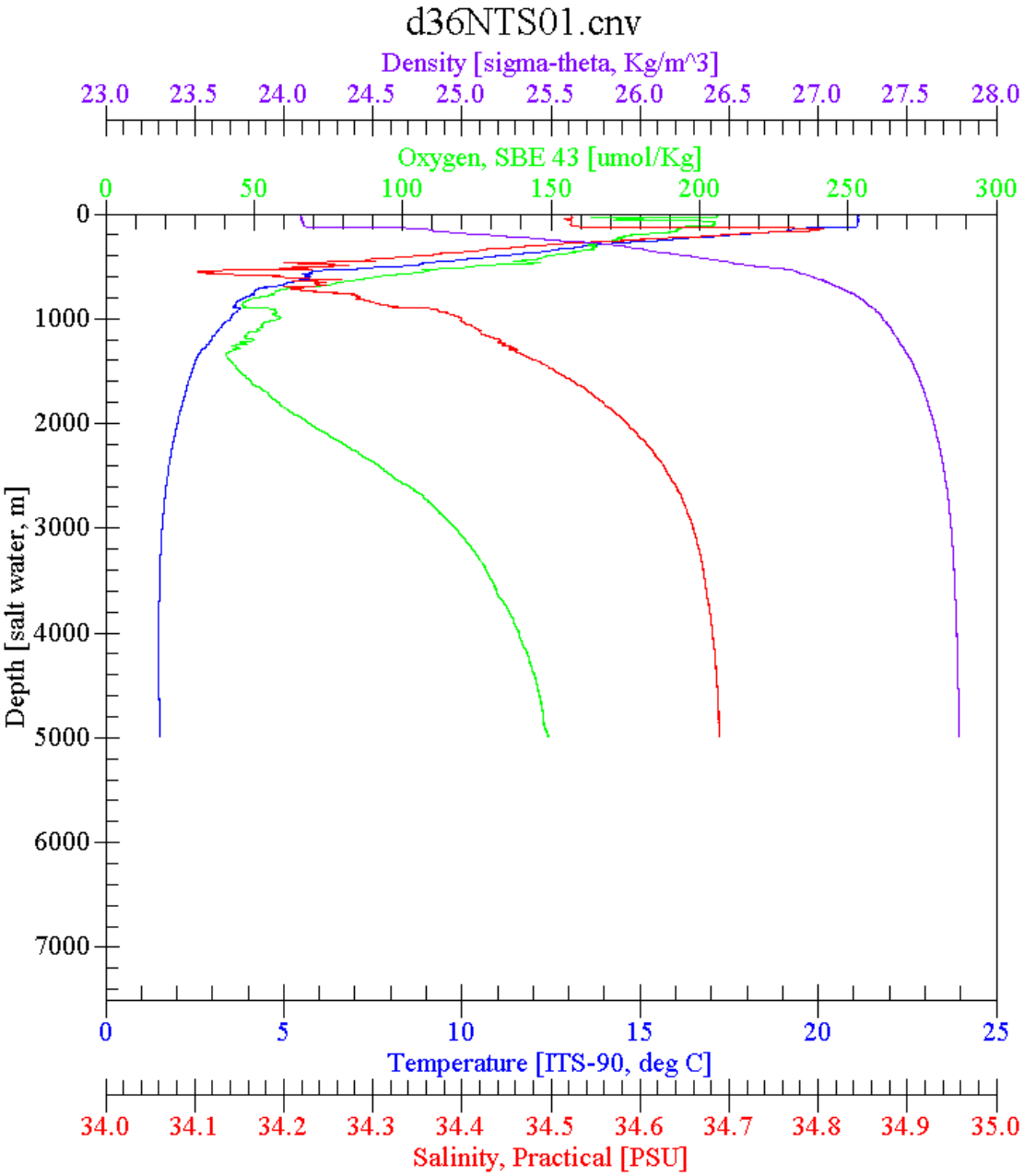
Start downcast: 36° 03.8' N, 142° 43.6 'E

