doi : 10.17596/0002491

R/V *Kaimei* /ROV *KM-ROV* Cruise Report

KM21-E04C Leg1

Nishi-Shichito Ridge

Oct. 12, 2021–Oct. 17, 2021

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

Contents

Abstract

A deep-sea research cruise was conducted at the Nishi-Shichito Ridge to develop novel monitoring methods for offshore seabed nature conservation areas and to acquire baseline data on the faunal diversity. Four ROV dives, one CTD water sampling cast, three baited camera casts, and one lander cast were conducted south of Genroku Seamount and on Hoei Seamount. A newly developed deep-sea lander equipped with three water sampling bottles, a mass filtration system for water, one sediment corer, several physico-chemical sensors, and a video camera was deployed at a depth of 2065 m south of Genroku Seamount and acquired 36-litter of seawater, four filters that filtered ca. 100 litters of seawater in total, a 23-min video footage, and environmental information such as current profiles, water temperature, salinity, pressure, turbidity, and dissolved oxygen concentration for 24 hours. About 150 biological samples, sediments, water sample, and hundreds of photographs were collected during ROV dives to obtain baseline information.

1. Participants aboard

1-1. Research group

FUJIWARA, Yoshihiro	(RIGC, JAMSTEC)
TSUCHIDA, Shinji	(RIGC, JAMSTEC)
FURUSHIMA, Yasuo	(RIGC, JAMSTEC)
YOSHIDA, Takao	(RIGC, JAMSTEC)
YAMAKITA, Takehisa	(RIGC, JAMSTEC)
KITAHASHI, Tomo	(RIGC, JAMSTEC)
KAWATO, Masaru	(RIGC, JAMSTEC)
OTA, Shuhei	(National Institute for Environmental Studies)
KISE, Hiroki	(Advanced Industrial Science and Technology)
HAMASAKI, Koji	(Atmosphere and Ocean Research Institute, The University of Tokyo)
SUNAMURA, Michinari	(Department of Earth and Planetary Science, Graduate school of Science, The University of Tokyo)
KOEDA, Keita	(The University Museum, The University of Tokyo)
HUANG, Can	(Graduate School of Frontier Sciences, The University of Tokyo)
HOOKABE, Natsumi	(Graduate School of Science, The University of Tokyo)
JIMI, Naoto	(Sugashima Marine Biological Laboratory, Graduate School of Science, Nagoya University)
WU Qianqian	(Graduate School of Human Development and Environment, Kobe University)
KOMAI, Tomoyuki	(Natural History Museum and Institute, Chiba)
YOKOOKA, Hiroyuki	(IDEA Consultants, Inc.)
TAKASHIMA, Soutarou	(IDEA Consultants, Inc.)
TAKATSUKI, Naoki	(IDEA Consultants, Inc.)
NISHIBAYASHI, Kenichiro	(IDEA Consultants, Inc.)

CTD Water Sampling Operator (MWJ)

Chief Marine Technician	TUN, Htet Aung
2nd technician	ENOKI, Masanori
3rd technician	NAKANO, Yukihiko

Multiple Core Sampling Operator (MWJ)

Chief Marine Technician	SHINOMIYA, Yuta

Observation Engineer (NME)

Chief Marine Technician	SERIZAWA, Kimiko
2nd technician	KODERA, Tohru

1-2. Operation team of *KM-ROV*

Operation Manager	MIURA, Atsumori
2nd ROV Operator	CHIDA, Yosuke
2nd ROV Operator	GOTO, Takuma
2nd ROV Operator	KUMAGAI, Shinnosuke
2rd ROV Operator	SUGIURA, Shuya
3rd ROV Operator	OKUHIRA, Yuto

1-3. Captain and crew of the R/V *Kaimei*

Captain	KIMURA, Naoto
Chief Officer	MURAMATSU, Takeshi
1nd Officer	SHISHIKURA, Takaaki
2nd Officer	YAMAGUCHI, Ryo
3rd Officer	ITO, Shun
Chief Engineer	KANEDA, Kazuhiko
1st Engineer	YAMAGUCHI, Katsuto
2nd Engineer	MIKAMI, Ryuzo
3rd Engineer	HAMAKAWA, Naoyuki
Chief Electronic Operator	ISHIWATA, Hiroki
2nd Electronic Operator	SUGIMOTO, Yohei
3rd Electronic Operator	IKEDA, Kohei
Boat Swain	OHATA, Masanori
Able Seaman	HIROSAKI, Kaname
Able Seaman	SHIMPO, Satoshi
Able Seaman	OHJIRI, Yuta
Able Seaman	KOJIMA, Shinya
Sailor	SUMOMOZAWA, Kazuya
Sailor	INOUE, Shinnosuke
No.1 Oiler	UEDA, Masanori
Oiler	HIGASHIGAWA, Yuji
Oiler	SUZUKI, Ryota
Oiler	HIDAKA, Toru
Chief Steward	MURAKAMI, Toru
Steward	WADA, Toru
Steward	KASHIWAGI, Koichiro
Steward	FUJIMOTO, Yuma

2. Purposes

On December 3, 2020, four areas were designated as the first offshore seabed nature conservation areas based on the Nature Conservation Act. Baseline data acquisition and continuous monitoring are required to understand the status of conservation areas, but due to their remote locations, such surveys are not easy to conduct. Human-occupied vehicles (HOVs) and remotely operated vehicles (ROVs) are commonly used for field observations of deep-sea ecosystems. Carousel-type water samplers and multiple corers are used to collect a large amount of seawater and sediments, respectively. Such equipment requires relatively large research vessels and well-trained operators, and the research expenses are quite high.

A research project “development of new biodiversity monitoring methods for offshore seabed nature conservation areas management”, funded by Environmental Restoration and Conservation Agency of Japan, was started in April, 2020, for sustainable monitoring of the conservation areas. The purposes of this cruise were as follows.

1. To test a newly developed deep-sea lander equipped with water sampling bottles, a mass filtration system for water, a core sampler, several physico-chemical sensors, and a video camera
2. To acquire the biodiversity baseline data for seamounts on the Nishi-Shichito Ridge
3. To compare the diversity acquired by two different methods: the traditional and newly developed methods

3. Results

3-1. ROV dives

3-1-1. Dive list

Date in 2021	Dive#	Site	Landing Leaving	Lat	Lon	Depth (m)	Comment
0ct. 13	KM-ROV#153	South of Genroku Seamount	13:07 15:32	30°39.5998'N 30°39.6010'N	139°02.6137'E 139°02.3352'E	2087 2083	Observation of benthic animals and sampling sediment, animals, deep-sea water
0ct. 15	KM-ROV#154	South of Genroku Seamount	13:44 14:12	30°39.5987'N 30°39.5935'N	139°02.4082'E 139°02.3568'E	2083 2084	Observation of benthic animals and baited trap recovering, and sampling, animals and mud
0ct. 16	KM-ROV#155	Houei Seamount	09:03 16:22	30°54.1541'N 30°54.4010'N	138°43.5368'E 138°43.3741'E	629 735	Observation of benthic animals and sampling sediment, animals, and deep-sea water
0ct. 17	KM-ROV#156	Anei Seamount	08:55 14:23	29°16.7312'N 29°17.0268'N	138°37.4482'E 138°37.8458'E	1149 771	Observation of benthic animals and sampling sediment, animals, and deep-sea water

3-1-2. Dive report

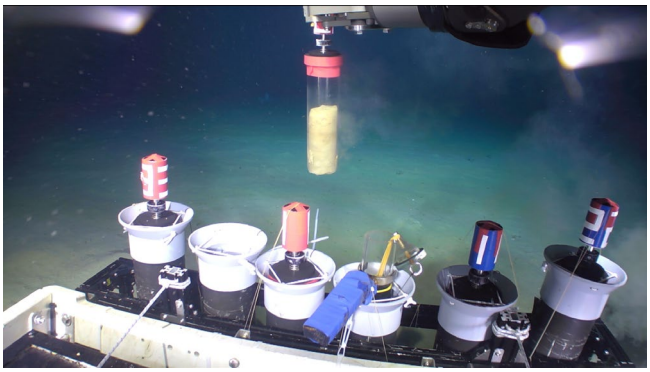
Dive Report KM-ROV#153

Date: October 13, 2021
Site: South of Genroku Seamount, Nishi-Shichito Ridge, ***Depth:*** 2087 m
Landing (Lat., Lon., Time, Depth): 30°39.600'N, 139°02.614'E, 13:07, 2087 m
Leaving (Lat., Lon., Time, Depth): 30°39.601'N, 139°02.335'E, 15:32, 2083 m
Observer: FUJIWARA, Yoshihiro

Theme: Development of deep-sea biodiversity monitoring technology for marine protected area management

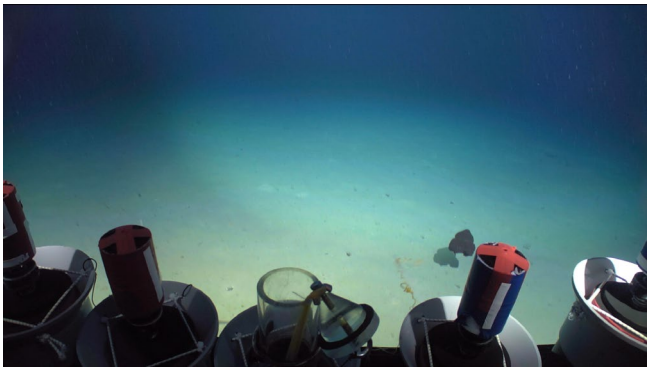
Purpose of dive:
Acquisition of biodiversity baseline data around deep-sea marine protected area, including observation of deep-sea fauna and topology and sampling of organisms, sediments, and water

Dive Summary
We landed at a depth of 2087 m south of Genroku Seamount and moved westward . We sampled bottom water by use of a Niskin bottle, collected sediments using five H-type push corers and a M-type sediment sampler, and collected benthic fauna with sediments using a suction sampler. Three times of "five-minute transect" were conducted for benthos mapping.

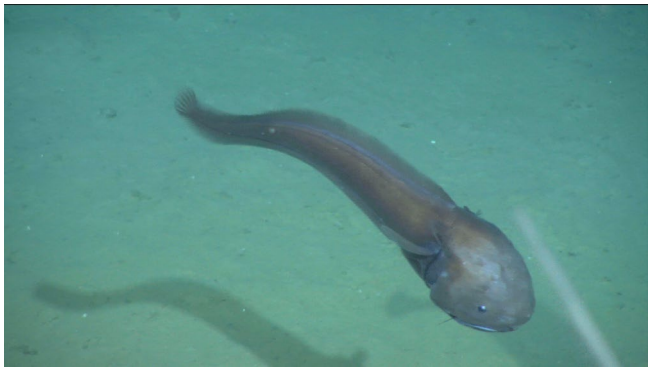


Sediment core sampling

Sunken debris

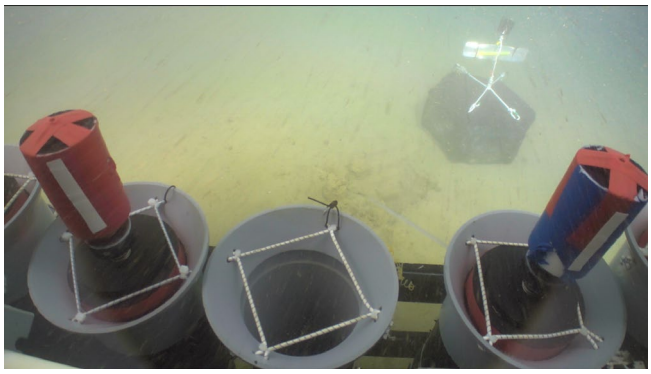


Jellyfish



Cusk-eel (*Acanthonus armatus*?)

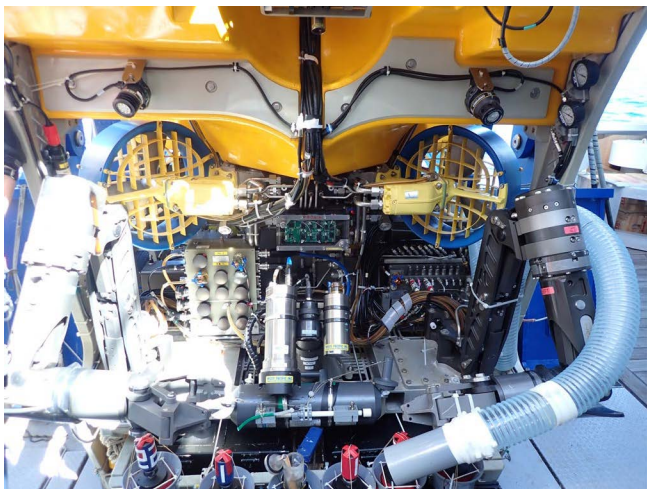
Sediment sampling using M-type corer



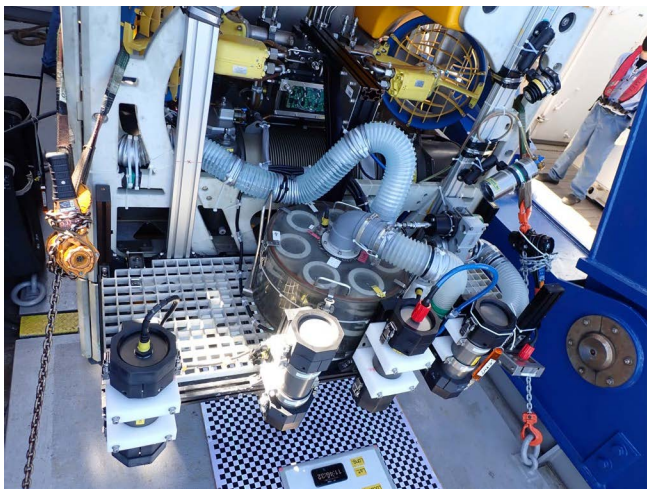
Deployment of fish trap

Payload Equipment:

H-type push corer x5 (front)
M-type sediment sampler x1 (front)
GoPro housing x1 (front)
KM-ROV box x2 (front)
Niskin bottle x1 (front)
Suction sampler and multi-bottled canister x1 (rear)
Bottom mapping camera x1 (rear)
Line laser x2 (rear)
24V LED light x1 and 100V LED light x 1 (both rear)



Front payload

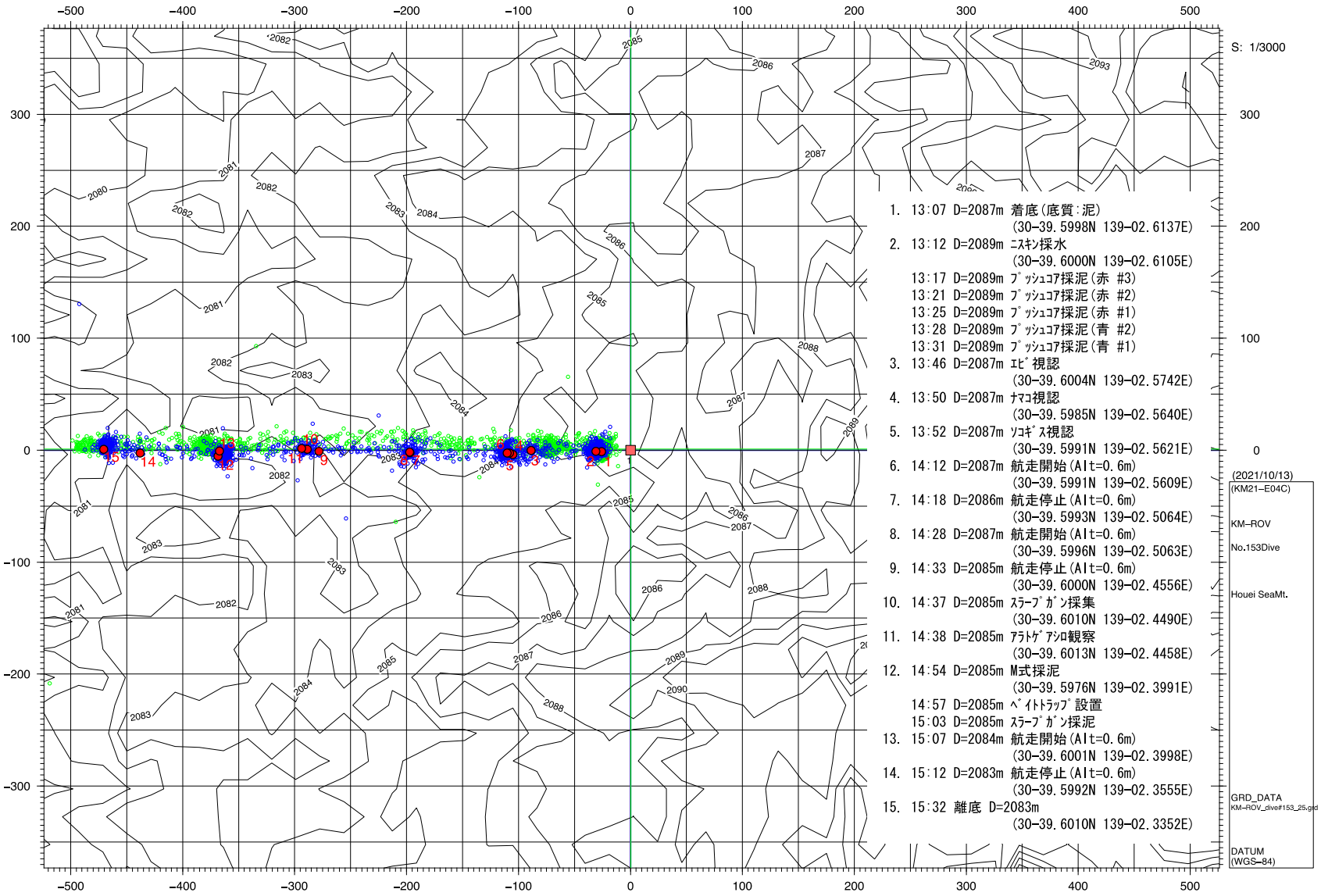


Rear Payload

Sampling Points and Markers:

Time	Position	Depth (m)	Events
13:26	30°39.600’N 139°02.611’E	2089	Water sampling using Niskin bottle
13:17	30°39.600’N 139°02.611’E	2089	Sediment core sampling
13:37	30°39.601’N, 139°02.449’E	2085	Biological sampling using suction sampler
14:54	30°39.598’N, 139°02.399’E	2085	Sediment sampling using M-type sediment sampler
14:57	30°39.598’N, 139°02.399’E	2085	Baited trap deployment
15:03	30°39.598’N, 139°02.399’E	2085	Biological sampling using suction sampler

Dive track KM-ROV#153



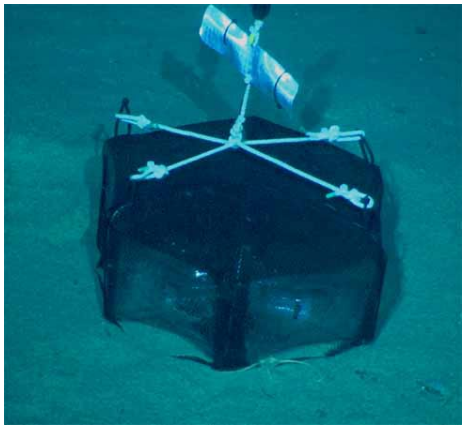
Dive Report KM-ROV#154

Date: October 15, 2021
Site: South of Genroku Seamount, Nishi-Shichito Ridge, ***Depth:*** 2087 m
Landing (Lat., Lon., Time, Depth): 30°39.599'N, 139°02.408'E, 13:44, 2083 m
Leaving (Lat., Lon., Time, Depth): 30°39.594'N, 139°02.357'E, 14:12, 2084 m
Observer: TSUCHIDA, Shinji

Theme: Development of deep-sea biodiversity monitoring technology for marine protected area management

Purpose of dive:
Acquisition of biodiversity baseline data around deep-sea marine protected area, including observation of deep-sea fauna and topology and sampling of organisms, sediments, and water

Dive Summary
We approached to the bottom at a depth of 2083 m south of Genroku Seamount near the point of baited trap deployed by the last dive. Before landing, we sampled bottom water by use of a Niskin bottle. Soon, the baited trap was found about 20m WSW from the landing site and successfully recovered in the sample box. Then we move to west and "five-minute transect" were conducted for benthos mapping. Around 80m west from the landing point, we sampled sediment by slurp gun and finished the half hour dive.



Baited trap

Sea urchin and moved trace

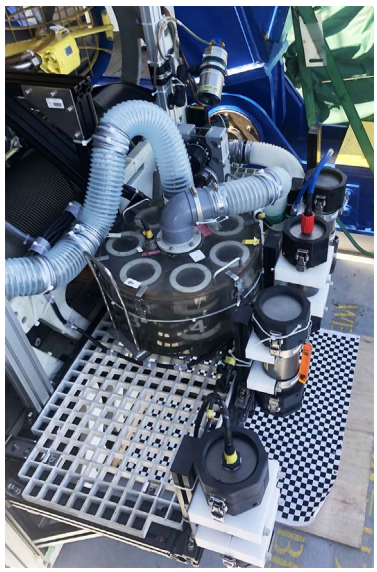
Plastic debris

Mud slurping

Payload Equipment:

H-type push corer x5 (front)
M-type sediment sampler x1 (front)
GoPro housing x1 (front)
KM-ROV box x2 (front)
Niskin bottle x1 (front)
Suction sampler and multi-bottled canister x1 (rear)
Bottom mapping camera x1 (rear)
Line laser x2 (rear)
24V LED light x1 and 100V LED light x 1 (both rear)

Front payload

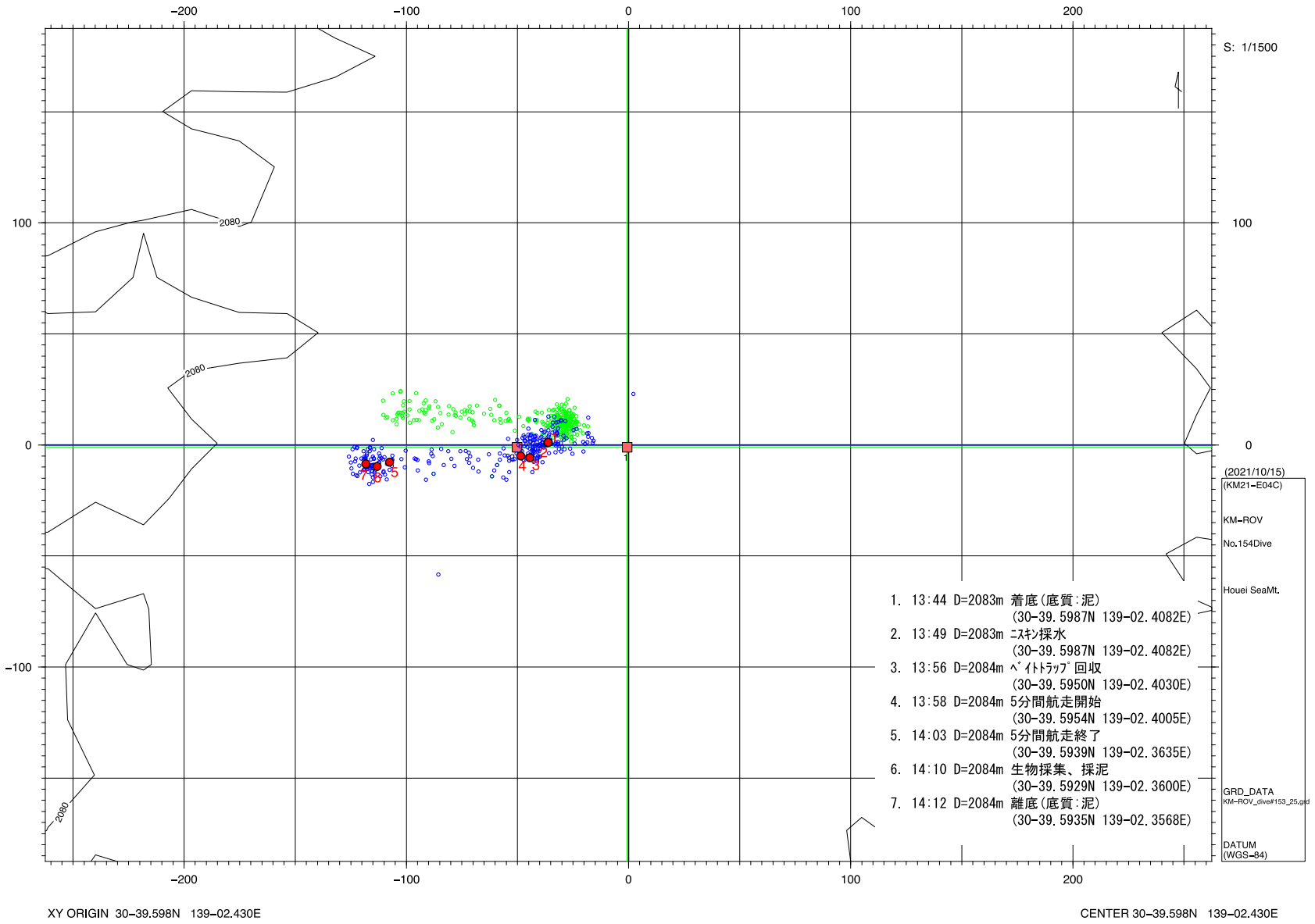


Rear Payload

Sampling Points and Markers:

Time	Position	Depth (m)	Events
13:44	30°39.5987’N, 139°02.4082’E	2083	Water sampling using Niskin bottle
13:56	30°39.5950’N, 139°02.4030’E	2084	Recovery of the baited trap
13:58	30°39.5954’N, 139°02.4005’E	2084	five-minute transect start
14:03	30°39.5939’N, 139°02.3635’E	2084	five-minute transect end
14:10	30°39.5929’N, 139°02.3600’E	504	Animal and seiment sampling using suction sampler
14:12	30°39.5935’N, 139°02.3568’E	2084	Left from the bottom

Dive track KM-ROV#154



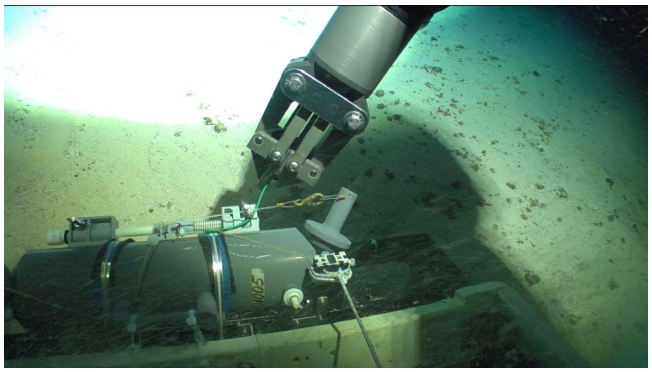
Dive Report KM-ROV#155

Date: October 16, 2021
Site: Houei Seamount, Nishi-Shichito Ridge, ***Depth:*** 735 m
Landing (Lat., Lon., Time, Depth): 30°54.1541'N, 138°43.5368'E, 9:03, 629 m
Leaving (Lat., Lon., Time, Depth): 30°54.4010'N, 138°43.3741'E, 16:22, 735 m
Observer: YOKOOKA, Hiroyuki

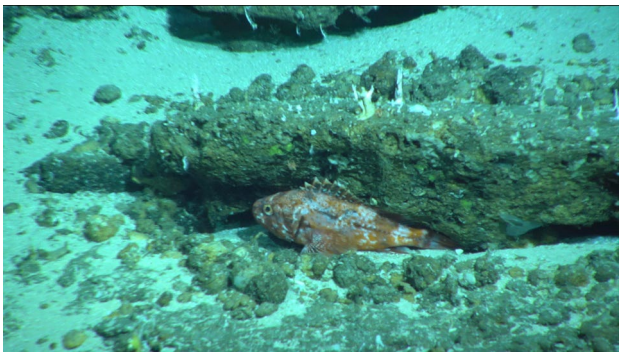
Theme: Development of deep-sea biodiversity monitoring technology for marine protected area management

Purpose of dive:
Acquisition of biodiversity baseline data around deep-sea marine protected area, including observation of deep-sea fauna and topology and sampling of organisms, sediments, and water

Dive Summary
We landed at a depth of 629 m Houei Seamount and moved southward. We sampled bottom water by use of two Niskin bottles, collected sediments using a M-type sediment sampler, and collected benthic fauna with sediments using a suction sampler and using a manipulator, and set a bait trap for collecting Fishes and Annilids. Two times of "five-minute transect" were conducted for benthos mapping. The battery powered, standalone stereo camera imaging system was installed on the ROV to make 3D reconstructions of the survey area.

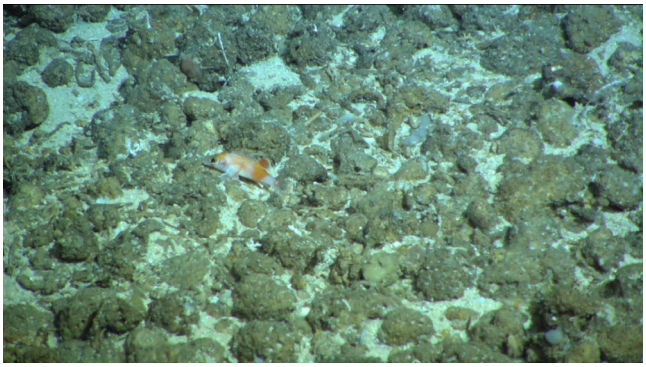


Niskin bottles sampling

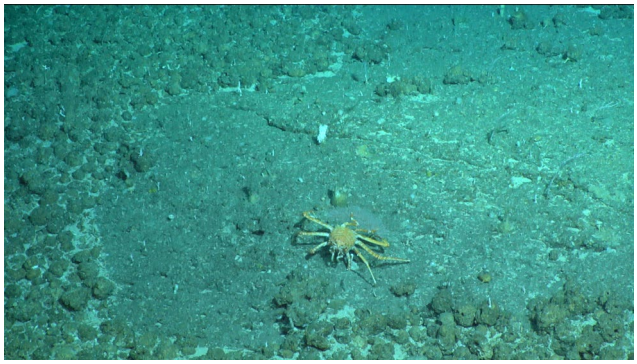


Rock fish

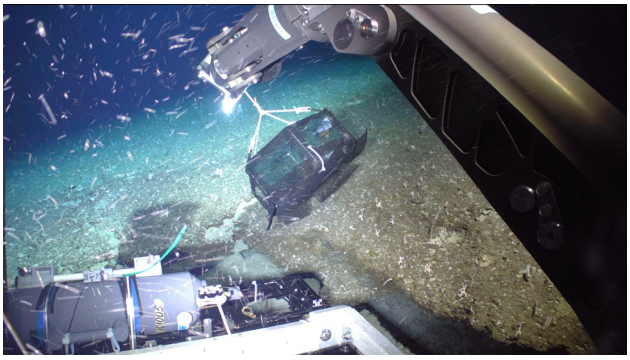
Shark



ヒメキチジ



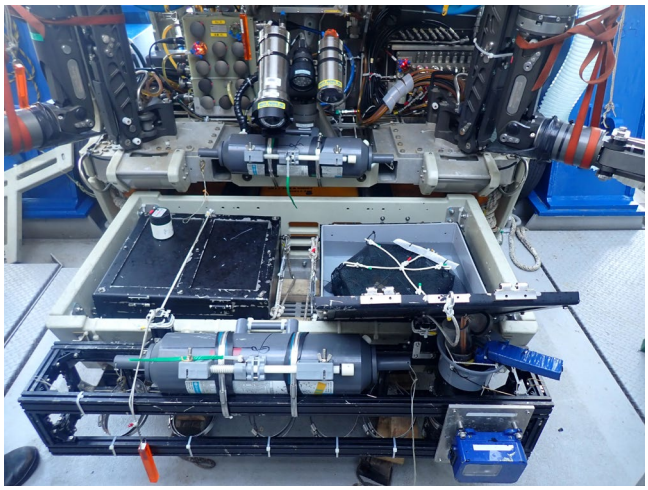
Crab



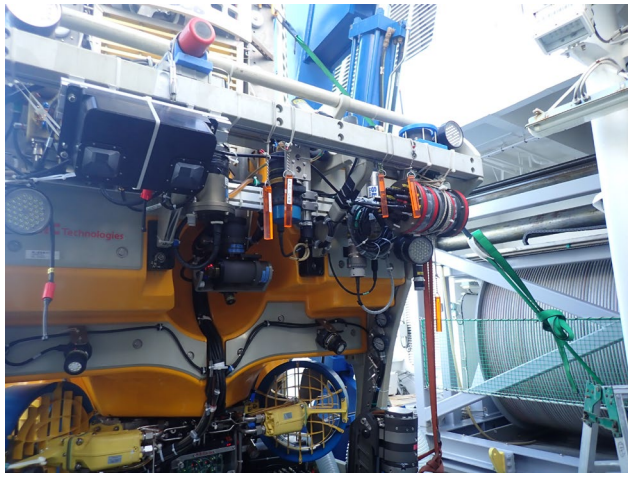
Deployment of fish trap

Payload Equipment:

GoPro housing x1 (front)
M-type sediment sampler x1 (front)
GoPro housing x1 (front)
KM-ROV box x2 (front)
Niskin bottle x2 (front)
Bait trap x1 (in KM-ROV box)
Poly Killer x2 (in Bait trap)
Suction sampler and multi-bottled canister x1 (rear)
Bottom mapping camera x1 (rear)
Line laser x2 (rear)
24V LED light x1 and 100V LED light x 1 (both rear)
Stereo camera imaging system (front upper)



