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R/V Yokosuka Cruise Report

YK13-12

In situ experimental & sampling study to understand abyssal biodiversity and biogeochemical cycles, western equatorial Pacific

Nov 7th, 2013 – Nov 30th,2013

Japan Agency for Marine-Earth Science and Technology

(JAMSTEC)

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1. Cruise Information

● Cruise ID: YK13-12

● Name of vessel: R/V Yokosuka

● Title of the cruise: *In situ experimental & sampling study to understand abyssal and hadal biodiversity and biogeochemical cycles*

● Title of proposal:

In situ experimental & sampling study to understand abyssal biodiversity and biogeochemical cycles (Hidetaka Nomaki)

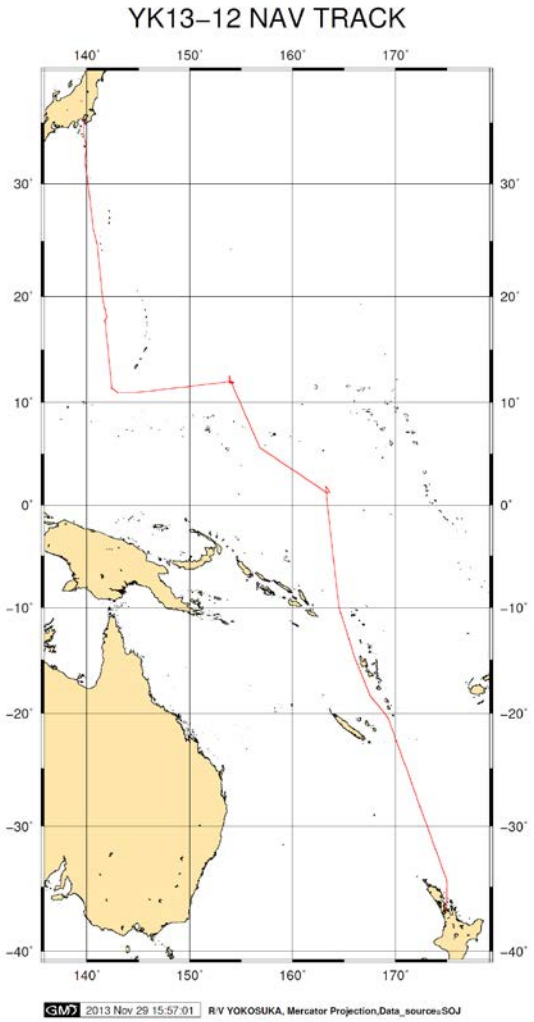
Capturing of *Hirondellea gigas* from Challenger Deep to isolate their DNA and RNA (Hideki Kobayashi)

● Cruise period: 7th Nov to 30th Nov, 2013

● Ports of call: Auckland to Yokosuka

● Research area: western equatorial Pacific

● Research Map



2. List of participants

2.1. Onboard scientists

● Chief scientist [Affiliation]: Hidetaka Nomaki [JAMSTEC]

● Representative of the science party [Affiliation]: Hidetaka Nomaki [JAMSTEC], Hideki Kobayashi [JAMSTEC]

● Science party (List) [Affiliation, assignment etc.]

Norio Miyamoto [JAMSTEC]

Takuro Nunoura [JAMSTEC]

Koji Seike [University of Tokyo]

Teresa Amaro [Hellenic Center of Marine Research]

Michael Tangherlini [Polytechnic University of Marche]

Hideki Kobayashi [JAMSTEC]

Satoshi Okada [Nippon Marine Enterprise]

2.2. Shinkai 6500 operation team

Submersible Op. Manager	Toshiaki Sakurai
Deputy Submersible Op. Manager	Yoshitaka Sasaki
1nd Submersible Staff	Kazuki Iijima
1nd Submersible Staff	Mitsuhiro Ueki
2nd Submersible Staff	Hitomi Ikeda
2nd Submersible Staff	Hirofumi Ueki
2nd Submersible Staff	Keigo Suzuki
2nd Submersible Staff	Masaya Katagiri

