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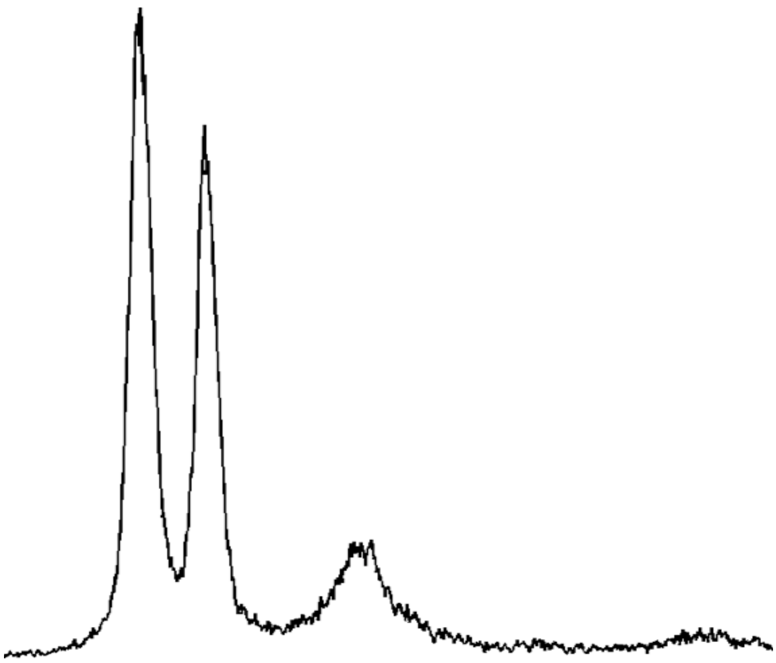
R/V Natsushima cruise report

NT12-07

Kagoshima Bay, Off Cape Noma-Misaki



24th - 29th March, 2012

Chief Scientist: Blair Thornton

Institute of Industrial Science, The University of Tokyo

Preface

This report describes the dives of the ROV Hyper-Dolphin at Kagoshima bay and off Cape Noma-Misaki, during the NT12-07 cruise of the R/V Natsushima between 24th and 29th March, 2012.

The NT12-07 cruise was conducted based on three separate proposals; #S11-56 "Experimental testing of instrumentation to perform in situ multi-element chemical analysis at a hydrothermal site", proposed by Blair Thornton of The University of Tokyo, #S11-10 "Towards the development of deep-sea organismal clocks", proposed by Yoichi Yusa of Nara Women's University, and #S11-76 "Study on reproductive and dispersal strategy of *Osedax japonicus*", proposed by Tomoko Yamamoto of Kagoshima University.

The main objectives of the cruise were as follows; to investigate the application of laser-induced plasmas as a mechanism to perform in situ multi-element analysis of both liquids and solids at sea, to collect biological samples to obtain information concerning barnacle growth to develop deep-sea clocks, and to establish a sampling method for *Osedax japonicus*, and evaluate their dispersal ability and reproductive period.

A total of four dives were performed with Hyper-Dolphin. Successful multi-element analysis of both liquids and solids was performed. Four frames of the *Osedax* colonization device "Namekujira_11" were recovered and biological samples, including *O. japonicas, S. stearnsii*, several hundred *Heteralepas* sp., *Octolasmis* sp., Scalpellidae sp., a wild *S. stearnsii* were collected during the dives.

The research party would like to thank the crewmembers of the R/V Natsushima lead by Captain Yoshiyuki Nakamura, the members of the ROV Hyper-Dolphin operation team lead by Kazuya Mitsufuji, marine technician Satomi Minamizawa, 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 (NT12-07 Chief Scientist)

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Contents

1. Cruise information

Cruise ID:	NT12-07
Research vessel:	R/V Natsushima
Title of cruise:	FY2011 Deep sea survey by the ROV Hyper-Dolphin at Kagoshima Bay and off Cape Noma-Misaki
Title of proposals:	“Experimental testing of instrumentation to perform in situ multi-element chemical analysis at a hydrothermal site”. “Towards the development of deep-sea organismal clocks”. “Study on reproductive and dispersal strategy of *Osedax japonicus*”.
Cruise period:	March 24, 2012 (Naha, Okinawa) March 29, 2012 (Kagoshima) (6days)
Survey sites:	Kagoshima Bay and Off Cape Noma-Misaki

