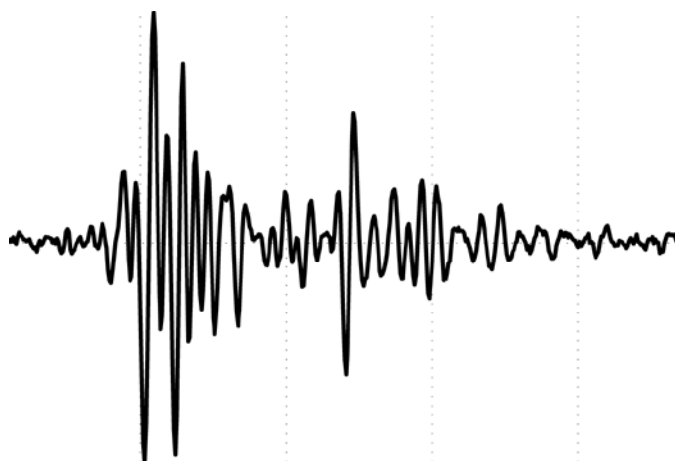


# R/V Natsushima cruise report

NT12-05

Takuyo-Daigo Seamount



27<sup>th</sup> February - 12<sup>th</sup> March, 2012

Chief Scientist: Blair Thornton

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## Preface

This report describes the dives of the ROV HyperDolphin at Takuyo-Daigo seamount during the NT12-05 cruise of the R/V Natsushima between 27th February and 12th March, 2012.

The NT12-05 cruise was conducted based on the proposal #S10-68 titled “A study on a novel hydrothermal ore mine survey by in situ multi parameter measurements”, proposed by Blair Thornton of The University of Tokyo.

The main objective of the cruise was to investigate the application of acoustic and visual instruments to perform in-situ measurements of the volumetric distribution of manganese crusts. In particular, the emphasis was to test newly developed instruments on the slopes of the seamount, to make continuous measurements over long distances and survey a large range of depths.

Due to unfavorable sea conditions, only 3 of the originally planned 7 dives could be performed. However, successful measurements of manganese crust thickness could be made on steep slopes of up to 30 degrees, and continuous measurements were made over a distance of 3.5km, covering a depth range of 700m (2100 to 1400m). The data obtained is not only valuable in terms of information concerning the distribution of manganese crusts on the seamount, but also provides valuable insights to guide future developments of the mapping instruments and sampling tools used during this cruise.

I would like to thank the crewmembers of the R/V Natsushima lead by Captain Yoshiyuki Nakamura, the members of the ROV HyperDolphin operation team lead by Yoshinari Ono, marine technician Masashi Ito and Yuta Yamamuro together with the staff of JAMSTEC and Nippon Marine Enterprise for their dedicated efforts which contributed greatly to the success of this cruise.

April 2012  
Blair Thornton (NT12-05 Chief Scientist)

## Notice on use

This cruise report is a preliminary document prepared during the cruise, and its content may be changed/corrected without notice. Regarding the use of information contained within this report, please contact the chief scientist for up to date details.

For further details concerning the methods and instruments used, please refer to the following publications or contact the chief scientist directly. A more detailed document concerning the instruments and methods is currently under journal review, and is expected to be published during 2012.

[1] Blair Thornton, Adrian Bodenmann, Akira Asada, Tamaki Ura, Mehul Sangekar, Katsumi, Ohira, Daigo Kirimura, 'Acoustic , visual survey of manganese crusts using an underwater vehicle at Takuyo-Daigo seamount', In. Proc. Underwater Technology 2011, Tokyo, 1028, 2011.

[2] Adrian Bodenmann, Blair Thornton, Takeshi Nakatani, Tamaki Ura, '3D colour reconstructions of a hydrothermally active area using an underwater robot', In Proc. IEEE/MTS Oceans'11 Kona, 110422-033, 2011.

[3] Blair Thornton, Akira Asada, Tamaki Ura, Katsumi Ohira, Daigo Kirimura, 'The development of an acoustic probe to measure the thickness of manganese crusts', In Proc. IEEE/MTS Oceans'10 Sydney, 2010.

[4] Adrian Bodenmann, Blair Thornton , Tamaki Ura, ' 3D Mapping of the Seafloor in Color Using a Single Camera: Benthic Mapping Based on Video Recordings , Laser Profiling To Generate Colored 3D Reconstructions of the Seafloor.' Sea technology, Vol. 51. No. 12, pp 51-53, 2010.

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## 1. Cruise information

<b>Cruise ID:</b>	NT12-05
<b>Research vessel:</b>	R/V Natsushima
<b>Title of cruise:</b>	FY2011 Deep sea survey by the ROV HyperDolphin at Takuyo-Daigo seamount
<b>Title of proposal:</b>	Investigation of acoustic and visual instruments to measure the distribution of manganese crusts using an underwater robot
<b>Cruise period:</b>	February 27, 2012 (Saipan) March 12, 2012 (Naha, Okinawa) (15days)
<b>Survey site:</b>	Takuyo-Daigo seamount

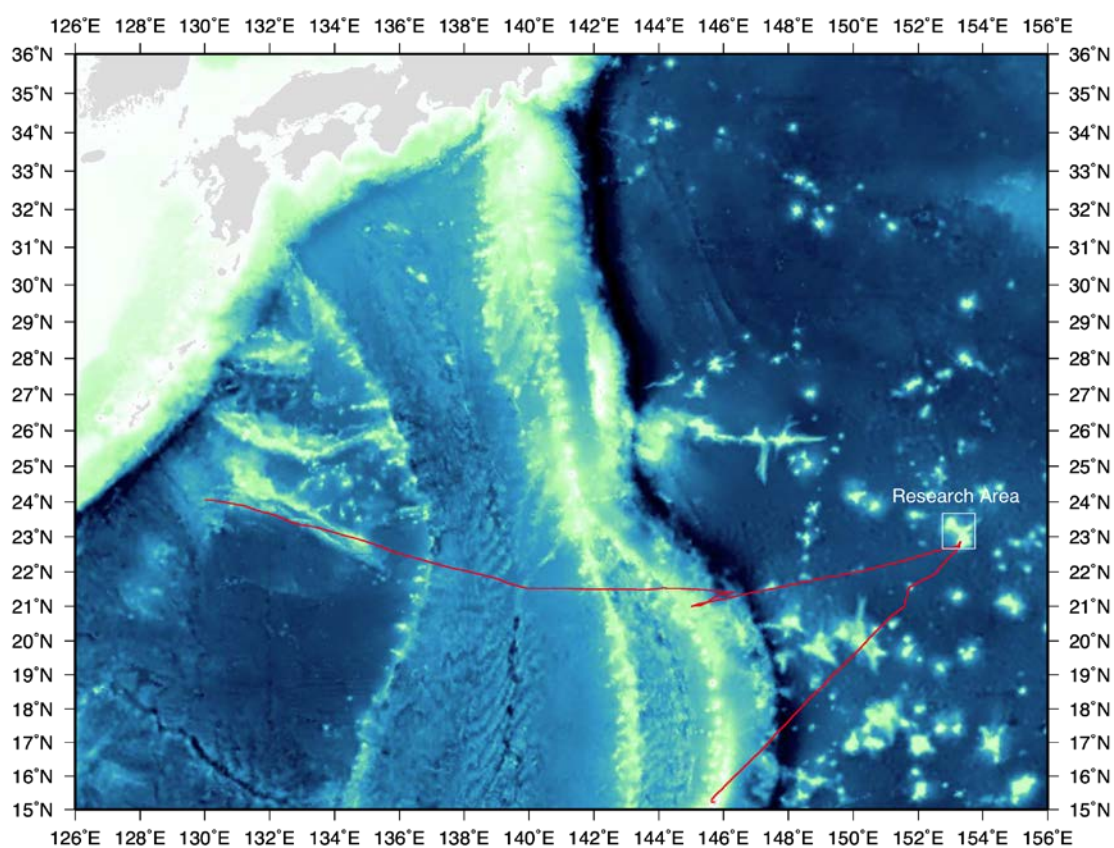


Fig. 1 R/V Natsushima track during NT12-05

## 1.1 Overview of observations

A survey of manganese crust deposits was performed at Takuyo-Daigo seamount (23°00'N, 153°20'E), northwest Pacific using the *ROV Hyper Dolphin* and the *R/V Natsushima* between February 27, 2012 and March 12, 2012. The main purpose of the cruise was to investigate the performance of acoustic and visual instruments, developed under the 'Program for the development of fundamental tools for the utilization of marine resources' of the Japanese Ministry of Education. The instruments have been developed to perform high resolution surveys of the volumetric distribution of manganese crusts from an underwater vehicle, and consist of an acoustic device, developed to perform continuous sub-surface measurements of manganese crust thickness at depths of up to 3000m, and a vision based mapping system that generates millimeter order resolution three-dimensional (3D) reconstructions of the seafloor in actual colours. The ultimate goal of the measurements is to accurately determine the volumetric distribution of exposed manganese crusts over a wide survey area. The instruments were mounted on-board the ROV Hyper-Dolphin of the Japan Agency for Marine-Earth Science and Technology (JAMSTEC), and were deployed at Takuyo-Daigo seamount. During the survey, the ROV was operated at depths between 1400m and 3000m at low altitudes of 1.5~2.5m on the slopes of the seamount to survey the area both acoustically and visually. It is considered that combining the proposed remote sensing system with conventional survey methods, such as shipboard multibeam, ROV sampling and drilling, can potentially accelerate manganese crust survey and lead to more accurate estimation of the volume of mineral resources over a large scale, as well as contribute to a better understanding of crust deposits from a scientific perspective on both local and regional scales.

During our previous cruise, NT10-11, acoustic measurements of the thickness of manganese crusts were successfully achieved for the first time. However, a number of important issues were also highlighted, which we aimed to overcome during this cruise. First, the previous acoustic system had a maximum range of 1m. This is sufficient for measurements on flat areas, but on steep slopes it is not possible to maintain such low altitudes with an ROV. To overcome this point, an acoustic probe with a range of 2.5m was developed and tested during this cruise. In addition to increased range, the system deployed employs dynamic beam focusing to achieve more robust measurements over rough terrains. Second, with our previous system acoustic measurements could not be made on slopes exceeding 10 degrees. In order to facilitate acoustic measurements on slopes, an actuated gimbal was introduced to actively tilt the acoustic probe so that its beam enters the surface of the seafloor with a zero incidence angle, even on steep slopes and also compensate for vehicle pitching motion.