Bottom position: 36° 03.99 N, 142˚ 43.97E ’E, 8022 m

End of Logging: Nov 2, 7:12 UTC

Summary of water samples

Bottle No.	Time (JST)	Depth (m)	Pressure (dp)	Temperature (˚C)	Conductivity (S/m)	Salinity (PSU)	Oxygen (μmol/kg)
1	14:34:38	5001.05	5097.93	1.529	3.20947	34.689	148.74
2	15:00:01	3498.55	3553.93	1.514	3.15489	34.674	130.14
3	15:00:14	3499.33	3554.73	1.514	3.15491	34.674	130.18
4	15:22:37	1995.18	2019.58	2.003	3.13214	34.588	70.51
5	15:34:18	1502.70	1519.29	2.435	3.14256	34.505	45.45
6	15:43:09	996.19	1005.96	3.503	3.20596	34.403	58.63
7	15:48:40	746.24	753.10	4.169	3.23958	34.236	56.02
8	15:53:19	546.80	551.56	6.676	3.45126	34.203	106.15
9	15:53:34	544.73	549.47	6.691	3.45232	34.201	106.84
10	16:00:17	192.76	194.27	18.094	4.57474	34.719	183.29
11	16:00:31	191.86	193.37	18.102	4.57565	34.720	183.61
12	16:08:05	102.31	103.09	21.100	4.85035	34.536	199.81


d36NTS01.cnv
Density [sigma-theta, Kg/m^3]
23.0 23.5 24.0 24.5 25.0 25.5 26.0 26.5 27.0 27.5 28.0
Oxygen, SBE 43 [umol/Kg]
0 50 100 150 200 250 300
Depth [salt water, m]
0 1000 2000 3000 4000 5000 6000 7000
0 5 10 15 20 25
Temperature [ITS-90, deg C]
34.0 34.1 34.2 34.3 34.4 34.5 34.6 34.7 34.8 34.9 35.0
Salinity, Practical [PSU]

CTD cast ID: 36NTS02

Date: Nov 5, 2012 (JST)

Site: Japan Trench (36N)