Front payload



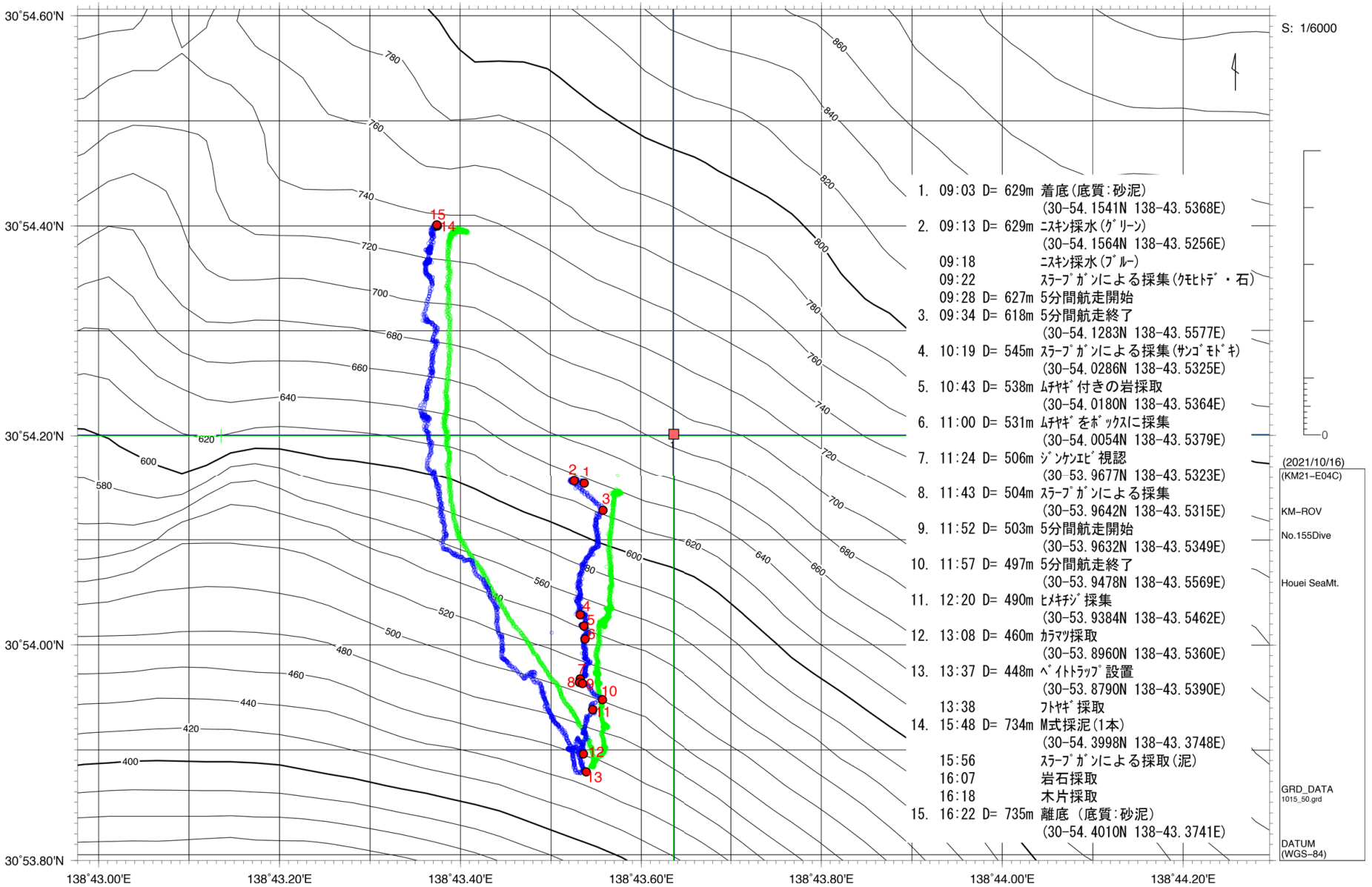
Front payload (upper)

Rear Payload

Sampling Points and Markers:

Time	Position	Depth (m)	Events
9:13	30°54.1564’N, 138°43.5256’E	629	Water sampling using Niskin bottle(Green)
9:18	ditto	ditto	Water sampling using Niskin bottle(Blue)
9:28	ditto	627	five-minute transect start
9:34	30°54.1283’N, 138°43.5577’E	618	five-minute transect end
11:43	30°53.9642’N, 138°43.5315’E	504	Biological sampling using suction sampler
11:52	30°53.9632’N, 138°43.5349’E	503	five-minute transect start
11:57	30°53.9478’N, 138°43.5569’E	497	five-minute transect end
12:20	30°53.9384’N, 138°43.5462’E	490	A Fish sampling using suction sampler
13:37	30°53.8790’N, 138°43.5390’E	448	Baited trap deployment
15:48	30°54.3998’N, 138°43.3748’E	734	Sediment sampling using M-type sediment sampler
16:22	30°54.4010’N, 138°43.3741E	735	Left from floor

Dive track KM-ROV#155



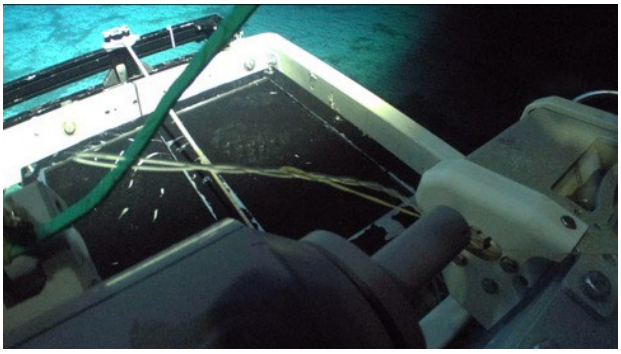
Dive Report KM-ROV#156

Date: October 17, 2021
Site: Anei Seamount, Nishi-Shichito Ridge, ***Depth:*** 1149 m
Landing (Lat., Lon., Time, Depth): 29°16.7312'N, 138°37.4482'E, 8:55, 1149 m
Leaving (Lat., Lon., Time, Depth): 29°17.0268'N, 138°37.8458'E, 14:23, 771 m
Observer: JIMI, Naoto

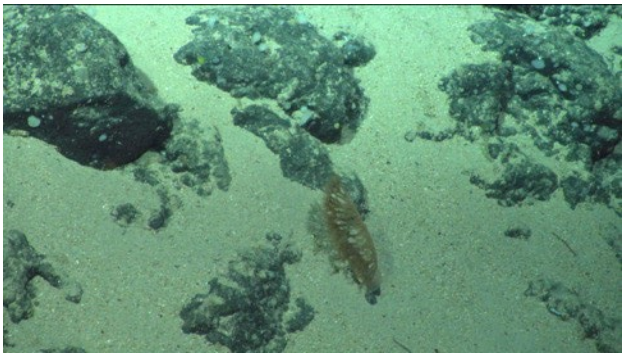
Theme: Development of deep-sea biodiversity monitoring technology for marine protected area management

Purpose of dive:
Acquisition of biodiversity baseline data around deep-sea marine protected area, including observation of deep-sea fauna and topology and sampling of organisms, sediments, and water

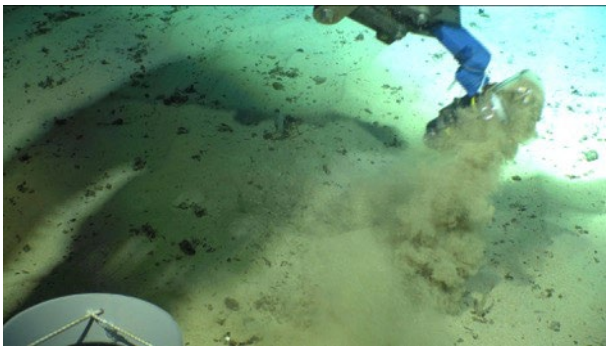
Dive Summary
We landed at a depth of 1149 m, Anei Seamount, and moved northward. We sampled bottom water by use of a Niskin bottle, collected sediments using a M-type sediment sampler, and collected benthic fauna and sediments with a suction sampler or manipulator. We also set a bait trap for collecting fishes and other benthic invertebrates. Five times of "five-minute transect" were conducted for benthos mapping. The battery powered standalone stereo camera imaging system was installed on the ROV to make 3D reconstructions of the survey area.


Niskin bottles sampling

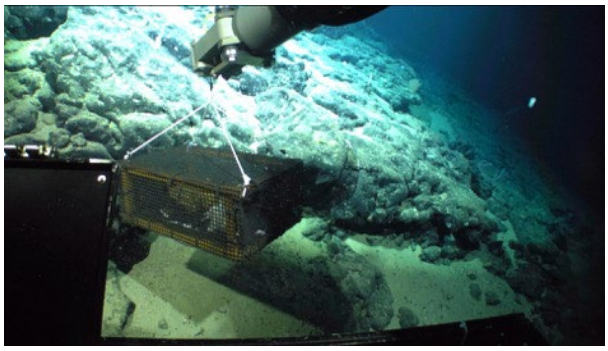

Zoanthairans with Hyalonematidae sp.


Pennatulacea sp.


Homeryon armarium



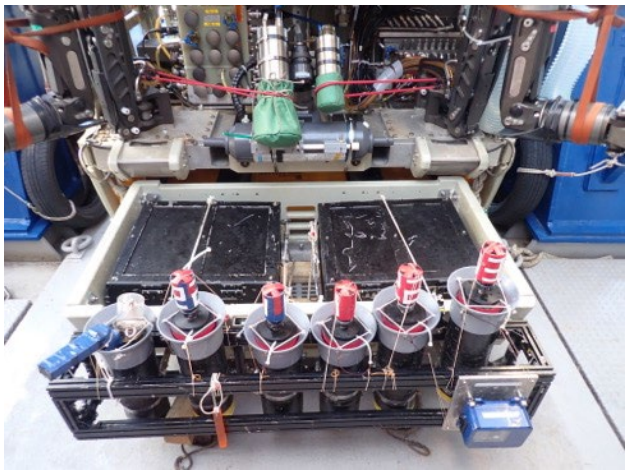
Sediment sampling using M-type corer



Deployment of fish trap

Payload Equipment:

GoPro housing x1 (front)
M-type sediment sampler x1 (front)
GoPro housing x1 (front)
KM-ROV box x2 (front)
Niskin bottle x1 (front)
Bait trap x1 (in KM-ROV box)
Polykiller x2 (in Bait trap)
Suction sampler and multi-bottled canister x1 (rear)
Bottom mapping camera x1 (rear)
Line laser x2 (rear)
24V LED light x1 and 100V LED light x 1 (both rear)
Stereo camera imaging system (front upper)



Front payload

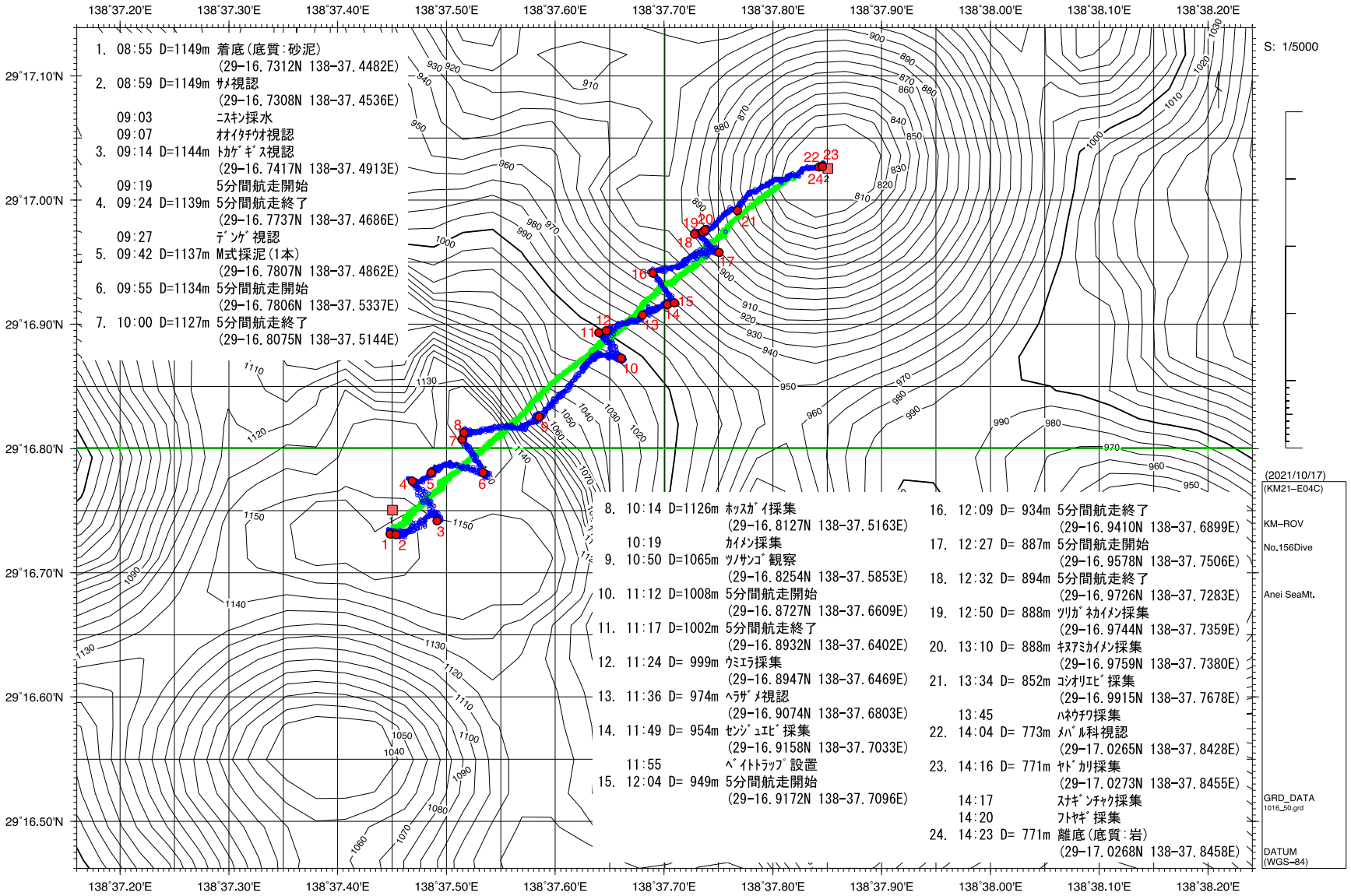
Front payload (upper)

Rear Payload

Sampling Points and Markers:

Time	Position	Depth (m)	Events
09:03	29°16.7308’N 138°37.4536’E	1149	Water sampling using Niskin bottle
09:42	29°16.7807’N 138°37.4862’E	1137	Sediment sampling using M-type sediment
10:14	29°16.7807’N 138°37.4862’E	1126	Biological sampling using suction sampler
11:24	29°16.8947’N 138°37.6469’E	999	Biological sampling using suction sampler
11:49	29°16.9158’N 138°37.7033’E	954	Biological sampling using suction sampler
11:55	29°16.9158’N 138°37.7033’E	954	Baited trap deployment
12:50	29°16.9744’N 138°37.7359’E	888	Biological sampling using suction sampler
13:10	29°16.9759’N 138°37.7380’E	888	Biological sampling using suction sampler
13:34	29°16.9915’N 138°37.7678’E	852	Biological sampling using suction sampler
14:16	29°17.0273’N 138°37.8455’E	771	Biological sampling using suction sampler

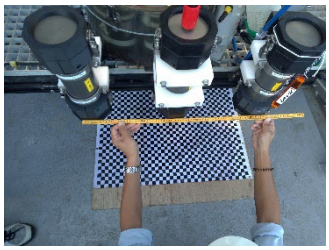
Dive track KM-ROV#156



3-1-3. Bottom mapping

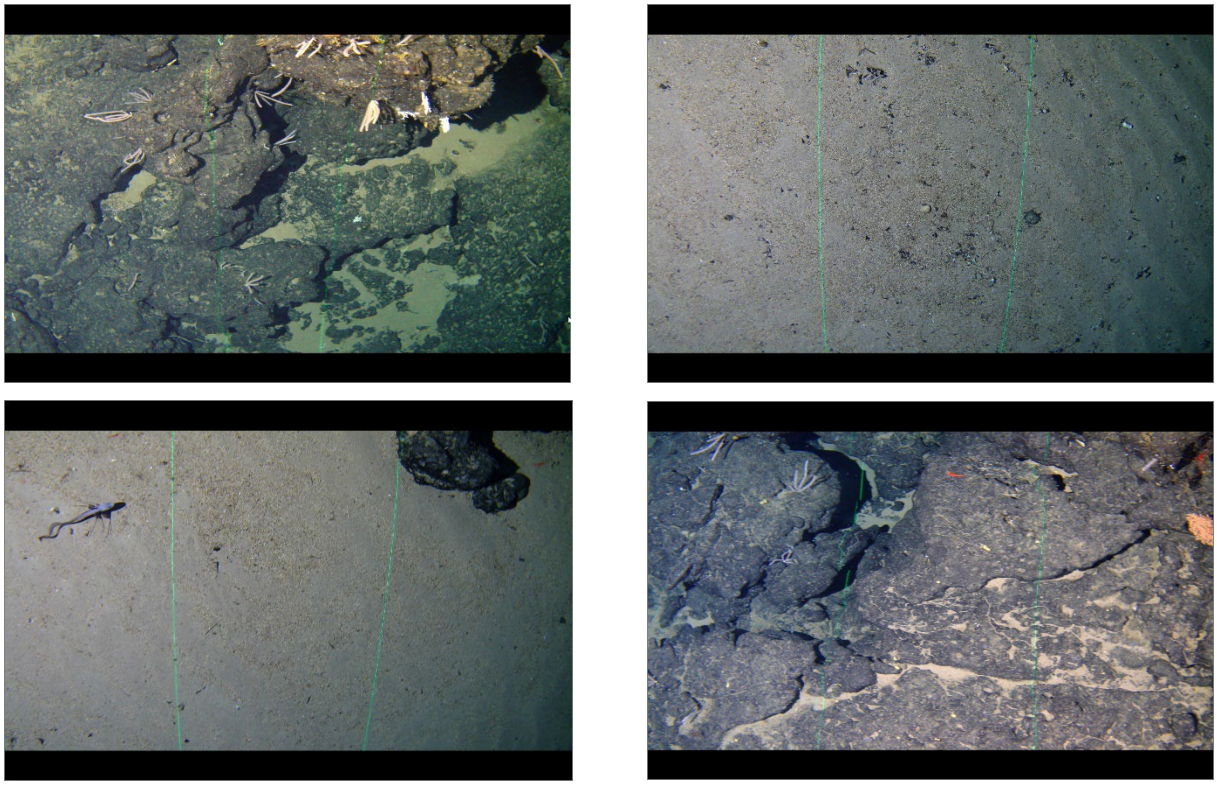
Seafloor imaging with a newly developed seafloor observation camera was examined. The Shooting range of the camera image (captured on a PC via Ethernet) was about 74.78cm x 51.51cm in 58.1cm height (at the edge of the lid) in the air when it is installed to the rear mount of the KMROV. The angle of view was approximately 63.8 x 46.4 degrees in air (assuming 2cm lid to focus). The lasers were also installed in the same rear mount of KMROV and set up almost parallel with a distance of 60.1 cm and it appeared approximately 59 cm distance in 58.1cm height (at the edge of the lid) in the air.