The acoustic and visual instruments were mounted on the ROV Hyper Dolphin together with an underwater rotary blade, designed to assist sampling of manganese crusts, a CTDO-pH sensor, fluorescence and turbidity meter and a water-sampling device. Three out of a total seven planned dives were performed, with four dives cancelled due to bad weather. A total of 13 crust/substrate samples, 3 sediment cores and 24 seawater samples were obtained from various depths on the seamount. These have been divided and prepared into samples for chemical, mineralogical and microbiological investigation. During the dives, measurements and sampling were performed on a ridge at the south west of the seamount between depths of 3000m and

1400m. However, due to limited time and failure of an underwater electrical cable at high pressure, it was not possible to obtain data over the full range of depths. During HPD#1352, an underwater cable failed at a depth of 2200m, which prevented visual mapping and vehicle navigation measurements. However, acoustic measurements and sampling could still be successfully performed. It was not possible to identify the cable that failed since the cable was only affected at pressure, so the electrical system was reconfigured so that the exact cable causing the problem could be identified during the next dive. During the first half of HPD#1353, measurements could be performed, but half way through the dive, the cable connected to the navigation system failed at around 1900m. After this dive it was possible to identify the cable causing trouble and replace it. During HPD#1354, data was obtained continuously between 2100m and 1400m depth, covering a lateral distance of distance of 3.5km.

The mapping data obtained during this cruise is not only valuable in terms of information concerning the distribution of manganese crusts on the seamount, but also demonstrates that the system developed is capable of performing acoustic measurements of manganese crust thickness on slopes up to 30 degrees. This was possible mainly due to the gimbal mechanism introduced to control the direction of the acoustic probe, and the increased measuring range and dynamic beam focusing capabilities of the acoustic probe itself. It could also be established that the 2.5m range of the probe was sufficient for operation of HyperDolphin on the slopes of the seamount. During this cruise, data was obtained through manual control of the gimbal mechanism based on measurements of the slope of the seafloor. Analysis of the mapping data will make it possible to develop an algorithm to automate this control based on realtime measurements of the slope of the seafloor. It is considered that an automatic gimbal control algorithm will further improve the quality of acoustic measurements as it will allow for more accurate gimbal control with significantly faster response compared to the manual control employed during this cruise. The combination of acoustic sub-surface measurements and the 3D visual reconstructions are found to be powerful tools to quantitatively assess the volumetric distribution of manganese crusts and it was demonstrated that this kind of acoustic and visual survey of manganese crusts, combined with sampling, forms an effective and powerful tool to better understand the nature of manganese crusts on a large scale. The experience gained during the dives will enable engineering feedback to make further improvements to the various payloads deployed during this cruise.

Finally, it should be mentioned that this cruise follows NT09-02 Leg 2 and NT10-11 as the third multi-disciplinary survey of manganese crust deposits at Takuyo-Daigo seamount. It is recognized that collaborative projects between ocean engineers, marine geophysicists and microbiologists such as on this cruise are highly productive for establishing a sound scientific and engineering background, and it is necessary that such collaborative cruises focusing on oceanic manganese crust deposits continue in the future.

## 1.2 Objectives

The main objective of this cruise was to test the performance of acoustic and visual mapping instruments designed to measure the thickness and distribution of manganese crusts. It is proposed that this system can be mounted on a mobile underwater platform, such as an autonomous underwater vehicle (AUV) or remotely operated vehicle (ROV), to continuously map the volumetric distribution of crusts remotely from low altitudes. The performance of the systems have previously been demonstrated during NT10-11 at Takuyo-Daigo seamount (Chief Scientist: Blair Thornton) on the flat top of the seamount.

On this cruise, the research party investigated the feasibility of extending the proposed survey strategy to the slopes of a seamount. The main motivation for this is to determine the distribution of crusts at different depths, since both changes in properties of seawater in the water column and the shape of the terrain itself are thought to play an important role in determining the thickness and distribution of manganese crusts. By combining the acoustic data with visual mapping data based on navigation information, it is possible to create a 3D colour reconstruction of the seafloor with multiple layers of information, as shown in figure 2, as a form of Geographic Information System (GIS). In addition to the investigation of the survey system, a major goal of this cruise was to perform sampling of manganese crust and their substrate from the outcrop of the seamount, and also obtain sediment and seawater samples over a range of depths for chemical, mineralogical and microbiological studies. For this, a rotary cutter was developed to aid in the sampling of manganese crusts. A CTDO-pH and a fluorescence and turbidity sensor were also deployed to measure the properties of the seawater close to the seafloor over the surveyed range of depths. Another major objective of this cruise was to deploy a series of in situ microbial colonization devices at various depths on the seamount. These will be used to perform a long-term experiment to help determine the contribution of the role played by microbes in manganese crust deposition. It is hoped that the efforts during this cruise, contribute to a better understanding of crust deposits from a scientific perspective and allow for more accurate estimation of the volume of mineral resources at Takuyo-Daigo seamount.

The research party consisted of four oceanic engineers, an acoustic engineer, a team of three geochemists and a microbiologist. The engineering group's main aim was to investigate the performance of the in-situ sensors and test the performance of the acoustic probe and mapping system in different seafloor morphologies in order to gain engineering feedback into future modifications for the systems. The aim of the geology team was to collect information to help describe the mode of geochemical and mineralogical variations on regional, small to microscopic scales in relation to depositional processes and geological environments. In order to achieve this, it is crucial that the rocks and manganese crusts are collected systematically by the ROV from known depths, and that the samples meet the following requisites;

- 1) the samples preserve their original structure,
- 2) seawater samples obtained in the same region are available for study,
- 3) the crust deposits are continuous from top to bottom,
- 4) the related topographic and oceanographic parameters such as CTDO-pH of the locations where the samples were obtained are known, and
- 5) the obtained samples are firmly rooted to the seafloor to be representative of the

region from which they were taken.

The samples will be subject to geochemical, mineralogical, and structural analyses at the University of Tokyo, Kochi University, and JAMSTEC, and it is planned that age dating will also be performed. The aim of the microbiologist was to obtain crust samples, and deploy colonization devices at various depths on the seamount. The colonization devices will remain on the seafloor for over a year, to perform long-term experiments to help determine the affinity of microbial species to crusts, and ultimately help determine the role played by microbes in the development of crusts.

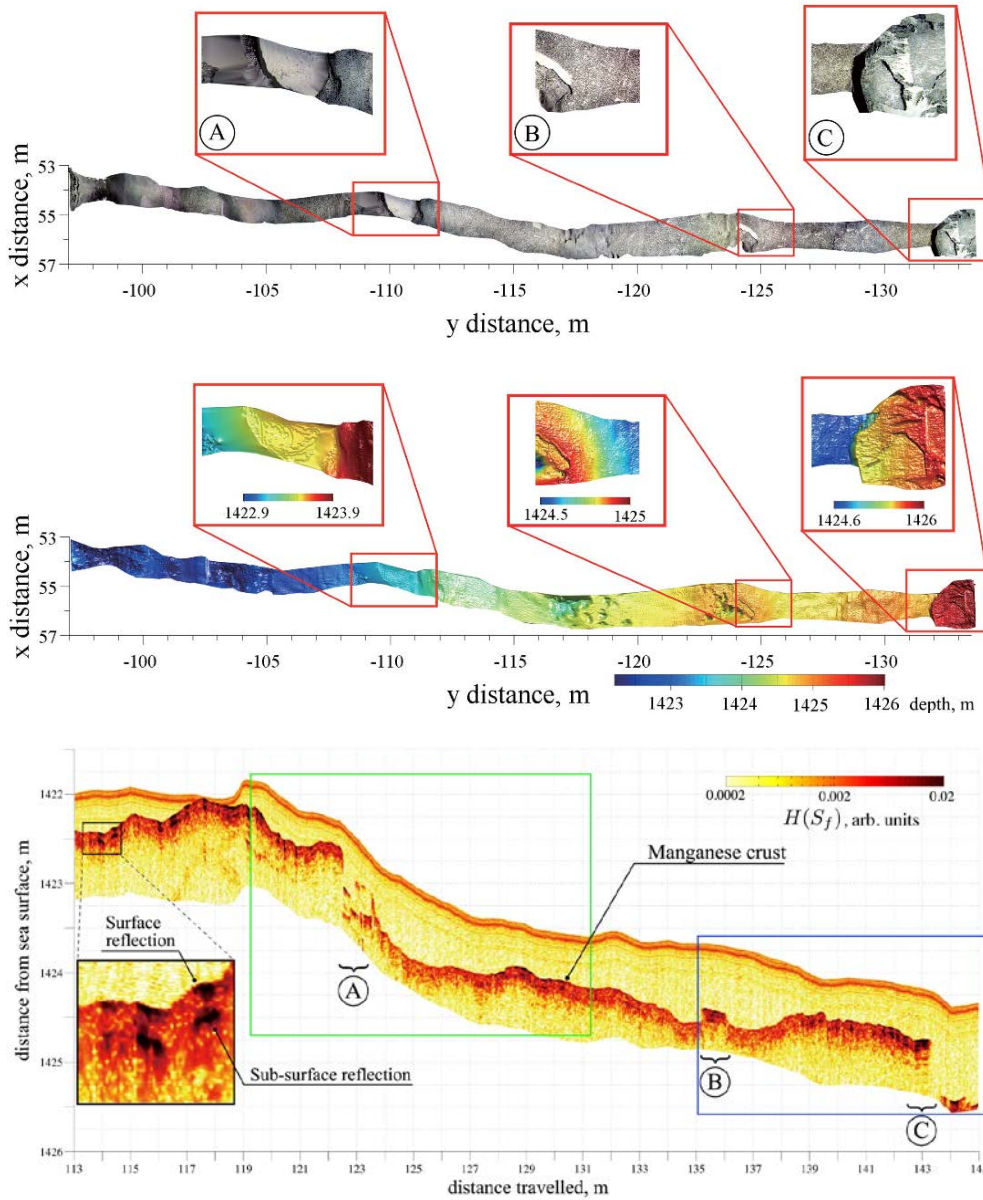


Fig. 2 3D reconstruction of manganese deposits based on acoustic sub-bottom and visual mapping data obtained during HPD#1144, NT10-11

## 2. Cruise Log

### 2.1 Survey area and time schedule

Three dives, HPD#1352 to HPD#1354 were performed in the areas shown in figure 3. A total of seven dives were originally planned, but could not be performed due to bad weather conditions. Table 1 shows the time schedule of the cruise.

