

Yokosuka Cruise Report YK17-17

***Shinkai6500* exploration Cruise
at Tarama Knoll in the Okinawa Trough**



**Elucidation of microbial ecosystems utilizing
iron and other environmental factors to support
their activity in “Iron-dominated Flocculent
Mats” of Tarama Knoll**

2 August 2017, Naha – 10 August 2017, Ishigaki Island

**Japan Agency for Marine-Earth Science & Technology
(JAMSTEC)**

Acknowledgements

We are grateful to Captain Mr. E. Ukekura, Chief Officer Mr. H. Kato, Chief Engineer Mr. E. Sakaguchi, Chief Electronic Operator Mr. Y. Komaki, Boatswain Mr. K. Hirosaki, No.1 Oiler Mr. Y. Yamaguchi, Chief Steward Mr. S. Sasaki and all of crews for their safe navigation and their skillful handling of “R/V Yokosuka”. Great thanks are directed to Submersible Operation Manager Mr. T. Sakurai and “Shinkai6500” operation team for their effort in operation and sampling. We also thank Mr. Y. Hashimoto, Nippon Marine Enterprise, Ltd., for his attentive supports.

Finally, we would like to express our gratitude to all who directly or indirectly supported this cruise.

Contents

Acknowledgements	2
1. Onboard members	
1.1 Yokosuka Crew	4
1.2 Shinkai 6500 Operation team	5
1.3 Research Party	6
2. Cruise Information	
2.1 Cruise Summary	9
2.2 Shipboard Log of YK17-17	13
2.3 Dive Report	
Shinkai6500 dive#1508 (H. Makita)	15
Shinkai6500 dive#1509 (T. Yamanaka)	20
3. Scientific Reports	
3.1. Elucidation of the microbial ecosystems utilizing iron and other environmental factors to support their activity in “Iron-dominated Flocculent Mats” of Tarama Knoll.	25
3.2. Geochemical studies of hydrothermal activity, mineralization and vent-associated animals obtained from Tarama Knoll and Hill in South Okinawa Trough, Japan.	26
3.3. Preliminary investigation to elucidate the deformation behavior of submarine mineral resources.	27
3.4. Durability evaluation of sealants for mining deep-seafloor ore deposits.	27
3.5. Vent megafaunal biodiversity and biogeography analyses	28
4. Notice on Using	29
5. Appendix	
5.1 Payload Pictures	30

1. Onboard members

1.1. Captain and crews of the R/V Yokosuka

Captain: **EIKO UKEKURA**

Chief Officer: **HIROYUKI KATO**

1st Officer: **TAKAFUMI AOKI**

2nd Officer: **TOSHIYO OHARA**

3rd Officer: **YUTA UOZUMI**

Chief Engineer: **EIJI SAKAGUCHI**

1st Engineer: **SHINICHI IKUTA**

2nd Engineer: **KATSUTO YAMAGUCHI**

3rd Engineer: **KEITO SHIMADA**

Chief Electronics Operator: **YOSUKE KOMAKI**

2nd Electronics Operator: **TAKATOMO SHIROZUME**

3rd Electronics Operator: **YOHEI SUGIMOTO**

Boat Swain: **KANAME HIROSAKI**

Quarter Master: **KAZUMI OGASAWARA, KAITO MURATA,
HIROAKI NAGAI, HIROTAKA SHIGETA**

Sailor: **TAKUMI MIURA** and **KEISUKE ISOBE**

No.1 Oiler: **YUKIHIRO YAMAGUCHI**

Oiler: **SHINYA SUGI, YUJI HIGASHIKAWA, TOSHINORI MATSUI**

Assistant Oiler: **TORU HIDAKA**

Chief Steward: **SUETO SASAKI**

Steward: **HIDEO FUKUMURA, HIRONOBU HODOKUMA,
TSUYOSHI NAGATOMO, SEIYA MATSUMOTO, KINA ABE**

1.2. Shinkai 6500 Operation Team

Submersible Op. Manager: **TOSHIAKI SAKURAI**

Deputy Submersible Op. Manager: **KAZUHIRO CHIBA**

1st Submersible Staff: **MITSUHIRO UEKI, KEITA MATSUMOTO**

2nd Submersible Staff: **HIROHUMI UEKI, YOUSUKE CHIDA,
KEIGO SUZUKI, TAKUMA ONISHI, JUNYA NIIKURA,
RYO SAIGO, YUDAI TAYAMA**

3rd Submersible Staff: **SATSUKI IIJIMA, TAIKEN YAMAGUCHI**

1.3. Research party

HIROKO MAKITA, *Chief Scientist*

Scientist (Geomicrobiology)

Department of Subsurface Geobiological Analysis and Research,
Japan Agency for Marine-Science and Technology (JAMSTEC)

TOSHIRO YAMANAKA, *Co-Chief Scientist*

Professor (Geochemistry)

Department of Ocean and Environmental Sciences,
School of Marine Resources and Environment,
Tokyo University of Marine Science and Technology (TUMSAT)

KEI OKAMURA

Professor (Geochemistry)

Center for Advanced Marine Core Research,
Kochi University

TAKUROH NOGUCHI

Associate Professor (Geochemistry)

Center for Advanced Marine Core Research,
Kochi University

TOMOHIRO TOKI

Associate Professor (Geochemistry)

Faculty of Science,
University of the Ryukyus

KENTARO NAKAMURA

Associate Professor (Geochemistry)

Department of System Innovation, Graduate School of Engineering,
The University of Tokyo

JUNICHI MIYAZAKI

Scientist (Geomicrobiology)

Department of Subsurface Geobiological Analysis and Research,
Japan Agency for Marine-Science and Technology (JAMSTEC)

CHONG CHEN

Postdoctoral researcher (Biology)

Department of Subsurface Geobiological Analysis and Research,
Japan Agency for Marine-Science and Technology (JAMSTEC)

HIROYUKI KASHIMA

Postdoctoral researcher (Microbiology)

Department of Subsurface Geobiological Analysis and Research,
Japan Agency for Marine-Science and Technology (JAMSTEC)

YOHEI MATSUI

Scientist (Geochemistry)

Project Team for Development of New-generation Research Protocol for
Submarine Resources Ore Genesis Research Unit,
Japan Agency for Marine-Science and Technology (JAMSTEC)

YUMI SUZUKI

Master's student (Microbiology)

Department of Applied Chemistry, Graduate School of Engineering,
Kanagawa Institute of Technology (KAIT); and
Department of Subsurface Geobiological Analysis and Research,
Japan Agency for Marine-Science and Technology (JAMSTEC)

TOMOHIKO SAKIYAMA

Master's student (Mine engineering)

Department of Earth Resources Engineering,
Graduate School of Engineering,
Kyushu University

KAORI KURABA

Undergraduate student (Geochemistry)
Department of Ocean and Environmental Sciences,
School of Marine Resources and Environment,
Tokyo University of Marine Science and Technology (TUMSAT)

ERI YOKOTA

Undergraduate student (Geochemistry)
Department of Ocean and Environmental Sciences,
School of Marine Resources and Environment,
Tokyo University of Marine Science and Technology (TUMSAT)

YASUSHI HASHIMOTO

Marine Technician
Marine Science Department, Nippon Marine Enterprises, Ltd.