2.3 Crew members

Captain	Shinya Ryono
Chief Officer	Naoto Kimura
2nd Officer	Tetsuo Shiroyama
3rd Officer	Hiroharu Omae
Chief Engineer	Eiji Sakaguchi
1st Engineer	Kazunori Noguchi
2nd Engineer	Kenichi Shirakata
3rd Engineer	Kota Kataoka
Chief Radio officer	Masamoto Takahashi

2nd Electronic Operator	Hiroki Ishiwata
3rd Electronic Operator	Ryosuke Komatsu
Boat Swain	Yoshiaki Kawamura
Quarter Master	Kazumi Ogasawara
Quarter Master	Jiro Hanazawa
Quarter Master	Daisuke Yanagitani
Sailor	Kazuho Ikeda
Sailor	Yoshihiro Ogawa
Sailor	Kenta Nasu
No.1 Oiler	Kazuaki Nakai
Oiler	Shinya Sugi
Oiler	Masayuki Fujiwara
Oiler	Tatsuomi Chino
Oiler	Toshinori Matsui
Chief Steward	Sueto Sasaki
Steward	Shinsuke Tanaka
Steward	Masanao Kunita
Steward	Kazuma Sonoda
Steward	Shiho Shimizu

3. Observation

3.1. General purpose

Abyssal plain covers roughly half areas of the Earth Surface, thus consist largest marine ecosystem on Earth and contains abundant benthic fauna living on and in the sediment. Abyssal plain ecosystem has been thought to be sustained by POM fluxes from the photic zone, which originated from photosynthesis. Since surface productivity of the ocean differs largely with latitude, distance from land, upwelling intensity, and so on, organic matter fluxes to the seafloor also varied with oceanic settings. Those differences in POM fluxes to the seafloor are believed to be major controlling factor of benthic diversity, biomass, sediment community oxygen consumption, etc. On the other hand, recent studies on sediment metagenomics reveal that some chemolithoautotrophic microbes may inhabit even in the "normal" deep-sea floor where no active hydrothermal vent or hydrocarbon seepage exist. However, there is no report on chemoautotrophic production rate at the deep-sea floor. In this study, we carried out sediment samplings and in situ incubation experiments to investigate diversity and biomass of benthos and relevant biogeochemical cycles at the deep-sea floor under different surface productivities.

3.2. In situ experiments to reveal autotrophic microbial activities at the abyssal plain

Hidetaka Nomaki and YK13-09, YK13-12 scientists

We carried out an in situ incubation experiment together with sediment geochemistry investigation at the 1N site (1°15N, 163°15E, water depth = 4277m) during YK 13-09 and YK13-12 cruises. The experiment is planned to reveal the chemoautotrophic carbon production rate at the deep-sea floor using in situ incubation cores. We prepared 5 different substrates which were labeled with either ^{13}C or ^{15}N. The incubation was carried out for 2 days and 51 days on the seafloor.

We collected sediment samples with push corer, water samples with Niskin water sampler, and megabenthos samples with slurp gun (suction sampler). Those samplings were also carried out at the east off Guam station (12°N, 154°E, water depth = 5920m), where surface water productivity is very low.

On board, sediment samples were sliced into different depth layers and subsampled for viral, microbial, geochemical, and meiofaunal analyses. Water samples were filtered on board and the filters were kept frozen. Megabenthos samples were preserved with either ethanol, formalin, or kept frozen.

3.3. In situ experiments for heterotrophic organism activities

Hidetaka Nomaki, Teresa Amaro, Michael Tangherlini, and YK13-09&YK13-12 scientists

We finished the 2 different in situ incubation experiments we had started 51 days ago at the 1N site (1°15N, 163°15E, water depth = 4277m) during dives #1375 on 16th November.

The 1st experiment objective was to look at the impact of key megafauna organisms on the uptake of carbon in macro/meio/microbial fauna. The 2nd experiment objective was to look at the physiological responses of key megafaunal organisms (holothurians) to a varying quality and quantity of food input. Both incubations were carried out for 2 days incubation and 51 days incubation.