Using these equipment, we were able to capture images of small organisms on the seafloor, such as shrimps and brittle stars, which could not be adequately seen in the horizontal image of the ROV due to insufficient resolution.



Location of the laser and camera

On the other hand, the information coming up via Ethernet has better resolution than the information stored in the SD card directly from the device. So, we used the software OBS Studio to capture the images, but we will consider the compression method in the future. In addition, image itself was very high without movement of ROV, when it move faster the blurred vision of video will recorded.



Images taken by the seafloor observation camera

3-2. CTD water sampling

(1) Personnel

Yoshihiro Fujiwara	(JAMSTEC)	*Principal Researcher
Tun Htet Aung	(MWJ)	*Operation Leader
Masanori Enoki	(MWJ)	
Yukihiko Nakano	(MWJ)	

(2) Objective

Investigation of oceanic structure and water sampling

(3) Parameters

Temperature
Salinity
Pressure
Dissolved Oxygen
Fluorescence
Beam Transmission
Turbidity
Photosynthetically Active Radiation (PAR)
Height Above Sea bottom （Altimeter, 100m range from bottom）

(4) Instruments and Methods

CTD/Carousel Water Sampling System, which is a 36-position Carousel Water Sampler (CWS) with Sea-Bird Electronics, Inc. CTD (SBE9plus), was used during this cruise. 12-liter sample bottles were used for sampling seawater. The sensors attached on the CTD were to measure temperature (primary and secondary), conductivity (primary and secondary), pressure, dissolved oxygen, fluorescence, beam transmission, turbidity, and photosynthetically active radiation and height above sea bottom (range is 100m from bottom). Mass Pump and Rinko Profiler were attached to CTD frame. The CTD/CWS was deployed from starboard on working deck. CTD system was kept at 10m above sea bottom for 3 hours to allow the Mass Pump to filter the sea water at near bottom to analyze environmental DNA.

Specifications of the sensors used are listed below.
CTD: SBE911plus CTD system
Under water unit:
SBE9plus (S/N: 09P84583-1235, Sea-Bird Electronics, Inc.)
Pressure sensor: Digiquartz pressure sensor (S/N: 134402)
Calibrated Date: 04 Mar. 2020

Carousel water sampler:
SBE32 (S/N: 324510-1086, Sea-Bird Electronics, Inc.)

Temperature sensors:
Primary: SBE03Plus (S/N: 03P2730, Sea-Bird Electronics, Inc.)
Calibrated Date: 28-Dec-2019

Secondary: SBE03 (S/N: 034818, Sea-Bird Electronics, Inc.)

Calibrated Date: 05-Jun-2021

Conductivity sensors:

Primary: SBE04C (S/N: 044450, Sea-Bird Electronics, Inc.)

Calibrated Date: 08-Jun-2021

Secondary: SBE04C (S/N: 043889, Sea-Bird Electronics, Inc.)

Calibrated Date: 08-Jun-2021

Dissolved Oxygen sensors:

Primary: RINKOIII (S/N: 0221, JFE Advantech Co., Ltd.)

SBE43(S/N: 433161, Sea-Bird Electronics, Inc.)

Calibrated Date: 25-May-2021

Fluorescence sensor:

Chlorophyll Fluorometer (S/N: 3701, Seapoint Sensors, Inc.)

Gain setting: 30X, 0-5 ug/l

Offset: 0.000

Transmission meter:

C-Star (S/N CST-1727DR, WET Labs, Inc.)

Calibrated Date: 15 May. 2021

Turbidity:

Turbidity Meter (S/N: 14954)

Gain setting: 100X

Scale factor: 1.000

Calibrated Date: None

Photosynthetically Active Radiation (PAR) sensor

PAR-Log ICSW (S/N: 1026, Satlantic, Inc.)

Calibrated Date: 6-Jul-2015

Altimeter:

Benthos PSA-916T (S/N: 52396, Teledyne Benthos, Inc.)

Submersible Pumps:

Primary: SBE5T (S/N: 058088, Sea-Bird Electronics, Inc.)

Secondary: SBE5T (S/N: 058145, Sea-Bird Electronics, Inc.)

Bottom contact switch: (Sea-Bird Electronics, Inc.)

Deck unit: SBE11plus (S/N 11-1033, Sea-Bird Electronics, Inc.)

The CTD raw data were acquired in real time using the Seasave (ver.7.26.7.121) provided by

Sea-Bird Electronics, Inc. and stored on the hard disk of the personal computer.

The bottles were fired after waiting for more than 30 seconds from the stop.

Data processing procedures and performed modules of SBE Data Processing-Win32 (ver.7.26.7.129) and their descriptions were as follows:

(The process in order)

DATCNV: Convert the binary raw data to engineering unit data. DATCNV also extracts bottle information where scans were marked with the bottle confirm bit during acquisition. The duration was set to 3 seconds, and the offset was set to 0.0 seconds.

RINKOCOR (original module): Correct the time dependent, pressure induced effect (hysteresis) of the RINKOIII profile data.

RINKOCORROS (original module): Correct the time dependent, pressure induced effect (hysteresis) of the RINKOIII bottle information data by using the hysteresis corrected profile data.

BOTTLESUM: Create a summary of the bottle data. The data were averaged over 3 seconds.

ALIGNCTD: Convert the time-sequence of sensor outputs into the pressure sequence to ensure that all calculations were made using measurements from the same parcel of water. Dissolved oxygen data are systematically delayed with respect to depth mainly because of the long time constant of the dissolved oxygen sensor and of an additional delay from the transit time of water in the pumped pluming line. This delay was compensated by 5 seconds advancing dissolved oxygen sensor (SBE43) output (dissolved oxygen voltage) relative to the temperature data. RINKOIII voltage (User polynomial 0) was advanced to 1 second.

WILDEDIT: Mark extreme outliers in the data files. The first pass of WILDEDIT obtained the accurate estimate of the true standard deviation of the data. The data were read in blocks of 1000 scans. Data greater than 10 standard deviations were flagged. The second pass computed a standard deviation over the same 1000 scans excluding the flagged values. Values greater than 20 standard deviations were marked bad. This process was applied to pressure, depth, temperature (primary and secondary) and conductivity (primary and secondary) dissolved oxygen voltage (SBE43).

CELLTM: Remove conductivity cell thermal mass effects from the measured conductivity. Typical values of thermal anomaly amplitude alpha = 0.03 and the time constant 1/beta = 7.0 were used.

FILTER: Perform a low pass filter on pressure and depth data with a time constant of 0.15 second. In order to produce zero phase lag (no time shift) the filter runs forward first then backward.

WFILTER: Perform a median filter to remove spikes. The window length is determined for a specific data value and the median value is calculated for each specified window, and the data value at the window`s center point is replaced by the median value. The window length is specified as 49 scans for the fluorescence data, beam transmission data, beam attenuation data, output voltage of Transmissometer and turbidity data.

SECTIONU (original module of SECTION): Select a time span of data based on scan number in order to reduce the file size. The minimum number was set to be the starting time when the CTD package was beneath the sea-surface after activation of the pump. The maximum number was set to be the end time when the package came up from the surface.

LOOPEDIT: Marked scans where the CTD was moving less than the minimum velocity of 0.0 m/s (traveling backwards due to ship roll).

DESPIKE (original module): Removed spikes of the data. A median and mean absolute deviation was calculated in 1-dbar pressure bins for both down and up cast, excluding the flagged values. Values greater than 4 mean absolute deviations from the median were marked bad for each bin. This process was performed twice for temperature, conductivity and dissolved oxygen (RINKOIII and SBE43) voltages.

DERIVE: Compute dissolved oxygen data (SBE43).

BINAVG: Averaged the data into 1 decibar pressure bins and 1 second time bins.

BOTTOMCUT (original module): Deleted discontinuous scan bottom data, when it’s created by BINAVG.

DERIVE: Compute salinity, potential temperature, and density (sigma-theta).

SPLIT: Separate the data from the input .cnv file into down cast and up cast files.

(5) Station list

1 cast of CTD measurement was carried out during Leg1 (Table 1) and 3 casts of CTD casts were carried out during Leg 2(Table 2).

(6) Preliminary Results

No problem found.

(7) Data archive

These data obtained in this cruise will be submitted to the Data Management Group of JAMSTEC, and will be opened to the public via “Data Research System for Whole Cruise Information in JAMSTEC (DARWIN)” in JAMSTEC web site.

<http://www.godac.jamstec.go.jp/darwin/e>

Table 1: KM21-E04C Leg1 CTD cast table

Stnnbr	Castno	Date(JST)	Time(JST)		BottomPosition		Depth	Wire	HT Above	Max	Max	CTD	Remark
		(mmddyy)	Start	End	Latitude	Longitude	(m)	Out (m)	Bottom (m)	Depth	Pressure	Filename	
G01	1	101421	04:16	09:00	30-39.60N	139-02.15E	2078.1	2079.0	8.5	2062.4	2087.0	G01M001	

3-3. MASS pump filtration

The “MASS Pump” is a large-scale *in situ* filtration system for seawater for environmental DNA research. It consists of three units: a filter unit (four Sterivex filters with 0.45 μm in pore size installed), a pump unit, and a control unit containing a system controller and a battery (Fig. 3-3-1). During this cruise, the pump was equipped on the CTD water sampling system (Fig. 3-3-2) and a lander (Fig. 3-3-3), and three trials of filtration were conducted at South off Genroku Seamount and Anei Seamount. When filtration was conducted by the MASS Pump installed on the lander at South off Genroku Seamount, Sterivex filters were clogged by the contaminated sediment and one of four tubes was burst during filtration (Fig. 3-3-4). The filtration durations and volumes were shown in Table 3-3-1.