Start of Logging: Nov 5, 7:15 UTC

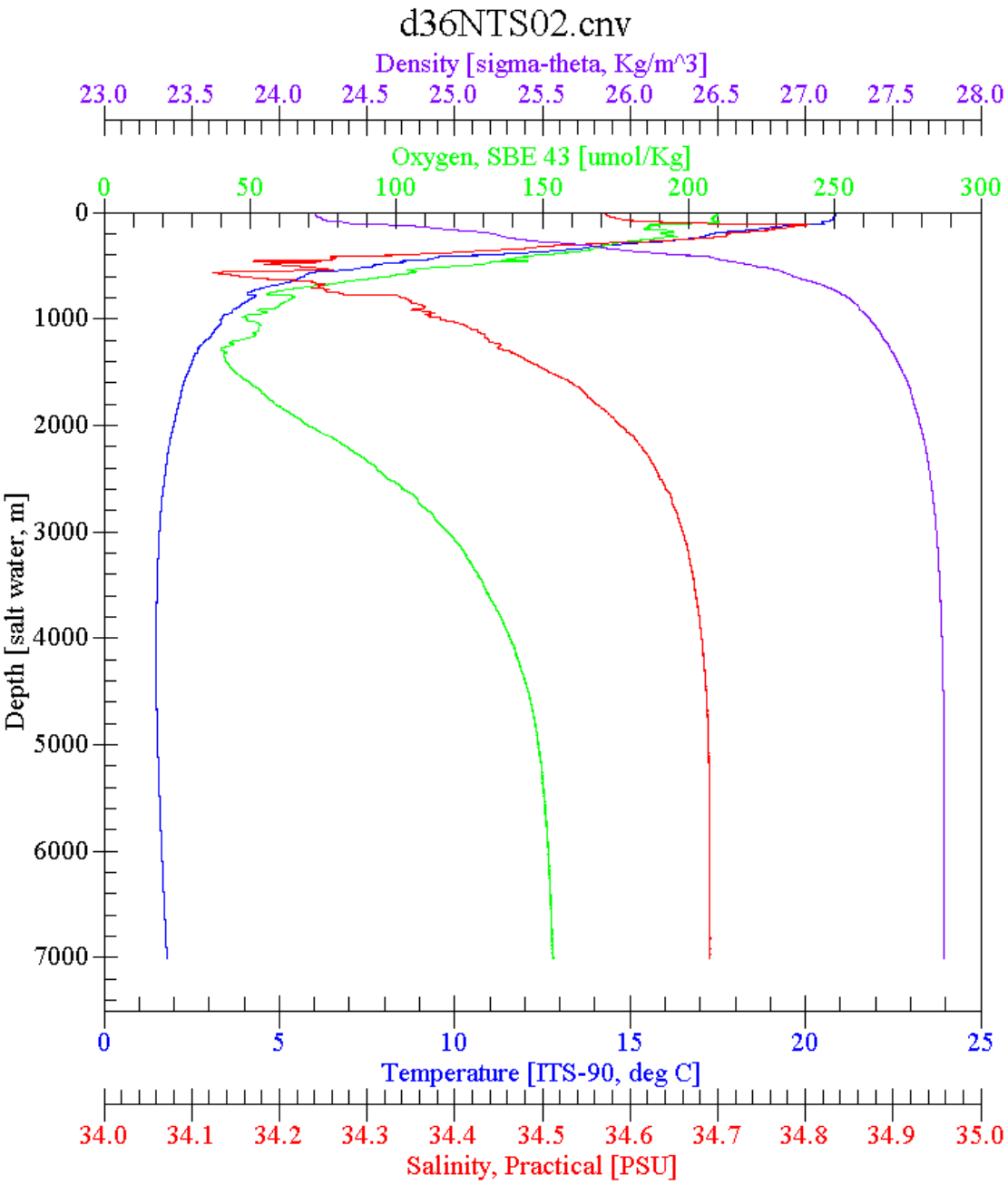
Start downcast: 36°06.3' N, 142°43.5 'E

Bottom position: 36° 05.88 N, 142˚ 45.91E ’E, 8012 m

End of Logging: Nov 5, 11:17 UTC

Summary of water samples

Bottle No.	Time (JST)	Depth (m)	Pressure (dp)	Temperature (˚C)	Conductivity (S/m)	Salinity (PSU)	Oxygen (µmol/kg)
1	18:11:54	7006.77	7175.04	1.796	3.29273	34.691	153.21
2	18:12:06	7007.88	7176.20	1.796	3.29278	34.691	153.17
3	18:12:32	7009.61	7178.00	1.797	3.29285	34.691	153.21
4	18:35:55	6000.49	6130.70	1.647	3.25101	34.691	151.23
5	18:54:56	5000.35	5097.22	1.530	3.20950	34.690	146.23
6	19:15:58	3497.34	3552.70	1.513	3.15472	34.674	127.46
7	19:41:54	1494.56	1511.03	2.404	3.13971	34.507	45.62
8	19:50:43	999.37	1009.18	3.407	3.19589	34.382	49.88
9	19:56:15	747.79	754.68	4.125	3.23610	34.240	54.35
10	20:00:42	551.43	556.24	7.261	3.50809	34.244	106.52
11	20:08:40	203.51	205.11	17.269	4.49244	34.712	193.42
12	20:13:07	98.55	99.30	20.525	4.80080	34.606	207.71


d36NTS02.cnv
Density [sigma-theta, Kg/m^3]
23.0 23.5 24.0 24.5 25.0 25.5 26.0 26.5 27.0 27.5 28.0
Oxygen, SBE 43 [umol/Kg]
0 50 100 150 200 250 300
Depth [salt water, m]
0 1000 2000 3000 4000 5000 6000 7000
0 5 10 15 20 25
Temperature [ITS-90, deg C]
34.0 34.1 34.2 34.3 34.4 34.5 34.6 34.7 34.8 34.9 35.0
Salinity, Practical [PSU]

8. 11K Free Fall Camera System observatory

Date: Nov 2, 2012 (JST)
Site: Japan Trench (36N)
Surface (in): 10:26
On the bottom: 36°04.3431' N, 142°44.6416 'E, 7963 m
On deck: 7:38

Objective:

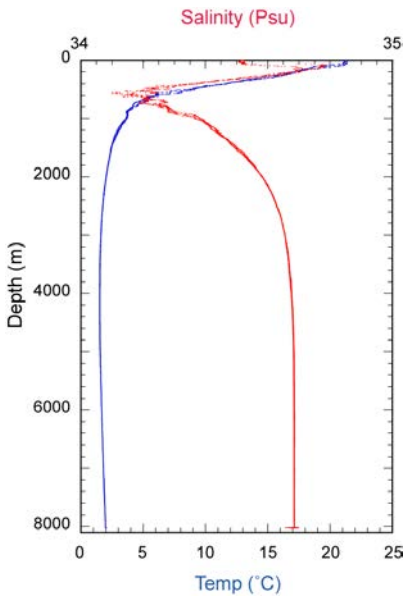
1) Taking trench bottom surface sediments without disturbance.

Payload:

1) CTD
2) 3 sediment samplers

Summary

A total of three sediment cores approximately 50 cm in length were successively obtained. The sediment consisted with fine silt and was extremely soft through the core column. Such sediment cores with high water content was apparently different from other trench bottom sediments obtained from the southern part of the Ogasawara Trench and the Mariana Trench.



9. ABISMO Dive Report

Dive Report ABISMO #18

Takuro Nunoura

Date: December 3, 2012

Site: Japan Trench

Location: 36°04.00' N, 142°44.00 'E, 8000 m

Objectives:

1) Taking abyssal plain sediments by a gravity corer in order to reveal nitrogen circulation by geochemical and microbiological analyses.
2) Taking trench bottom waters

Payloads:

1) CTD
2) 4 Water samplers (5L Niskin bottles)
3) 8 Water samplers (50ml pressure tight bottles)

Summary

Due to a mechanical trouble, the dive was canceled at a depth of 150 m below sea surface.

10. Scientific objectives

A. Major objectives

Geochemical cycles and microbial ecosystems in hadopelagic environments have not been investigated. The *ROV ABISMO* that harbors water samplers and a gravity corer can take both water and sediment samples from 10000 m below the sea surface. In the previous cruises for the *ROV ABISMO* conducted in 2007, 2008 and 2011 (KR07-17, KR08-5 and KR11-11), we obtained waters and sediments from the Challenger Deep in the Mariana Trench and the Izu-Ogasawara Trench. In addition, we took a sediment core from the abyssal plain adjacent to the Izu-Ogasawara Trench. These sediments and waters provided unprecedented views of microbial ecosystems in hadopelagic environments described below.

1. Oceanic trench biosphere

Molecular microbiological analyses (clone analyses and quantitative analyses for SSU rRNA gene and functional genes) revealed that microbial communities in trench waters were distinct from abyssal waters above the trench.

2. Sedimentary trench biosphere

Microbial ecosystem and geochemical features of the trench bottom sediment in the Ogasawara Trench was also different from that on the abyssal plain. This suggests the presence of the unique system that accumulates organic compounds in the trench environments.

One of the major objectives of this cruise is to know the microbial and geochemical interactions between the Japan Trench and the Izu-Ogasawara Trench. The other is to verify the generality of the trench biosphere that was discovered in the Challenger Deep, and clarify the role of developing trench biosphere especially for nitrifiers communities.

B. Scientific objectives of participants

Molecular analyses for hado- and abysso-pelagic waters and sediments

Takuro Nunoura and Miho Hirai (JAMSTEC)

In order to know the nitrogen cycles in abysso-pelagic waters and sediments, we will examine clone analysis and quantitative PCR targeting functional genes and SSU rRNA genes related to aerobic and anaerobic nitrification for both sediment and water samples. In addition, metagenomic and metaproteomic analyses and single cell genomic analysis targeting water samples will be conducted to know the adaptation mechanisms for had-pelagic environments and the role of trench microbial ecosystems in water.

Viral abundance and diversity in hado- and abysso-pelagic waters

Yukari Yoshida (JAMSTEC)

Viruses are now recognized as significant components of marine surface ecosystems. It has been suggested that they regulate microbial cellular and functional abundances and, consequently, affect global nutrient and energy cycles. Viruses can also mediate lateral gene transfers and drive the co-evolution between viruses and hosts. However, in contrast to the extensively studied marine surface environments, viral functions and ecology in deep-sea environments remain poorly characterized. In this cruise, viral and prokaryotic abundances in deep-sea environments were investigated. Furthermore, we will perform viral metagenome analysis to reveal viral diversity in deep-sea.