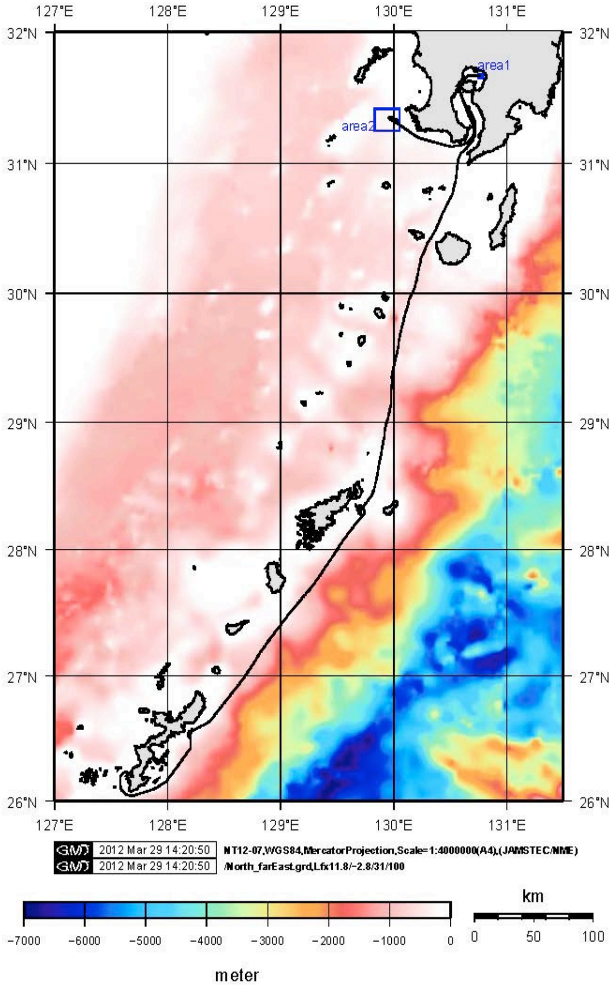

Fig. 1 R/V Natsushima track during NT12-07

1.1 Overview of observations

The NT12-07 cruise was conducted based on three separate proposals; #S11-56 “Experimental testing of instrumentation to perform in situ multi-element chemical analysis at a hydrothermal site”, proposed by Blair Thornton of The University of Tokyo, #S11-10 “Towards the development of deep-sea organismal clocks”, proposed by Yoichi Yusa of Nara Women's University, and #S11-76 “Study on reproductive and dispersal strategy of *Osedax japonicus*”, proposed by Tomoko Yamamoto of Kagoshima University.

A total of four dives were conducted in Kagoshima bay and off Cape Noma-misaki, using the ROV Hyper-Dolphin and the R/V Natsushima between March 24, 2012 and March 29, 2012. The main objectives of the cruise were as follows; to investigate the application of laser-induced plasmas as a mechanism to perform in situ multi-element analysis of both liquids and solids at sea, to collect biological samples to obtain information concerning barnacle growth to develop deep-sea clocks, and to establish a sampling method for *Osedax japonicus*, and evaluate their dispersal ability and reproductive period.

Dives HPD#1359 and HPD#1362 were conducted based on proposal #S11-56 in Wakamiko caldera at a depth of approximately 200m in Kagoshima Bay. During the dives, the ROV was instrumented with sensors to perform in situ multi-element chemical analysis, long range seafloor mapping, seafloor radiation measurements and continuous pH measurements. Sampling of seawater and sediments was performed together with temperature measurements of vent fluids. During the first part of HPD#1359, plasma based multi-element analysis of seawater composition was performed using the I-SEA, where the laser used to induce the plasmas was focused directly into the seawater to map the composition of a 100m by 100m area at a depth of approximately 200m. Hyper-Dolphin performed a lawnmower pattern supported by a GUI developed by the research team, to show the pilots the ROV’s position measured by the DVL and the path to be followed. Visual mapping and seawater sampling were performed at an altitude of 6m. Gamma radiation and pH measurements were also continuously performed. During the second part of the dive, a long term temperature measurement device was deployed near an active vent, and sediment sampling was performed. Radiation measurements and high resolution spectroscopy were performed near several active hydrothermal vents. During HPD#1362, experiments were performed in the same area, where this time chemical analysis was performed via a fibre optic cable to enable the plasma to be generated on specific targets. Visual mapping, radiation measurements and pH measurements were performed as in the HPD#1359. During the first half of the dive, Hyper-Dolphin followed lawnmower pattern covering an area of 70m by 70m and measurements of seawater composition were performed. During the second half of the dive, LIBS measurements were performed on solids. During these dives, successful in situ multi-element analysis was achieved for the first time, and it was demonstrated that plasmas can be used as a mechanism for in situ chemical analysis of both liquids and solids at sea. A number of measurements made in seawater exhibited distinct spectral signatures, which it is thought may originate from suspended particulate matter.