2. Cruise Information

2.1. Cruise Summary

Iron is the most abundant sub-ground element and the fourth most abundant element on Earth; most organisms use iron as an essential nutrient. Recent studies have demonstrated ecological changes caused by the lack of iron. In addition, iron dissemination is considered to be a good strategy for controlling and keeping an ecological environment. In these ways, the great importance of iron for Earth's organisms was recognized. During the recent years, the culture –dependent and –independent microbiological characterization revealed that the zeta-proteobacteria “*Mariprofundus ferrooxidans*” (Emerson D., *et al.*, 2007), an choemolithoautotrophic microorganism utilizing ferrous iron, is commonly found in some deep-sea low-temperature hydrothermal fields; rocks alteration regions, and iron mat sites (Davis and Moyer, 2008, Kato *et al.*, 2009, Makita *et al.*, 2016). This type of iron-utilizing chemolithoautotroph microorganism has significant ecological roles, such as iron and carbon cycling, in microbial communities occurring in low-temperature hydrothermal fields in the deep-sea (Bach *et al.*, 2003). Research about such microorganisms and their habitat, such as their biodiversity, the dominant species in each site, their true role in their natural habitats, and their mechanisms of interaction with other microorganisms as well as rocks, has begun to progress in the recent years.

At the summit of Tarama Knoll, an iron mat dominated area named ‘Fox site’ has been observed since 2009 in NT09-10 Leg2 cruise. This site has since been investigated in 2010 by the cruise NT10-06 Leg2 and in 2011 by the cruise NT11-18. Microbiology of Fox site is thus already under investigation (Makita *et al.*, 2016). However, the previous study was unable to show the role and contribution of iron-utilizing microorganisms in shaping the observed surrounding environment. In this study, we will attempt to demonstrate and elucidate the association between the iron-utilizing microorganisms and the nature of the Fox site microbiological ecosystems. Then, we will be able to compare the microbial ecosystems at Fox site with other known iron mat sites occurring at low-temperature hydrothermal systems around the globe to further our understanding of the linkage between microbiology and chemistry in these sites with their geological and tectonic background. The main aims of the present cruise includes, 1) recovery and installation of GALI

(Genba-Atryoku Lithoautotroph) *in-situ* colonization systems in the iron mat area at Fox site, 2) to collect hydrothermal vent fluids, sea water and pore water from the iron mat, 3) to collect iron mat itself and the surrounding sediment.

Cruise ID: YK17-17

Research vessel: R/V Yokosuka and the DSV Shinkai6500

Title of the cruise: Elucidation of microbial ecosystems utilizing iron and other environmental factors to support their activity in "Iron-dominated Flocculent Mats" at Tarama Knoll

Title of proposal: Elucidation of microbial ecosystems utilizing iron and other environmental factors to support their activity in "Iron-dominated Flocculent Mats" at Tarama Knoll

Chief Scientist & Proponent of the cruise: Hiroko Makita (JAMSTEC)

Cruise period and Port of call:

August 2, 2017 (Naha)-August 10, 2017 (Ishigaki Island)

Research area: Okinawa, Japan

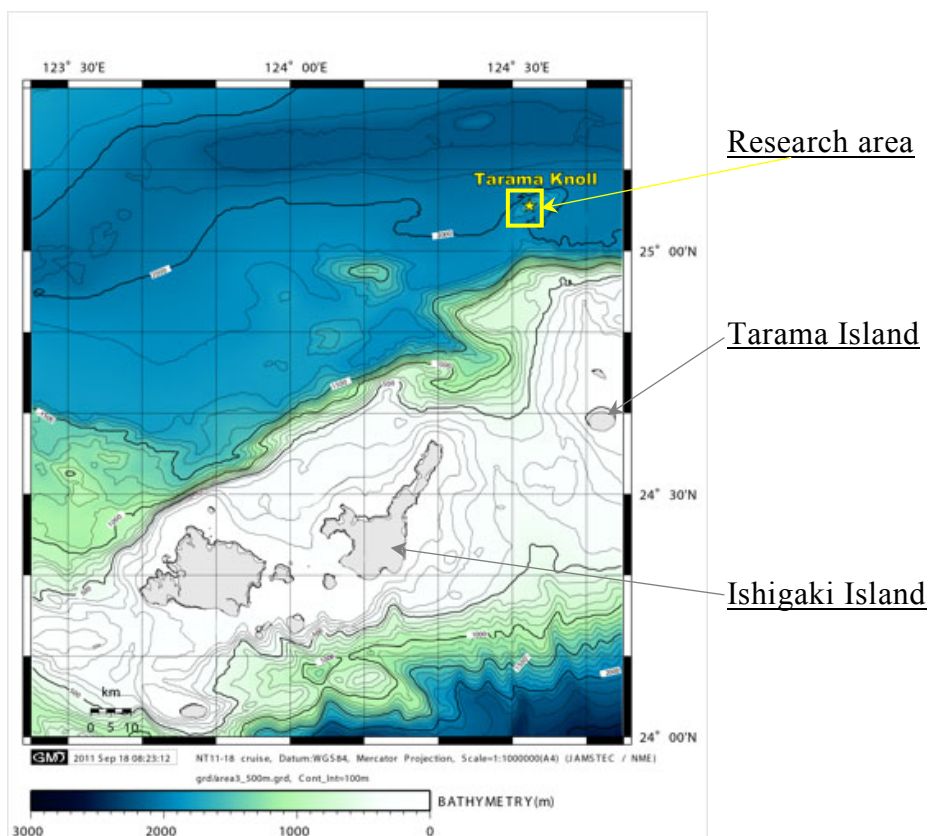


Fig. 2-1. Index map showing location of the research area.

(This map was created by Ms. Kyoko Tanaka for NT11-18 cruise.)

Water depth: 1,500-2,000 m

Overview of the Investigation:

We carried out following *in-situ* experiments and samplings.

1. Recovery of *in-situ* colonization system 'GALI'
2. Sampling of sediments, benthos, fluid
3. Turbidity, DO, Methane, Hydrogen and ORP measurements

Dives:

6K Dive#1508 (Aug. 3, 2017)

6K Dive#1509 (Aug. 4, 2017)

