



KAIREI Cruise Report
KR18-15

Universal adaptive traits for extreme environment;
Isolation of host nonspecific viruses targeting harmful
bacteria toward environmental and medical technology

Iheya North hydrothermal field;
Area off southeastern coast of Miyako Island

Nov.15,2018-Nov.20,2018

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

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Cruise Abstract

The research cruise KR18-15 was carried out for two research projects “Universal adaptive traits for extreme environment” proposed by Keiichi Kakui (Hokkaido University; Chief Scientist) and “Isolation of host nonspecific viruses targeting harmful bacteria toward environmental and medical technology” by Akihiro Okamoto (National Institute for Materials Science (NIMS); Deputy Chief Scientist). Two dives for the former and one dive for the latter had been originally planned, but all three dives were conducted as collaborative dives for two projects.

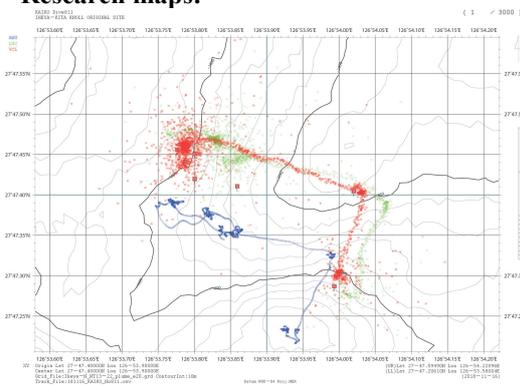
The objective of the former project is to reveal universal adaptive morphological traits for deep-sea environments in marine animals. To achieve this goal, we planned to collect many deep-sea animals in various taxa as possible. Since whale-bones and wood logs on deep-sea bottom are good habitat and shelter for deep-sea animals, we planned to collect on/in/around these substrates which were deployed during 2008, 2009 and 2012 cruises.

The objective of the latter project aims to exploit deep-sea-specific phage resources which can suppress iron corrosion and pathogenic bacteria by microorganisms. For this purpose, we planned to collect (1) large invertebrates in chemosynthetic communities such as bivalves in genera *Bathymodiolus* and *Calyptogena*, the galatheid decapod *Shinkaia crosnieri*, and tube worms (siboglinid annelids), (2) chimneys, (3) environmental water in large biological colonies, hot water - seawater mixed zone (mixing zone), and plume, and (4) sediments.

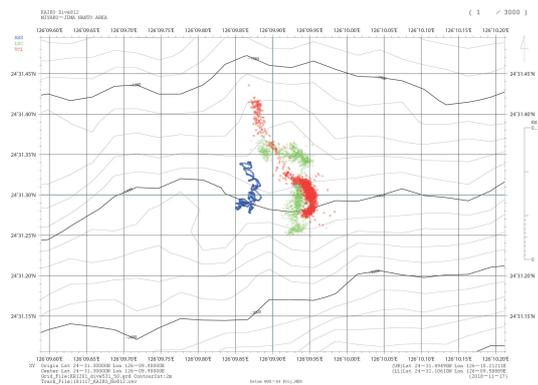
Three KAIKO dives carried out during this cruise were Dive #811 in the Iheya North hydrothermal field (ca. 1000 m deep), Dive #812 in the area off southeastern coast of Miyako Island (ca. 2000 m deep), and Dive #813 in the area off SE coast of Miyako Island (ca. 5000 m deep). In the Dives #811 and 812, almost all planned samplings were successfully carried out. In Dive #813, we failed to find whale-bone and woods; only mud sampling by rake was done.

1. Cruise Information

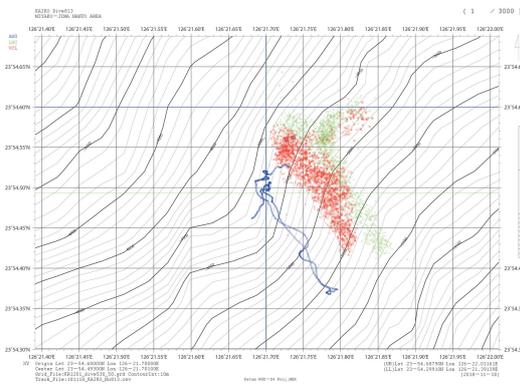
- **Cruise ID:** KR18-15
- **Name of vessel:** RV KAIREI with ROV KAIKO Mk IV
- **Title of cruise:** Universal adaptive traits for extreme environment; Isolation of host nonspecific viruses targeting harmful bacteria toward environmental and medical technology
- **Chief Scientist:** Keiichi Kakui [Hokkaido University]
- **Captain of KAIREI:** Yoshiyuki Nakamura
- **Submersible operation manager in KAIKO team:** Tomoe Kondo
- **Marine technician:** Mitsuteru Kuno [Nippon Marine Enterprises]
- **Cruise period:** from Nov. 15, 2018 to Nov. 20, 2018
- **Ports of departure / arrival:** Naha-shinkou / Naha-shinkou
- **Research areas:** Iheya North hydrothermal field; Area off southeastern coast of Miyako Island
- **Research maps:**



Dive #811 (Iheya North hydrothermal field)



Dive #812 (Area off SE coast of Miyako Island)



Dive #813 (Area off SE coast of Miyako Island)

2. Research Proposal and Science Party

- Title of proposals:

“Universal adaptive traits for extreme environment” by Keiichi Kakui [Hokkaido University]
“Isolation of host nonspecific viruses targeting harmful bacteria toward environmental and medical technology” by Akihiro Okamoto [National Institute for Materials Science (NIMS)]

- Representatives of Science Party:

Keiichi Kakui [Hokkaido University]
Akihiro Okamoto [NIMS]

- Science Party:

Chief Scientist	Keiichi Kakui [Hokkaido University]*
Deputy Chief Scientist	Akihiro Okamoto [NIMS]†
Onboard Researchers	Akinori Yabuki [JAMSTEC]*
	Yoshihiro Fujiwara [JAMSTEC]*
	Michitaka Shimomura [Kyoto University]*
	Kensuke Yanagi [Natural History Museum and Institute, Chiba]*
	Daisuke Uyeno [Kagoshima University]*
	Masato Hirose [Kitasato University]*
	Hayato Tanaka [Tokyo Sea Life Park]*
	Shinta Fujimoto [Tohoku University]*
	Naoto Jimi [Hokkaido University]*
	Akito Ogawa [The University of Tokyo]*
	Yuki Oya [Hokkaido University]*
	Satoshi Nakagawa [Kyoto University]†
	Masaaki Kitajima [Hokkaido University]†
	Junichi Miyazaki [JAMSTEC]†
	Jumpei Uchiyama [Azabu University]†
	Tomohiro Mochizuki [Tokyo Institute of Technology]†
	Xiao Deng [NIMS]†
	Pauliina Rajala [Nanyang Technological University]†
	Makoto Sugimura [Enoshima Aquarium]†
Marine technician	Mitsuteru Kuno [Nippon Marine Enterprises]

*, †: Members for Kakui’s and Okamoto’s projects, respectively.

3. Research Activities

3-1. Overview of Projects

A. Universal adaptive traits for extreme environment

Responsible personnel: Keiichi Kakui [Hokkaido University]

Objective

Deep sea is under the dark, low-temperature, high-pressure, and poor nutritional conditions, and one of the most extreme environments for animal life; however, very diverse taxa successfully colonized and adapted to this environment. Currently, our knowledge about their general adaptive traits for the deep-sea environment is fragmentary. One of reasons for this situation must be that, although the investigation of general traits needs samples from various animal groups, preparing such samples for detailed morphological observations is difficult; many animal groups require special processing techniques which is difficult for non-experts. This project is proposed by a team of experts (taxonomists) of various animal groups, and is to reveal universal adaptive morphological traits for the deep-sea environment in marine animals.