Dives HPD#1360 and HPD#1361 were conducted based on proposals #S11-10 and

#S11-76. During HPD#1360, the *Osedax* colonization device Namekujira_11 was found at the depth of 229 m off Noma-Misaki. Four frames (KDM-09 - 12) were recovered and water was also sampled. Small individuals of *Osedax* sp. were found on at least pig bones in the frames. In addition, an individual of *S. stearnsii* originally attached to the frames on Namekujira_11 when it was released on 13th April 2011 was recovered, and several hundred individuals of *Heteralepas* sp., one individual of *Octolasmis* sp. and one individual of *Scalpellidae* sp. settled on the artificial substrata of Namekujira_11 were recovered. During HPD#1362, the #6 whale fall was observed. Four small pieces of whale bone, and two crinoids, some sea anemones, and water and sediment samples were obtained. Then the ROV moved towards the #7 whale fall, and on the course we observed animal community on the sand bottom. We collected a fish, a ctenophoran (*Lyrocteis imperatoris*), a barnacle (*Scalpellum streansii*), and three individuals of octocorals. For the one individual of *S. stearnsii* recollected, we will count the growth lines, and try to check the growth after staining. We fixed most individuals of *Heteralepas* sp. and will measure their sizes later. This will give us the information on their growth in maximum 1 year. In NT10-07, we also collected many individuals of the same species of *Heteralepas* that had been attached within 4 months. Thus, we have two calibrating time points for estimating their growth curves. In addition, we will rear the remaining (ca. 100) individuals of *Heteralepas* collected this time, to further observe their growth curves in the aquaria kept at 15 ^{o}C. The *Osedax* will be cultured on a whale bone and pig bones in JAMSTEC and Nara women's university to observe their reproductive trait.

1.2 Objectives

The main objectives of the cruise were as follows;

Subject 1: to investigate the application of laser-induced plasmas as a mechanism to perform in situ multi-element analysis of both liquids and solids at sea,

Subject 2: to collect biological samples to obtain information concerning barnacle growth to develop deep-sea clocks, and

Subject 3: to establish a sampling method for *Osedax japonicus*, and evaluate their dispersal ability and reproductive period.

The main objective of the Subject 1 is to test the principle of using a laser-induced plasma as a mechanism to perform in situ analysis of the chemical composition of liquids and solids on the seafloor. A 3000m depth rated prototype device, I-SEA (In-situ Seafloor Element Analyzer) has been developed under the 'Program for the development of fundamental tools for the utilization of marine resources' of the Japanese Ministry of Education. The devices employs a technique known as laser-induced breakdown spectroscopy (LIBS), which is a form of atomic emission spectroscopy, that works by focusing a high power laser pulse onto a target to create a plume of excited material that emits light containing spectral lines that correspond to the atoms and ions that compose the plume. Since the laser-ablated materials from which the optical emissions occur are in the form of atoms and ions in a plume, elemental analysis of gases, liquids and solids immersed in a transparent liquid, such as water, should be possible. At the University of Tokyo, it has been demonstrated that well resolved emission spectra can be observed both from bulk liquids and solids immersed in liquids at high pressures of up to 30MPa. This cruise is the first time I-SEA has been tested at sea, and the first application of LIBS at sea. In addition to testing of I-SEA, a long range 3D seafloor mapping device has been tested for the first time during this cruise. Visual mapping of the seafloor is typically performed from low altitudes of about 2~3m, which greatly limits the areas that can be mapped during a single dive. However, by using high sensitivity cameras and an array of flashes for illumination, it thought that colour imaging can be performed from altitudes of 6~10m to map wider areas. In addition to these two systems, in situ radiation measurements of hydrothermally actives seafloor have been carried out together with high sensitivity pH measurements of seawater. Sampling of both seawater and sediments for laboratory based chemical and mineralogical analyses, and temperature measurements of hydrothermal vent fluids were performed during this cruise.

The main objective of Subject 2 is to collect barnacle samples to investigate their potential application as deep-sea clocks. Barnacles are sedentary animals attached to various substrata. They are common not only in shallow waters but also in deep seas worldwide. Their growth speed varies depending on the species, and their shells often have distinct growth lines. These characteristics may facilitate them to be used as "organismal clocks": by knowing the growth speed or the time needed to add a growth line, we can estimate the minimum time their substrata exist in the sea. Although there are some studies estimating growth speed in the shallow species, almost none on deep-sea species (except for one rather fast-growing species *Poecilasma kaempferi*). *Scalpellum stearnsii* and *Heteralepas* sp. are common in the district off Cape Noma-Misaki. *Scalpellum* has distinct growth lines and is a slow-growing species;

Heteralepas is apparently fast-growing. The purpose of this study was to collect information on barnacle growth to develop deep-sea clocks.