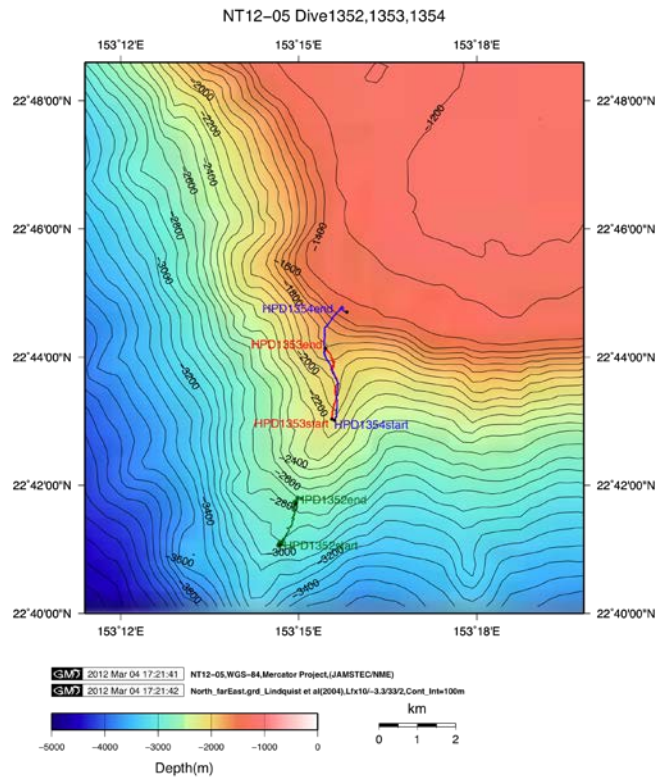


Fig. 3 Trajectories of HyperDolphin during NT12-05

Date	Local Time	Note
27.Feb.2012	10:00	Leave SAIPAN and proceed to research area
	11:00-11:30	Onboard education and safety training.
28 to 29.Feb.2012		Proceeding to research area
1.Mar.2012	6:30	Arrived at resaerch area.
	8:35 ~ 15:31	HPD#1352
2.Mar.2012	8:16 ~ 16:02	HPD#1353
3.Mar.2012	7:20 ~ 16:13	HPD#1354
	17:30	Suspended research work due to sea state
4 to 5.Mar.2012		Proceeding to 21-00.0N 145-00.0E
6 to 11.Mar.2012		Proceeding to NAHA
12.Mar.2012	8:00	Arrived at NAHA

Table. 1 Time schedule of NT12-05

## 2.2 Research party

Name	Affiliation
Blair Thornton*	Institute of Industrial Science, The University of Tokyo
Shingo Kato	Japan Collection of Microorganisms, RIKEN BioResource Center
Hisaaki Sato	Department of Earth Science, Kochi University
Ayaka Tokumaru	Department of Earth and Planetary Science, The University of Tokyo
Adrian Bodenmann	Institute of Industrial Science, The University of Tokyo
Takumi Sato	Department of Ocean Technology, Policy and Environment, The University of Tokyo
Daigo Kirimura	Link Laboratory Inc.
Yusuke Yano	Hakuyodo Co.Ltd
Koichi Iijima	Submarine Resources Research Project, Japan Agency for Marine-Earth Science and Technology (JAMSTEC)

Table. 2 Research party (\*chief scientist)

### 3. Instrumentation and methods

During this cruise, 7 different payloads were mounted on HyperDolphin. These can be grouped into sensors for realtime measurements, tools to facilitate sample and experimental setups to be deployment on the seafloor. During the dives the readings of the various sensors were monitored in the robot control room by the research party. Figure 4 shows HyperDolphin with the various payloads attached. Figure 5 shows the gimbaled acoustic probe and visual mapping system (seaXerocks) during a tank test.

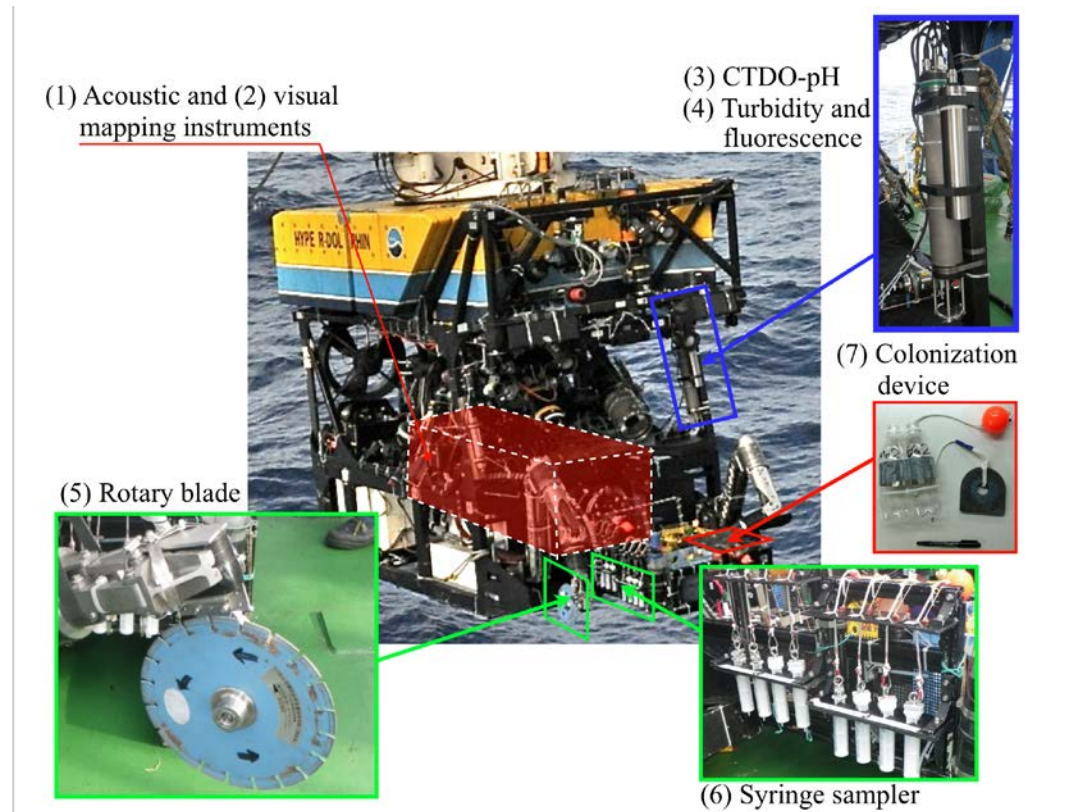


Fig. 4 Payloads mounted on HyperDolphin during NT12-05. (1) Acoustic probe for measurement of manganese crust thickness and (2) seaXerocks mapping device for 3D colour reconstruction of the seafloor and measurement of vehicle position mounted in the robot's payload box, see Fig. 5. (3) CTDO-pH and (4) Turbidity and fluorescence sensor. (5) Rotary blade to aid sampling of crusts, and (6) syringe water sampler. (7) Microbial colonization device.

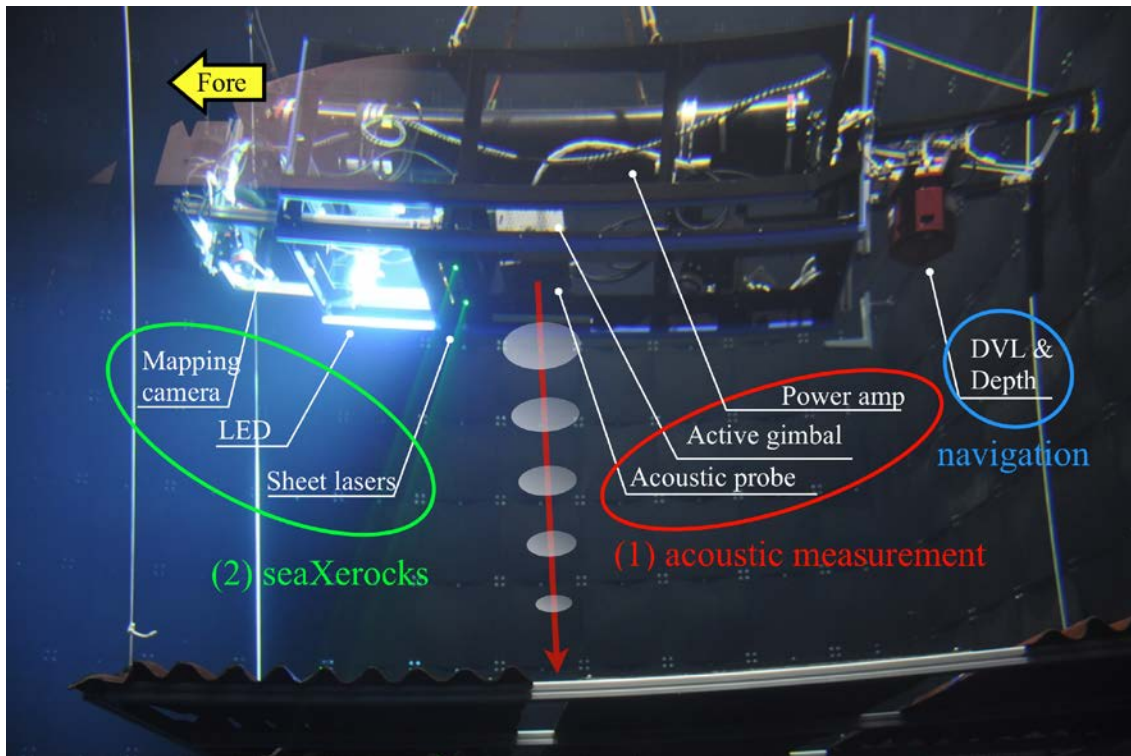


Fig. 5 Configuration of payload box showing, (1) the acoustic measurement system that consists of an acoustic probe, and active gimbal and power amplifier. (2) the seaXerocks 3D visual mapping system that consists of 2 mapping cameras, 2 LED panels and 2 sheet lasers. Both systems use a DVL and depth sensor information to determine the vehicle's position and orientation.

### 3.1 In situ measurement devices

#### 3.1.1 Gimbaled sub-surface acoustic probe

An acoustic probe has been developed to perform remote acoustic measurements of manganese crust thickness. The probe, pictured in figure 6, has a diameter of 220mm, a height of 200mm and weighs 12kgs in air (6kgs in water).



Fig. 6 Acoustic probe developed to measure manganese crust thickness

The transducer consists of an annular array of 2MHz 1-3 piezoelectric composite elements for transmission, with a 200kHz receiver array built-in along the same axis. The probe emits a 2MHz, high frequency amplitude modulated signal in order to generate a narrow 200kHz beam that penetrates the target. This penetrating beam has a footprint of diameter 18mm on a target 1500mm away, and has no side lobes. By performing dynamic beam focusing with the array, it is possible to automatically focus the acoustic beam onto targets at a range of between 500 and 2500mm. The echoes of the low frequency component are detected using the on-axis 200kHz receiver. The oil-filled probe design is pressure compensated to operate at depths of up to 3000m. The housing, which can be seen in figure 5 contains the control electronics and power amplifier, used to generate a 15kW signal to drive the acoustic probe. The acoustic probe was mounted on an actively controlled gimbal mechanism, as shown in Figure 7. During this cruise, the gimbal was operated manually based on measurements of range using HyperDolphin's range sensor, located at the fore end of the vehicle, and the DVL range measured at the aft end of the vehicle. Based on these range measurements, the relative slope of the seafloor was computed in realtime and the gimbal mechanism remotely controlled accordingly. Figure 8 shows some examples of single acoustic measurements made during HPD#1354 on various steepnesses of slope on the seamount. These measurements shown in the figure were made at various depths at remote locations on the seamount. Due to the active control of the gimbal and dynamic ranging of the acoustic beam, it was possible to obtain high quality reflections from seafloors sloped by up to 30 degrees. Further details of the system can be found in refs [1,3].