2.2. Shipboard Log of YK17-17

Date	Local Time	Note	Position/Weather/Wind/Sea condition
2-Aug-17	8:00	Scientists onboard.	12:00 (UTC+9h)
	9:00	Let go all shore lines & left NAHA for research area	South of Kumejima
		"TARAMA KNOLL"	25-59.5N, 126-58.5E
	10:00-10:40	On board education & training for scientists.	Fine but cloudy
	11:00-11:30	Cruise meeting, 6k team & scientists.	North-3 (Gentle breeze)
	16:40	Konpira ceremony	2 (Smooth)
	18:00-18:30	Scientist meeting	1 (Low swell short)
			Visibly: 8'
3-Aug-17	1:30	Arrived at research area & com'ced drifting	12:00 (UTC+9h)
	5:09	Released XBT at <25-02.1166N, 124-32.3017E>	Tarama knoll
		Released underwater sensor	25-05.5N,124-32.7E
	05:37-06:26	Carried out MBES site survey	Fine but cloudy
	8:58	Hoisted up 'SHINKAI 6500'	Calm-0
	9:05	Launched 'SHINKAI 6500'	1 (Calm)
	9:16	SHINKAI 6500' dove & Started her operation #1508(30)	1 (Low swell short)
	09:38-09:40	surface water sampling	Visibly: 8'
	10:15	'SHINKAI 6500' landed on the sea bottom (D=1,723m)	
	15:41	'SHINKAI 6500' left the sea bottom (D=1,506m)	
	16:14	Refloated 'SHINKAI 6500'	
	16:31	Hoisted up 'SHINKAI 6500'	
	16:41	Recovered 'SHINKAI 6500' & finished above operation	
	17:16-20:01	Carried out MBES plume investigation	
	19:30-20:00	Scientist meeting	
	23:00-23:30	Shifted to SW ward	
4-Aug-17	03:15-03:30	Shifted to south ward	12:00 (UTC+9h)
	9:02	Hoisted up 'SHINKAI 6500'	Tarama knoll
	9:08	Launched 'SHINKAI 6500'	25-05.5N,124-32.7E
	9:17	SHINKAI 6500' dove & Started her operation #1509(31)	Fine but cloudy
	10:15	'SHINKAI 6500' landed on the sea bottom (D=1,839m)	NNW-2 (Light breeze)
	16:15	'SHINKAI 6500' left the sea bottom (D=1,943m)	1 (Calm)
	16:57	Refloated 'SHINKAI 6500'	1 (Low swell short)
	17:15	Hoisted up 'SHINKAI 6500'	Visibly: 8'
	17:24	Recoverd 'SHINKAI 6500' & finished above operation	
	18:15	Left research area & com'ced proceeding to ISHIGAKI island	

5-Aug-17	8:30	Arrived at ISHIGAKI-KO	12:00 (UTC+9h)
	11:00	3 scientists disembarked.	ISHIGAKI-KO
			24-20.0N,124-08.8E
			Fine but cloudy
			SW-3 (Gentle breeze)
			1 (Calm)
			0 (No swell)
			Visibly: 8'
6-Aug-17	8:00	All scientists disembarked.	

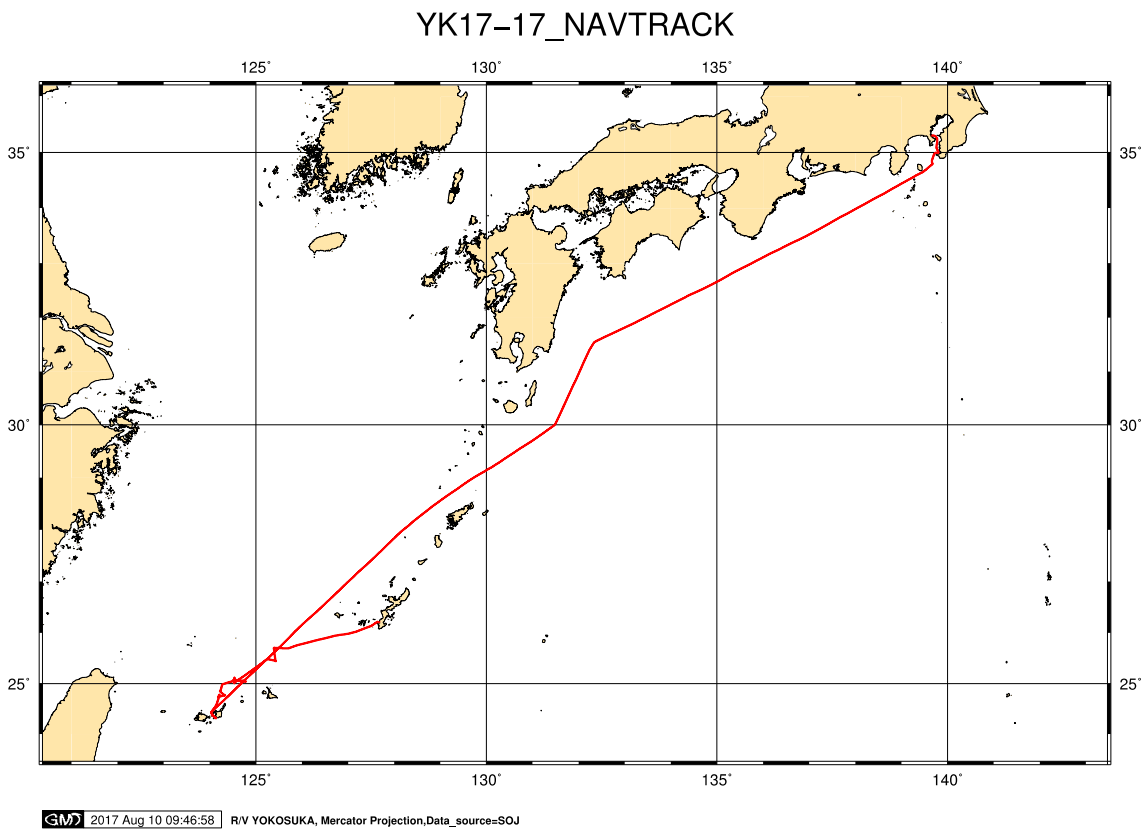


Fig. 2-2. Navigation track of YK17-17 cruise.

2.3. Dive Reports

Dive Report: Shinkai6500 Dive #1508

Date: August 3, 2017

Site: Tarama Knoll hydrothermal field

Landing: 10:16:12; 25° 5.4397'N, 124°32.749'E; 1,728 m

Leaving: 15:41:13; 25°5.5975 'N, 124°32.3666 'E; 1,507 m

Observer: Hiroko Makita (JAMSTEC)

Pilot: Kazuhiro Chiba, **Co-Pilot:** Yousuke Chida

Objectives:

Objectives of this dive were;

- 1) Search and recover the *in-situ* colonization system 'GALI-2', which was placed at Fox site during the NT10-06 Leg2 cruise in 2010.
- 2) Search for a new hydrothermal vent site at the southeastern part of the Tarama Knoll, as indicated by an AUV *Urashima* dive during YK14-16 cruise.
- 3) Collect hydrothermal/biological samples at the iron mat site (fox site) and the new hydrothermal site in Tarama Knoll.

Dive Summary:

We landed southwest of Swan site at the depth of 1,728 m, and headed northwest, discovering active vent chimneys at the new hydrothermal site. Soon after, we found massive animal colonies at an area several tens of meters from the landing point. After that, we found many chimneys covered by megafaunal animals. Most of them were active chimneys, either clear or gray smoker. These findings clearly proved that there is a previously undiscovered hydrothermal vent site at the area as suggest by AUV *Urashima* in the YK14-16 cruise. The gray smoker chimney was accompanied by many animals (e.g. *Shinkaia crosnieri*). We named one of the characteristic chimneys "Komainu Chimney". We carried out sampling of chimney, animals and fluid. Then, we set #224 marker near the gray smoker chimney. We named this new hydrothermal site as the YZ site.

Then, after going over several arrays of active chimneys, we successfully

found the 'Experimental set' at the depth of 1678m, and the *in-situ* colonization system 'GALI-2' was also successfully recovered at Fox site near the summit of Tarama knoll. This site has a thick iron mat with low temperature hydrothermal flow. We carried out sediment sampling and several sensor measurements at this site. After taking samples *Shikai6500* left the bottom at 15:40 pm at a water depth of 1,512 m.