The main objective of Subject 3 was to study reproductive and dispersal strategy of *Osedax japonicus*. Once large marine mammals die and their carcasses sink into deep sea, they supply a large amount of unusual organic matter to deep-sea ecosystems. After consumption of soft tissue by scavengers, bacterial decomposition of oil-rich skeleton produces an anaerobic environment. This is followed by a sulphophilic stage with sulfide efflux and carbon fixation by chemosynthetic bacteria. Thus, deep-sea whale falls are expected to support a widespread and characteristic fauna including chemosymbiotic species (see Smith and Baco 2003). *Osedax* polecats have been discovered from only whale bones naturally. Since they apparently feed on some components (lipids, collagen) of the bones, they should have an important function to the succession of whale fall community. Whale fall is temporal and specific habitat, which occurs fortuitously in association with the death of whale. To be endemic in this unique habitat, *Osedax* species should have special strategy for reproduction and dispersal. In *Osedax*, males are dwarf and remain as small as larvae. This characteristic seems to be one of their reproductive and dispersal strategies. We observed the sperm whale-fall ecosystems off Cape Noma-Misaki, Kagoshima, on the southwestern coast of Kyushu, Japan at depths of 219-254m from 2003 to 2008. Twelve sperm whale (*Physeter macrocephalus*) bodies were sunk on February 1, 2002 after the mass stranding of 14 whales on January 22, 2002. They are located in waters shallower than that of whale-falls reported previously (Fujiwara et al. 2007). *Osedax japonicus* has been discovered and described here. It is one of only two species, which are reported from depths shallower than 250m (*O. japonicus* in the Pacific Ocean and *O. mucofloris* in the Atlantic Ocean). We think that this species should be an ideal model animal for studying reproductive and dispersal strategy of endemic species in this unique habitat. Off Noma-Misaki is only one place where we can sample *O. japonicus* and their population seems to have decreased year by year. The main objective of this cruise is to establish the sampling method of this species, and to evaluate their dispersal ability and reproductive period.

2. Cruise log

2.1 Survey area and time schedule

The cruise started in Naha, Okinawa and four dives were carried out near Kyushu. Dives HPD#1359 and HPD#1362 were performed in Kagoshima Bay. Dives HPD#1360 and HPD#1361 were carried both on the same day off Cape Noma-Misaki. The trajectories of HyperDolphin during the four dives are shown in Fig. 2. Due to bad weather conditions and high waves, the first dive site was reached one day later than planned. However, on the last day of the cruise where initially no dive was planned, Hyper-Dolphin was deployed in the morning, and although time was limited during the final day, all scheduled dives could be carried out. Table 1 shows the time schedule of the cruise.