Proposal

Although animal densities on the plain deep-sea floor are generally low, those around substrates are relatively high. Thus we plan animal samplings on/in/around whale bones and sunken wood logs in this project. We will dive at ca 2000 m and 5000 m sites in the area off southeastern coast of Miyako Island, Ryukyu Islands, where several sets of whale bones and wood logs were deployed at different depths during past cruises.

During the dives, we will collect (1) animals on/under whale bones and wood logs by slurp gun, (2) one whale bone and wood log by a manipulator, and (3) sediments under and/or around whale bones and wood logs by a rake and MBARI corer. Collected sediments will be filtered with sieves (1 mm, 0.45 mm, and 0.063 mm) to retrieve small organisms. Obtained animals will be fixed in fixatives suitable for DNA extraction (e.g., absolute ethanol, RNAlater) or detailed morphological observations (e.g., paraformaldehyde, glutaraldehyde, etc.).

Brief Results

During Dive #811 (Iheya North), invertebrates such as crustaceans, nematodes, and polychaetes were collected around hydrothermal vents (numerous *Shinkaia crosnieri* individuals were found there) and tubeworm colony with a slurp gun and manipulator. During Dive #812 (area off SE coast of Miyako Island, ca 2000 m deep), we successfully found deployed whale bones/wood logs, thus retrieved one whale bone and one Keyaki (*Zelkova* sp.) log, and collected sediments and invertebrates such as crustaceans, nematodes, polychaetes, and molluscs by using a slurp gun, rake, and MBARI corers. In Dive #813 (area off SE coast of Miyako Island, ca 5000 m deep), we failed to find whale bones and wood logs; thus we collected sediments by rake. The detail morphological observation will be carried out after this cruise.

B. Isolation of host nonspecific viruses targeting harmful bacteria toward environmental and medical technology

Responsible personnel: Akihiro Okamoto [NIMS]

Objective

Prokaryotes living in deep sea environments such as hydrothermal vents and seafloor sediments are attracting great interest from the viewpoint of drug discovery and the like. Meanwhile, there was no application-oriented research or technical development proposal focusing on use of “deep sea lytic bacteriophage” as a tool for controlling harmful bacteria, while phage exists several times more number than prokaryotes. In this research, we aim to exploit deep-sea-specific phage resources which can suppress iron corrosion and pathogenic bacteria by microorganisms. Specifically, we sampled from the deep seabed hydrothermal activity area and marine sediments that contain our target host bacteria that have been already isolated. Furthermore, the effect of such a new phage on microbial ecosystem is evaluated by combining microflora analysis and metagenome analysis of bacteriophage.

Proposals

Analogue of *H. pylori*, ϵ proteobacteria, lives in the vicinity of hydrothermal vent in the Iheya Northern deep sea bottom hydrothermal activity area, but it is 10 m away from the jet hole. Since ϵ proteobacteria is a chemoautotroph that uses hydrogen or a sulfur compound as an energy source, it grows actively in the vicinity of the hydrothermal vent (source of energy) and decreases as it moves away from the ejection hole. However, although the energy source is diluted about 10 m away from the hydrothermal vent hole, there is a degree to which colonies can be made by large organisms such as tube worms. In such an environment, the type of hydrogen / sulfur oxidizing bacteria is replaced by ϵ proteobacteria from γ proteobacteria. Although the transition of such biota is generally explained by the characteristics of the microorganism (difference in affinity to the substrate etc.), there is almost no basis for the explanation, and we suspect that one cause may be in the phage is considered extremely high.

In this application task, we will investigate the environments where the number of these bacteria varies in order to acquire the desired phage. We conduct a dive survey at Iheya North hot water activity area located in the central Okinawa trough. Major work items during the dive are: (1) bivalves (members in *Bathymodiolus* and *Calypptogena*), tubeworms, and chimneys (to sample box) using a manipulator, (2) collection of large organisms such as *Shinkaia crosnieri* using a slurp gun, (3) environmental water in large biological colonies, hot water - seawater mixed zone (mixing zone) and plume using Niskin water sampler.

Brief Results

In addition to work items in the proposal, we added sediment samplings by MBARI corers. In Dive #811, all planned sampling except living bivalves sampling was successfully carried out. In Dive #812, water samplings in water column and near whale bones by Niskin water sampler and sediment samplings under whale bones and wood logs by MBARI corer were done. In Dive #813, almost all dive time was used to search whale bones and wood logs (but failed), so no sample for this project was obtained. Culturing of bacteria and viruses will be started after this cruise.

3-2. Dive Logs

A. Dive #811

Date: Nov. 16, 2018

Site: Iheya North Original Site

Landing: 10:00; 27°47.4357'N, 126°53.8147'E, D=989 m

Leaving: 14:32; 27°47.3103'N, 126°54.0001'E, D=989 m

Observer: Satoshi Nakagawa [Kyoto University]

Objectives:

To take a variety of hydrothermal samples, including seawater, chimneys, sediments, and animals.

Dive summary:

Slightly turbid seawater (potentially hydrothermal plume) was collected at the altitude of 172 m (launcher depth 856.4m) by Niskin bottle #1. The vehicle landed on the SBC, and then headed to NBC. At the top of NBC, we tried to collect a chimney structure but failed (too fragile). We successfully collected a blackish chimney structure near a *Shinkaia* colony (several meters below the NBC top). In front of the *Shinkaia* colony, we collected seawater by using a Niskin sampler (#5). Then, we collected about 200 individuals of *Shinkaia*. We left the NBC and headed to C0016B. At C0016B, we successfully collected pieces of chimney structure. Near event mark #1, we took seawater using a Niskin bottle (#2). At event mark #5, we found dead *Calyptogena* shells. Near shells, we collected a MBARI core (red) and Niskin bottle (#3). We headed event #6 (tubeworm colony). At event #6, we collected water sample using Niskin bottle #4. A sediment core sample was also collected by using a MBARI (yellow). Additionally, we collected tubeworm individuals, and then, the vehicle left the bottom.

Payloads:

Niskin water sampler (5L) x 6

Canister (single)

Slurp gun pump

Multi sensor

Methane sensor

Turbidity sensor

MBARI corer 35 cm x 2

Sample box x 2

Event list:

Time (Local)	Launcher Dep. (m)	Vehicle Dep. (m)	Description
9:40:25	856.4	-	Niskin bottle #1 (slightly turbid, plume?)
10:00:18	892.1	988.6	Landed on SBC (event mark 2)
10:10:32	868.4	963.8	Found CBC (tubeworm)
10:21:49	861.5	958.1	Arrived at NBC
10:24:02	872	966.7	Niskin bottle #6 (NBC top, mixing)
10:34:33	864.5	959.7	Failed chimney sampling
10:53:30	867.6	957.4	Collected a chimney structure (blackish, right box)
11:02:43	802.1	902.3	Niskin bottle #5 (in front of <i>Shinkaia</i> colony)
11:02:50	866.2	966.4	Collected <i>Shinkaia</i> (approx 200 individuals)
12:30:28	865.8	984.7	Collected chimney structures (C0016B)
12:51:12	868.8	1018.9	Niskin bottle #2 (Near No. 1 point)
13:21:55	913.8	1048.6	Found dead <i>Calyptogena</i>
13:24:39	922.7	1047.7	Niskin bottle #3 (White mat near dead <i>Calyptogena</i>)
13:35:05	922.8	1044.8	MBARI core (red)
13:56:35	857	989	Niskin bottle #4 (tubeworm site)
14:15:09	865.1	989.9	MBARI core (yellow)
14:25:51	863.9	989.3	Collected tubeworm

B. Dive #812

Date: Nov. 17, 2018

Site: Area off southeastern coast of Miyako Island

Landing: 10:36; 24°31.3270'N, 126°09.9164'E, D=1976 m

Leaving: 14:38; 24°31.3901'N, 126°09.8794'E, D=1975 m

Observer: Keiichi Kakui [Hokkaido University]

Objectives:

To collect animals on/under deployed whale bones and wood logs (“substrates”), substrates, and sediments under and/or around substrates.