We collected sediment samples with push corer, water samples with Niskin water sampler, and megabenthos samples with slurp gun (suction sampler).

On board, sediment samples were sliced into different depth layers (0-1, 1-2, 2-3, 3-5, 5-10 cm) and subsampled for viral, microbial, geochemical, meiofaunal, macrofauna analyses. Water samples were filtered on board and the filters were kept frozen. Megabenthos samples were preserved at -80°C freezer.

To characterize an oligotrophic area, we moved to another station at 12N site (12°N, 154°E, water depth = 5920 m). We made one dive #1376 on the 20th of November where we took 3 push cores. On board, sediment samples were sliced into different depth layers (0-1, 1-2, 2-3, 3-5, 5-10 cm) and sampled for meiofaunal and macrofauna analyses.

3.4. Significance of nitrification in oligotrophic deep sea sediments

Takuro Nunoura, Manabu Nishizawa (JAMSTEC)

Nitrification; oxidation of ammonia and nitrite to nitrite and nitrate, respectively, is one of the most dominant chemolithotrophic metabolisms in deep sea environments, and likely play significant roles in nitrogen cycle in marine sedimentary habitats. In fact, the presence and predominance of putative ammonia-oxidizing thaumarchaeotes in benthic archaeal communities has been reported. However, little is known about their in situ activity and significance in benthic nitrogen cycle. In this cruise, we took abyssal plain sediments from the north pacific gyre and equatorial pacific sites, and will examine the significance of nitrifier communities using molecular biological and stable isotopic approaches. Moreover, we conducted in situ incubation analyses to examine nitrification activity at the equatorial pacific site. The stable isotopic pore water chemistry and molecular biological approaches to the samples obtained by the in situ incubation will provide novel insights into the benthic nitrogen cycle under the relatively oligotrophic ocean.

3.5.Unveiling the drivers of virus-prokaryotes activity and interactions in abyssal ecosystems

Michael Tangherlini

(*Department of Life and Environmental Sciences, Polytechnic University of Marche, Italy)*

Introduction: Viruses are 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 about 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 YK13-12 cruise organised by JAMSTEC (11-2013), the goal of the Italian research group (UNIVPM) was to investigate the dynamics in virus – prokaryotes activity and interactions in the abyssal ecosystem in the Pacific Ocean and to compare the results with the data acquired during the previous year's cruises in the Ogasawara trench and abyssal plain (11-2011) and Japan trench (12-2012). Additional experiments using *in situ* incubations were conducted in collaboration with JAMSTEC to study the response of benthic communities to food inputs and prokaryotic chemosynthetic processes.

Materials and methods: Seawater samples were collected during the YK13-15 cruise in stations 1N and 12N, from the sea surface and the bottom of the abyssal ecosystem in the Pacific Ocean. Surface and sub-surface sediments were collected from the same stations.
Additional experiments (*in situ* temperature incubations of replicated mesocosms) were conducted on board and *in situ* for sediment samples.
On these samples, UNIVPM research team will conduct analyses for the determination of:
Viral abundance; total prokaryotic abundance; relative importance of Bacteria and Archaea on total prokaryotic abundance; viral production; prokaryotic heterotrophic production; extracellular enzymatic activities; organic matter composition and turnover; prokaryotic benthic diversity through next generation sequencing; DNA metagenomics; RNA analysis and transcriptomics.
Moreover, several antibiotics were used to inhibit Archaeal or Bacterial activity in selected sets of samples, in order to study the effects of the host inhibition on viral production.

Future work: The comparison of the new results with those obtained previously during JAMSTEC cruises in the Ogasawara trench and Abyssal plain (2011) and Japan trench (2012) and from future cruises (Mariana Trench, 2014) will allow to gain more information about the functioning of the

trench ecosystem in relation to the surrounding abyssal environment. Discussion and collaboration with the other groups of scientists will lead to a more complete knowledge of the connectivity between different trenches and will shed new lights on the deep-sea ecology and ecosystem functioning of these areas.