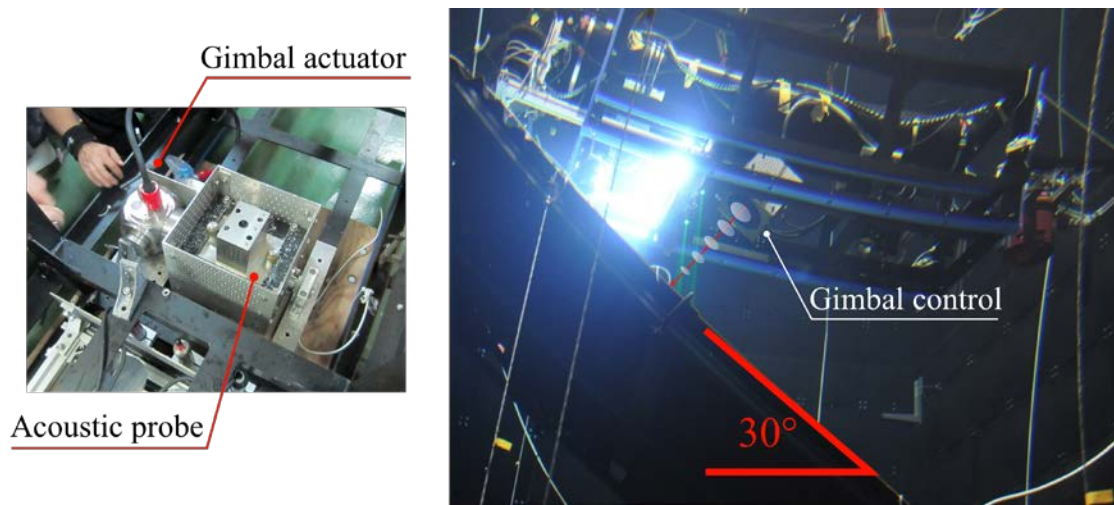


Fig. 7 Active gimbal control mechanism (left) and tank testing (right) demonstrating how the acoustic probe can be aligned so that its incident beam is perpendicular to the seafloor.

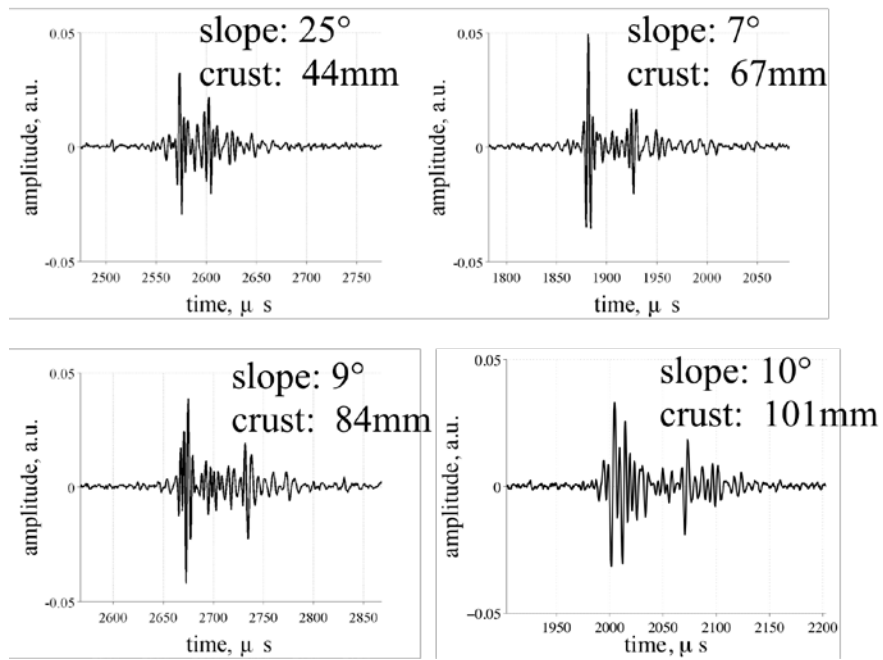


Fig. 8 Examples of acoustic measurements made on different slopes during HPD#1354. The measurements are from different depths and remote locations with respect to each other.

### 3.1.2 seaXerocks 3D visual mapping system

A mapping device was mounted on HyperDolphin in order to create high resolution 3D colour digital reconstructions of the seafloor. This data forms a basis to visualize the data recorded by the other payload sensors, i.e. the acoustic probe and the CTDO-pH sensor measurements, as a form of underwater GIS. The system consists of two sheet lasers, two cameras and two LED arrays mounted on HyperDolphin's payload box, configured as in figure 9. The sheet lasers project a single green laser line onto the seafloor perpendicular to the direction of motion of the vehicle and the projected images are captured by colour cameras mounted with translational and angular offsets with respect to the laser. The two cameras were mounted fore and aft of the lasers in order to prevent occlusions occurring on the steep, bumpy slopes of the seamount.

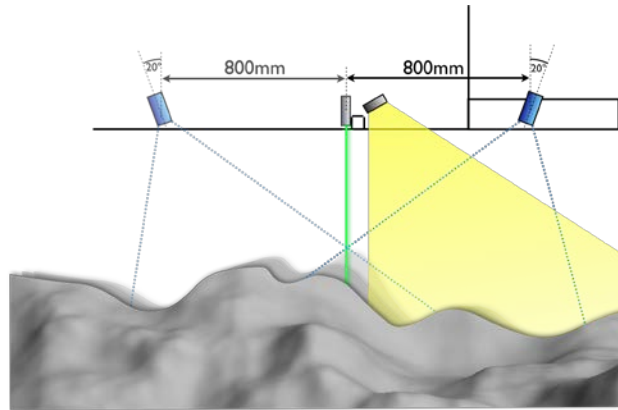


Fig. 9 Configuration of mapping device used for generation of digital 3D colour reconstructions of the seafloor

As the vehicle moves forwards, the projected laser line scans the shape of seafloor, and through triangulation of the laser projections captured by each camera, it is possible to generate a detailed 3D bathymetry of the seafloor based on the vehicles position. This system has been previously deployed on HyperDolphin during NT10-11 and KY11-02 leg 2 for manganese crust surveys at Takuyo-Daigo seamount and Ryusei seamount. It has also been applied to survey of active Hydrothermal vents in Okinawa (NT11-17) and on the AUV Tuna-Sand of the University of Tokyo in Kagoshima bay, see Figure 10.

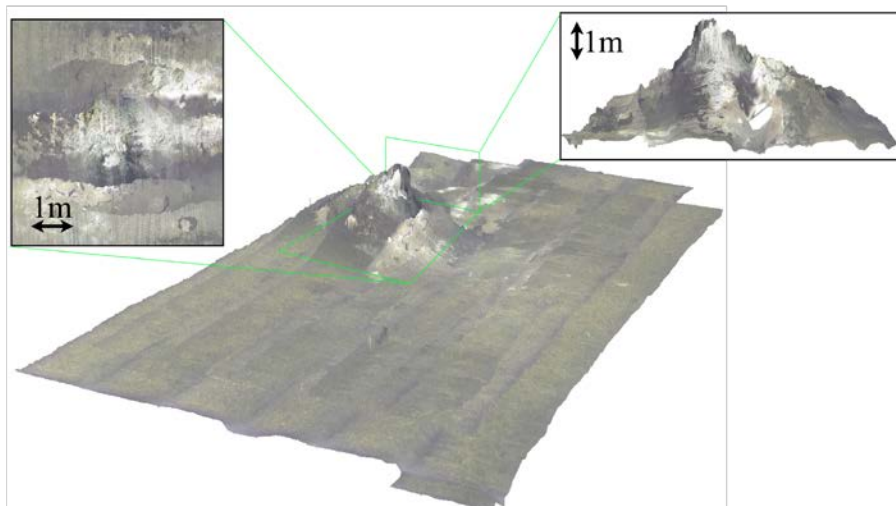


Fig. 10 3D reconstruction of White Cone Chimney at Kagoshima bay. The data was obtained using seaXerocks mounted on the AUV Tuna-Sand in 2010 [2]

Figure 11 shows examples of the raw images measured by the system, and figure 12 shows examples of some of the reconstructions of data measured during HPD#1354.

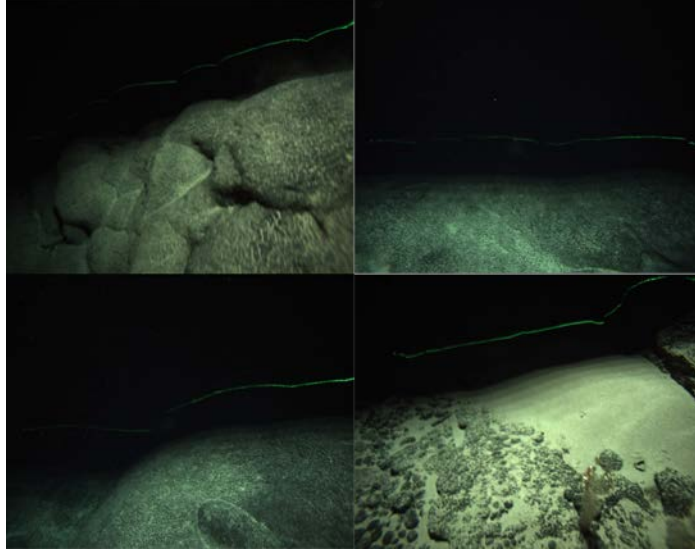


Fig. 11 Raw images obtained by seaXerocks during HPD#1354. Millimeter resolution bathymetry is obtained through triangulation of the projected laser line and colour information is obtained from the illuminated portion of the image using the pixel mapping algorithm developed by our group [4].