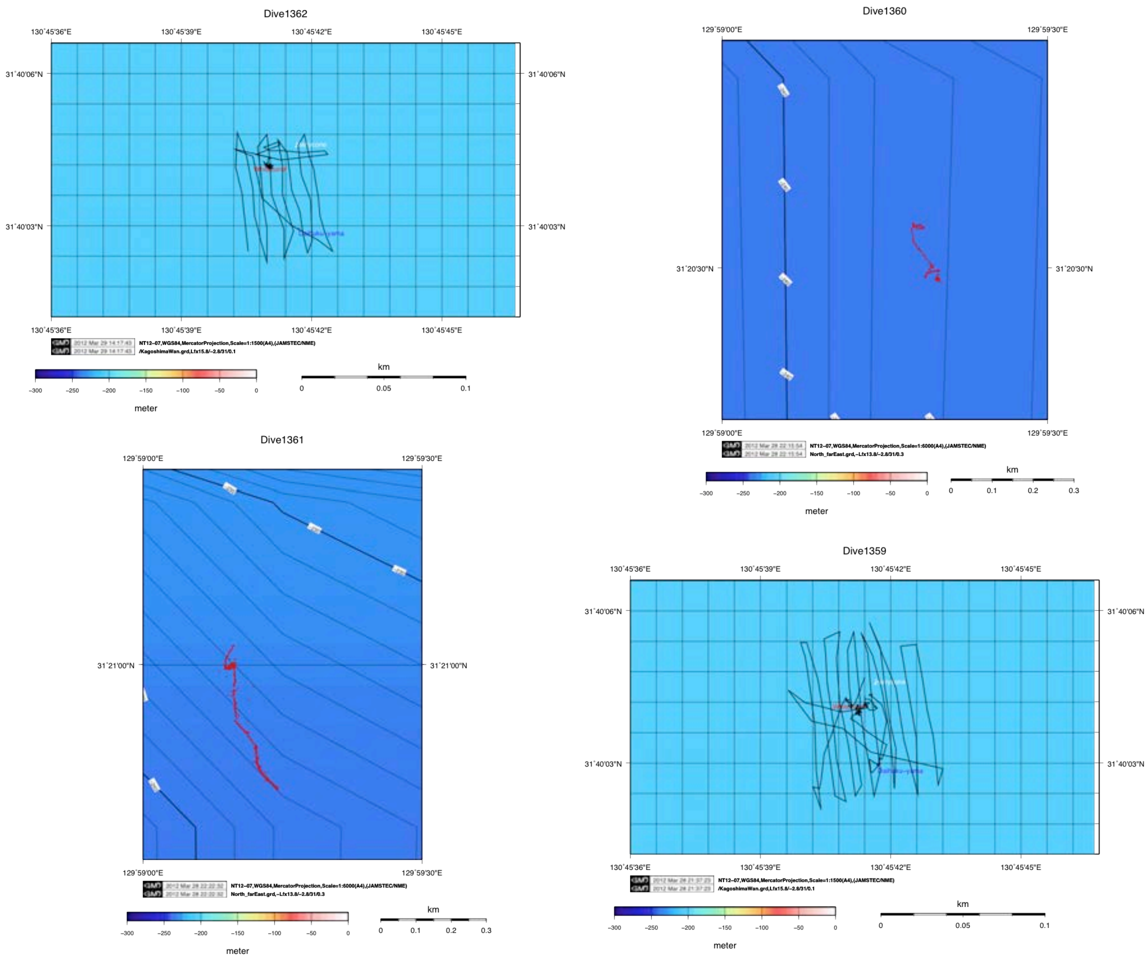

Fig. 2 Hyper-Dolphin dive trajectories during NT12-07

Date	Local Time	Note
24. Mar. 2012	9:00	Leave NAHA and sail to east side of Okinawa
	11:00-11:30	Onboard education and safety training
25. to 26 Mar. 2012		Proceeding to research area
27. Mar. 2012	8:26-14:29	HPD#1359
28. Mar. 2012	8:17-10:03	HPD#1360
	12:57-16:21	HPD#1361
29. Mar. 2012	8:18-11:22	HPD#1362
	15:00	Arrival at KAGOSHIMA

Table 1: Time schedule of NT12-07

2.2 Research party

Name	Affiliation
Blair Thornton*	Institute of Industrial Sciences, The University of Tokyo
Yoichi Yusa	Nara Women's University
Tomoko Yamamoto	Kagoshima University
Toshiro Yamanaka	Okayama University
Adrian Bodenmann	Institute of Industrial Sciences, The University of Tokyo
Tomoko Takahashi	The University of Tokyo
Noriko Yasuoka	Nara Women's University
Natsumi Yasuda	Nara Women's University
Yoshihiro Fujiwara	Japan Agency for Marine-Earth Science and Technology
Norio Miyamoto	Japan Agency for Marine-Earth Science and Technology
Masayuki Miyazaki	Japan Agency for Marine-Earth Science and Technology
Mai Ookubo	Japan Agency for Marine-Earth Science and Technology
Tamaki Ura#	Institute of Industrial Sciences, The University of Tokyo#
Satomi Minamizawa	Nippon Marine Enterprises, LTD.

Table 2: Research Party (* chief scientist) (#24th Mar. 2012 07:00-15:55)

3. Instruments and methods

During this cruise, 12 different payloads were mounted on Hyper-Dolphin. These can be grouped into sensors for realtime measurements and tools to facilitate sampling. During the dives the readings of the various sensors were monitored in the ROV control room by members of the research party. Figure 3 shows Hyper-Dolphin with the various payloads attached for dives HPD#1359, HPD#1362, and Figure 4 shows the payloads for dives #1360, #1361 respectively.