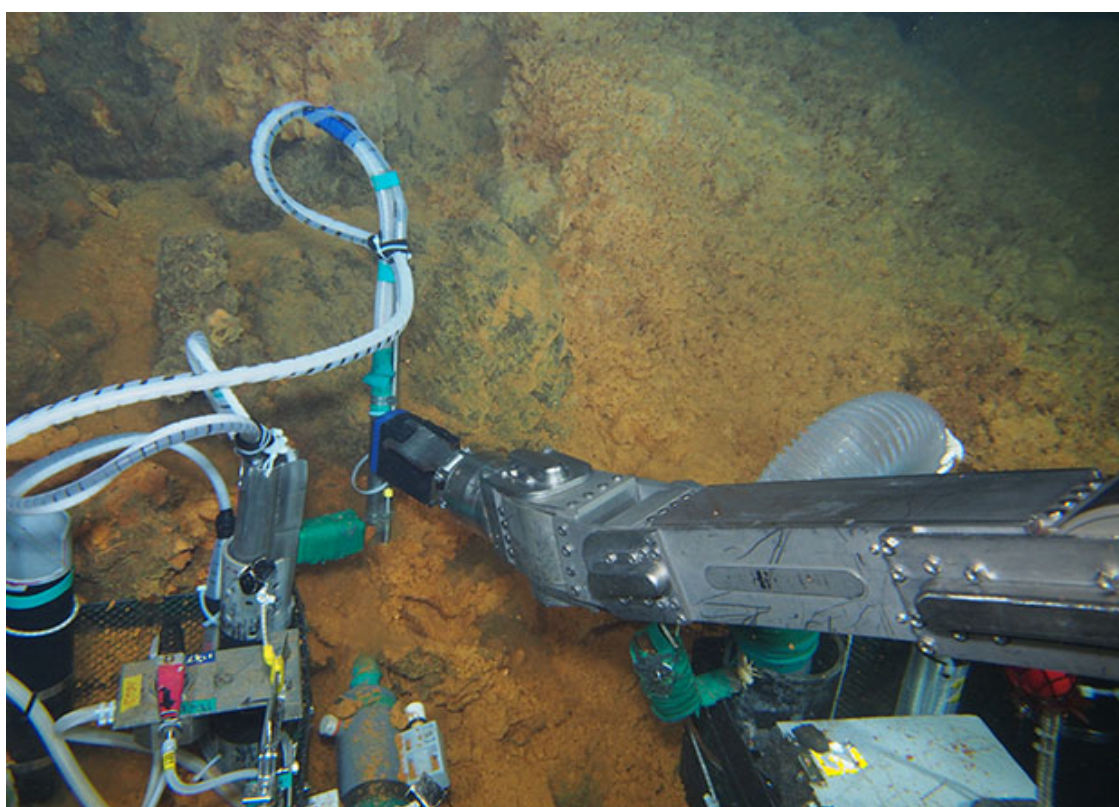


Fig. 2-3. Seafloor at Fox site near the summit of the Tarama Knoll.

Payloads:

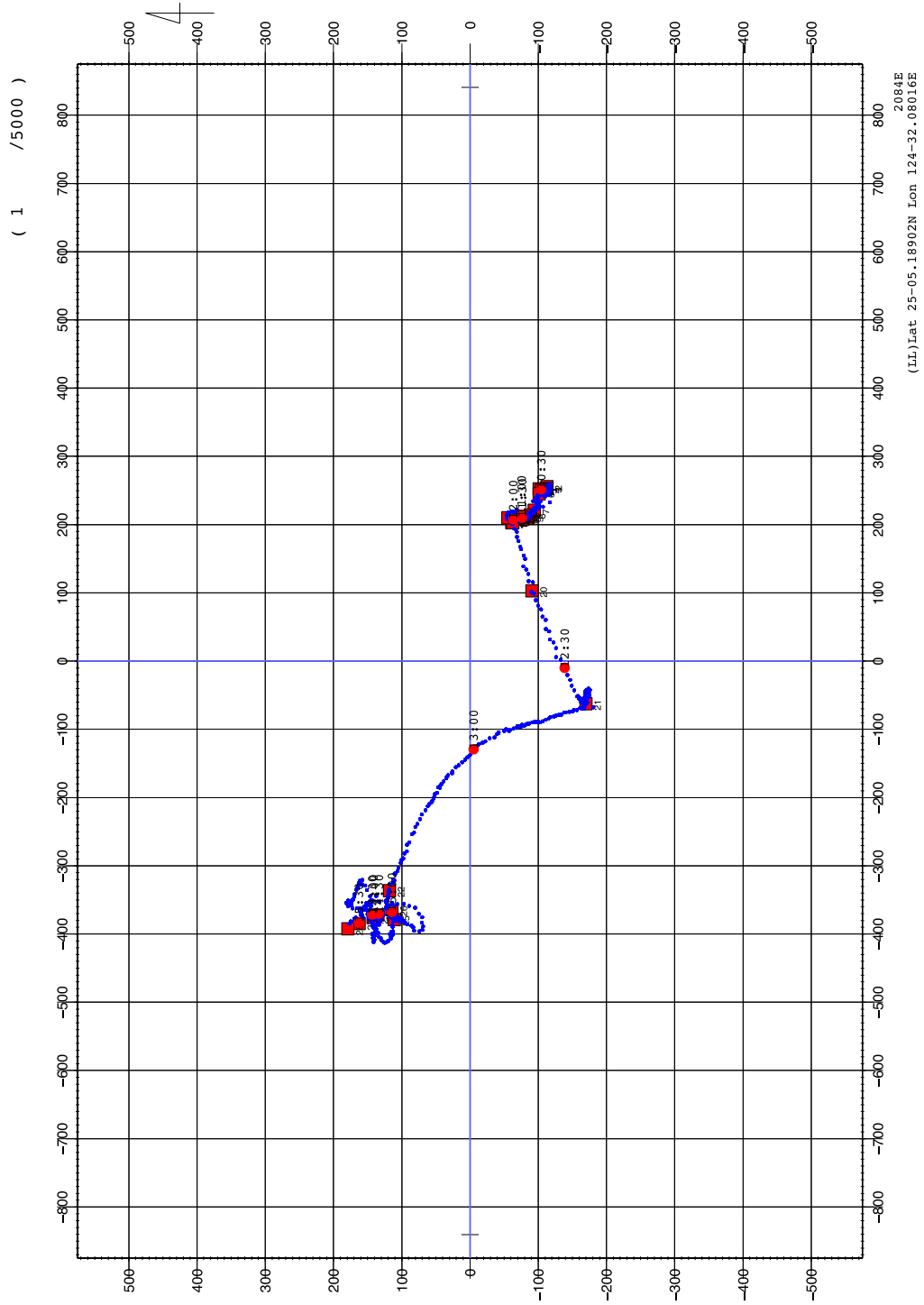
- 1) WHATS III with a temperature probe
- 2) M-type sediment sampler
- 3) Iron mat sediment sampler
- 2) Niskin water sampler
- 3) Bag sampler
- 4) Pile Bunker pore water sampler
- 5) D-Pote
- 6) METs Methane sensor

- 7) Multi sensor
- 8) DO meter
- 9) Turbidity meter
- 10) Electric Potentiometer
- 11) Suction sampler with single canister
- 12) Sample box
- 13) Shinkai6500 marker