References

Danovaro, R. et al (2008). Nature 454: 1084-1087; Corinaldesi, C. et al (2011). Mol. Ecol. 20: 642-654. 5. Dell'Anno, A. & Danovaro, R. (2005). Science 309: 2179; Sogin, M. et al (2006). PNAS 32: 12115-12120; Suttle, C.A. (2007). Nature Rev. Microbiol. 5: 801–812;

3.6. Capturing of *Hirondellea gigas* from Challenger Deep to isolate their DNA and RNA

Hideki Kobayashi (Biogeos, JAMSTEC)

- Purpose, Objectives, background

We reported *Hirondellea gigas* of Challenger Deep had a novel cellulase, which was very useful for production of glucose from cellulose. However, we cannot isolate complete DNA or RNA, because of degradation caused by leaving at room temperature for a while. We need to isolate undigested DNA and RNA from amphipods for cloning of cellulase gene. In this cruise, one of purposes is capturing *H. gigas* to check their digestive enzymes.

- Research results

We sank a mooring system with 11 baited traps (3: balk, 5: covered by insulating materials, 3: automatically sealed after 9 h) into the Challenger Deep (11-21.9082N, 142-25.7606E D=10,896m) on 23 Nov. 2013. After 8 h 46 m stay at the bottom, we captured about 90 individuals (large: 70, small: 20). We stored about 10 amphipods in 50 ml of All Protection Solution (QIAGEN; Germantown, MD, USA), and 50 ml of RNAlater (QIAGEN; Germantown, MD, USA). 7 individuals were stored in 2.5% glutaraldehyde solution for electron microscopic observation. Other amphipods are frozen in liquid nitrogen and stored in -80°C freezer (about 40 individuals and small individuals). We did not have time to count exact number of amphipods in order to avoid digestion of DNA, RNA and protein by internal DNase, RNase and protease. There is no amphipods in baited traps sealed automatically after 9 h.

- Future study

First of all, we isolate DNA and RNA from amphipods, and check whether DNA or RNA was digested. When RNA is not digested, we will decide all RNA sequence after construction of cDNA library. We search all RNA sequence for cellulase gene. If we fortunately find whole length of cellulase gene sequence, we will try getting cellulase gene from cDNA library to obtain the recombinant enzyme. When only partial cellulase gene sequences were obtained, in most cases, we will try whole gene from

genomic DNA by PCR technology.

3.7. Acknowledgment

We thank to the captain and crew members of R/V Yokosuka for their strong supports during the cruise. We also thank to the submersible operation manager and the Shinkai 6500 operation team for their skilful operation of the submersible.

3.8. List of observation equipments

In situ incubation core (inner diameter: 8.2cm, length: 32cm)
In situ incubation box (Surface area: 900 cm^2)
Holothurian cage (Surface area: ~600 cm^2)
Niskin water sampler (5L)
H-type push corer (inner diameter: 8.2cm, length: 32cm)
Suction sampler with multiple canister

3.9. Cruise log

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
07-Nov-13		**Sail out & started YK13-12**		11/7 12:00 (UTC+13h)
	10:20	Let go all shore lines,left Auckland for research area(Equator vicinity).		36-36.6S 174-59.1E
	11:30-11:40	On board education for scientists.		cloudy
	13:00-13:30	SHINKAI 6500 meeting.		SSW-4(Moderate breeze)
	16:40-17:00	KONPIRA pray for safety cruise.		3(Sea Slight)
				1(Low Swell)
				Visibly: 8'
08-Nov-13		**Proceeding to research area**		11/8 12:00(UTC+13h)
				31-45.5S 173-48.9E
				Fine but cloudy
				WSW-5(Fresh breeze)