Dive summary:

Water collection was carried out at the launcher depth 1547 m (water depth 1986 m) by Niskin bottle #1. The vehicle landed on the bottom, and then started to search substrates; soon after, substrates were found. After short observation of the overview of substrates, the vehicle moved in front of the whale bones, and collected water by Niskin bottle #2, animals on the bones by a slurp gun, a whale bone (B10) by a manipulator, two MBARI cores (#1, 4), and sediments around the bones by a rake. Then the vehicle moved in front of the Keyaki (*Zelkova* sp.) logs, and collected animals on the logs by a slurp gun, a Keyaki log (L07) by a manipulator, two MBARI cores (#3, 2), and sediments under/around the log by a rake. After sampling around substrates, we started animal survey in the surrounding area; we collected three *Eynpniastes* sea cucumbers by a slurp gun. After leaving the bottom, the hose pipe of left slurp gun was cut off by accident, and several samples including all sea cucumbers were lost. On board, one of the MBARI cores (#3) was found with a large crack and the surface water was missing (also the surface sediment seems to have been lost).

Payloads:

Niskin water sampler (5L) x 2

Canister (single) x 2

Slurp gun pump

Multi sensor

Methane sensor

Turbidity sensor

MBARI corer 35 cm x 4

Sample box x 4 (1 long, 2 short, 1 cylindrical)

Event list:

Time (Local)	Launcher Dep. (m)	Vehicle Dep. (m)	Description
10:02:10	1547.3	0	Niskin #1 in water column; 1986 m deep
10:36:07	1848.2	1973.5	Landed on bottom
10:45:47	1849.3	1982	Photographed whale bones and woods
10:58:34	1848.5	1978.4	Arrived in front of whale bones
10:59:34	1849	1979.1	Niskin #2 near whale bones
11:09:43	1848.7	1977.6	Collected animals on bones by slurp gun
11:14:49	1849.1	1979.8	Recovered deployed whale bone (B10)
11:31:15	1849.2	1982.6	MBARI 1: Just under the whale bone (B10)
11:34:44	1848.5	1979.8	MBARI 4: Just under the whale bone (B10)
11:38:48	1847.7	1977.9	Collected brown galatheid decapod by slurp gun
11:47:26	1848.6	1978.6	Collected surface of bottom sediment around B10 by rake
11:50:46	1849.5	1982.5	Collected an disk-shaped stuff (an echinoderm?) by rake.
12:20:21	1849	1980.7	Collected surface of bottom sediment around B10 by rake
12:51:55	1849.8	1976.3	Arrived in front of Keyaki logs
13:05:27	1847.7	1977.2	Collected animals on logs by slurp gun
13:17:56	1848.3	1978.9	Recovered deployed Keyaki log (L07)
13:31:27	1849.4	1978.5	MBARI 3: just under the wood (L07)
13:37:22	1849.5	1976.2	MBARI 2: just under the wood (L07)
13:55:33	1847.9	1975.8	Collected bottom sediment under/around L07 by rake.
14:12:23	1848.3	1974.8	Started animal survey
14:23:17	1850.1	1971.7	Collected <i>Eynpniastes</i> sea cucumber by slurp gun
14:26:32	1849.4	1970.1	Collected <i>Eynpniastes</i> sea cucumber by slurp gun
14:27:52	1848.4	1968.2	Collected <i>Eynpniastes</i> sea cucumber by slurp gun
14:29:45	1849.2	1967.5	Finished animal survey
14:38:37	1850.1	1967	Left the bottom

C. Dive #813

Date: Nov. 18, 2018

Site: Area off southeastern coast of Miyako Island

Landing: 11:29; 23°54.5893'N, 126°21.8280'E, D=4960 m

Leaving: 14:44; 23°54.5096'N, 126°21.7564'E, D=4967 m

Observer: Keiichi Kakui [Hokkaido University]

Objectives:

To collect animals on/under deployed whale-bones and wood logs (“substrates”), substrates, and sediments under and/or around substrates.

Dive summary:

Water collections were carried out at the launcher depth 3080 m (water depth 4919 m) by Niskin bottle #1 and at the altitude of 14 m (water depth 4970 m) by Niskin bottle #2. The launcher arrived at the planned depth at 10:03; after adjusting the position and direction of KAIKO, the vehicle was detached at 11:12. The vehicle landed on the slope bottom, and then started to search substrates. Although we took almost all dive time to search, we failed to find the substrates. Just before leaving the bottom, we collected sediments by a rake.

Payloads:

Niskin water sampler (5L) x 2

Canister (single) x 2

Slurp gun pump

Multi sensor

Methane sensor

Turbidity sensor

MBARI corer 35 cm x 4

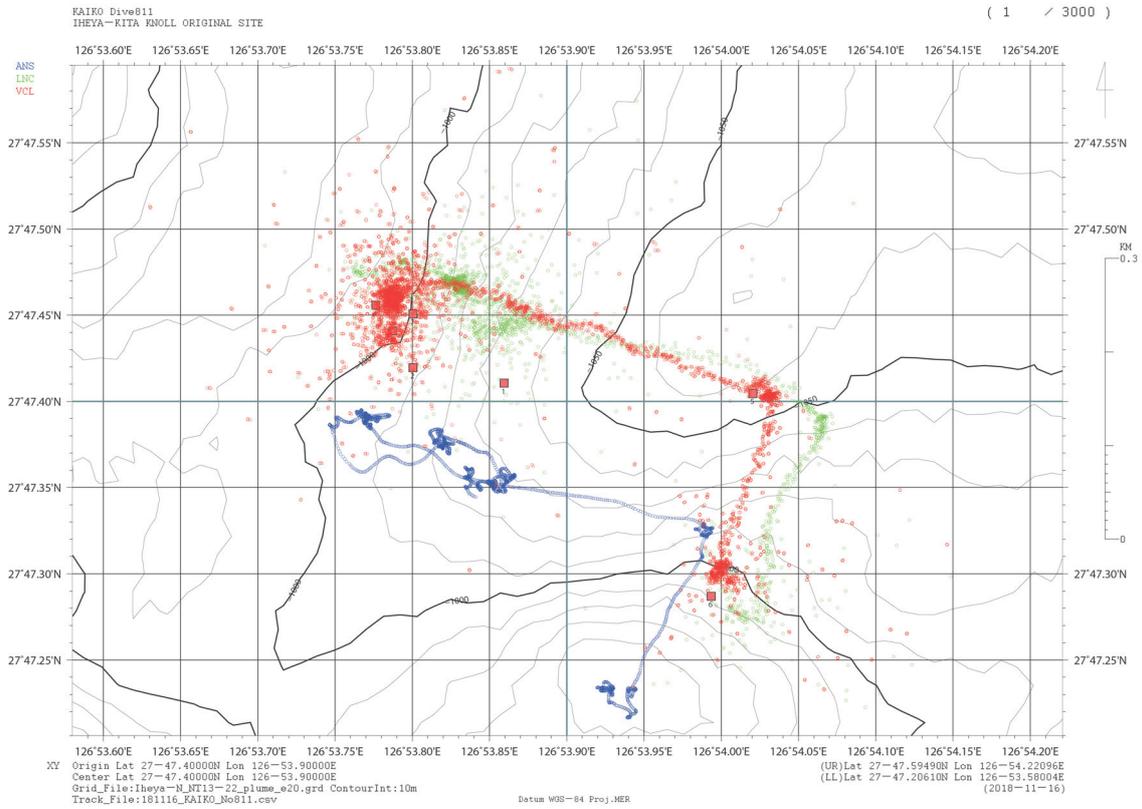
Sample box x 4 (1 long, 2 short, 1 cylindrical)