Location of Events of the Shinkai6500#1508:

Time (Local)	Dep. (m)	Pos. Lat.			Pos. Lon.			Event
10:12:14	1730.8	25	5.4386	N	124	32.7526	E	Sea water sampling, Niskin (Red)
10:16:12	1728.2	25	5.4397	N	124	32.749	E	Landing Target (Temp.: 3.83°C)
10:27:30	1724.1	25	5.4418	N	124	32.7484	E	Sediment sampling, M-type sediment sampler (Black)
10:35:05	1708.7	25	5.4425	N	124	32.7346	E	Find active chimneys and massive animal colony
10:55:48	1686.6	25	5.468	N	124	32.7249	E	Sea water sampling, Niskin (Green)
11:08:50	1688.8	25	5.461	N	124	32.725	E	Sampling of Komainu Chimney's fluid, WHATS-III (1,2), Max Temp.: 281°C
11:30:51	1689.7	25	5.4585	N	124	32.7249	E	DO measurement on the colony of animals
11:33:35	1689.4	25	5.4594	N	124	32.7262	E	Multi-sensor measurement on the colony of animals
11:59:11	1687.2	25	5.4664	N	124	32.7205	E	Fluid sampling, WHATS-III (4), Max Temp.: 310°C
12:03:42	1686.6	25	5.467	N	124	32.7197	E	Setting Marker #224
12:05:50	1686.8	25	5.4667	N	124	32.7198	E	Chimney sampling, Box2
12:09:21	1687.9	25	5.4695	N	124	32.7241	E	Animal sampling, single canister
12:10:21	1689.1	25	5.4699	N	124	32.723	E	DO measurement on the colony of animals
12:15:16	1688.6	25	5.4706	N	124	32.7255	E	Multi-sensor measurement on the colony of animals
12:19:25	1688.7	25	5.4712	N	124	32.7238	E	Animal sampling, single canister
12:38:07	1678.5	25	5.41	N	124	32.5594	E	Recovery of NT15-22 experiment set
13:54:18	1532.4	25	5.5766	N	124	32.3774	E	Fluid sampling, Bag sampler
13:58:11	1531.9	25	5.5765	N	124	32.3785	E	D-pote measurement for iron mat
14:00:37	1532.1	25	5.5772	N	124	32.3785	E	Multi-sensor measurement on surface of the iron mat
14:01:52	1532.2	25	5.5777	N	124	32.3763	E	Sediment sampling, Iron mat sediment sampler
14:09:17	1533.1	25	5.5766	N	124	32.3781	E	Sediment sampling, M-type sediment sampler (Green)
14:13:17	1532.4	25	5.5759	N	124	32.3775	E	DO measurement on surface of the iron mat
14:27:41	1533.7	25	5.5733	N	124	32.3782	E	Recovery of HPD maker
15:01:15	1535.2	25	5.5626	N	124	32.3831	E	Find HPD#1107-1 maker
15:28:08	1523	25	5.5891	N	124	32.3718	E	Recovery of GALI-2
15:31:56	1523.7	25	5.5877	N	124	32.3719	E	Sediment sampling, M-type sediment sampler (Red)
15:39:17	1522.4	25	5.5881	N	124	32.3711	E	DO measurement on surface of the iron mat
15:40:33	1512.5	25	5.5954	N	124	32.3709	E	Left the bottom

Dive track of the Shinkai6500#1508



Dive Report: Shinkai6500 Dive #1509

Date: August 4, 2017

Site: Tarama knoll hydrothermal field

Landing: 10:11:39; 25° 5.4397'N, 124°32.749'E; 1,728 m

Leaving: 15:41:13; 25°5.5975 'N, 124°32.3666 'E; 1,507 m

Observer: Toshiro Yamanaka (TUMSAT)

Pilot: Keigo Suzuki, **Co-Pilot:** Ryo Saigo

Objectives:

Objectives of this dive were;

- 1) Collect hydrothermal/biological samples at the iron mat site (Fox site) and the new hydrothermal site (YZ site) in Tarama Knoll.
- 2) Deploying the new *in-situ* colonization system 'GALI-21' in Fox site.
- 3) Search for a new hydrothermal vent site at northeastn part of Tarama Hill, as suggested by the multi-beam survey by R/V Yokosuka in the YK14-16.

Dive Summary:

We landed near YZ site at a depth of 1,842 m, and proceeded to find the Komainu Chimney and other active chimneys in YZ site. We found Komainu Chimney quickly. After that, we carried out sampling of chimney, animals, as well as fluid. Then, we headed for Fox site located at the summit of Tarama Knoll. Upon reaching Fox site, we successfully installed the new *in-situ* colonization system 'GALI-21' in Fox site. Then, we set #225 marker near the 'GALI-21' system. We carried out sediment sampling and several sensor measurements at this site. After that, we began cruising to Tarama hill, where we tried to find a new hydrothermal site. After about two hours of cruising, we landed at the target site. A lot of white discoloration was seen on the seafloor. We confirmed a small number of animals characteristic of hydrothermally influenced sites (e.g., tubeworms) in this place. However, we could not find a chimney emitting high temperature fluid flow. After taking sediment and animal samples *Shinkai6500* left the bottom at 16:15 pm at a water depth of 1,948 m.

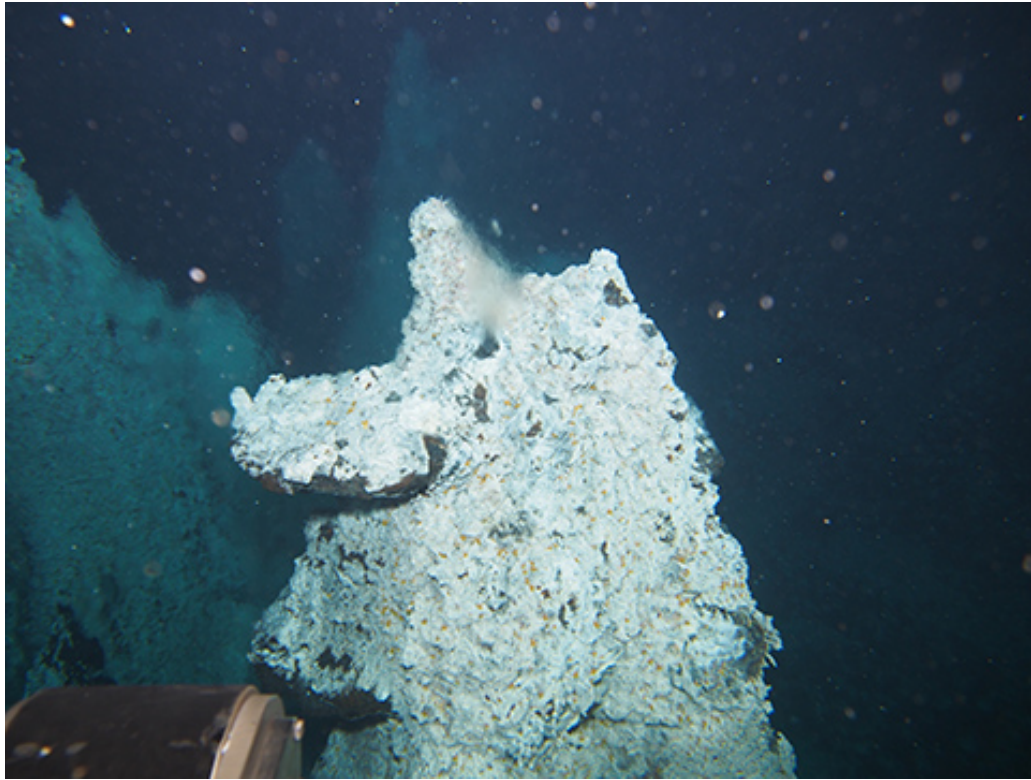


Fig. 2-4. Komainu Chimney at YZ site in Tarama Knoll.