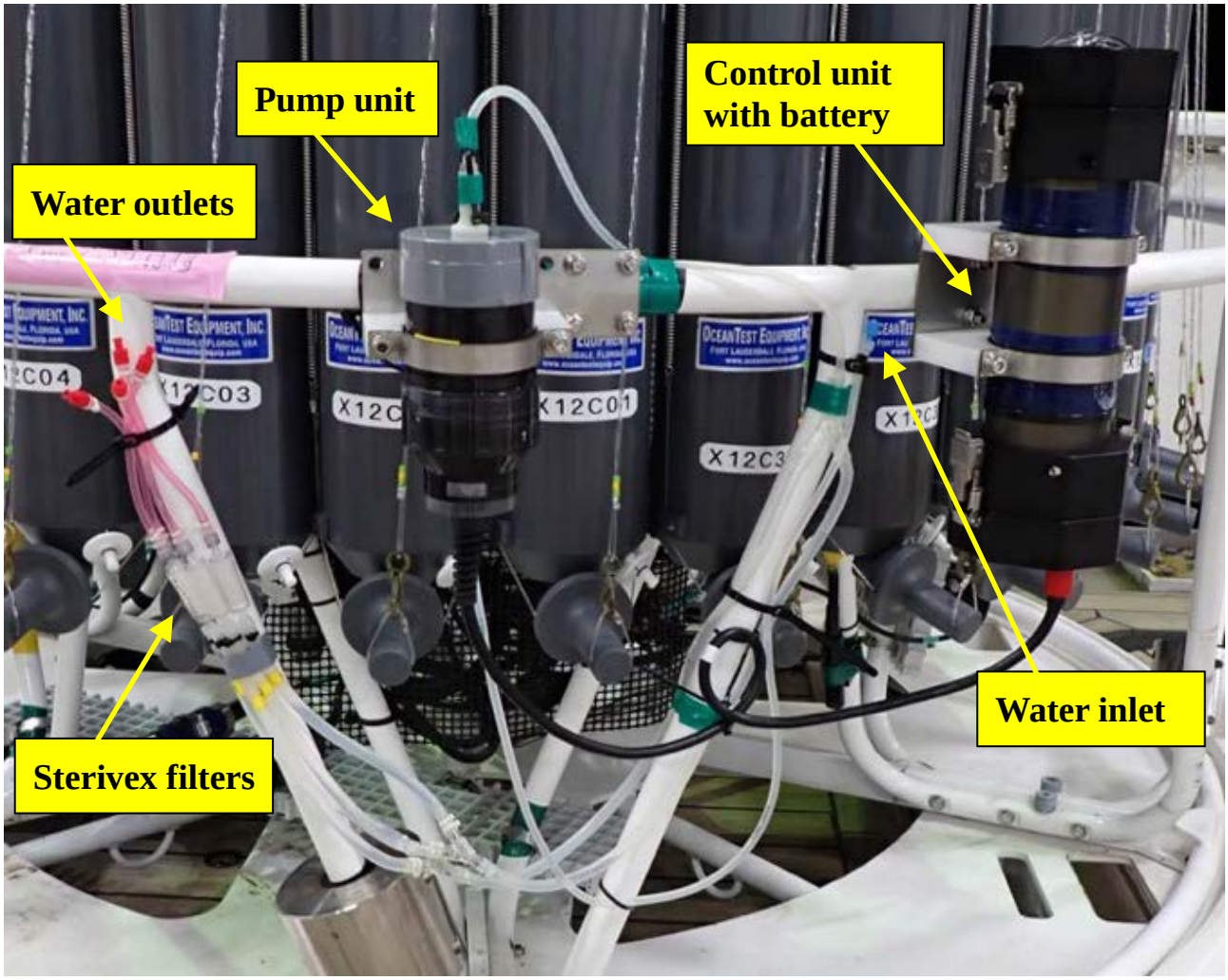


Fig. 3-3-1. MASS Pump

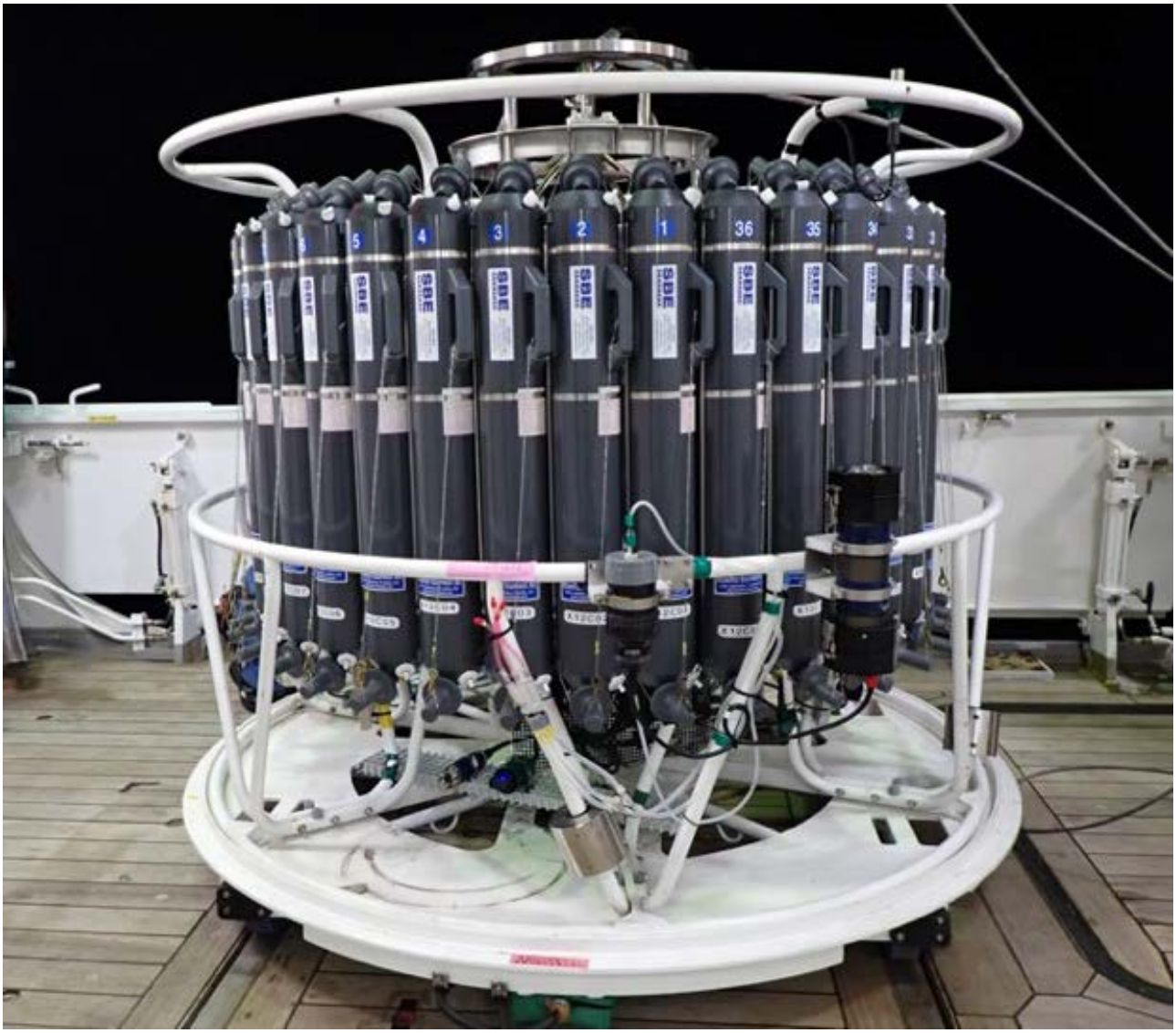


Fig. 3-3-2. MASS Pump installed on a CTD water sampling system

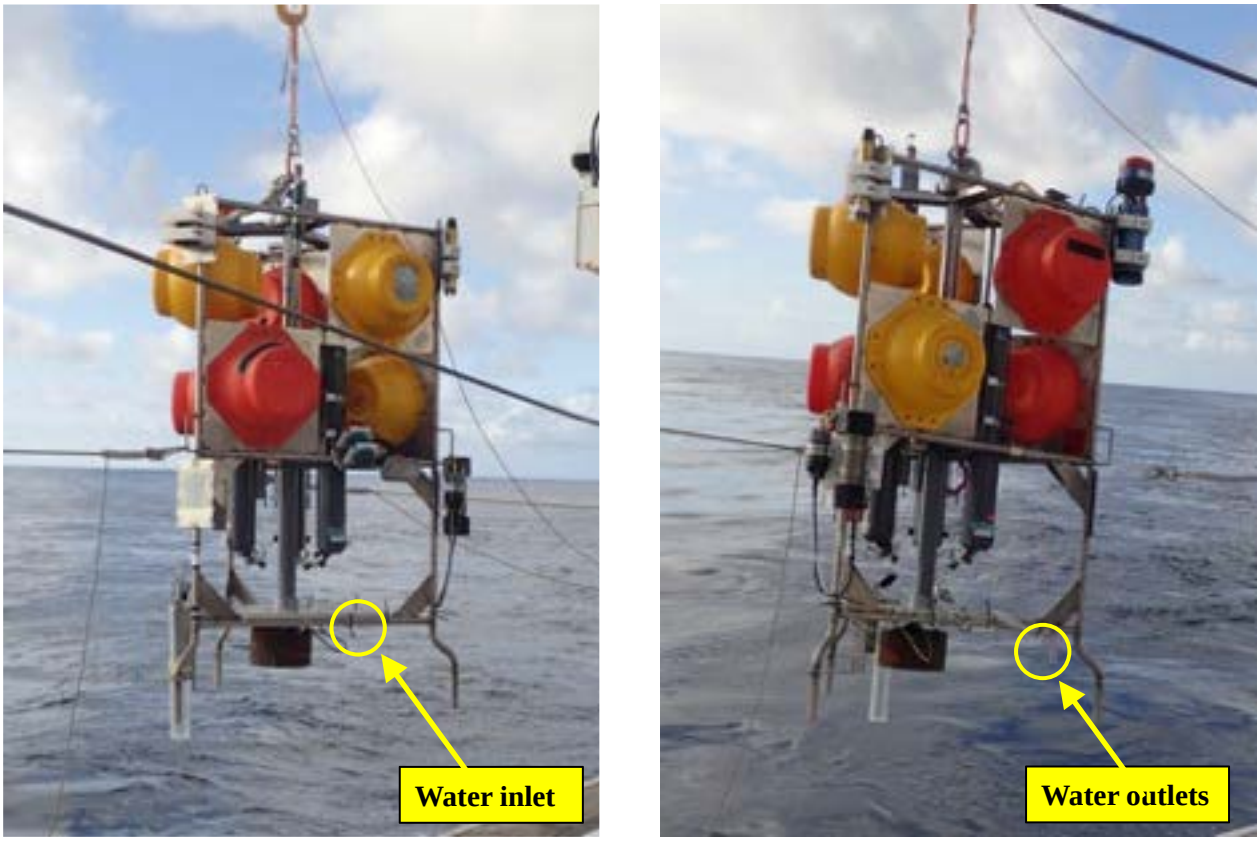


Fig. 3-3-3. MASS Pump installed on a lander

Fig. 3-3-4. Burst tube (left) and clogged filter (right)

Table 3-3-1. Filtration results using MASS Pump

Date	Filtering duration (h)	Platform	Cast No.	Location	Cordinate	Depth (m)	Filtered water volume (L)	No. of Sterivex	Remarks
2021/10/14	3 hrs	CTD	G01-M001	South off Genroku Seamount	30°39.6000'N 139°02.1525'E	2060.4	104.9	KM21-C1P1	Pump: BP01, Controller: 2
								KM21-C1P2	
								KM21-C1P3	
								KM21-C1P4	
2021/10/15	3 hrs	Lander	#1	South off Genroku Seamount	30°38.7671'N 139°01.5733'E	2065.0	ND (105.2 L from flow count)	KM21-L1P1	Pump: BP02, Controller: 2, tubing broken due to filter clogged
								KM21-L1P2	
								KM21-L1P3	
								KM21-L1P4	
								KM21-C2P4	

3-4. Baited camera deployment

Three baited cameras were operated by free-fall deploying from the vessel and recovering by acoustic release command of the transponder. Video recorded more than ten hours in each cast and the total of video image acquired more than a hundred hours. Species diversity of scavengers and predators appeared in the image and the first arrival times of each animal will be measured. Baited cameras used in this cruise were illustrated (Fig. 3-4-1) and equipment loaded on the camera was listed (table 3-4-1) as below. The weight of the camera is 136.4 kg in air and 20.0 kg in water. Total of nine casts were listed as table 3-4-2.

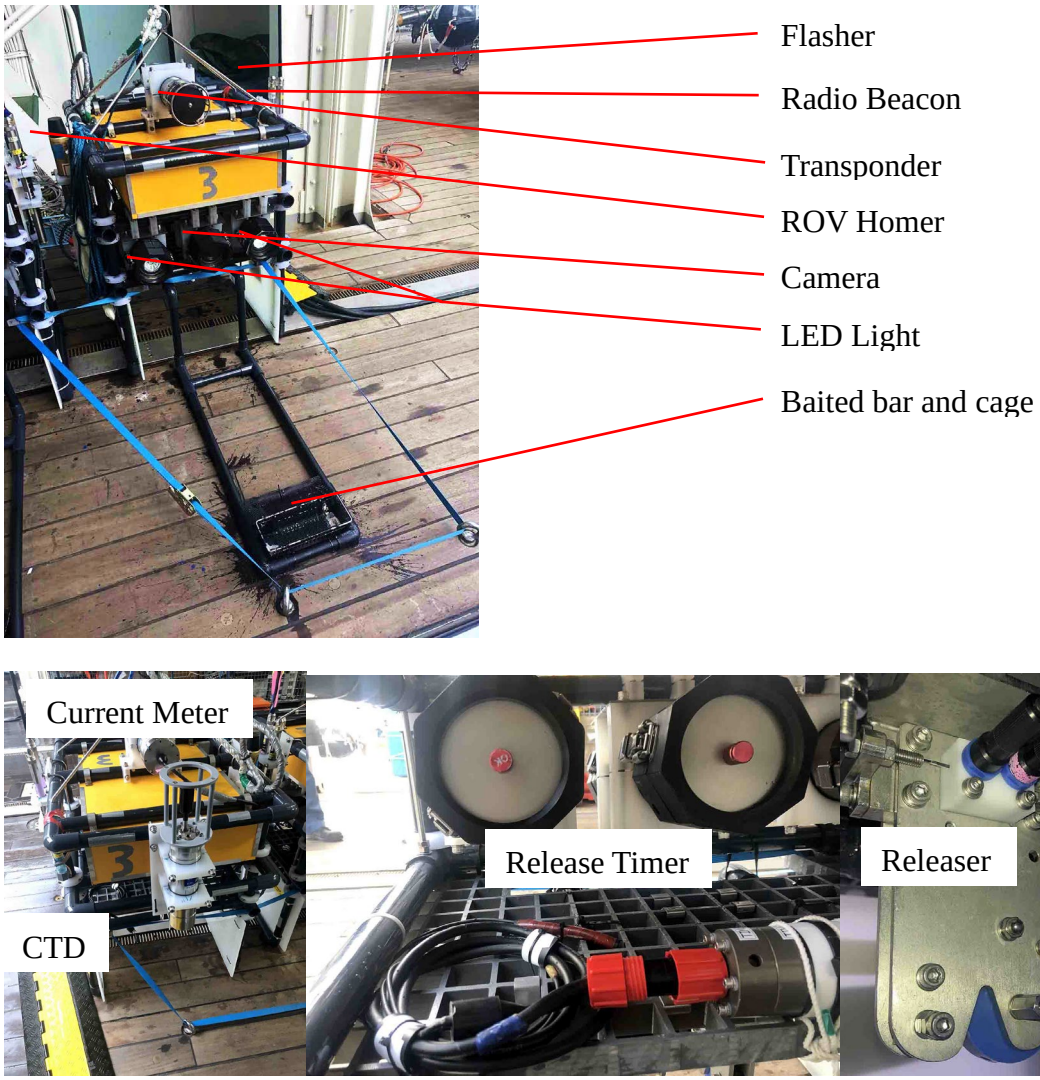


Fig. 3-4-1. Baited camera used in this cruise

Table 3-5-1. Equipment loaded on the camera.

Camera ID	POP1	POP2	POP3
Transponder	4E-4	4D-1	4B-2
ROV Homer	35	49	33
Current meter Infinity-Deep	94	89	93
CTD	9270	9271	9272
Radio Beacon	JS2164	JS2111	JS2165
Flasher	B02-038	B02-037	C01-090

Table 3-4-2. Cast list of the baited camera during KM21-E04C Leg1

Cast No.	Casting Point	Casting Date	Casting Time	Landing Time	Descent speed (m/m)	Landing Point Lat./Lon.		CTD Depth (m)	Recovery Date	Release command	Surfaced	Ascent speed (m/m)
POP1-1	Houei Seamount N	2021 10.14	11:06	-	-	30-40.3519N	139-02.4976E	2091	2021 10.15	7:17	8:24	31.2
POP2-1	E		13:45	15:05	26.20	30-39.4237N	139-02.8315E	2092		8:10	9:14	32.7
POP3-1	W		14:32	15:50	25.93	30-39.4577N	139-01.9974E	2000		6:11	7:15	31.3
POP1-2	Anei Seamount W	2021 10.17	15:36	16:22	24.13	29-16.8714N	138-37.3290E	1110	2021 10.18	9:20	9:57	30
POP2-2	E		15:58	16:41	24.45	29-16.4605N	138-37.8897E	1073		8:35	9:09	31.6
POP3-2	N		16:28	17:11	26.40	29-17.3989N	138-38.0519E	1135		10:11	10:45	33.4

3-5. Free-fall lander deployment

■ Introductions

As part of the development of technology for simple and inexpensive monitoring of the sea bottom environment, a free-fall lander (hereinafter referred to as "lander") has been developed. In this cruise, we used this system to monitor the sea bottom environment for one day and one night at a depth of 2,000 m near the Genroku Seamount (Fig.1), which is one of the offshore natural environment conservation areas. The lander was installed on the seafloor (at a depth of about 2000 m) near 30°38.6007N, 139°01.4617E in free fall from R/V KAIMEI. The installation time was 16:07 on October 14, 2021, and the retrieval time was 15:49 on October 14, 2021 (time of detachment). The location of the installation on the seafloor was determined by checking the transponder response from the ship.

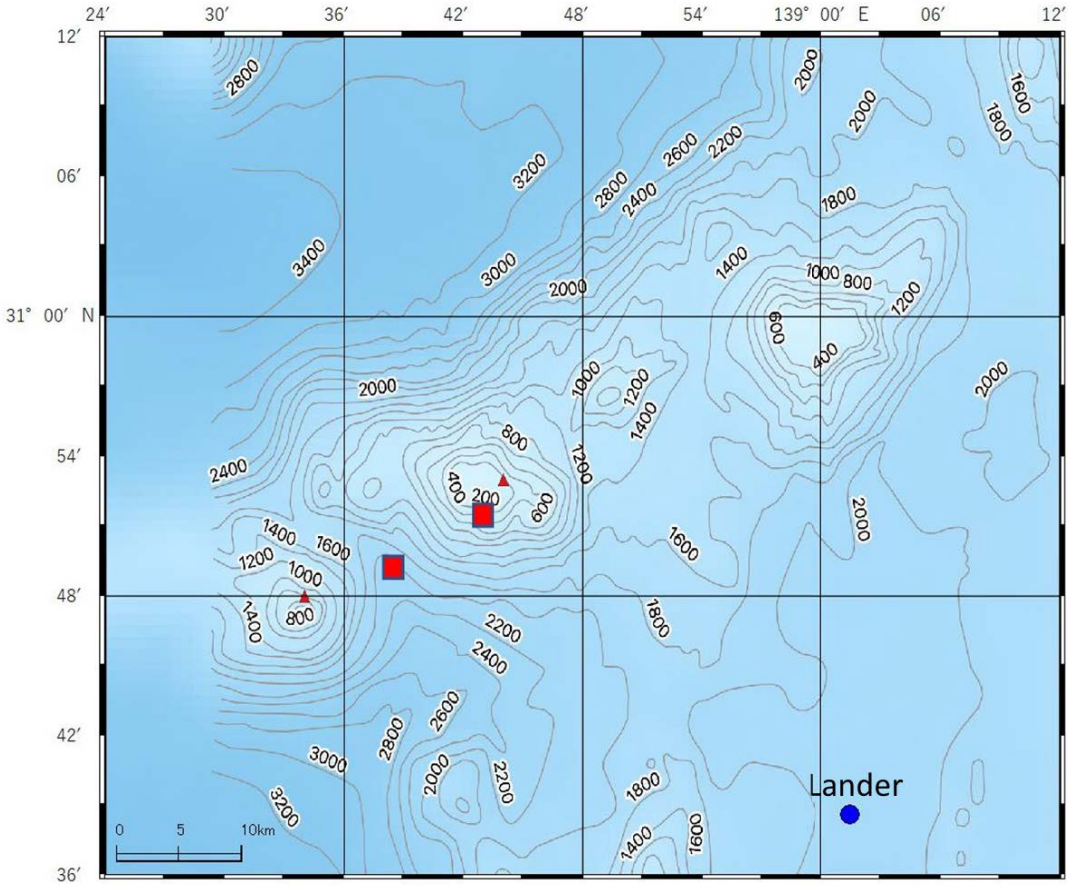


Fig.1 Installation point of Lander

■ Configuration of the Lander System

The lander system consists of a 4K video camera with interval shooting capability, an ultrasonic Doppler current meter with environmental sensor (AANDERAA, RDCP600), three Niskin water samplers (12L), a MASS pump type filtration system, and a core sampler.