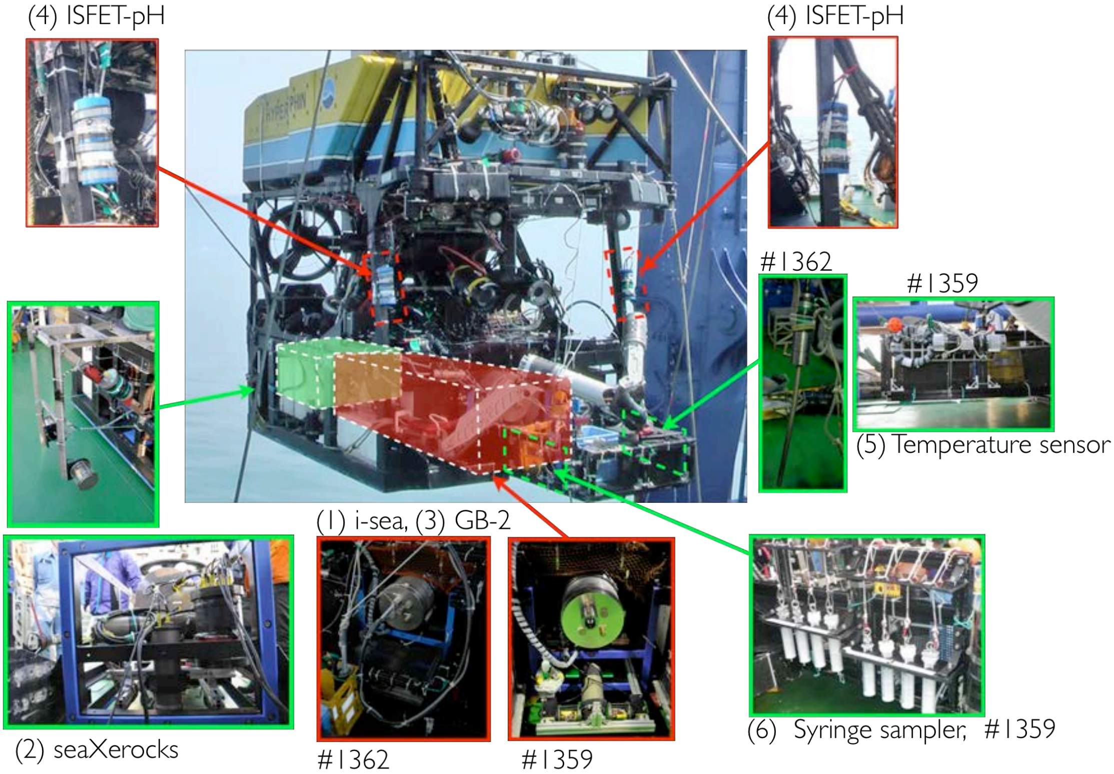

Fig. 3 Payloads mounted on Hyper-Dolphin for dives HPD#1359, HPD#1362. (1) I-SEA multi-element analyzer with direct optic for measurement of liquids (HPD#1359) and fibre optic for measurement of solids (HPD#1362). (2) seaXerocks mapping device for 3D colour reconstruction of the seafloor and measurement of vehicle position (HPD#1359, HPD#1362). (3) GB-2 gamma radiation sensor mounted under I-SEA (HPD#1359, HPD#1362). (4) ISFET-pH sensor mounted on the port and starboard of Hyper-Dolphin (HPD#1359, HPD#1362). (5) Temperature sensor for long term observation (HPD#1359) and for realtime measurement (HPD#1362). (6) Syringe water sampler (HPD#1359).