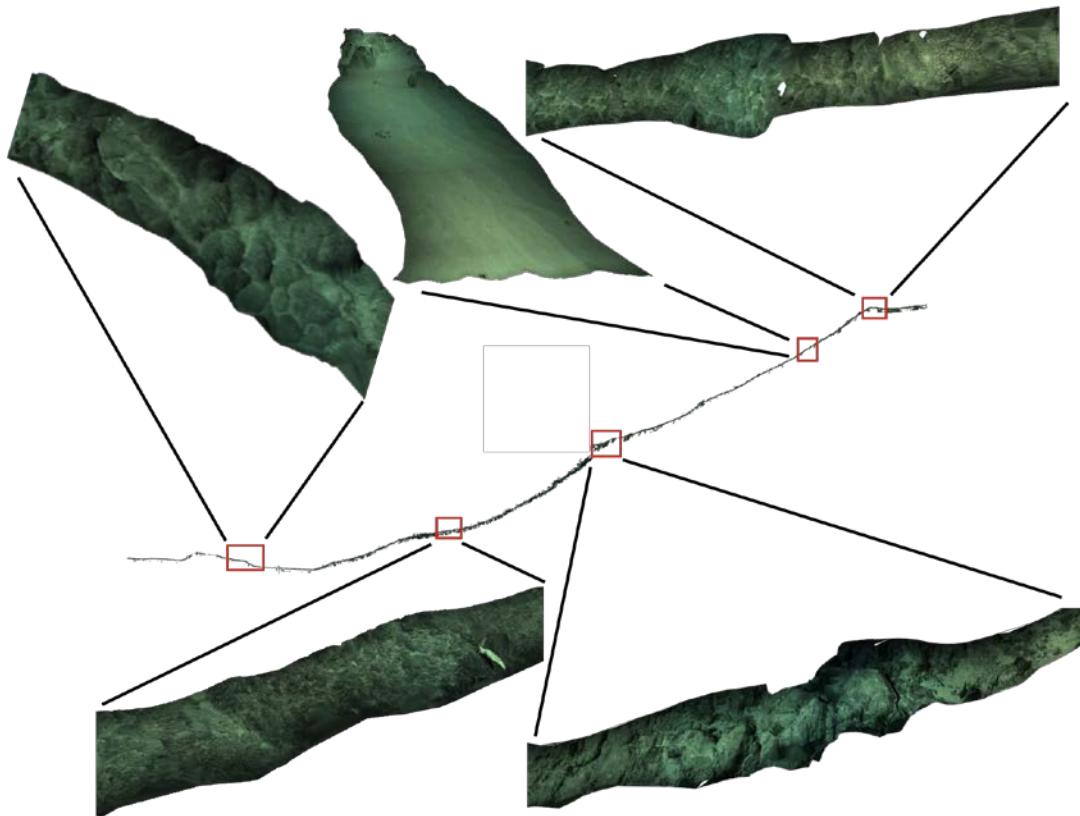


Fig. 12 Preliminary 3D reconstructions generated on-board the ship using data measured during HPD#1354. The data has not been filtered for noise and the colour correction, described in [2] has not been applied.

### 3.1.3 CTDO-pH

Two instruments were used to measure conductivity, temperature, depth, dissolved oxygen and pH. The Ocean seven 316Plus CTD multi-parameter probe was mounted to measure pressure, temperature, conductivity, oxygen and pH. It was attached on the front left side of HyperDolphin where there is no obstruction to the flow of seawater. Calibration with pH7 (6.86) buffer solution was conducted before the dives, at the beginning of the cruise. The pH sensor was also soaked in pH7 buffer solution, just before and after each of the dives for more accurate calibration. The RBR XR420 was attached to the other side of Hyper Dolphin to measure pressure and temperature. An optional sensor (Seapoint) was equipped to measure turbidity. It was attached where there was unobstructed flow of seawater and the effect of the lights of HyperDolphin are minimal (on front right side of the ROV). Vertical profiles of the seawater characteristics were obtained along the slope of the seamount and in the water column from the seawater surface to 3000m depth.

### 3.1.4 Fluorescence and turbidity

A C-Star Transmissometer for turbidity measurement and CDOM ECO-FL(RT)D (Environmental Characterization Optics (ECO) channel fluorometers) for fluorescence measurement (an optional sensors of the Ocean seven), were attached to front left side of HyperDolphin to minimize the effects of the ROV's lights. The Seapoint, an optional sensor of RBR, was used for turbidity measurement. Vertical profiles of the seawater characteristics were obtained along the slope of the seamount and in the water column from the seawater surface to 3000m depth.

## 3.2 Sampling tools

### 3.2.1 Rotary blade

A rotary blade has been developed to aid sampling of manganese crusts. The blade is used to make incisions into the crust and substrate, and a crowbar was used to extract samples directly from the outcrop. The main hydraulic unit of the ROV HyperDolphin supplies hydraulic pressure, and the flow can be controlled from the control room of the ROV. The specifications of the rotary blade are as shown in table 3.

Hydraulic pressure	13.7MPa (2,000psi)
Flow rate	20L/min
Maximum rotation	2,500rpm
Torque	15Nm
Power	1.5kW
Maximum blade diameter	300mm

Table 3 Specifications of the rotary blade

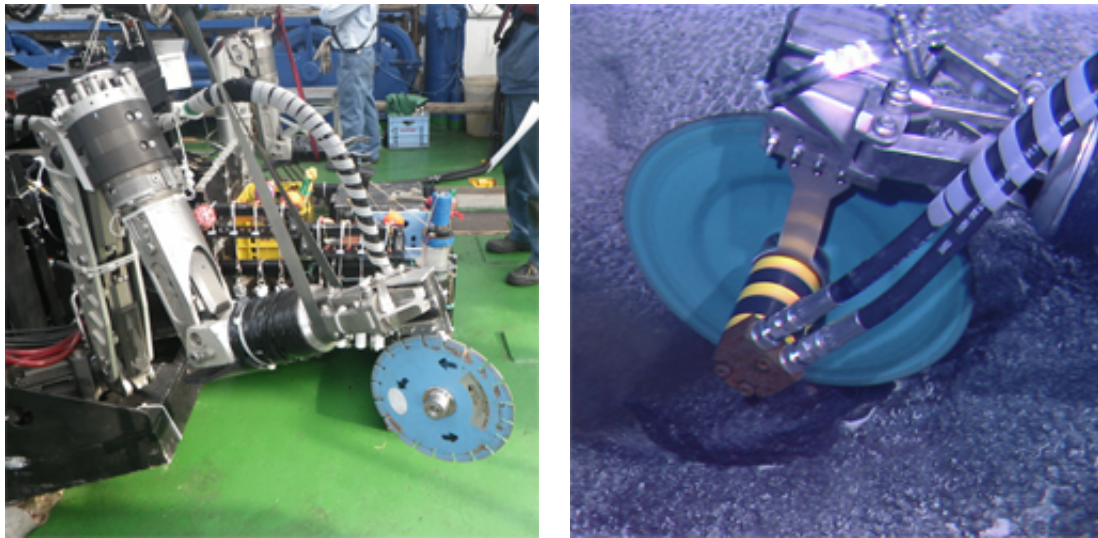


Fig. 13 Rotary cutter

In the first dive of this cruise (HPD #1352), a rotary blade with a diameter of 250mm was used in order to test the torque of hydraulic motor. For the second and third dives (HPD #1353-#1354) a rotary blade with a diameter of 300mm was adopted to allow for deeper incisions to be made. Although rock sampling using the rotary cutter was successful and allowed samples to be obtained from the outcrop, some modifications are necessary in the future. The major issue is that the blade jams and stops rotating when load from the side acts on it. This occurs when the manipulator holding the rock cutter moves laterally with respect to the incision. It is important to move the manipulator only in the cutting plane, which is difficult with a multi-degree of freedom manipulator such as those of HyperDolphin. In order to overcome this problem, a linear actuation mechanism will be introduced in our future work to enable the blade to be moved linearly while the manipulator itself remains fixed.

### 3.2.2 Syringe sampler

A syringe sampler, consisting of 8, all plastic 60ml syringes was attached to HyperDolphin to obtain water samples at various depths at low altitudes from the seamount. The device is operated using HyperDolphin's manipulators to pull a cord connected to each syringe to sample seawater. One of the major objectives of the water sampling was to study potential mechanisms for manganese crust formation. It is considered that manganese crusts grow either through precipitation of manganese oxidization particles at the surface of the crusts, or by sedimentation of manganese oxidization particles precipitated in seawater the promote the growth of the crusts. Deep seawater, except for hydrothermal plumes or in ridges, are thought to have little turbidity. However, if manganese crusts are formed by sedimentation of particles, the seawater near the seamount should contain nanoparticles. In addition, chemical and physical information of the seawater near the seamount has not been studied sufficiently to understand the relation between manganese crusts and seawater.

The main purpose of the water sampling on this cruise was (1) to try to collect nanoparticles which potentially form manganese crusts and (2) compare the REEs concentration of manganese crusts and seawater near the outcrop, which would lead to a better understanding of the formation of manganese crusts.

### 3.3 Long-term microbial observations

Microorganisms may play an important role on metal cycling between seawater and crusts. Our previous study indicated that the abundant and diverse prokaryotes are present on the manganese crusts collected from Takuyo-Daigo Seamount in NT09-02 cruise by culture-independent molecular biological analyses (Nitahara et al., 2011). However, the physiological characteristics of the detected prokaryotes cannot be identified from their phylogeny. It is not yet known if they are Fe/Mn-oxidizing bacteria or not, and if they are, it is not known how they may be associated with the formation of manganese crusts. In addition, many uncultured bacteria were detected in the manganese crusts, which potentially include novel Fe/Mn-oxidizing bacteria and, moreover, Fe/Mn-reducing bacteria (Nitahara et al., 2011). If there are Fe/Mn-reducing bacteria within the manganese crusts, Fe and Mn can be released from the crusts by their metabolic activity. Therefore, their activity potentially affects the growth rate of the crusts. Our goal is to identify which microorganisms are actively associated with the growth of the manganese crusts by culture-dependent analysis. Considering the wide distribution of the manganese crusts on the seafloor, this study will provide novel insights into the significance of microbial activity on global metal cycling in ocean.

#### 3.3.1 Microbial colonization device

To identify what microorganisms initially attach on manganese crusts and to assess accumulation of elements on the microorganisms, in situ microbial colonization devices made from 500 ml PET bottles were deployed (Fig. 14). The bottles have several small holes to allow seawater to flow freely. Square chips (1 cm x 1 cm x 1 mm) were cut from the samples of manganese crusts and basaltic rocks, which were collected during the NT09-02 cruise. These chips were washed by EtOH and placed in each PET bottle. Five colonization devices were put on the seafloor (Fig. 15) at depths of 2900, 2738, 1979, 1768 and 1425 m. The devices will remain on the seafloor for more than a year before being recovered.



Fig. 14. Photo of a microbial colonization device

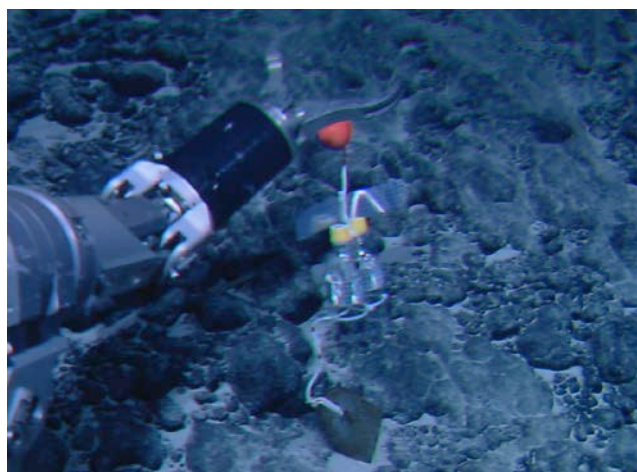


Fig. 15. On-site photo of the device

### 3.4 Preparation of samples for Geochemical analysis

During past Hyper-Dolphin cruises, manganese crusts were carefully sampled, together with measurements of seabed topography, geological characteristics and water chemistry. The samples proved suitable for characterization of variation patterns within the crust layer. The objective of the mapping and sampling is to study the effects of changes in composition with the local environment, as well as with water depths and the regional geology.