Event list:

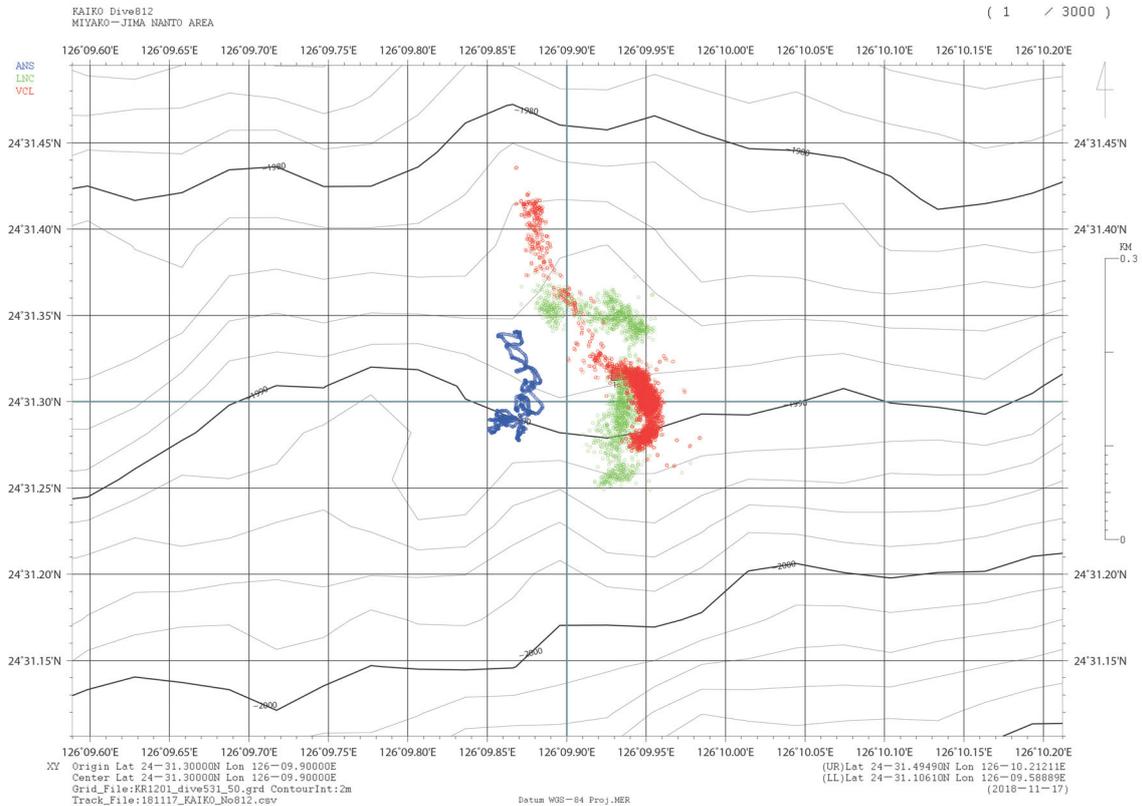
Time (Local)	Launcher Dep. (m)	Vehicle Dep. (m)	Description
9:31:00	3080		Niskin bottle #1, right side; 4914 m deep
11:11:52	4829.2	4831.1	Vehicle departure
11:25:50	4829.7	4948.8	Found bottom
11:27:10	4831.7	4953.5	Niskin bottle #2, left side; 4970 m deep
11:28:54	4829.3	4958.4	Landed on bottom
12:03:37	4807.8	4918.6	Trouble in bathometer and chart
12:16:13	4792.2	4896.6	Landed on bottom
12:21:40	4791.6	4898.2	Restarted survey
12:26:28	4791.2	4902.4	Landed on bottom; checked past records
12:31:34	4791.3	4904.4	Amended the target site; restarted survey
13:19:17	4868.8	4991.4	Arrived the amended target site
13:20:12	4870.9	4997	Restarted survey
14:26:14	4851.5	4962.3	Gave up searching of deployed substrates
14:28:37	4851.3	4967.4	Landed on bottom
14:36:36	4851.9	4963.7	Collected sediments by rake
14:44:27	4850.5	4961.8	Leaving the bottom