Fig. 2-5. Seafloor of Tarama Hill.

Payloads:

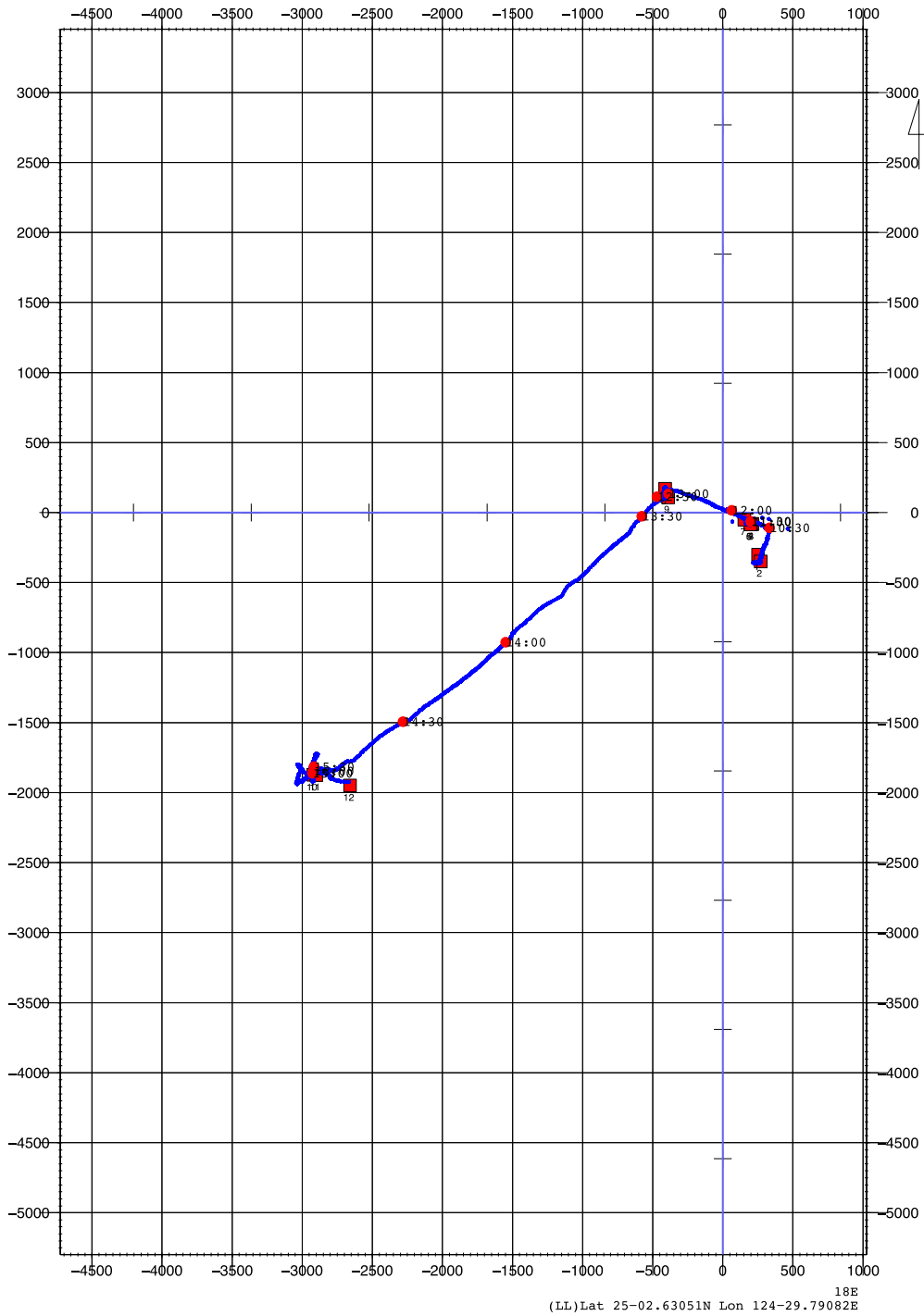
- 1) WHATS III with a temperature probe
- 2) M-type sediment sampler
- 3) Iron mat sediment sampler
- 2) Niskin water sampler
- 3) DO meter
- 4) Turbidity meter
- 5) Electric Potentiometer
- 6) Suction sampler with single canister
- 7) Sample box
- 8) Shinkai6500 marker

Location of Events of the Shinkai6500#1509:

Time (Local)	Dep. (m)	Pos. Lat.			Pos. Lon.			Event
10:11:39	1842.2	25	5.3077	N	124	32.7582	E	Landing Target
10:40:17	1702.1	25	5.4628	N	124	32.7348	E	Find animals
10:47:42	1687.2	25	5.459	N	124	32.7266	E	Find many active chimneys
10:53:14	1687.8	25	5.4588	N	124	32.7257	E	Approaching to 'Komainu Chimney'
10:57:53	1688.5	25	5.4586	N	124	32.7226	E	Temp. measurement (Max.: 270°C)
11:05:40	1690.4	25	5.455	N	124	32.7168	E	Closing to another chimney and measuring Temp. (Max.: 307°C)
11:20:16	1690	25	5.4541	N	124	32.7186	E	Fluid sampling, WHATS-III (1)
11:28:11	1691.4	25	5.4515	N	124	32.7167	E	Chimney sampling (Box 2)
11:30:42	1690.2	25	5.4522	N	124	32.7177	E	Closing to another chimney and measuring Temp. (Max.: 150°C)
11:48:31	1674.2	25	5.4667	N	124	32.6984	E	Animal sampling, single canister
12:24:51	1520.3	25	5.5928	N	124	32.3562	E	Find 6k#1508 ballast
12:47:17	1542.5	25	5.5579	N	124	32.3699	E	DO measurement on surface of the iron mat
13:00:21	1542.5	25	5.5597	N	124	32.3688	E	Sediment sampling, M-type sediment sampler (Black)
13:11:53	1544.1	25	5.558	N	124	32.3703	E	Sediment sampling, M-type sediment sampler (Green)
13:19:33	1543.9	25	5.5595	N	124	32.3686	E	Deployed GALI-21 and 6K#225 Marker
15:51:09	1963.3	25	4.4846	N	124	30.8629	E	Sediment sampling, M-type sediment sampler (Red)
15:57:47	1962.1	25	4.4857	N	124	30.8627	E	Animal sampling, single canister
16:01:31	1964.9	25	4.4844	N	124	30.8751	E	Sea water sampling, Niskin (Red)
16:15:33	1948.2	25	4.4447	N	124	31.0187	E	Left the bottom

Dive track of the Shinkai6500#1509

(1 / 25000)



3. Scientific Reports

3.1. Elucidation of the microbial ecosystems utilizing iron and other environmental factors to support their activity in “Iron-dominated Flocculent Mats” of Tarama Knoll

The purpose of this cruise is to obtain sediment, iron mat and fluid samples to examine the associations between endolithic microorganisms, especially iron-utilizing microorganisms and environmental factor at a deep-sea hydrothermal field in Tarama Knoll.