Hydrogenetic manganese crusts are potential archives of paleoceanographic and geological environments as well as a potential mineral resources in the future. However, the oceanographic or geological parameters controlling the mode of elemental variations are not yet well understood. We aim to identify the parameters that control their chemical and mineralogical diversities in space and time by comparing geochemical, mineralogical, physical and microbiological characteristics of manganese crusts on small to microscopic scales. For this purpose, the key technique is a delicate sampling method that should provide us undisturbed manganese crusts and on-site measurement. This cruise is a part of our program to characterize geological occurrences of manganese crusts from two typical areas (Minami-Torishima Island area and Okino-Torishima Island area) over the northwestern Pacific Ocean. We plan to describe microscopic scale geochemical, mineralogical, structural properties with reliable time scales, as we did at the Takuyo-Daigo seamount and Kyushu-Palau seamounts during the earlier cruises NT09-02, NT10-11, KY10-11, and NT11-10. Another objective of rock sampling on this cruise is to obtain thick crusts with a fresh substrate rock to reveal detailed growth rate of crusts using Re-Os isotope chronology and also to determine the age of substrate rock. Efforts were made to recover samples without damage together with continuous measurement of C-T-D-pH-DO along the track on the bottom. In-situ samples were taken for geochemistry, mineralogy, microbiology, and physical engineering studies on the manganese crusts. At each station, 0.7-10.5 kg samples were taken, and then slice and carefully kept wet cool in a refrigerator after packing in air-tight plastic bags. To avoid chemical biological damage, air-tight, wet and cool conditions are requisites for analysis samples.

Deep-sea sediments with highly concentrated REEs in the Pacific Ocean has drawn special attention after the publication of Kato et al. (2011) [5]. JAMSTEC has immediately started to search available cores recovered in the Pacific, which are stored in Kochi Institute for Core Sample Research (core repository of JAMSTEC) to fill additional data in the empty area in the paper, especially within the exclusive economical zone (EEZ) and surroundings of Japan. In the search, it was found that coring has not been carried out by JAMSTEC in the EEZ around Minami-Torishima Island for more than 15 years. Though Ocean Drilling Program had implemented some legs around this area in the past, recovered sediments just beneath the seafloor (mud-line) are not in sufficiently good condition for studies to be performed as the top of the soft sediment may have been heavily disturbed while execution of hydraulic piston coring. In addition, the cores recovered in the legs were not suitable for continuous depth profiling of REEs due to poor recovery rates. Thus, undisturbed surface sediment samples from this area are important to fill the gaps of information for spatial REEs concentration patterns in sediments.

[5] Kato, Y., Fujinaga, K., Nakamura, K., Takaya, Y., Kitamura, K., Ohta, J., Toda, R., Nakashima, T. and Iwamori, H., 2011, Deep-sea mud in the Pacific Ocean as a potential resource for rare-earth elements, *Nature Geoscience*, DOI: 10.1038/NGEO1185

#### *3.4.1 Manganese crusts*

Once retrieved on-board the R/V Natsushima, all rock and manganese crust samples were prepared while still wet to avoid dissolution or fragmentation and to maintain their original physical conditions. Manganese crusts or nodules with hard substrates or nuclei were split using a diamond blade saw while still wet, then sliced or faceted for geological analysis. Sub-samples for land-based analysis and measurements were selected from each rock sample, packed in air-tight plastic bags, and then kept in a refrigerator at about 4°C or lower. Large samples were delivered to Kochi University and JAMSTEC, and the rest was kept at JAMSTEC for archiving.

#### *3.4.2 Sediments*

Sediments on the Takuyo-Daigo Seamount collected by MBARI-type push corers were sub-sampled dependant on the condition of the cores. If a core was completely retrieved from the corer sleeve, a hand-made core pusher was attached beneath the core bottom so that the core could be extracted preserving its original structure. Subsamples for microbiological study were collected from top surface of the sediment using a clean spatula. The sediments were sliced at every 5cm depth intervals and each slice was packed into a polyethylene bag. For sliced samples below 5cm core depth, about 5mm width of margin of the core was trimmed using a spatula to exclude contaminated sediment during coring and extraction of the cor. If a core was not completely retrieved from the sleeve, which means that some part of core remained in the sleeve, the retrieved core was extracted on a stainless steel tray using a spatula and gentle vibration. A subsample for microbiological study was collected from middle part of the core because the top part of the core becomes disturbed when the corer is laid. Then the core was divided into 5cm length (in core length), trimmed 5mm width of margin, and packed into a polyethylene bag. The remaining sediment in the sleeve was scooped by spatula and put into a polyethylene bag without trimming. In the same way, if all sediment was remained in the sleeve when the corer was pulled out, the remaining core was scooped by spatula and put into a polyethylene bag.

All sediment samples were carefully handled using clean spatulas to avoid microbiological and geochemical contamination. After the sub-sampling, the polyethylene bags were stored in a refrigerator at 4°C. Basic information of the cores such as core length, sediment color, lithology and sedimentary structure were collected during the sub-sampling. However, as described above, the cores were not halved so detailed lithological descriptions were not carried out.

#### *3.4.3 Water*

Seawater samples collected by the syringe sampler and NISKIN sampler were stored in plastic bottles after filtering with 1M nitric acid at 4°C for chemical measurement. The pH values of seawater samples collected by the syringe sampler were measured by a handy-type pH meter (TPX-999i, Toko chemical research) with a glass electrode (PCE108CW-SR, Toko chemical research). The results are shown below.

Depth (m)	pH	T when measured °C
2899	7.777	25.4
2842	7.761	25.7
2743	7.734	25.3
2738	7.756	25.6
2117	7.722	25.2
2051	7.656	25.3
1911	7.673	25.3
1773	7.687	25.2
1597	7.649	25.5
1466	7.658	25.4
1423	7.72	25.3

Table 4. pH of seawater samples

### 3.5 Preparation of samples for microbial characterization

For microbial analyses, manganese nodules, sediments and bottom seawater were collected. These samples were stored in suitable condition for each analysis as described below. Several samples were inoculated into the culture media prepared for cultivation of Fe/Mn-oxidizing bacteria and Fe/Mn-reducing bacteria.

#### 3.5.1 *Manganese crusts*

The collected manganese nodules were divided into each portion of manganese oxides or basement rocks using a clean hammer. Large subsamples were stored in plastic tubes at  $-80^{\circ}\text{C}$  for DNA/RNA analysis. Cells attached with small subsamples were fixed in plastic tubes with PBS buffer containing formalin (final 3.7% v/v) at  $4^{\circ}\text{C}$  until microscopic observation. Small subsamples were also stored in filtered seawater with glycerol (final 15% v/v) in plastic tubes at  $-80^{\circ}\text{C}$  for cultivation.

#### 3.5.2 *Sediments*

A part (approximately ~1 g) of the sediments collected by the MBARI sampler were sampled using a clean spatula. Subsamples were stored in plastic tubes at  $-80^{\circ}\text{C}$  for DNA/RNA analysis. Cells attached with small subsamples were fixed in plastic tubes with PBS buffer containing formalin (final 3.7% v/v) at  $4^{\circ}\text{C}$  until microscopic observation. Small subsamples were also stored in filtered seawater with glycerol (final 15% v/v) in plastic tubes at  $-80^{\circ}\text{C}$  for cultivation.

#### 3.5.3 *Water*

Seawater samples collected by Syringe sampler and NISKIN were stored in plastic bottles at  $4^{\circ}\text{C}$  for cultivation. Some of the samples collected by Syringe sampler were stored in plastic bottles with formalin or Gly-TE at  $-80^{\circ}\text{C}$  for microbial analysis.

## 4. Dive summary

### 4.1 HPD #1352

Measurements were performed on the slope of the south western flank of the seamount, at depths between 2903m and 2743m. A lateral distance of 1.4km was covered, in an area that had already been surveyed during NT09-02 Leg 2. Due to failure of an electrical cable during the descent, it was not possible to perform any visual mapping and navigational data could not be obtained. However, acoustic measurements could be made and the acoustic probe was manually control to point perpendicular to the seafloor. Although a large number of samples were obtained during NT09-02 Leg 2, in most cases only a single sample was obtained at each depth. During this dive, 6 samples were collected from roughly the same depth (2900m) from locations several tens of meters apart. Even at the same depth the appearance of the surface of the seafloor was found to be variable. Microbial cultivation cells were deployed at 2 points (2900m and 2738m) and water samples were taken at regular depth intervals. Along the transect, the surface of seafloor was variable with bumpy crusts, pillowy crusts, pebble to boulder size of nodules, and areas covered with nodules covered sediments. The event log of this dive is shown in Table 5. With regard to the failed cable, since the problem only manifested itself under pressure, it was not possible to identify the problematic cable even after the dive.

Time	Depth	Remark
8:35	0m	Start of dive
	2200m	Failure of underwater cable: Mapping and Navigation
10:09	2903m	Reach the seafloor
10:14	2899m	Syringe seawater sample S1, S2
10:17	2900m	Deploy microbial colonization device B1
10:23	" "	Manganese crust sample R1
10:31	" "	Manganese crust sample R2
11:52	2896m	Manganese crust sample R3, R4
13:01	" "	Manganese crust sample R5, R6
14:16	2871	Sediment sample C1
15:03	2824m	Syringe seawater sample S3, S4
15:25	2743m	Syringe seawater sample S5, S6
15:27	2738m	Syringe seawater sample S7, S8
15:30	" "	Deploy microbial colonization device B2
15:31	2737m	End of dive

Table 5. Event log for HPD #1352

### 4.2 HPD #1353

Measurements were performed on the slope of the south western flank of the seamount, at depths between 2117 and 1768m. A lateral distance of just over of 2km was covered during the dive, where the route was slightly offset from that surveyed during NT09-02 Leg 2 to follow the ridge of the seamount. Steep slopes of 20 to 30 degrees where almost entirely covered with smooth crusts were frequently observed between 1950m and 1800m depth. Many ripple marks (around 10cm interval) were observed on the

surface of sediment pools during this dive. During the first half of the dive acoustic and visual mapping measurements could be performed, however at 1930m the underwater cable connected to the navigation device (DVL) failed and navigation data could not be measured from this point. However, acoustic and visual measurements could still be made to the end of the dive. Sporadic dense appearance of white sponges and remains of gorgonian were recognized. A total of 5 crust samples were obtained from the outcrop at 1917m and 1775m depth and water samples were obtained at regular depth intervals. A microbial colonization device was deployed at a depth of 1768m. Due to reconfiguration of the electronic system after the previous dive, it was possible to identify and replace the problematic cable after this dive.