				4(Sea Moderate)
				3(Moderate Short)
				Visibly: 8'
09-Nov-13		**Proceeding to research area**		11/9 12:00(UTC+12h)
	24:00	Put ship's clocks back 1hour.		27-01.1S 171-51.9E
				Fine but cloudy
				SE-4(Moderate breeze)
				3(Sea Slight)
				3(Moderate Short)
				Visibly: 8'
10-Nov-13		**Proceeding to research area**		11/10 12:00(UTC+12h)
				22-02.1S 169-54.0E
				Cloudy
				ESE-4(Moderate breeze)
				3(Sea Slight)
				1(Low Swell)
				Visibly: 8'
11-Nov-13		**Proceeding to research area**		11/11 12:00(UTC+12h)
				17-57.8S 167-22.5E
				Fine but cloudy
				ESE-5(Fresh breeze)
				3(Sea Slight)
				1(Low Swell)
				Visibly: 8'
12-Nov-13		**Proceeding to research area**		11/12 12:00(UTC+12h)
	09:30～10:30	On board fire drill.		13-22.6S 165-35.2E
	24:00	Put ship's clocks back 1hour.		Fine but cloudy
				ESE-4(Moderate breeze)
				3(Sea Slight)

				1(Low Swell)
				Visibly: 8'
13-Nov-13		**Proceeding to research area**		11/13 12:00(UTC+11h)
				08-40.6S 164-21.6E
				Fine but cloudy
				E-1(Light air)
				1(Calm)
				1(Low Swell)
				Visibly:8'
14-Nov-13		**Proceeding to research area**		11/14 12:00(UTC+11h)
				03-54.9S 163-49.6E
				Fine but cloudy
				ENE-2(Light breeze)
				2(Sea smooth)
				1(Low Swell)
				Visibly:8'
15-Nov-13		**Arrived at research area(Equator vicinity)**		11/15 12:00(UTC+11h)
	14:00	Arrived at research area.		01-15.0N 163-18.9E
	14:32～15:02	Carried out MBES mapping survey.		Fine but cloudy
	15:07～15:12	Carried out water sampling by backet.	01-14.84N,163-12.76E	ENE-2(Light breeze)
				2(Sea smooth)
				1(Low Swell)
				Visibly:8'
16-Nov-13		**Operation "SHINKAI6500" Dive#1375**		11/16 12:00(UTC+11h)
	08:59	"SHINKAI6500" dove & started her operation # 1375.		01-15.2N 163-14.6E
	10:52	"SHINKAI 6500" landed on the sea bottom (Depth=4277m).		Fine but cloudy

	14:55	"SHINKAI 6500" left the sea bottom (D=4278m).		SE-3(Gentle breeze)
	16:46	Surfaced "SHINKAI 6500"		2(Sea smooth)
	17:13	Recovered "SHINKAI 6500" & finished the operation.		1(Low Swell)
	19:00	Left research area to Guam eastward.		Visibly:8'
17-Nov-13		**Proceeding to research area**		11/17 12:00(UTC+11h)
				03-36.4N 159-45.4E
				ESE-4(Moderate breeze)
				3(Sea Slight)
				1(Low Swell)
				Visibly: 8'
18-Nov-13		**Proceeding to research area**		11/18 12:00(UTC+11h)
				07-20.8N 156-03.4E
				Fine but cloudy
				ESE-4(Moderate breeze)
				3(Sea Slight)
				3(Moderate Short)
				Visibly: 8'
19-Nov-13		**Arrived at research area(Guam eastward)**		11/19 12:00(UTC+11h)
	13:00	Released XBT.		11-47.9N 154-05.8E
	13:11	Carried out water sampling by backet.	11-58.57N,154-00.78E	Fine but cloudy
	13:22	Released proton magnetometer.		E-4(Moderate breeze)
	13:34～13:54	Carried out figure eight running.		3(Sea Slight)
	14:06～14:43	Carried out MBES mapping survey.		3(Moderate Short)
	15:04	Com'ced MBES mapping survey.		Visibly: 8'