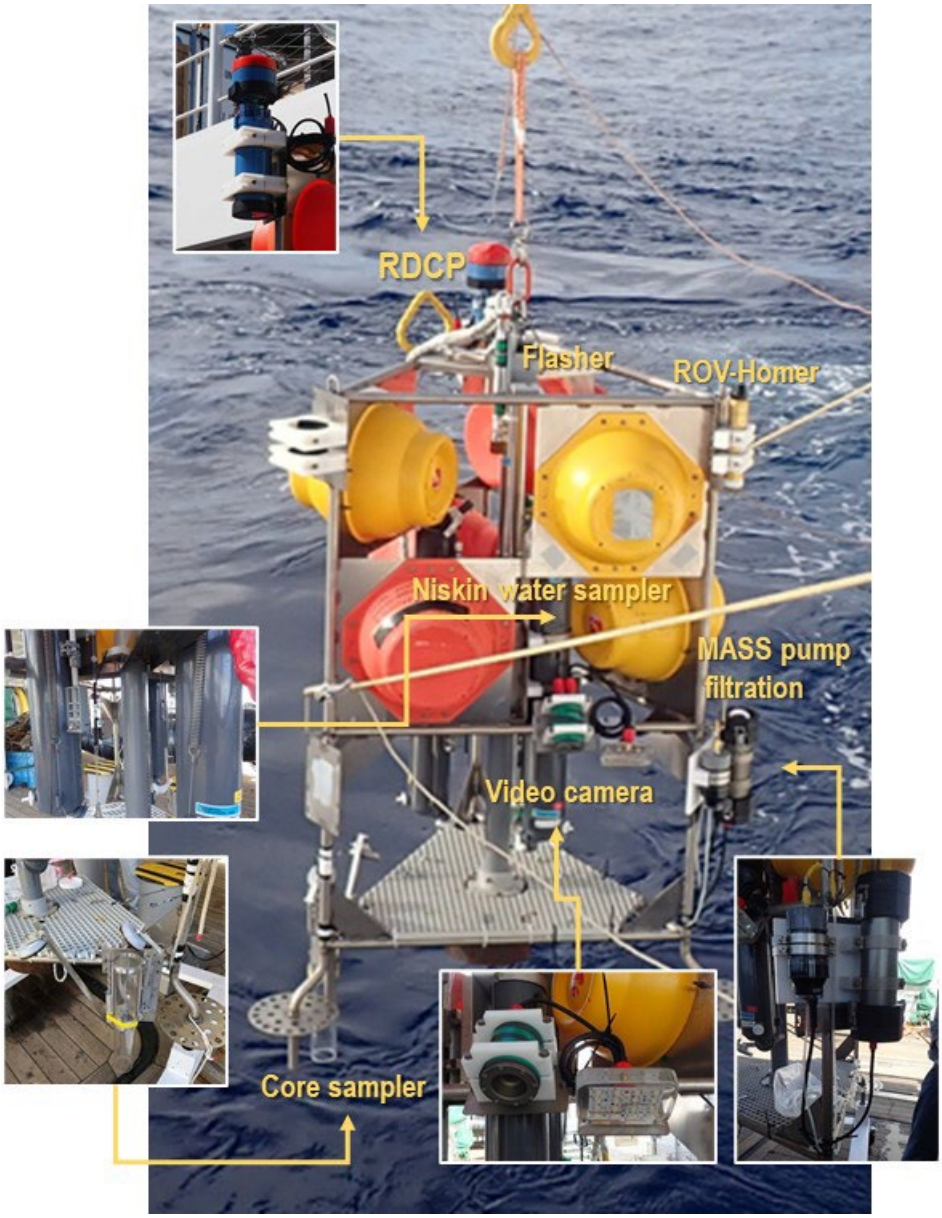


Fig.2 Configuration of the Lander System

The lander is installed by free-fall from the ship, and the weight (about 90 kg) is detached by a releaser (KAIYO DENSHI Co., Ltd., model: BX-1003) installed in the center of the lander to raise it to the sea surface. The total weight of the main body of the lander used this time was about 400 kg, and the underwater weight was about 25.0 kg (both with weights).

The lander was newly equipped with one core sampler to enable mud sampling. For water sampling, “Galvanic Timed Releases” were used to simplify the setting of the water sampling trigger. The water sampling was set to be done 6 to 8 hours after the lander was deployed. The same "Galvanic Timed Releases" were used to collect the core sampler. The "Galvanic Timed Releases" used were the AA2 type from International Fishing Devices Inc (Fig. 3).

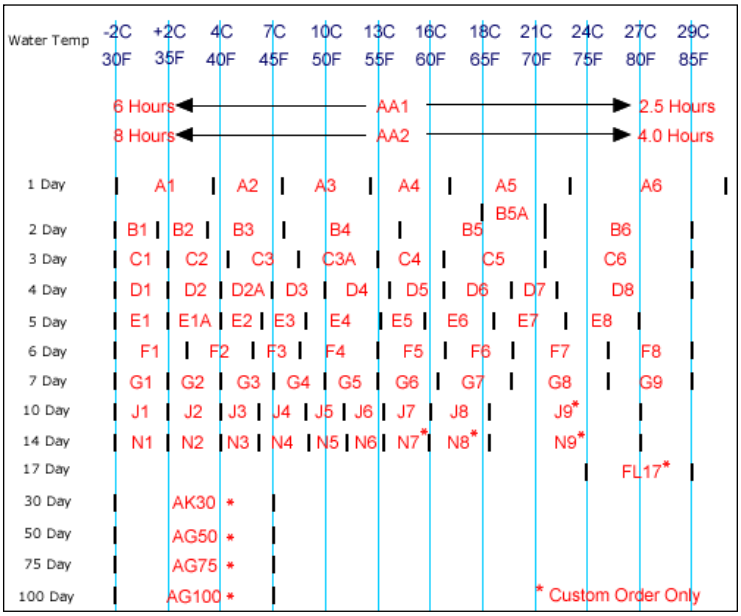


Fig.3 The Time/Temperature Chart of Galvanic Timed Releases.

The observation settings for each environmental measurement device are as follows.

- Video camera: once per hour for one minute.
- Current direction and velocity: measured every 10 minutes, 80 layers with a layer thickness of 2 m
- Environmental sensors (water temperature, salinity, pressure, turbidity and DO): measured every 5 minutes
- Niskin water sampling: 6 to 8 hours after lander insertion (3 bottles)
- MASS pump filtration: 4 hours before retrieving the lander
- Core Sampling: at the time of landing the lande

■ Function of core sampling attached to the lander

Core sampling in the lander was performed at the time of bottoming by a core sampler attached to the foot of the main body. After landing, the core is inserted into the seabed by the weight of the lander. The upper and lower lids are closed after a few hours by Galvanic Timed Release. The lower lid, however, remains on the seafloor and is closed during recovery (when the lander is surfaced) to secure the sample. An image of the core sampling is shown in Fig.4. In this case, only one core was used due to the force to pull out the core and buoyancy.

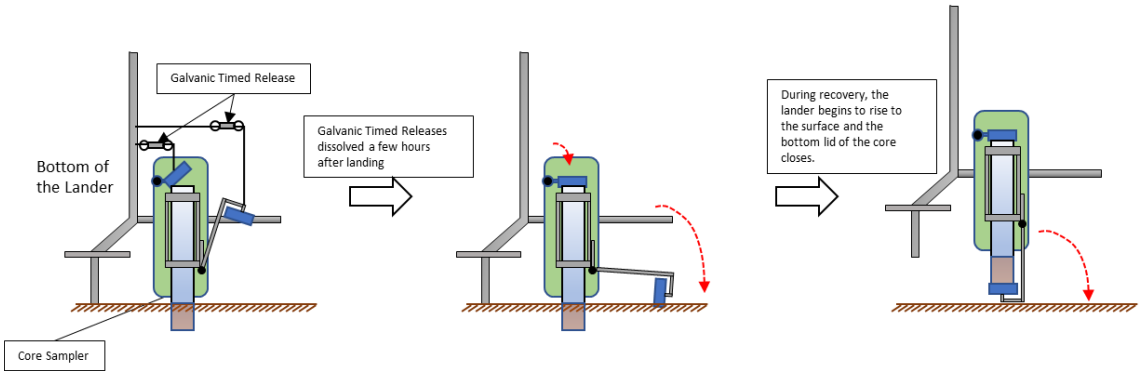
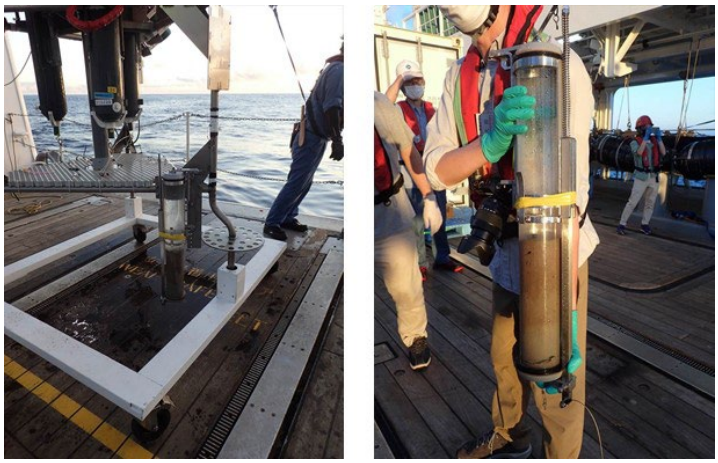


Fig.4 Image of Lander Mud Extraction

Fig.5 shows an image of the core taken by the lander. Because the seafloor was muddy, it was estimated that more than 20 cm of core could be collected. If a large number of core samples are needed, we believe that three cores can be collected by attaching a similar core sampler to the remaining leg of the lander.

Fig.5 Core sampling using Lander



■ Result of RDCP

Fig.6(a)-(d) show the results of time series fluctuations of (a) velocity, (b) velocity of east-west component, (c) velocity of north-south component, and (d) velocity of vertical component obtained by RDCP, respectively. Current data were successfully recovered for the layers at depths between the seafloor and 70m above the bottom (about the 30th layer). The data is now under scrutiny.

From the results of current measurements, the velocity near the seafloor was about 7.0 cm/sec, and the dominant current direction was toward the southwest. The periodicity of the flow will be calculated in detail in the future.

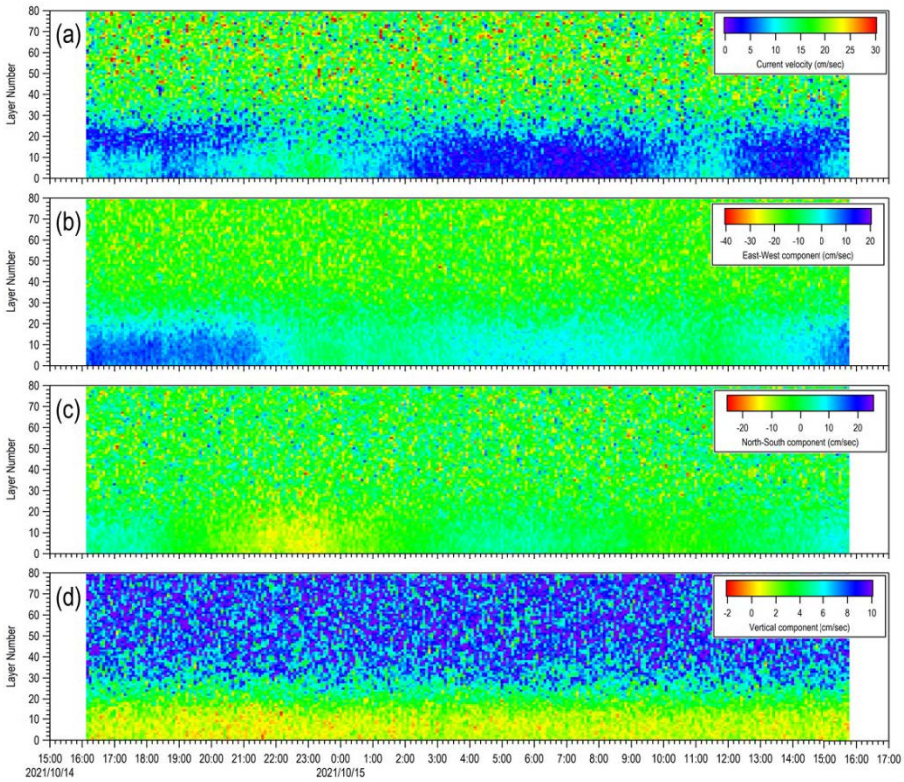


Fig.6 Results of current speed measurement using an acoustic Doppler current profiler (RDCP600): (a) Current velocity, (b) East-West component current velocity, (c) North-South component current velocity, (d) Vertical component current velocity.

■ Result of environmental sensors

Fig.7(a)-(e) shows the time series of the environmental measurement sensors installed in the RDCP. From the top, (a) dissolved oxygen, (b) salinity, (c) water temperature, (d) turbidity, and (e) water depth are shown. These data are currently under scrutiny, including confirmation of sensor operation. Therefore, the values in the time series are not absolute values. The data will be corrected and converted to actual measurement values in the future.

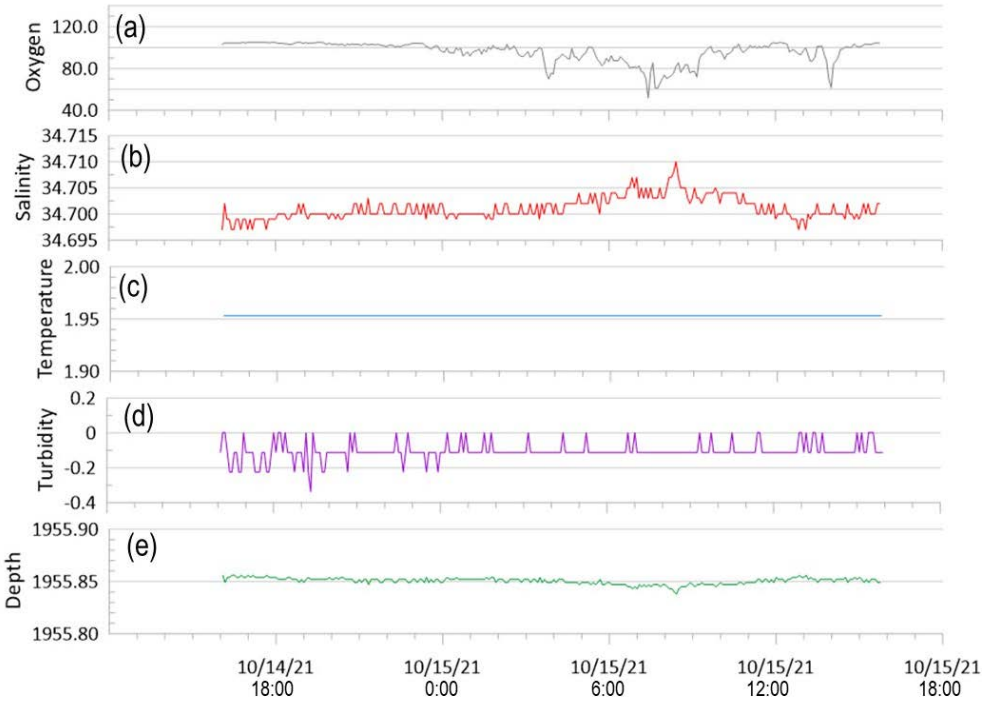


Fig.7 Time series fluctuation of environmental data by each sensor attached to RDCP:

(a) DO (Dissolved oxygen), (b) Salinity, (c) water temperature, (d) turbidity, (e) Depth.

■ Capture image of video camera on Lander system

The video camera system attached to the lander system succeeded in shooting a one-minute sea bottom video image every hour from 17:00 on October 14th, 2021 to 15:00 on October 15th, 2021. Fig.8 shows sample captured images of the sea bottom video image. In each video, we can observe the situation near the sea bottom. By comparing with the RDCP data, it will be possible to capture the numerical changes of the current and the changes in the field environment. In the image in the lower part of Fig.8, we were able to capture the movement of a hermit crab carrying an anemone on its back.

Fig.8 Capture image of video camera on Lander system:

(a) 2021.10.14 17:00, (b) 2021.10.15 02:00.

■ Water sampling with Niskin water sampler

On this cruise, we used Galvanic Timed Release as a water sampling trigger for Niskin sampling. By using Galvanic Timed Release, we were able to simplify the setting of the water sampler. However, it is not possible to obtain an accurate water sampling time with galvanic water sampling. The approximate time the water was collected after installation can be estimated from the water temperature. Fig.9 shows a diagram of the Galvanic Timed Release set in the water sampler.

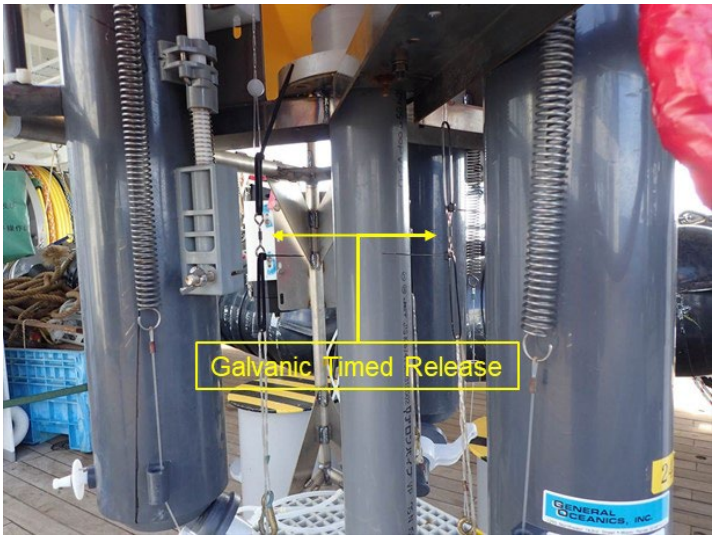


Fig.9 Galvanic Timed Release set in a Niskin water sample.