3-3. Dive Tracks

A. Dive #811



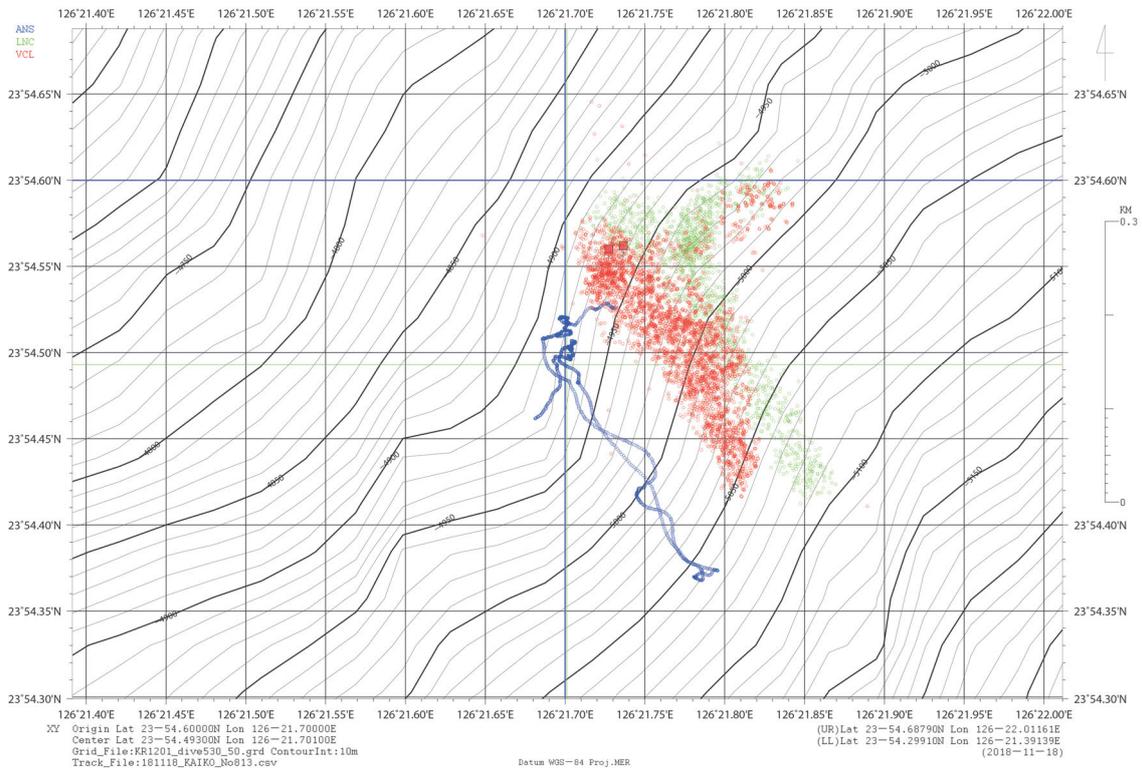
B. Dive #812



C. Dive #813

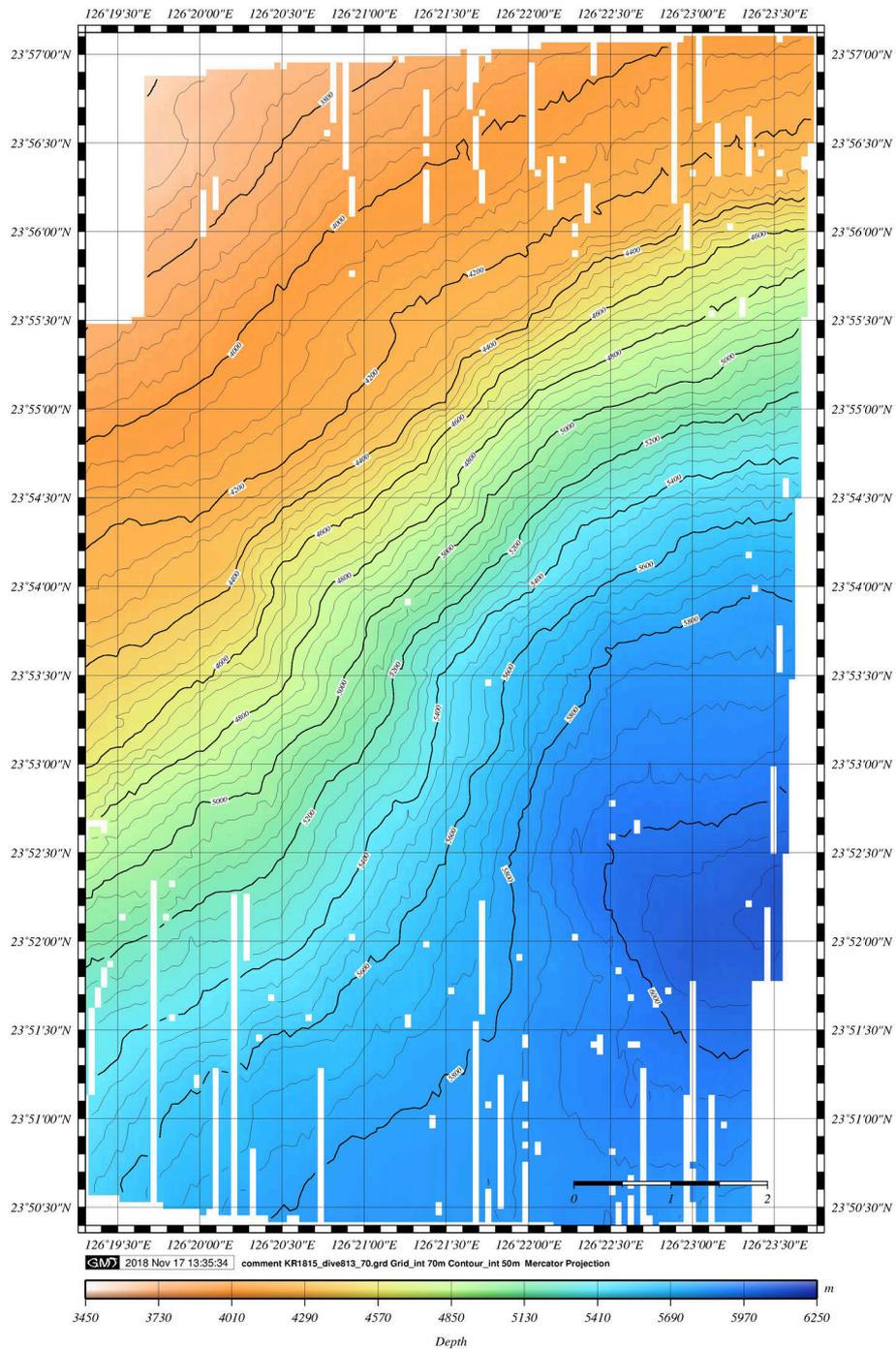
RAIKO Dive#13
MIVARO-JIMA NANTO AREA

(1 / 3000)



3-4. Geophysical Survey Result (Dive #813)

KR1815_dive813_70_cl50A3.ps



4. Cruise Log

Nov. 15: 14:00 On boarding KAIREI
15:00 Leaving Naha-shinkou port
KAIKO briefing
15:30 KAIREI briefing
18:00 Scientists meeting