Cultivation of heterotrophic microbes

Takaaki Kubota and Mitsuhiro Yoshida (JAMSTEC)

As with other environments, microorganisms are known to play an important role in deep-sea ecosystems. Microbial communities in deep-ocean, such as Japan Trench, are still unexplored and hold great potential for both science and industry.

In this cruise, heterotrophic microbial communities, including prokaryotes and viruses in the trench environments were investigated to know the microbial diversity and to explore the microorganisms capable of the industrial application use.

Water samples were collected from different depths in the trench location. The 100 μl of each sample was directly added to the culture media for cultivating

heterotrophic bacteria. Sediment samples were taken from different depths in the trench location, and we will try to isolate bacteria. In addition, we will try to isolate the viruses with the cultivated bacterial hosts.

Prokaryotic activity throughout the water column of the Japan Trench.

Katsunori Yanagawa (The University of Tokyo) and Taichi Yokokawa (CMES, Ehime University)

Purpose

Marine prokaryotes play important roles on organic matter fluxes in the oceans. Despite evolving understanding about the importance and the diversity of prokaryotes in marine ecosystems, the data are scarce regarding prokaryotic activity in the water columns of open oceans, particularly in deep water realm. In this cruise, we focused on the prokaryotic abilities to utilize substrates in meso- and bathypelagic waters. Substrates uptakes were evaluated using radio-labeled tracer experiments. To estimate the contribution of *Archaea* and *Bacteria* to total prokaryotic substrate incorporations, we use an inhibitor approach.

Furthermore, we will determine microbial community structure by using fluorescence in situ hybridization (CARD-FISH) and elucidate the vertical dynamics of prokaryotic community composition in the water column of the trench. The comparison of these results will provide important insight into the role of prokaryotes in terms of organic matter flux in the deep-water realm.

Materials and Methods

Immediately after sampling of 12L-Niskin water, we transfer seawater sample into the bottles, added radio-labeled substrates (bicarbonate, glucose, pyruvate, leucine, glutamine, D-aspartic acid, L-aspartic acid, and amino acids mixture), and incubated at in situ temperature (± 2 ºC) in the dark. One trichloroacetic acid (TCA) killed blank was prepared for each sample. Incubation periods were one hour and two to three days for the upper (0 – 250 m) and deeper (300 – bottom) water layers, respectively.

To estimate the contribution of *Archaea* and *Bacteria* to total prokaryotic substrate incorporations, six inhibitors were used to inhibit their activity. Erythromycin

specifically inhibits protein synthesis in Bacteria but should not affect archaeal protein synthesis, whereas diphtheria toxin and GC_7 inhibit protein synthesis in Archaea but does not affect Bacteria. To stop the growth of ammonia oxidizing, inhibitors (i.e., PTIO, ATU and Acetylene) to ammonia oxidation were added into a part of the samples.

The filter method

For each substrate, duplicate subsamples (10 mL) dispensed into screw-capped glass tubes amended with ^{14}C labeled substrates. After the incubation with the substrates, microbial activity was stopped by addition formalin. Then the microbial cells were filtered onto 0.2-μm-pore sized polycarbonate membrane. The filtrated water, which passed through a filter with a nominal pore size of 0.2 μm, was used for carbon isotope analysis of dissolved organic carbon (DOC) to clarify the carbon flow from microbial cells into extracellular materials. These samples were stored at -80°C until future onshore analysis.

The microfuge method

For each substrate, triplicate subsamples (1.5 mL) dispensed into screw-capped centrifuge tubes amended with tritiated substrates and incubated at in situ temperature (± 2ºC) in the dark. One trichloroacetic acid (TCA) killed blank was prepared for each sample. After the incubation, proteins were TCA (final conc. 5%) extracted twice by centrifugation (14000 rpm, 10 min), followed by the extraction with ice-cold 80% ethanol.