■ Pitch and roll fluctuations obtained by RDC

Fig.10(a) and (b) shows the pitch and roll data obtained by RDCP, which showed that the lander was shaking from around 11:00 to 14:00 on October 15, 2021. This result was consistent with the time when the Mass Pump was working.

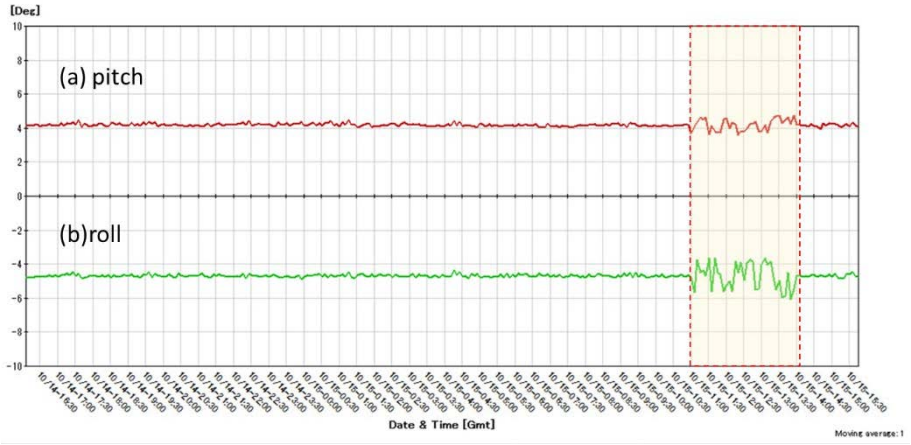
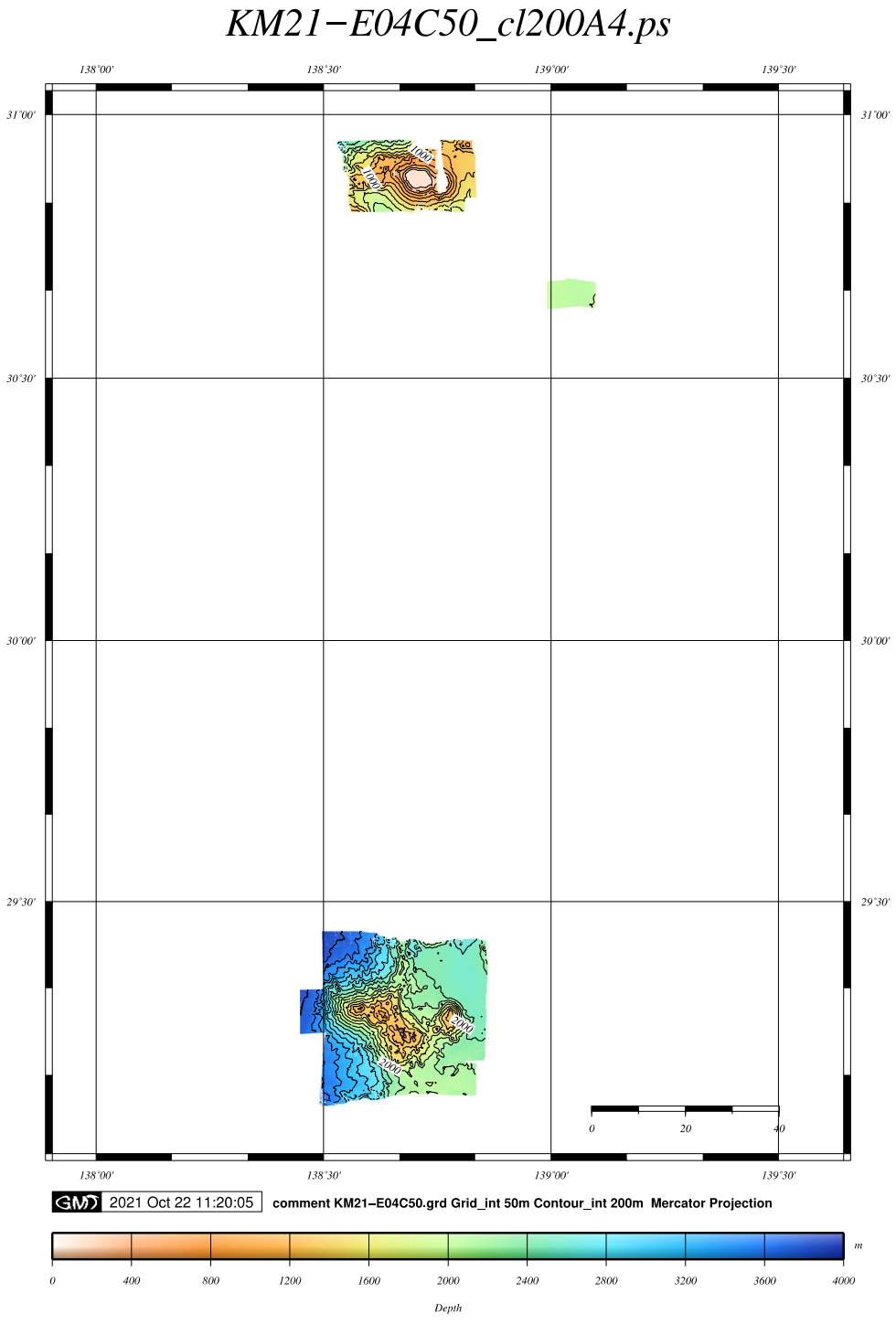


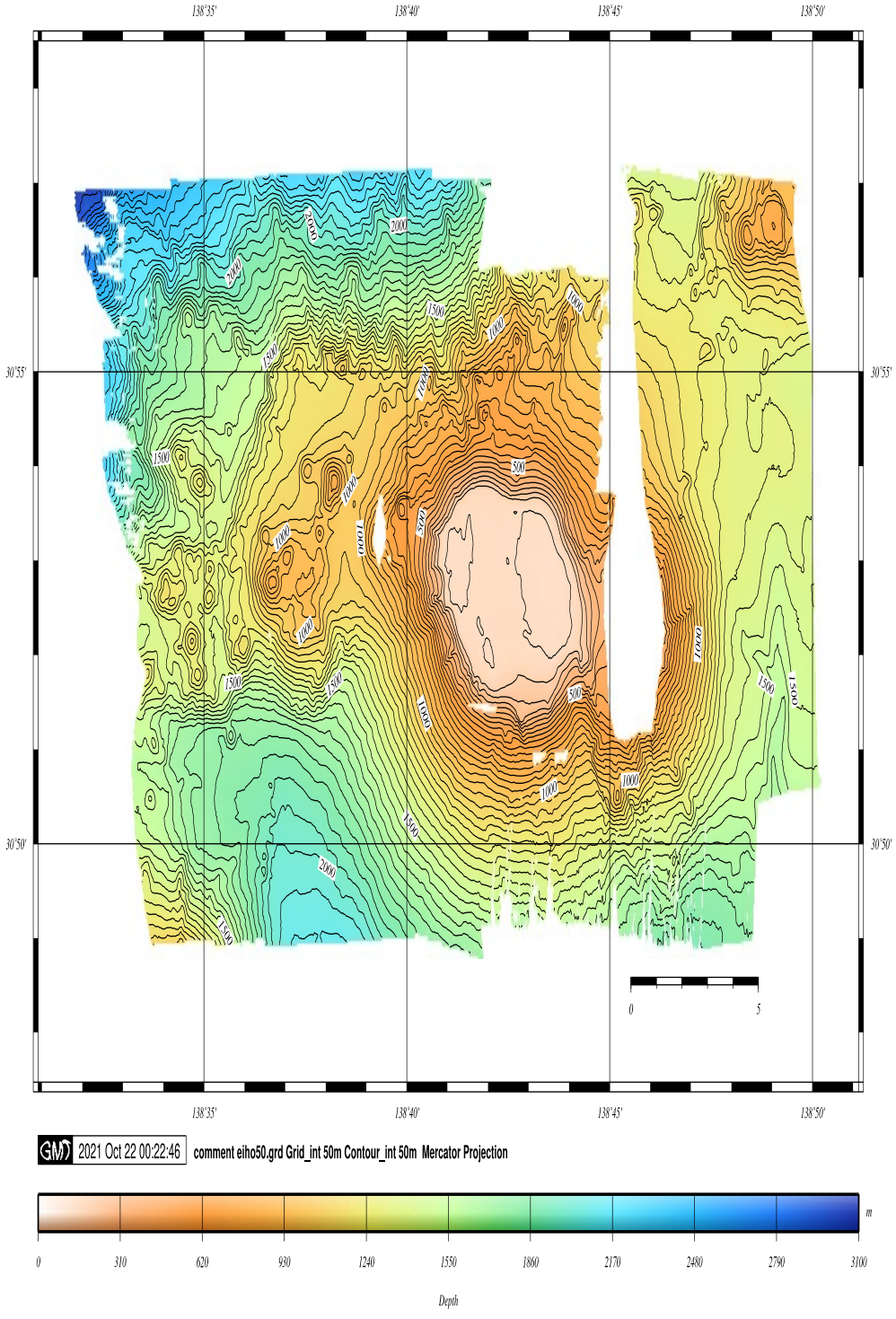
Fig.10 Fluctuation of (a) pitch and (b) roll

3-6. Geophysical survey results

Houei and Anei Seamounts

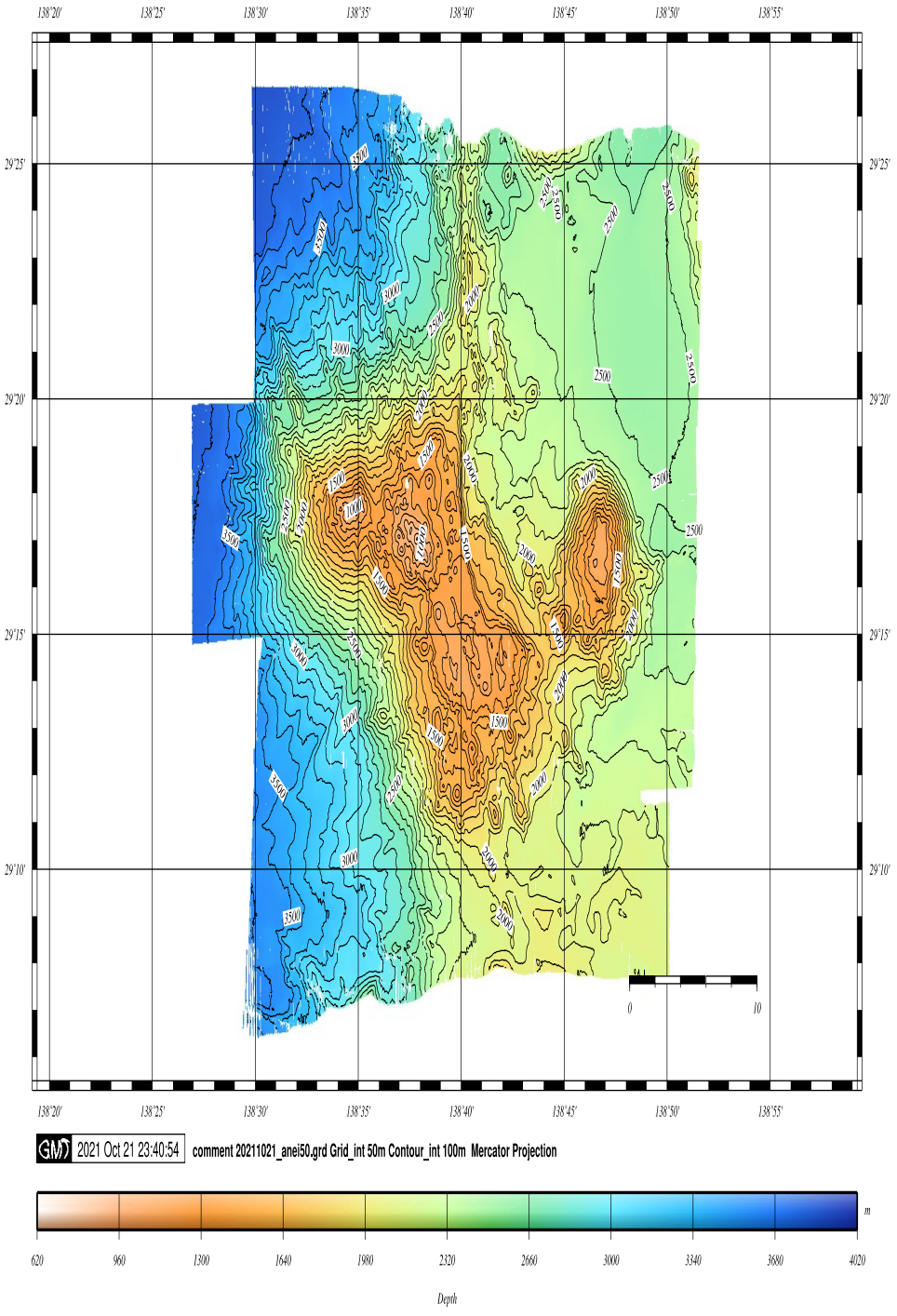


eiho50_cl50A4.ps



Hoei Seamount

20211021_anei50_cl100A4.ps

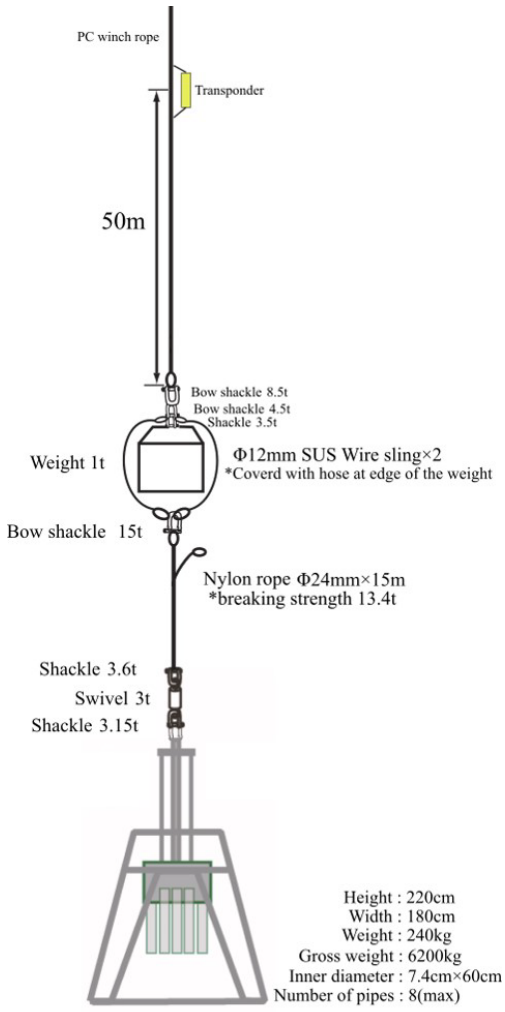


Anei Seamount

3-7. Multiple corer sampling

Date: October 15, 2021
Site: Genroku Seamount, Nishi-Shichito Ridge
Position: 30°39.5747’N, 139°02.6818’E
Depth: 2,090 m

We deployed a multiple corer, which collects up to eight sediment cores simultaneously, at the Genroku Seamount (Water depth: 2,090 m) to collect sediment samples. Each core covers 43.0 cm^2 of seafloor surface area. MC hit the bottom at 11:21 pm (JST) but failed to collect samples because the trigger didn’t work.



MULTIPLE CORER System

4. Proposals for the future studies

FUJIWARA, Yoshihiro

1. Development of "MASS Pump" – a mass filtration system of deep seawater for environmental DNA analyses
   Fujiwara, Y., Masuda, K., Kawato, M. et al.
2. Detection of rare species using environmental DNA in deep sea
   Fujiwara, Y., Tsuchida, S., Kawato, M., Yoshida, T. et al.
3. Comparison of predator/scavenger diversity and biomass between seamounts in offshore marine protected area in Japanese waters
   Fujiwara, Y., Tsuchida, S., Koeda, K., et al.

HOOKABE, Natsumi

1. A new species of *Cryptonemertes* (Monostilifera: Nemertea) beneath the pedal disks of sea anemone from Annei seamount
   Natsumi Hookabe, Kensuke Yanagi, James Davis Reimer, Yoshihiro Fujiwara, Rei Ueshima.
2. Three new species of monostiliferous nemerteans (Nemertea) from Annei seamount
   Natsumi Hookabe, Yoshihiro Fujiwara, Rei Ueshima.
3. Three new species of palaeonemerteans (Nemertea) from Annei and Genroku seamounts
   Natsumi Hookabe, Yoshihiro Fujiwara, Rei Ueshima.
4. Five new species of Polynoidae (Annelida) from sea mounts
   Naoto Jimi, Hiroki Kise, Yoshihiro Fujiwara.
5. A new species of Sinagogidae from sea mounts
   Naoto Jimi, Itaru Kobayashi, Yoshihiro Fujiwara.