Nov. 16: 08:39 Dive #811 (Iheya North hydrothermal field) started
10:00 Vehicle on bottom (996 m deep)
14:28 Vehicle off bottom (987 m deep)
15:38 Dive #811 finished
18:00 Scientists meeting

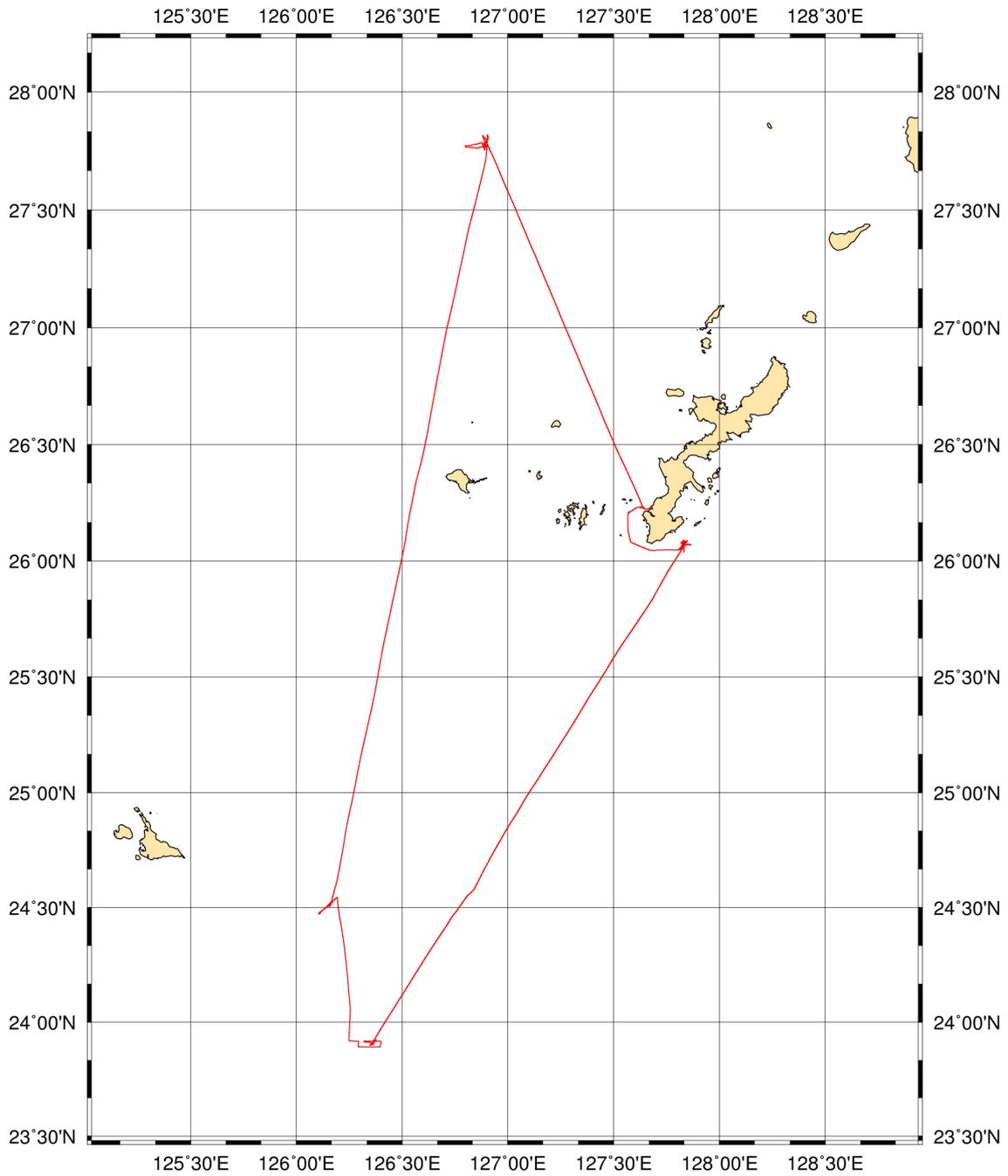
Nov. 17: 08:38 Dive #812 (Area off SE coast of Miyako Island) started
10:36 Vehicle on bottom (1976 m deep)
14:38 Vehicle off bottom (1975 m deep)
16:18 Dive #812 finished