Time	Depth	Remark
8:29	0m	Start of dive
9:40	2117m	Reach the seafloor
9:42	" "	Niskin N1, Syringe seawater sample S1, S2
10:23	2051m	Syringe seawater sample S3, S4
12:18	1930m	Failure of underwater cable: Navigation
13:02	1917m	Manganese crust sample R1
13:56	" "	Manganese crust sample R2
14:05	" "	Manganese crust sample R3
14:16	1911m	Syringe seawater sample S5, S6
15:48	1773m	Syringe seawater sample S7, S8, Niskin N2
15:53	1775m	Manganese crust sample R4
15:57	1768m	Deploy microbial colonization device B1
16:01	" "	Manganese crust sample R5
16:02	" "	End of dive

Table 6. Event log for HPD #1353

#### 4.3 HPD #1354

Measurements were performed on the slope of the south western flank of the seamount, at depths between 2111 and 1410m. A lateral distance of just of 3.5km was covered. Despite being only a few hundred meters from the route taken during NT09-02 Leg 2, the spectacular crust overhangs seen during NT09-02 Leg 2 at 1440m depth were not observed during this dive. On the boundary between the slope and flat terrace around 1425m crusts were scarcely observed and sediments with pebble to cobble size nodules were spread above this depth. Rock sampling was carried out using the rotary blade and a relatively fresh basalt sample was collected around this depth. Sediment and water samples were obtained at regular depth intervals. During this dive continuous acoustic and visual mapping information over the entire 3.5km length for the duration of the dive coving a depth range of 701m. The acoustic measurements confirmed that despite their being no visible crust overhangs, the thickness of the crusts on the shoulder of the seamount in this region was around 10cm thick.

Time	Depth	Remark
7:20	0m	Start of dive
8:25	2111m	Reach the seafloor
10:42	1980m	Sediment sample C1, Deploy microbial colonization device B1
10:50	1975m	Niskin N1
13:40	1597m	Syringe seawater sample S1, S2
14:19	1466m	Syringe seawater sample S3, S4
14:46	1425m	Sediment sample C2, Deploy microbial colonization device B2
14:54	" "	Manganese crust sample R1
15:05	1423	Syringe seawater sample S5, S6, Niskin N2
16:12	1429m	Manganese crust sample R2
16:13	" "	End of dive

Table 7. Event log for HPD #1354

## 5 Sample list

### 5.1 Rock/crust table

Dive	Sample	Depth	Description	Dimensions (mm)	Mass (kg)
HPD#1352	R1	2900	nodule		
	R2	2900	crust	230×140×130	3.1
	R3	2896	crust	190×180×140	3.4
	R4	2896	crust	120×110×6	0.7
	R5	2896	crust	280×190×90	3.3
	R6	2896	crust	250×150×110	3.2
HPD#1353	R1	1917	crust	200×70×100	1.1
	R2	1917	crust	170×140×170	1
	R3	1917	crust	400×230×140	10.5
	R4	1775	crust	300×230×110	5.7
	R5	1768	nodule		
HPD#1354	R1	1425	nodule		
	R2	1429	crust	180×100×100	1.8

Table 8. Sample list

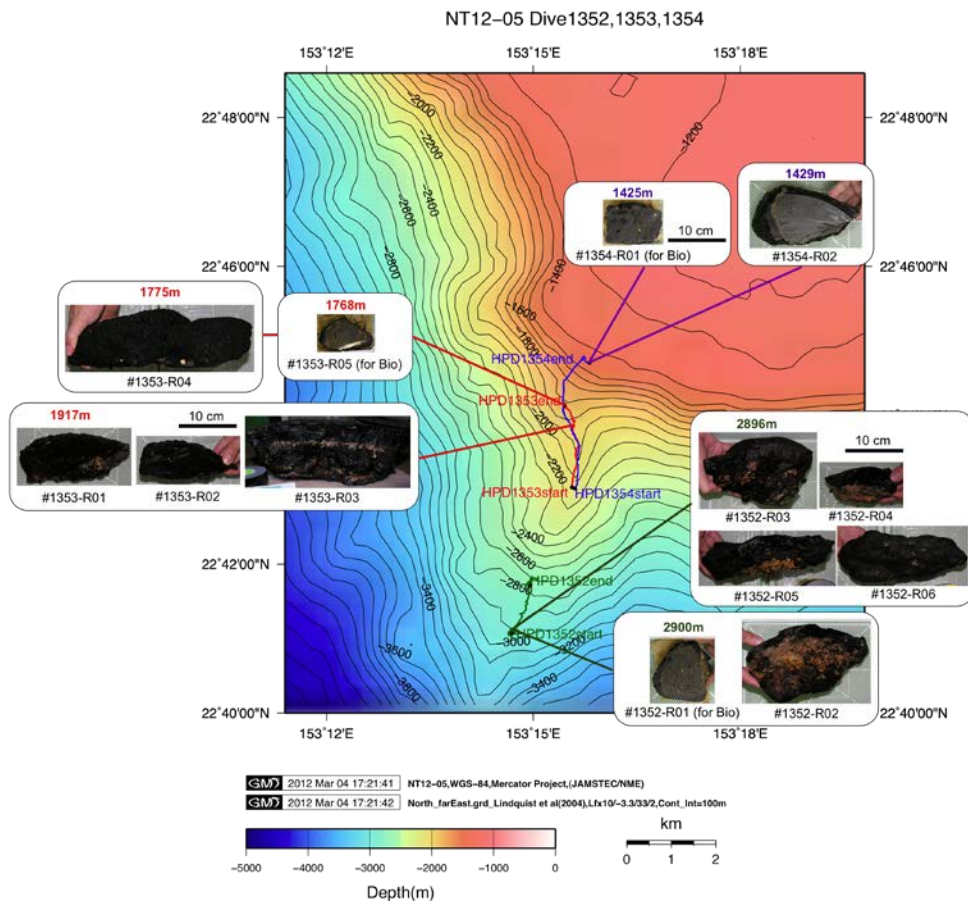


Fig. 16 Samples obtained during this cruise

## 5.2 Description of samples

13 samples of manganese crust and nodule, with masses ranging from 1.0 to 10.5kg, were collected from various depths during three dives (see Table 8). The rocks were completely covered by a layer of manganese oxide which thickness between 10 to 65mm. The original objectives to sample from different water depth and obtain more than one sample from the same depth were successfully achieved.

The dive HPD#1352 was carried out at the southern ridge of the seamount, at water depths between 2903 and 2737m, along same route with the dive HPD#953 in cruise NT09-02 leg2. In this dive, plates and fragments of crust with 10 to 20mm in thickness were collected at 2900m and 2896m depth. Except for samples HPD#1352-R1 and R6, the collected crust samples were firmly rooted to the seafloor and are thought to be representative of the locality. The substrate rock types were phosphatized limestone and calcareous conglomerates. Sample HPD#1352-R3 was 190mm wide, sub-rounded plate with calcareous conglomerate covered by 20 mm layer of manganese oxide (Fig.17 and 18). Samples HPD#1352-R2, R4 and R5 had phosphatized limestone substrates with 10 to 20mm manganese crust layer. A nodule sample HPD#1352-R1 collected for microbial analysis was weathered breccia nuclei covered by 10mm layer of manganese oxide. Sample HPD#1352-R6, a 250mm wide plate covered by manganese crust, was a loose cobble existed above sediment, not rooted to the seafloor.



Fig.17. Surface-view photo of manganese crust sample, HPD#1352-R3



Fig.18. Side-view photo of manganese crust sample, HPD#1352-R3

Dive HPD#1353 was carried out at the southern ridge of the seamount, at depths of between 2117 and 1768m. The dive route was planned to trace the top of the ridge where was distant about 200m west of the HPD#955 dive track. In this dive, plates and fragments with crust layers of 20 to 65mm in thickness were collected at 1917m and 1775m depth. Most of the substrate rocks were phosphatized calcareous conglomerate and limestone. Samples HPD#1353-R1 and R2 were obtained using the rotary blade to cut the samples from the outcrop. These sample had calcareous conglomerate substrate and nuclei covered by 20 to 30mm manganese crust layer. Sample HPD#1353-R3 was 400mm wide plate with calcareous conglomerate substrate covered by a 65mm layer of manganese crust (Fig.19 and 20). Sample HPD#1353-R4, a 300mm wide plate covered by manganese crust was a loose boulder contacted onto the surface of sediment. A nodule sample HPD#1353-R5 collected for microbial analysis was highly-altered basalt nuclei covered by 2 to 10mm layer of manganese oxide.



Fig.19. Surface-view photo of manganese crust sample, HPD#1353 R3



Fig.20. Side-view photo of manganese crust sample, HPD#1353 R3

The dive HPD#1354 was carried out at the southern ridge of the seamount, at water depths between 2111 and 1429m. The dive route traced the HPD#1353 dive track and continued to the shoulder of the seamount. In this dive, relatively fresh basalt substrate rock and manganese nodule for microbial analysis were collected at 1429m and 1425m depth, respectively. Sample HPD#1354-R2, obtained from a depth of 1429m, was 180mm wide, relatively fresh basalt associated with chilled margin, covered with 10mm layer of manganese oxide (Fig.21 and 22). This fresh, hard rock could only be collected thanks to the deep incision made by rotary blade. Fig.21 shows the plane formed by multiple operation of the cutter, for more than 20 minutes. A nodule sample HPD#1354-R1 collected for microbial analysis had 30mm layer of manganese crust with calcareous fragment nuclei.



Fig.21. Surface-view photo of manganese crust sample, HPD#1354 R2



Fig.22. Side-view photo of manganese crust sample, HPD#1352 R2

In addition to the crust samples, sediments were sampled at depths of 2871m, 1980m, and 1425m. The lithologies of sediment samples were similar for the different depths; the lithology was dull yellow orange (10YR6/4) homogenous foraminifer ooze (Fig.23 and 24). The oozes consisted of well sorted foraminifer tests and fine fraction as matrix was rarely recognized in macroscopic observation. The foraminifer species were all planktic, such as *Orbulina* spp., *Goroborotalia* spp. and *Globigerinoides* spp.. Some of these foraminifer tests have stained color (pale ~ light brown). In all cores, 0.5~3 cm in

diameter, sub-rounded to sub-angular black manganese nodules were contained at surface of the sediments. Sediment sample information is summarized in Table 9.