KAWATO, Masaru

Masaru KAWATO, Takao YOSHIDA et al.
eDNA metabarcoding of deep-sea fish using optimized DNA extraction and amplification methods for deep-sea water

During KM21-E04C cruise, several water samplings (CTD/Niskin, CTD/MASS Pump, Lander/Niskin and Lander/MASS pump) at two sites (South off Genroku Seamount and Anei Seamount) and following water filtration procedure were conducted. Using these filtrated water samples, we will carry out eDNA metabarcoding of fish by means of modified DNA extraction and PCR amplification protocols optimized for eDNA derived from deep-sea water (Kawato et al., 2021). Objectives of this barcoding were 1) to ascertain the extent of contamination of exogenous DNA during the filtration procedure on board, 2) to evaluate the effect of inhibitory substances on PCR amplification, 3) to compare the barcoding data among eDNA samples collected by different sampling devices and 4) to provide diversity information of deep-sea fish living at each site based on eDNA metabarcoding data using MiFish universal primers.

Masaru KAWATO, Takao YOSHIDA, Yoshihiro FUJIWARA, Shinji TSUCHIDA et al.
Search for an 'unknown' alepocephalid fish detected by MiFish eDNA metabarcoding using water samples collected at the bottom of Shotoku Seamount

A remarkable fish DNA sequence which has quite low homology with all alepocephalid species, was detected in MiFish eDNA metabarcoding from deep-water samples collected at the bottom of Shotoku Seamount in the previous KM20-10C cruise. In the next survey around this site, we will try to clarify existence and identity of this unknown alepocephalid fish by means of both eDNA metabarcoding and in situ observations (baited camera and ROV camera).

KISE, Hiroki

***Taxonomic study of* the subclass Anthozoa collected by ROV during KM21-E04C cruise**

Seamounts are topologically complex and influence the distribution and abundance of marine organisms (Rowden et al. 2010). In addition, seamounts are considered as biodiversity hotspot with high levels of endemism (Chivers et al. 2013). However, few studies on assemblage of marine invertebrates in the Nishi-shichi-tou Ridge in Japanese waters have performed. Basic information such as taxonomy is needed to better understand total diversity in this regioin.

In this study, we focused on the class Anthozoa (Cnidaria) and phylum Porifera. The KM ROV on the RV Kaimei collected > 30 specimens consist of subclass Hexacorallia (Zoantharia, Actiniaria and Cerianthariа) and Octocorallia (Alcyonacea and Pennatulacea) and > 10 specimens of Hexactinellida sponge. Some specimens of Abyssoanthidae and Epizoanthidae (Hexacorallia: Zoantharia), Actiniaria (Hexacorallia), and Stolonifera (Octocorallia) are considered as undescribed species, requiring further examination. Following list is potential papers from KM21-E04C.

1. Two new species of Zoantharia from seamounts in the Japanese waters.
2. Anthozoa fauna of the Nishi-shichi-tou Ridge.
3. Dominant Stylasteridae communities among seamounts in the Nishi-shichi-tou Ridge.
4. Cerianthidae from seamounts near Japan with unusual epibiotic associations.
5. A new species of Stolonifera from Anei Seamount.
6. New record of Anthoptilidae from the northwest Pacific Ocean.
7. A new species of glass sponge-associated actiniarians from Anei Seamount.

References

Chivers AJ, Narayanaswamy BE, Lamont PA, Dale A, Turnewitsch R (2013) Changes in polychaete standing stock and diversity on the northern side of Senghor Seamount (NE Atlantic). Biogeosciences 10: 3535– 3546.

Rowden AA, Dower JF, Schlacher TA, Consalvey M, Clark MR (2010) Paradigms in seamount ecology: fact, fiction, and future. Marine Ecology 31: 226–241.

KITAHASHI, Tomo

Meiofaunal diversity

Tomo Kitahashi (JAMSTEC), Shuhei Ota (NIES), Hiroshi Koshikawa (NIES), Masanobu Kawachi (NIES), Motohiro Shimanaga (Kumamoto Univ.), Shigeaki Kojima (AORI)

Meiofauna, defined as small benthic invertebrates that pass through a 500–1000 mm sieve and are retained on a 32–63 mm sieve, are important components of deep-sea benthic ecosystems because they are more abundant than larger macro- and megafauna and have a considerable influence on sediment nutrient cycling and stability. Therefore, they are good biological indicators of anthropogenic and natural disturbances. However, traditional methods of investigating meiofauna include individually counting and identifying small specimens under a microscope. This approach is both labor intensive and time consuming. Therefore, alternative methods that allow high volumes of meiobenthic samples to be processed are needed, particularly when monitoring spatial and temporal variations in ocean ecosystems. Metabarcoding analysis focused on environmental DNA (eDNA) have recently been used to examine deep-sea meiofaunal diversity and communities (e.g., Kitahashi et al. 2020). In addition, a new method for investigating meiofauna using an imaging flow cytometer (FlowCAM) was developed (Kitahashi et al. 2018).

During this cruise, we obtained 3 sediment samples using a push corer which was operated by the manipulator of KM-ROV and a corer installed on a Lander system at the Genroku and Anei Seamounts. We will investigate meiofaunal assemblages using traditional, metabarcoding, and imaging methods and compare the results. This study will lead to establish a rapid and readily methods to investigate meiofaunal diversity.

Reference:

Kitahashi, T., Sugime, S., Inomata, K., Nishijima, M., Kato, S., Yamamoto, H., 2020. Meiofaunal diversity at a seamount in the Pacific Ocean: A comprehensive study using environmental DNA and RNA. Deep-Sea Research I. 160, 103253.

Kitahashi, T., Watanabe-Kayama, H., Tsuchiya, M., Yamamoto, H., Yamamoto, H., 2018. A new method for acquiring images of meiobenthic images using the FlowCAM. MethodsX 5, 1330–1335.

KOEDA, Keita

On the basis of the cruse approximately 50 species of deep-sea fishes were observed by using KM-ROV, AUV “YOUZAN”, and baited camera. A single specimen of *Plectrogenium* sp. (Plectrogenidae) was collected by ROV in rocky bottom at 491 m depth on the Houei Seamount. A single specimen of *Ceratoscopelus townsendi* (Myctophidae), 17 specimens of *Simenchelys parasitica* (Synaphobranchidae) were collected as bycatch of CTD, baited camera, respectively. In

addition, a tissue sample of Synaphobranchidae was collected in the ROV operation. The movies, captured photographs, and specimens will be identified to the lower level as possible, to clarify the biodiversity of the seamounts. Therefore, four projects are planned herein by using these materials.

Project 1: Fish fauna of Japanese seamounts
Keita Koeda and Yoshihiro Fujiwara

Project 2: New distributional records of deep-sea fishes from Japanese seamounts
Keita Koeda, Mizuki Matsunuma, and Yoshihiro Fujiwara

Project 3: Ecology of *Hydrolagus purpurescens* (Chimaeridae)
Keita Koeda, Akinori Teramura, Shinji Tsuchida, and Yoshihiro Fujiwara

Project 4: Population structure analysis of *Simenchelys parasitica* (Synaphobranchidae)
Keita Koeda, Nozomu Muto, and Yoshihiro Fujiwara

KOMAI, Tomoyuki

1. Taxonomic study of decapod crustaceans collected by ROV during KM20-10 cruise

The decapod crustacean fauna of the Nishi-shichi-tou Ridge is poorly known. The Kaimei cruises will contribute to document the fauna on the basis of material collected by ROV operation and video records and images made by the Kaimei ROV and AUV "Youzan". Material of decapod crustaceans collected during KM21-E04C cruise has been preliminary identified. The following 12 species are identified amongst specimens collected by ROV operation. The unidentified species might represent undescribed species, requiring further examination. Some rarely collected species are also included, of which two individuals representing two species, *Homeryon armarium* and *Sternostylus* sp. are kept alive and will be transferred to the Shin-Enoshima Aquarium for rearing. Specimens for DNA extraction have been preserved for representatives of every species for accumulation of sequence data for species identification and eDNA metabarcoding.

Infraorder Stenopodidea
Family Stenopodidae
***Spongicoloides* sp.**: Kaimei ROV dive #157, 1 male, 1 ovigerous female.
Remarks. A hererosexual pair was collected from a large colony of hexactinellid sponge *Euprectella* sp. It is likely that the specimens represent *S. wejiaensis* Xu, Zhou & Wang, 2017, described from near the Weijia Guyot in the Magellan Seamount Chain, North-West Pacific, at depth of 2279 m (Xu et al. 2017).

Infraorder Polychelida
***Homeryon armarium* Galil, 2000:** Kaimei ROV dive #156, 1 specimen.
Remarks. This species has been rarely collected, represented by the two type specimens from the Kyushu-Palau Ridge (Baba et al. 1986; Galil 2000) and one additional specimen from the Nikko

Seamount, North Mariana Ridge (Komai & Tsuchida 2014). Individuals of this species are occasionally seen in video records by the AUV "Youzan" made during the present cruise, suggesting that the species normally inhabits rocky bottom.

Infraorder Axiidea
***Eiconaxius* sp.**: Kaimei ROV dive #156, 1 ovigerous female.
Remarks. The present specimen inhabited in colony of an unidentified species of farreid sponge.

Infraorder Anomura
Superfamily Chirostyloidea
Family Eumunididae
***Eumunida* sp.**: Kaimei ROV dive #156, 1 specimen.
Remarks. The present specimen was collected together with colony of an unidentified farreid sponge.

Family Sternostylidae
***Sternostylus* sp.**: Kaimei ROV dive #156, 1 specimen.

Superfamily Galatheoidea
Family Munidopsidae
***Munidopsis* sp. 1**: Kaimei ROV dive #155, 1 specimen.

***Munidopsis* sp. 2:** Kaimei ROV dive #156, 1 specimen.
Remarks. The present specimen was associated with colony of an unidentified species of farreid sponge.

***Munidopsis* sp. 3:** Kaimei ROV dive #157, 1 specimen.

Superfamily Paguroidea
Family Parapaguridae
***Parapagurus* sp. 1**: Kaimei ROV dive #153, 2 ovigerous females.

***Parapagurus* sp. 2**: Kaimei ROV dive #156, 1 ovigerous female.

Family Paguridae
***Michelopagurus limaturus* (Henderson, 1888)**: Kaimei ROV dive #156, 1 specimen.

***Paguridae* gen. sp.**: Kaimei ROV dive #155, 1 specimen.

2. eDNA metabarcoding for malacostracan crustaceans

Filtered sea-water samples were collected at four stations by means of CTD and at one station by means of mass pump installed on a lander for eDNA metabarcoding of macro-invertebrates and fishes. We will test the possibility to detect eDNA of malacostracan crustaceans by using MiDeca primers (Komai et al. 2019) from these sea water samples.

Preliminary analyses using CTD samples made during KM20-10C cruise failed to detect benthic malacostracan species except for the bythograeid crab *Gandalfus yonohana*, which is endemic to hydrothermal vents. It is urgent need to establish how to effectively sample sea-water near bottom for detection of macro- or mega benthos.

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Baba, K., Hayashi, K.-I. & Toriyama, M. (1986) Decapod Crustaceans from Continental Shelf and Slope around Japan: The Intensive Research of Unexploited Fishery Resources on Continental Slopes. Japan Fisheries Resource Conservation Association, Tokyo, 336 pp.

Galil, B. (2000) Crustacea Decapoda: Review of the genera and species of the family Polychelidae Wood-Mason, 1874. In: Crosnier, A. (Ed.), Résultats des Campagnes MUSORSTOM. Vol. 21. Mémoires du Muséum national d'Histoire naturelle 184: 285–387.

Komai, T., R. Gotoh, T. Sado & M. Miya (2019) Development of a new set of PCR primers for eDNA metabarcoding from decapod crustaceans. Metabarcoding and Metagenomics 3: 1–19. DOI 10.3897/mbmg.3.33835.

Komai, T. & S. Tsuchida (2014) Deep-sea decapod crustaceans (Caridea, Polychelida, Anomura and Brachyura) collected from the Nikko Seamounts, Mariana Arc, using a remote operated vehicle (ROV) "Hyper-Dolphin". Zootaxa 3764: 279–316.

Xu, P., Zhou, Y. & Wang, C. (2017) A new species of deep-sea sponge-associated shrimp from the North-West Pacific (Decapoda, Stenopodidea, Spongicolidae). ZooKeys 685: 1–14.

SUNAMURA, Michinari

Diversity and functional gene characteristics of microbial community in bottom water and sediment around seamounts in the Izu-Shichito Ridge, Japan, as a reflection of ecosystems of deepsea marine protected area

Koji Hamasaki, Michinari Sunamura, Kazutoshi Yoshitake and Hideto Takami

The Aichi Targets, agreed at the 10th Conference of the Parties to the Convention on Biological Diversity, set the goal of properly managing and conserving at least 10% of coastal and marine areas by 2020. Recently, Japan's marine protected areas reached 13.3% after newly designating offshore marine protected areas based on the revised Natural Environment Conservation Law enacted in April 2019. Four areas were designated at the Izu-Shichito Ridge, the Izu-Ogasawara Trench, Mariana Ridge and Mariana Trench and required for the management and conservation of biodiversity. It's become clear that the deep-sea far from the land is also affected by climate change caused by human activities. In addition, various anthropogenic factors such as the development of seafloor resources (hydrothermal deposits, methane hydrate etc.), deep-sea towing net fishing, decrease in dissolved oxygen concentration due to stagnation of deep circulation, and accumulation of garbage are threatening the deep-sea ecosystems and biodiversity. Offshore marine protected areas are being established to mitigate the deterioration of the deep-sea environment caused by such human activities, but it is necessary to monitor the secular change of biodiversity in order to

evaluate its effectiveness. However, the offshore/deep-sea seafloor area is more difficult to investigate and observe than the coastal area and offshore surface area, and it is currently difficult to efficiently grasp such fluctuations.

Prokaryotes such as bacteria and archaea that live in the environment have overwhelming biomass and functional diversity and play a major role in the maintenance of ecosystems. In addition, changes in its composition and function are also good indicators of environmental changes. In recent years, as a method for grasping their diversity and function, an analysis of microbial community genomes has been widely used, in which DNA is directly extracted from biological cells contained in seawater or sediments and the number of species and functions are estimated from the sequence information. Hence, the analysis of prokaryote community genomes can be used as monitoring the change of ecosystems and inhabiting macroorganisms with these microbes. For marine prokaryotes in surface water, the database for identifying taxa and functions is being enhanced due to the rapid accumulation of data. However, the data of deep-sea prokaryotes is overwhelmingly insufficient, that is the preparation of baseline data showing the current situation and reference data such as gene sequences that identify individual species are still largely limited.

Here we address the question whether we can relate prokaryote community diversity to macrofaunal biodiversity in seamount areas. The purpose of this study is to describe the diversity and functional gene characteristics of prokaryotic community in bottom water and sediment and compare them with biodiversity of benthic macrofauna around seamounts located at Izu-Shichito Ridge, one of the four offshore marine protected areas of Japan. We describe the diversity, community structure, and population density of prokaryotic community in the water samples collected from 1-100 m above the bottom and sediments samples by means of SSU rRNA gene amplicon sequencing. Also, the functional gene characteristics of these samples are described by means of metagenomic sequencing. Together with the physical and chemical environment of sediment samples, these data will also contribute to characterize microbial community of this area and will be baseline datasets to evaluate spaciotemporal change in microbial community during the future monitoring of deep-sea marine protected areas.

WU, Qianqian

Evaluating the diversity deep-sea invertebrates of each seamount using environmental DNA (eDNA) metabarcoding analysis

To estimate the diversity of deep-sea invertebrates of each seamount using eDNA metabarcoding analysis, deep-sea water was collected using CTD with Niskin water samplers. Approximately 30 L of each water sample was filtered through a sterivex (pore size, 45 nm) using a peristaltic pump. After filtration, sterivex was filled with ATL and then stored at -80°C. In addition, the biological specimens at each sampling site were collected through the slurp gun installed on the ROV.
To evaluate the diversity of deep-sea invertebrates of each seamount, the following projects for each taxonomic group (include: Scyphozoa; Anthozoa; Cephalopoda, Crinoidea; Ophiuroidea) will be conducted in the future.

Project 1: To supplement the database for eDNA metabarcoding analysis, the sequence of biological specimens

collected during this voyage will be investigated.
Qianqian Wu, Hiroki Kise, Tomoyuki Komai, Yoshihiro Fujiwara, Masanori Okanishi, Kensuke Yanagi, James Davis Reimer, Masaki Miya, Toshifumi Minamoto

Project 2: Evaluate the biodiversity of each seamount via the universal primers of each taxonomic group with eDNA metabarcoding analysis.
Qianqian Wu, Hiroki Kise, Masanori Okanishi, Kensuke Yanagi, James Davis Reimer, Tomoyuki Komai, Takao Yoshida, Yoshihiro Fujiwara, Masaki Miya, Toshifumi Minamoto

5. Acknowledgement

We thank the captain and crew of the R/V *Kaimei* and the operation team of ROV *KM-ROV* for organizing the cruise and conducting the diving research and sampling. We also thank marine technicians from Nippon Marine Enterprises and Marine Work Japan for research support and ground stuff from Japan Agency for Marine-Earth Science and Technology for all the procedures to establish this cruise. This cruise was supported by the Environment Research and Technology Development Fund (JPMEERF20S20700) of the Environmental Restoration and Conservation Agency of Japan.

6. Notice on Using