20-Nov-13		**Operation "SHINKAI6500" Dive#1376**		11/20 12:00(UTC+11h)
	09:00	"SHINKAI6500" dove & started her operation # 1376.		12-00.0N 154-00.0E
	11:33	"SHINKAI 6500" landed on the sea bottom (Depth=5920m).		Fine but cloudy
	12:45	"SHINKAI 6500" left the sea bottom (D=5920m).		NE-5(Fresh breeze)
	14:58	Surfaced "SHINKAI 6500"		3(Sea Slight)
	15:27	Recovered "SHINKAI 6500" & finished the operation.		3(Moderate Short)
	16:10	Left research area to Mariana trench.		Visibly: 8'
21-Nov-13		**Proceeding to research area**		11/21 12:00(UTC+11h)
	24:00	Put ship's clocks back 1hour.		11-32.8N 150-05.2E
				Fine but cloudy
				ENE-5(Fresh breeze)
				4(Sea moderate)
				3(Moderate Short)
				Visibly:8'
22-Nov-13		**Proceeding to research area**		11/22 12:00(UTC+10h)
				11-00.6N 145-23.3E
				Fine but cloudy
				ENE-4(Moderate breeze)
				3(Sea Slight)
				1(Low Swell)
				Visibly:8'
23-Nov-13		**Deployment of the bait trap lander**		11/23 12:00(UTC+10h)
	08:00	Released XBT.		11-20.6N 142-22.0E

	08:31～09:10	Carried out MBES mapping survey.		Fine but cloudy
	17:46	Deployed the bait trap lander.		E-5(Fresh breeze)
	20:40	The bait trap lander landed on the sea bottom (Depth=10,896m).		4(Sea moderate)
	20:50～21:40	Carried out calibration for position of the bait trap lander.		3(Moderate Short)
				Visibly:8'
24-Nov-13		**Recovery of the bait trap lander**		11/24 12:00(UTC+10h)
	05:26	The bait trap lander left the sea bottom (D=10,896m).		11-49.8N 142-23.2E
	08:35	Surfaced the bait trap lander.		Fine but cloudy
	09:10	Recovered the bait trap lander.		E-4(Moderate breeze)
	10:00	Left research area to Yokosuka.		3(Sea Slight)
				3(Moderate Short)
				Visibly: 8'
25-Nov-13		**Proceeding to Yokosuka**		11/25 12:00(UTC+10h)
	24:00	Put ship's clocks back 1hour.		16-49.4N 141-51.6E
				Fine but cloudy
				E-5(Fresh breeze)
				4(Sea moderate)
				3(Moderate short)
				Visibly:8'
26-Nov-13		**Proceeding to Yokosuka**		11/26 12:00(UTC+9h)
				21-48.5N,141-19.2E
				Cloudy
				SSE-4(Moderate breeze)
				3(Sea Slight)
				3(Moderate Short)
				Visibly: 8'
27-Nov-13		**Proceeding to Yokosuka**		11/27 12:00(UTC+9h)

				26-26.9N,140-33.3E
				Overcast
				NE-3(Gentle breeze)
				2(Sea smooth)
				3(Moderate short)
				Visibly: 8'
28-Nov-13		**Proceeding to Yokosuka**		11/28 12:00(UTC+9h)
				31-06.8N,139-55.5E
				Overcast
				SSW-5(Fresh breeze)
				5(Sea rough)
				4(Moderate average)
				3(Moderate short)
				Visibly:8'
29-Nov-13		**Proceeding to Yokosuka**		11/29 12:00(UTC+9h)
	15:00	Casted anchor at Yokosuka No.4 section.		34-51.6N,139-41.0E
				Blue sky
				WSW-8(Gale)
				6(Very rough)
				4(Moderate average)
				Visibly:8'
30-Nov-13		**Arrived at Yokosuka,then concluded YK13-12**		11/30 12:00(UTC+9h)
	08:20	Recovered anchor.		SUMITOMO HMI
	09:00	Arrived at Yokosuka(SUMITOMO HMI).		

4. Notice on Using

Notice on using: Insert the following notice to users regarding the data and samples obtained.

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.