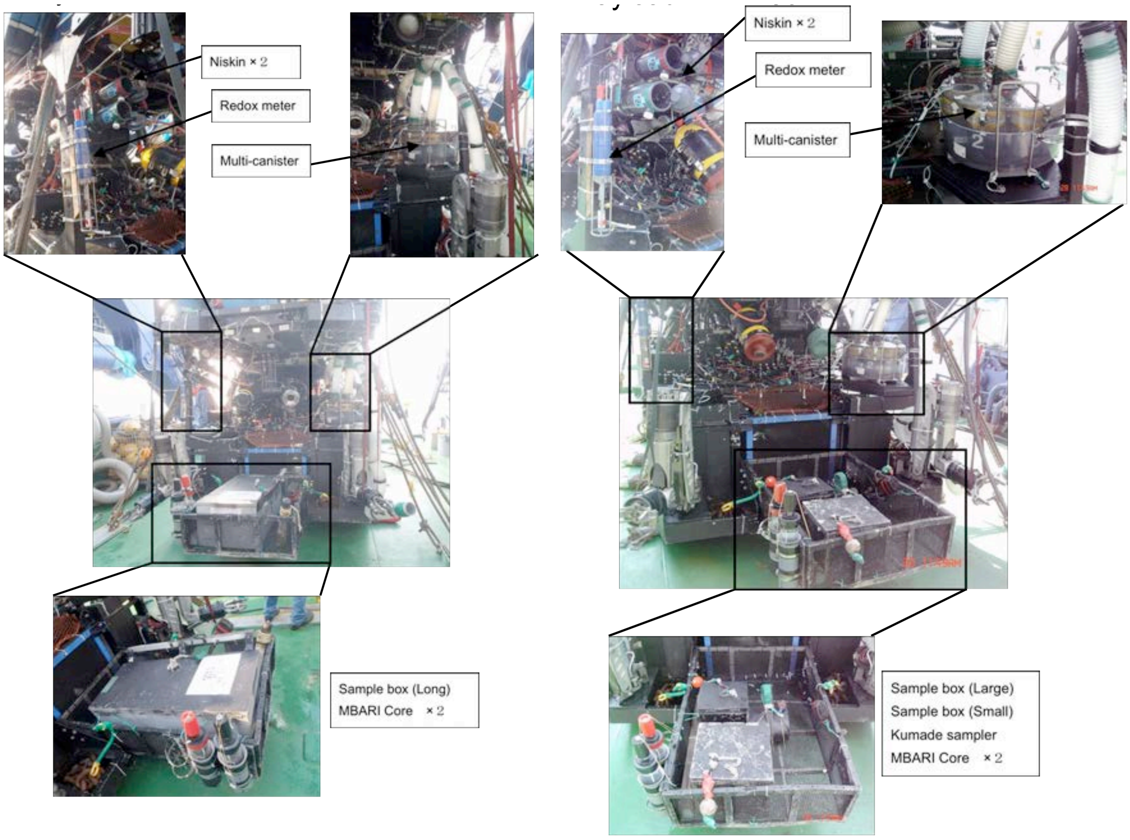

Fig. 4 Payloads mounted on Hyper-Dolphin for dives #1360 (left) and #1361 (right)

3.1 I-SEA multi-element analyzer

The I-SEA (In situ Seafloor Element Analyzer) is a chemical sensor developed to perform in situ, multi-element analysis of the composition of liquid and solids at depths of up to 3000m. The principle of measurement is based on LIBS (Laser-Induced Breakdown Spectroscopy), which is a form of atomic emission spectroscopy that performs measurements on plasmas generated by focusing a high power pulse laser (6 MW, 8ns) onto a liquid, or solid target. This cruise is the first deployment of this device, and it is the first time this measurement technique has been applied at sea. The sensor pictured in figure 5, consists of two parts, a main body and a probe connected with a 2.5m optical fiber cable. The main body of the sensor contains lasers to generate the plasmas and instruments to perform spectroscopic measurements. The probe focuses the laser and the observation optics onto the target. The main body of I-SEA has a diameter of 303mm, a length of 1500mm and weighs 110kgs in air (0kgs in water) and is composed of a power supply, two lasers, a spectrograph and a detector. Figure 6 shows the internal configuration of the main body. The probe, which makes contact with the target to be measured has a diameter of 50mm and a length of 150mm.