Objectives of our microbiological studies include, (1) the evaluation of microbial diversity and distribution by using DNA and RNA approaches (e.g. amplicon-sequencing), Fluorescence in situ hybridization (FISH), quantitative polymerase chain reaction (Q-PCR), (2) the measurement of microbial activity by using cultivation-, enzymatic-, DNA and RNA approaches, and metabolic product analysis.

We collected some iron mat, sediment and fluid samples during YK17-17. Samples were prepared on-board for future studies. A part of iron rich mat samples was slurred with filter-sterilized seawater and then stored in glass bottle at 4°C for cultivation. The remaining pieces were stored in plastic tubes at -80 °C for DNA/RNA analysis. Samples of hydrothermal sediments were also stored as described above. The slurry of some samples with filter-sterilized seawater was inoculated into culture media for cultivation of iron-, nitrogen- or methane- utilizing bacteria. These culture media were incubated at several temperatures in the dark.

Microbial cells attached to the samples were fixed in plastic tubes filled with filter-sterilized seawater containing formalin (final 3~4 % v/v) at 4°C for microscopic observation.

Water samples were collected using Bag and Niskin samplers. For cell counts, the water samples were fixed by adding formaldehyde (final 3~4 % v/v) at 4°C. For DNA extraction, the prokaryotic cells in the water samples were collected on 0.22- μ m-pore-size cellulose nitrate filters and then stored at -80°C.

3.2. Geochemical studies of hydrothermal activity, mineralization and vent-associated animals obtained from Tarama Knoll and Hill in South Okinawa Trough, Japan.

To reveal geochemical characteristics of venting fluids and associated hydrothermal minerals at the hydrothermal system of Tarama Knoll, major chemical and isotopic compositions and mineral assemblages of the collected fluid and chimney samples will be analyzed.

To reveal ecology and nutrient sources of animals inhabiting the hydrothermal venting area of Tarama Knoll and the weakly bubbling area of Tarama Hill, isotopic compositions (C, N, S) of the collected animal samples will be analyzed.

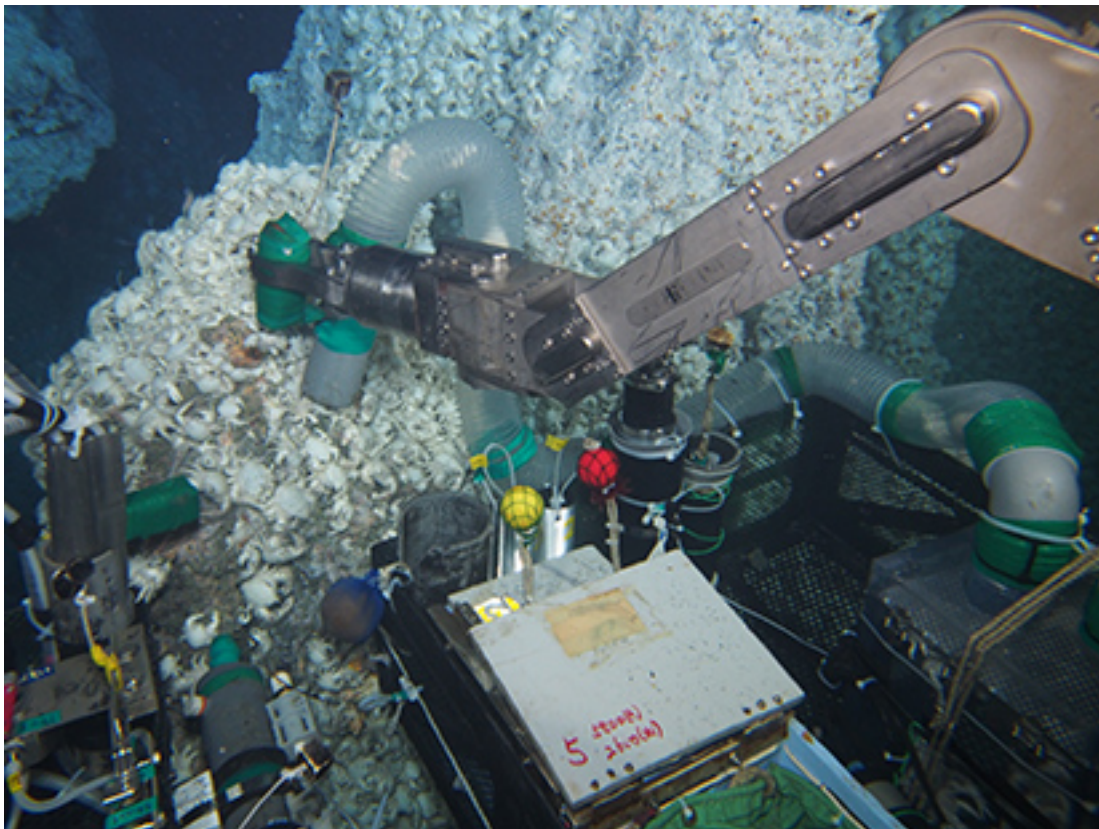


Fig. 3-1. Animal sampling at Tarama Knoll.

3.3. Preliminary investigation to elucidate the deformation behavior of submarine mineral resources.

In order to reveal and control the deformation behavior of sea-bottom mineral resources potentially available for seabed mining, sediment samples were collected at Tarama Knoll during YK17-17. We will measure particle size, density, consistency and other physical properties to evaluate the applicability of suction mining and its environmental impact.

3.4. Durability evaluation of sealants for mining deep-seafloor ore deposits.

For durability evaluation of sealants planned for use for deep-seafloor mining, four types of existing sealants were deployed on the seafloor of Tarama knoll on December 2015 (water depth *c.* 1680 m). During this cruise, we successfully recovered the sealants and we will measure the changes in size, strength, and other physical properties of them for developing a suitable sealant for deep-sea use.

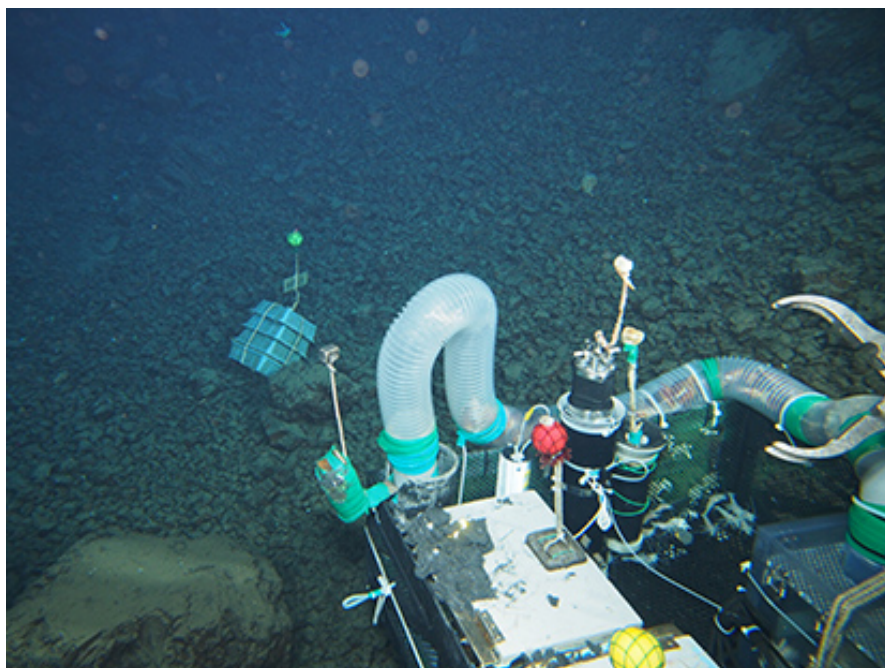


Fig. 3-2. Experimental set deployed at Tarama Knoll.