Nov. 18: 07:38 Dive #813 (Area off SE coast of Miyako Island) started
11:29 Vehicle on bottom (4960 m deep)
14:44 Vehicle off bottom (4967 m deep)
17:26 Dive #813 finished

Nov. 19: Staying around southern coast of Okinawa Island
Nov. 20: 09:00 Arriving at Naha-shinkou port

Appendix
I. Ship Track

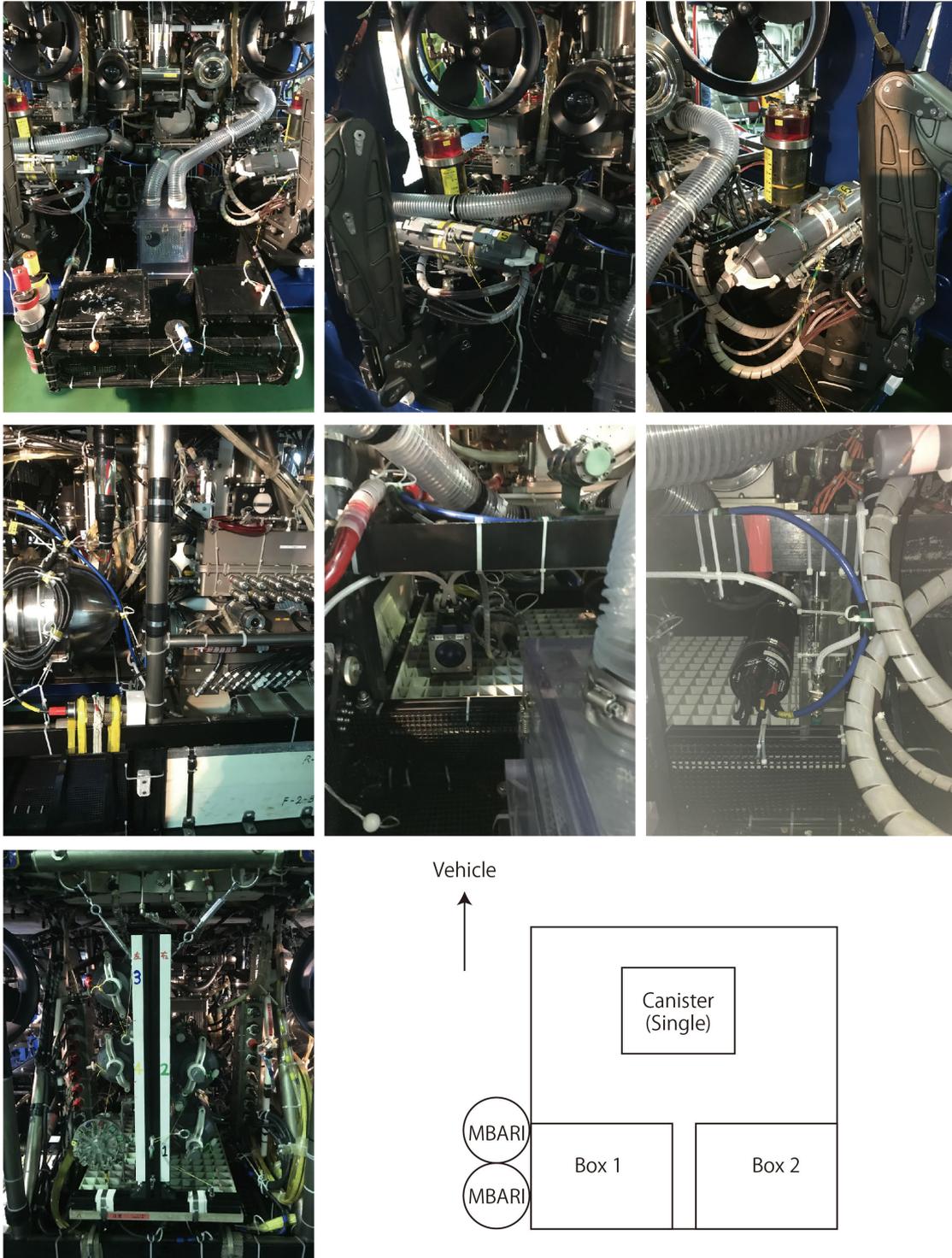
KR18-15 Nav Track



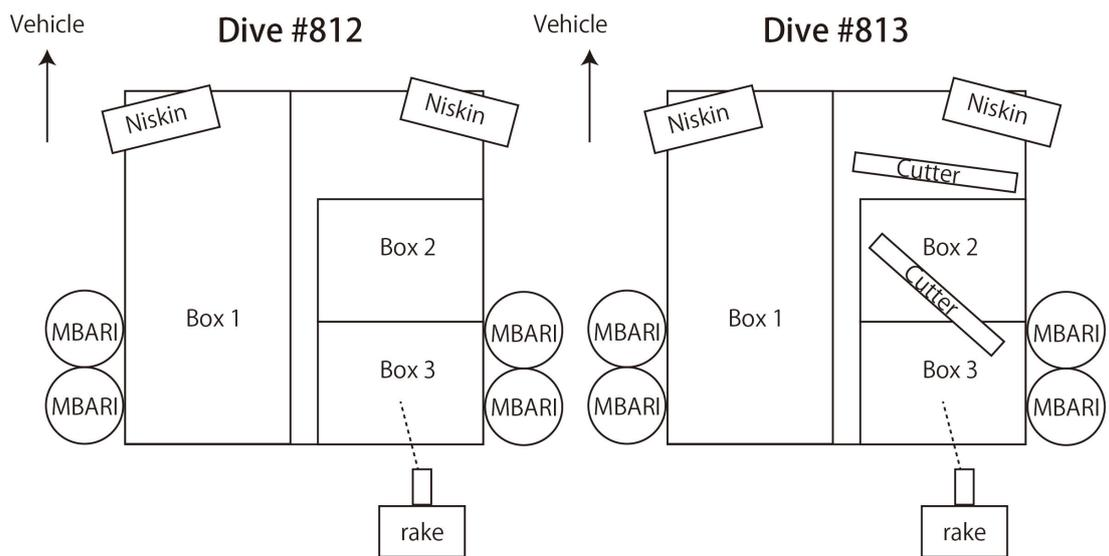
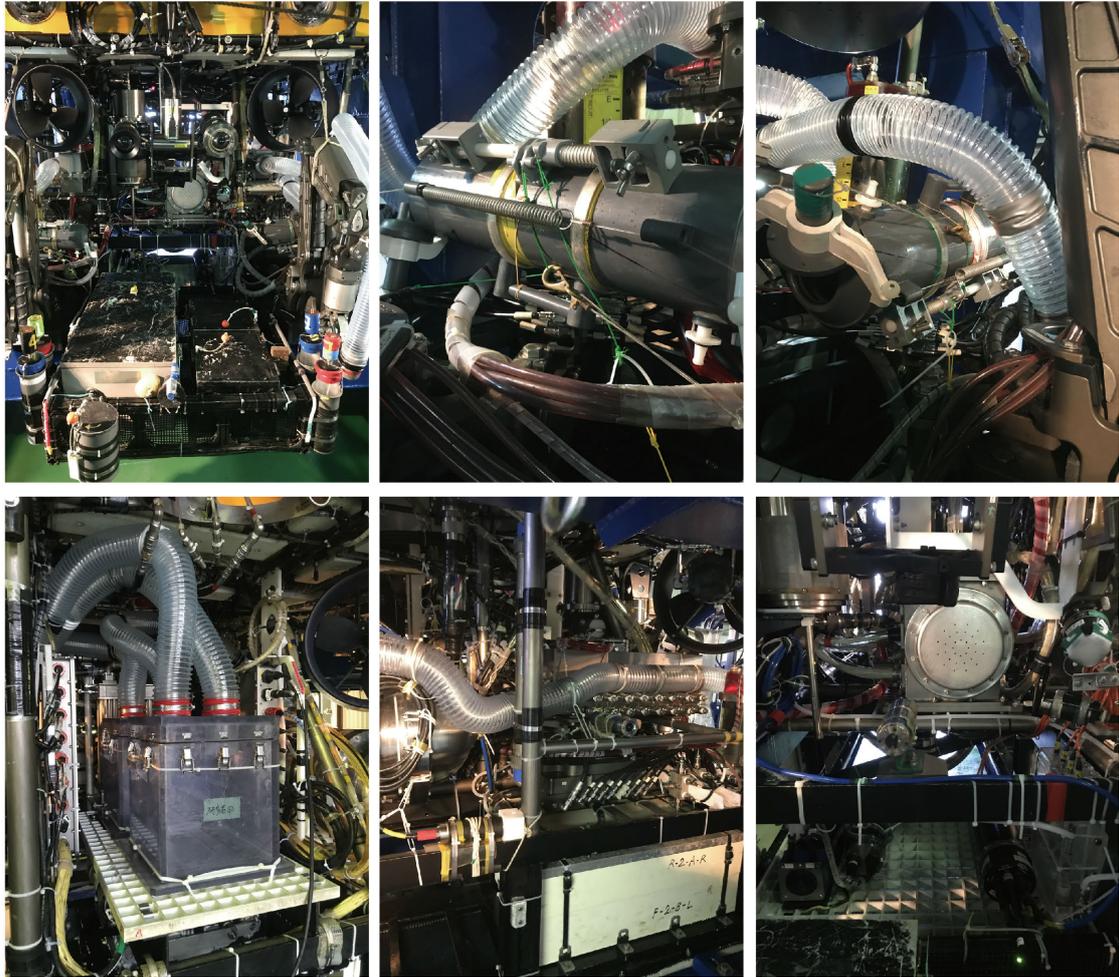
GM 2018 Nov 20 00:02:53 R/V KAIREI, Mercator Projection, Data_source=SOJ