Measurement of radioactivity

The samples were radioassayed with a liquid scintillation counter (Tri-Carb 3100TR, PerkinElmer) using Ultima-GOLD (Packard) as scintillation cocktail. Quenching was corrected by External standard channel ratio. The disintegrations per minute (DPM) of the TCA- and formalin-killed blank was subtracted from the average DPM of the samples, and the resulting DPM was converted into substrates incorporation rates

Viral ecology in hadopelagic environments

Eugenio Rastelli and Marco Lo Martire (*Department of Life and Environmental Sciences, Polytechnic University of Marche, Italy)*

Introduction:

Viruses are by far the most abundant biological entities in the world's oceans (approximately 4×10^{30}, Suttle 2007). Recent estimates suggest that every kg of deep-sea sediment contains 10^{12} viruses and 10^{11} prokaryotes (Sogin et al 2006). Recent studies revealed that viral infection in aquatic sediments can be the major cause of mortality for benthic prokaryotes (Danovaro et al 2008). Viral lysis transforms infected microbes into organic detritus, which can then be used again by non-infected prokaryotes and/or contribute to biogeochemical cycles. Extracellular DNA is likely to play a key role in both processes (Dell'Anno & Danovaro 2005) and can be a reservoir of genes (1 kg of deep-sea sediment can contain 10^{13} copies of 16S rRNA genes; Corinaldesi et al 2011).

Purpose:

During the KR12-19 cruise organised by JAMSTEC (12-2012), the goal of the italian research team (UNIVPM) was to investigate the dynamics in virus - prokaryotes interactions in the Japan trench and compare the results with the data acquired during the previous year's cruise in the Ogasawara trench (KR11-11).

Materials and methods:

Seawater samples were collected during the KR12-19 trench cruise in one station, from the sea surface to the bottom of the Japan trench. The aim of the water sampling was to study viral production and extracellular enzymatic activities at different quotes of the water column.

Surface and sub-surface sediments were collected from the same station. On these sediment samples, UNIVPM research team will conduct analyses for the determination of viral abundance and production, total prokaryotic abundance, abundance of Bacteria and Archaea, extracellular enzymatic activities, extracellular and intracellular DNA concentration.

Additional experiments (*in situ* temperature incubations of replicated mesocosms) were conducted onboard to study viral impact on Bacteria and on Archaea, both for seawater

and sediment samples.

Several antibiotics were used to inhibit Archaeal or Bacteral activity, in order to study the effects of this selective inhibition on viral production.

Future work:

The comparison of the new results with those obtained previously from the Ogasawara trench (KR11-11 cruise, 2011) will allow to gain more information about the functioning of the trench ecosystem in relation to the surrounding environment. Discussion and collaboration with the other groups of scientists working on different aspects of deep-sea ecology will lead to a more complete knowledge of the trench ecology and functioning.

References

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Marine nitrogen dynamics in hadopelagic environments

Tomoko Makita (TUAT), Takuro Nunoura (JAMSTEC) and onshore scientists (TUAT and JAMSTEC)

Our purpose is to understand nitrogen dynamics in hadopelagic environments, including water column and sediments.

1) In order to determine microbial activities associates with nitrogen cycles using stable isotopic analyses, we preserve sediment, gas and interstitial water samples.
2) In order to examine the nitrification activity through the water column, we conducted in situ nitrification activity measurement using pressure tight water samplers and stable isotopic analysis. In addition, we tested nitrification activity measurements using diverse substrates including ammonium and urea for water samples from the specific depths (bottom of euphotic zone, oxygen minimum zone-mesopelagic waters, deep water and trench waters) on board.

Protists in deep-ocean

Ryuji Kondo (Fukui Prefecture Univ)

The ‘microbial loop’ is known as a dynamic component of the planktonic food web in marine systems. Heterotrophic nanoflagellates (HNF) are significant consumers of bacterioplankton in aquatic environments. Labyrinthulids play a role in degradation of particulate/high molecule organic matter in marine and estuarine environments. In this cruise, vertical profiles of HNF and labyrinthulids abundances will be investigated throughout the water column of deep-ocean, Japan and Ogasawara Trenches. We will also try to isolate and cultivate the eukaryotic microbes in order to know their physiology.

11. Appendix

Notice on Using

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.