3.5. Vent megafaunal biodiversity and biogeography analyses

During the YK17-17 research cruise, two geological features near Tarama Island were explored: Tarama Knoll and Tarama Hill. During DSV *Shinkai6500* dive #1508, a hydrothermal vent site with numerous active high-fluid venting chimneys (mostly clear fluid venting but also a couple orifices venting greyish fluids) was discovered on the southeastern part of Tarama Knoll. Dive #1509 continued to sample from this site but also explored Tarama Hill for hydrothermal activity. Although no high-temperature venting was found in Tarama Hill, a small apparently hydrothermally-influenced patch was found.

Specimens for biological investigations were taken from these two hydrothermal-influenced sites. The main method of collection was using a suction sampler (slurp gun with single canister), although some specimens were also recovered from surface of chimney fragments, GARI-2, or from the sample basket. Upon recovery, specimens from each sampling location were sorted by eye, with all animals above 0.5mm in body size retained. Specimens were identified to the lowest taxonomic level possible post-sorting and counted (if possible). Most specimens were immediately fixed for taxonomy (10% seawater buffered formalin or 4% paraformaldehyde), genetic analyses (99.5% ethanol or -20/-80°C frozen), and RNA extraction (RNA later solution).

For groups which are difficult to identify to species level using facilities available on-board the ship (e.g., small bivalves, polychaetes), further taxonomic work will take place in JAMSTEC to better understand the biodiversity of these new sites, and Okinawa Trough in general. These will likely reveal species previously unknown to science, and genetic barcoding and molecular taxonomy using genes such as ITS-2, H3, and COI will aid such traditional morphology work. Specimens collected on-board the present cruise will contribute to the larger-scale population genetics study of the Okinawa Trough and north-western Pacific in general, to shed light on how the biogeography and life-history of various vent-reliant animal species differ. Investigation of the stable isotopic ratio of various vent animals is also planned (using specimens frozen in -80°C) for comparison between species and with other sites. The results from these planned analyses will no doubt help further to better our understanding of deep-sea biodiversity in the Okinawa Trough as a whole.

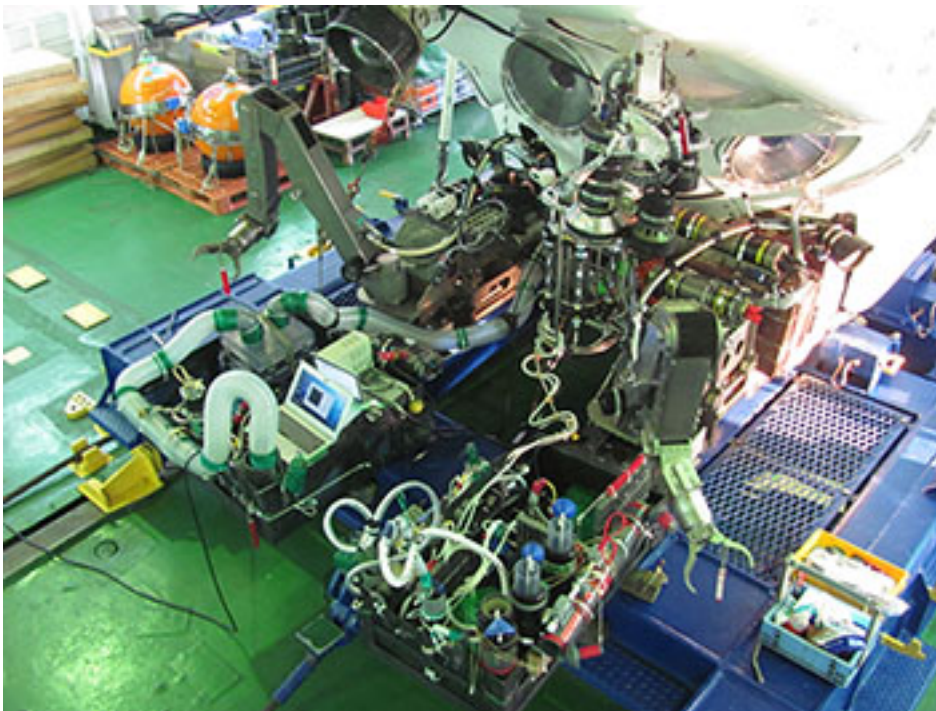
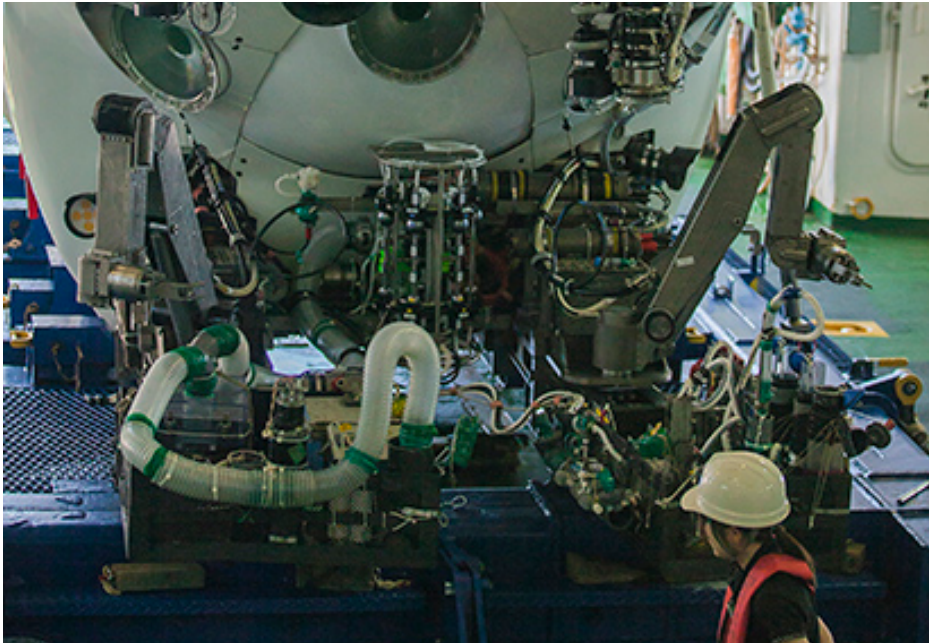
4. Notice on Using

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.

5. Appendix

5.1. Payload picture

6K#1508 (20170803), Tarama Knoll



6K#1509 (20170804), Tarama Knoll & Tarama Hill