II. Payload Settings

A. Dive #811

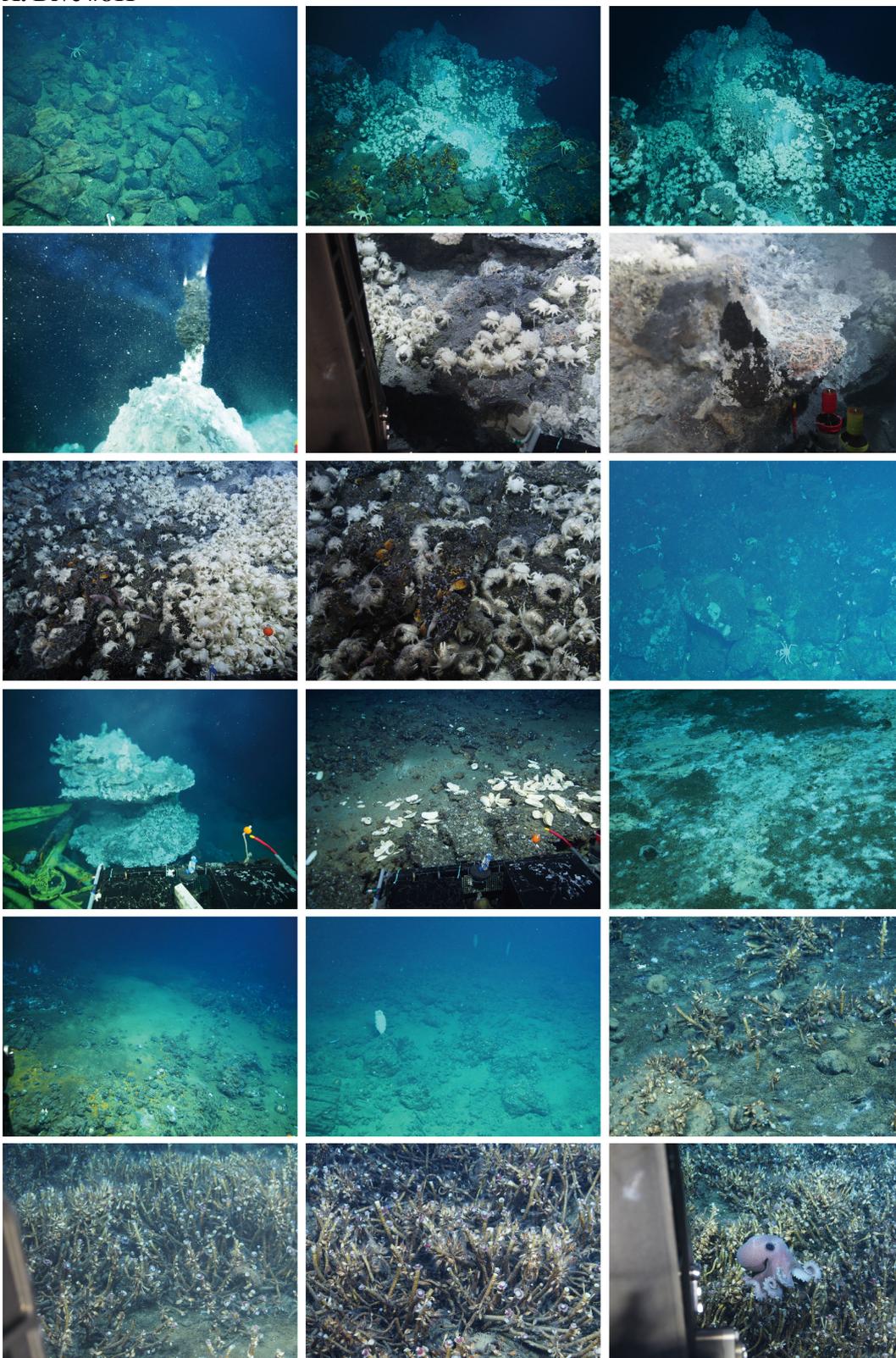


B. Dives #812, 813

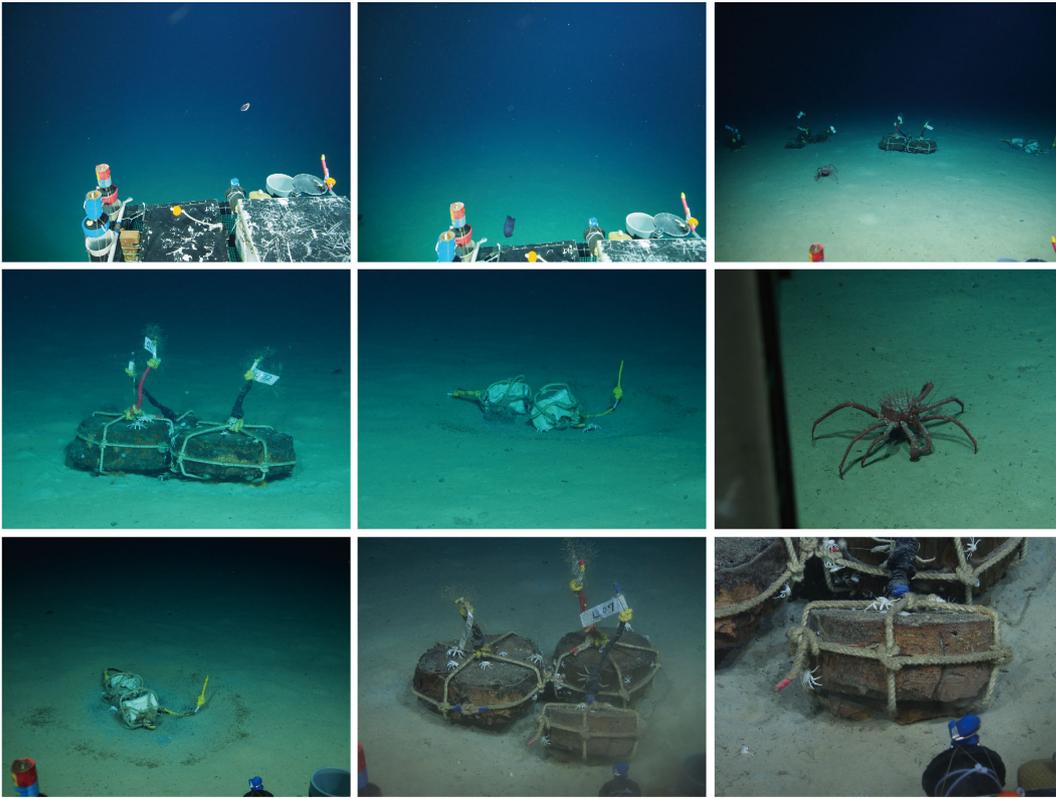


III. Still Images from Each Dive

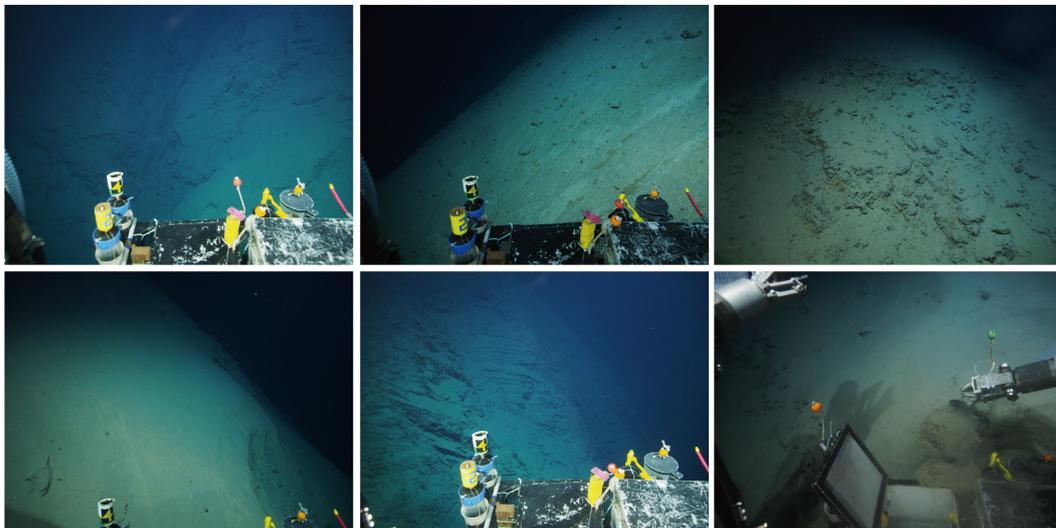
A. Dive #811



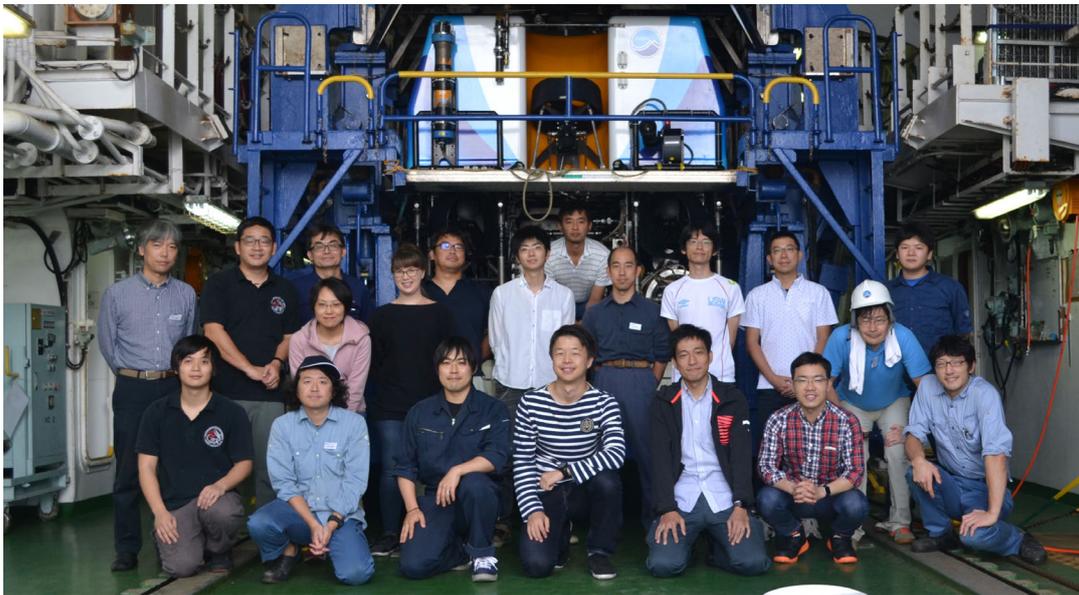
B. Dive #812



C. Dive #813



IV. Group Portrait



Notice on Using

This cruise report is a preliminary documentation as of the end of cruise.
This report is not necessarily corrected even if there is any inaccurate description (i.e. taxonomic classifications). This report is subject to be revised without notice. Some data on this report may be raw or unprocessed. If you are going to use or refer the data on this report, it is recommended to ask the Chief Scientist for latest status.
Users of information on this report are requested to submit Publication Report to JAMSTEC.

<http://www.godac.jamstec.go.jp/darwin/explain/1/e#report>

E-mail: submit-rv-cruise@jamstec.go.jp