Dive	Sample	Depth (m)	Lithology	Length (mm)
HPD#1352	C1	2871	Foram. ooze	160
HPD#1354	C1	1980	Foram. ooze	110
	C2	1425	Foram. ooze	45

Table 9. Sediment sample list

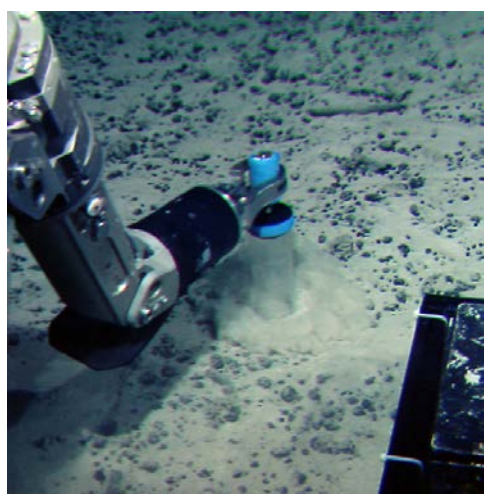


Fig. 23. Photo of push corer sampling of HPD#1354 C1

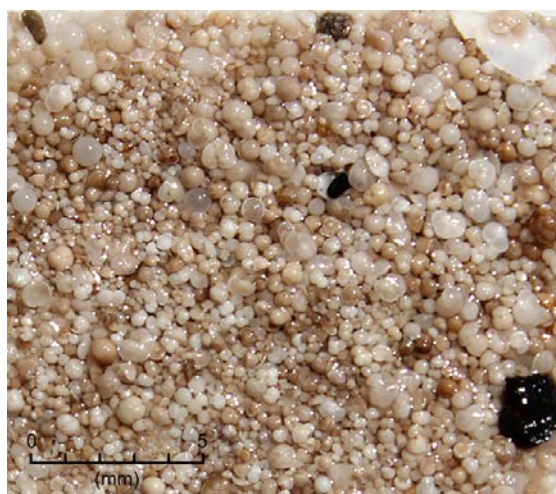


Fig. 24. Close-up photo of the foraminifer ooze from HPD#1354 C1 0~5cm

## 6. Future Plans

### 6.1 Instrumentation and methods

During this cruise, it was demonstrated that the introduction of a gimbal mechanism can enable acoustic measurement of crust thickness to be made on steep slopes. The gimbal mechanism used was designed with a range of  $\pm 30$  degrees, however, during this cruise HyperDolphin's nose was pitched downwards by 10 degrees on average, which is unfavourable for acoustic measurements while climbing the slope of the seamount, and meant that the relative slope of the seafloor often exceeded the range of motion of the gimbal mechanism, and it will be necessary to increase the range of the gimbal in our future work. During this cruise, the gimbal was controlled manually based on realtime measurements of the slope of the seafloor relative to the ROV. This caused a delay in response to changes in slope and vehicle motion. It is recommended that in the future, control of the gimbal is achieved automatically based on realtime analysis of the laser line projection of the visual mapping system and measurements of range from the DVL mounted at the aft of the vehicle. The goal of the algorithm is to find the direction normal to the seafloor in realtime and point the acoustic probe into this direction. Processing the entire bathymetry measured by the visual mapping system is an option, but it not efficient since ultimately the direction vector can only be calculated with respect to a planar approximation of the seafloor. In order to improve the computational efficiency we are developing an algorithm that determines just two points from the laser projection, the average of the port and starboard side of the projection, with the DVL range information forming a third point to define the relative angle of the seafloor plane under the vehicle. The acoustic and bathymetric data obtained during this cruise will allow our group to develop and optimize such an algorithm, as well as perform a detailed assessment of its ability to accurately control the probe in realtime. Figure 25 shows preliminary simulation results of a realtime control algorithm applied using data measured during this cruise.

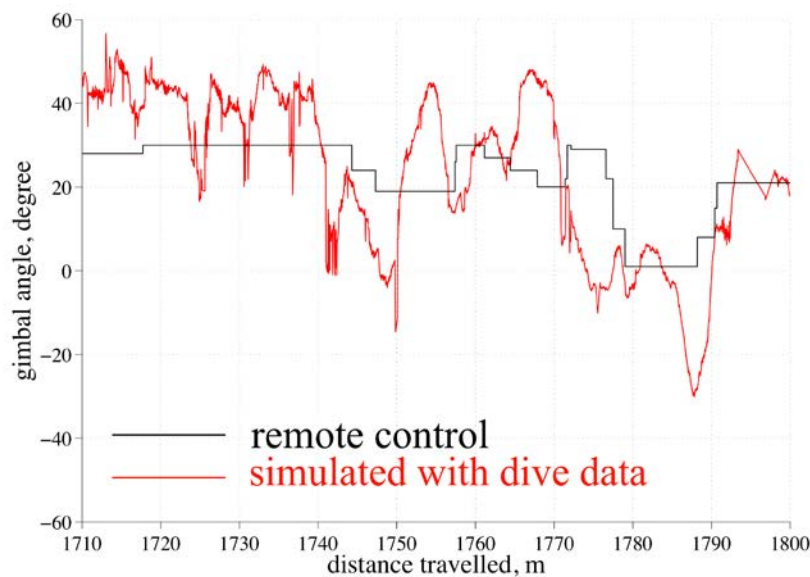


Fig. 25. Simulated realtime control and actual remote control gimbal angles

During this cruise, all ROV tracklines climbed the seamount in the direction normal to its contours. However, even though the routes taken were only a few hundred meters from the routes taken during NT09-02 Leg 2, there were significant differences in the appearance of the crust deposits. In the future, it is recommended that some tracks are surveyed along contours of the seamount to isolate the effect of topology from effects of changing properties of seawater in the vertical water column. In order to perform effective measurements, it will be necessary to introduce a double gimbal mechanism for the acoustic probe.

With regard to the range and operating frequency of the acoustic probe, the increased measurement range of the acoustic system ( $1.5\text{m} \pm 1\text{m}$ ) was found to be sufficient to record continuous data while operating an ROV on steep slopes. The increased range is achieved by operating the probe at higher frequencies to form a more focused beam from long distances. Although this increases both the range and resolution of the probe, it also raised concerns about the levels of attenuation in the crusts. This was found not to be a problem and it is noted that there was little attenuation of the sound waves in the crusts, in accordance with observations made during NT10-11.

The measurements of the seaXerocks mapping device have been used to generate several, high resolution 3d reconstructions of the seafloor. Though noise filtering and colour correction for attenuation have not been performed at this point, the 3D reconstructions shown in this report can be generated onboard the ship on a standard laptop PC. The operating range of the mapping system was matched to the acoustic system, but can be extended for other applications. The 3D reconstructions, when studied together with the acoustic measurements of crust thickness, provide an effective method to assess the volumetric distribution of the crust deposits. By locating the mapping device towards the front of the ROV and increasing altitude, it was possible to solve the problems encountered during our previous NT10-11 cruise concerning agitation of the seafloor, and during this cruise continuous data could be obtained over the entire length of the dives with a swath of 1.5 to 3m. By introducing a second camera observing the laser line projection from a different angle to the first, it is possible to obtain mapping data with no occlusions, even on rough and rugged terrains.

Sampling of the crusts was performed principally with the ROV manipulator and the rotary blade. In order to sample crusts directly from the outcrops, it was necessary to use the rotary blade to make incisions in the crusts. The 300mm blade was found to be more effective during this cruise. However, there are still issues concerning the operation of this device, mainly concerning lateral loads causing the blade jam when moving the blade to extend the incisions. It is suggested that a single degree of freedom actuator can be introduced to advance the blade more efficiently. Also, once an incision is made, removing the crust sample is still an issue. Efforts were made using a crowbar, but this was found to be ineffective, and an alternative method will be necessary to improve sampling efficiency in the future.

For sedimentary study about REEs-rich sediments to reveal its spatial concentration pattern, it is necessary to obtain more sediment samples from deeper part and surroundings of the seamount. Fortunately a research cruise to visit Takuyo-Daigo seamount using the HyperDolphin 4500 is planned in December 2012, and another research cruise to obtain deep sea sediment using piston corer around Minami-Torishima Island is also planned in January 2013. Surface sediment sampling

will be carried out along depth transects in the former cruise, which is expected to cross the calcium compensation depth and get mud (or sand consisted of authigenic small nodule) samples which is non-diluted by biogenic components. If the mud sample is obtained at the shoulder of the seamount, it will be interesting to compare to deep sea sediment which will be obtained in the latter cruise about REEs concentration and mineralogy.

## 6.2 Analysis of samples

Secular variations in the major and minor element chemistry, mineralogy, and microstructures within manganese oxide layers will be investigated with respect to distance from the surface, or to the bottom at the boundary with substrate rocks, with millimeter order resolution. The wet and dry density and porosity will be measured at intervals of a few millimeters. The aim of these studies is to date the age of each thin layer of the crusts and determine the growth rates. Detailed Re-Os isotopes dating will be also carried out using thick crust samples, and Ar-Ar dating will be carried out to determine the age of substrate basalt of the seamount. Possible chronological correlation will be examined between thick crusts found in remote locations within this seamount and between different seamounts. Furthermore, correlations will be examined between structural and compositional features of the external surface and geochemical, sedimentary and oceanographic conditions of present-day seawaters, such as, basement geology, depth, currents and redox potential.

Another major aim of research is the chemical and mineralogical speciation of metal elements, such as Co, Ni, REE, and Pt, since the crusts are believed to yield high potential as a mineral resource. Some samples may be assessed using micron-scale sensitive analysis instruments, such as EPMA, LA-ICP/MS, SIMS, XANES etc. Some samples will also be used as test pieces in the development of in-situ element analysis techniques.

‘Stained’ foraminifer tests were recognized in the sediments. This color may be caused by a precipitation of manganese oxide. Thus EPMA and Raman spectroscopic analysis will be carried out to reveal elemental sequence and crystallography of the materials. Simultaneously, REEs concentration measurement will be measured for bulk sediment sample.

Concerning microbiological analysis, phylogenetic diversity, composition and metabolic functions of microorganisms in the collected samples will be determined by DNA/RNA analysis targeting 16S rRNA and functional genes and their transcripts, which are related to carbon fixation, sulfide oxidization, manganese oxidation, ammonia and nitrite oxidation and nitrate and sulfate reduction. The abundance of microorganisms in the samples will be determined by FISH analysis. Microbial growth in the culture media inoculated on board will be confirmed by microscopic observation. If the growth will be confirmed, isolation and characterization of cultured species will be performed. Cultivation in several growth conditions (temperature, pH, nutrients, aerobic/anaerobic) will be tested using the glycerol stock samples. The colonization devices will be recovered, possible during the sampling cruise in December 2012. Microbiological (DNA/RNA analysis, FISH, cultivation) and mineralogical (EPMA, XRF, SEM, HRTEM) characterization for the chips in the devices will be performed.

## Acknowledgements

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