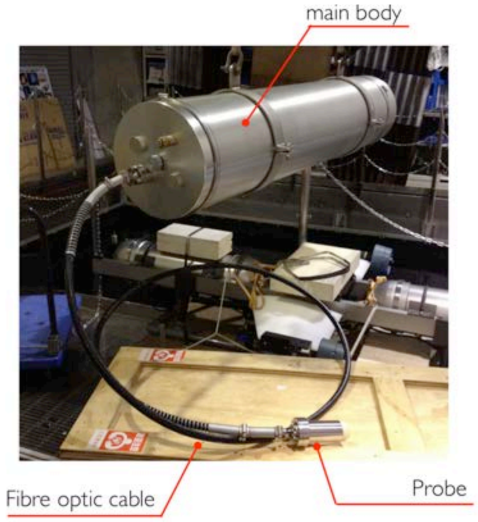

Fig. 5 I-SEA multi element analyzer

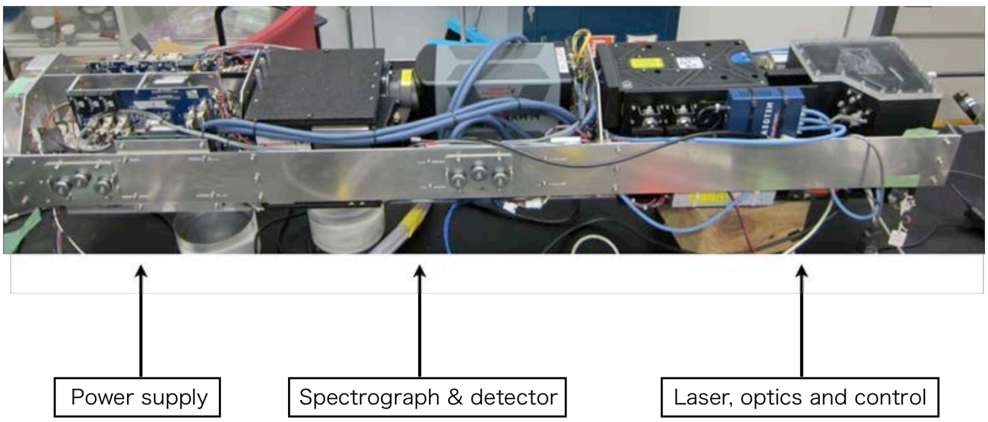

Fig. 6 Inside the main body of I-SEA

The device was attached to the payload box located at the center of Hyper-Dolphin during dives HPD#1359 and HPD#1362. During HPD#1359, analysis of the composition of seawater was successfully performed. During HPD#1362, measurements of the chemical composition of solids was successfully performed. Figure 7 shows details of the spectra for calcium, lithium and potassium measured during HPD#1359. During measurements of seawater, signals thought to originate from particulate matter were observed, as show in figure 8, which shows part of a C_2 swan band. The deployment during this cruise is the first time LIBS has been applied at sea, and the measurements made during this cruise are the first time plasmas have been used as a mechanism for in situ multi-element analysis at sea.

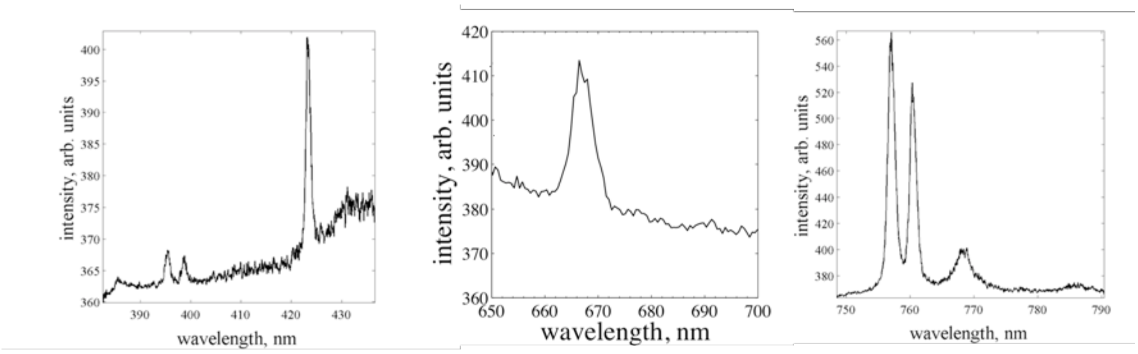

Fig. 7 Detailed views of spectra observed from seawater measurements at 200m depth showing calcium (left), lithium (center) and potassium (right)

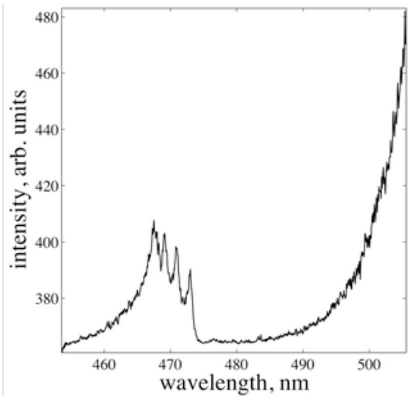

Fig. 8 Detailed view of a C_2 swan band measured at 200m depth. The signal is thought to originate from particulate matter suspended in the seawater.

3.2 Long range seaXerocks 3D visual mapping system

Typically visual mapping of the seafloor is carried out from altitudes of 2~3m, as with the mapping performed using seaXerocks during NT10-11, KY11-02 and NT12-05. The long range seaXerocks is a newly developed long range vision based mapping device, which was mounted on Hyper-Dolphin and tested for the first time during this cruise. The newly developed system consists of a high sensitivity camera, a sheet laser and multiple flashes, which were mounted on the payload box and at the back of the vehicle. The sheet laser projects a line onto the seafloor perpendicular to the direction of motion of the vehicle and the colour camera is mounted 145cm behind the sheet laser and directed vertically downwards.

As the ROV moves forwards, the projected laser line scans the shape of the seafloor and through triangulation of the laser projection captured by the camera, it is possible to generate a topographic map of the scanned seafloor. In order to map the data from the vehicle's frame of reference to a global frame of reference, a DVL with compass and attitude sensor as well as a depth sensor were used to measure the vehicle's position and orientation throughout the dive. Both triggering of the flashes and the camera are controlled by the computer. Most of the time, one image was recorded per second and the flashes triggered every six seconds, resulting in five out of six photos showing the laser line projection and the sixth one showing the seafloor illuminated by the flashes. The camera records raw Bayer filtered images, which are converted to colour images in post processing. This gives control over the white balance by applying optimised software gains on the three colour channels without losing quality in the process. The photos can then be used for generating a 3D reconstruction of the seafloor.

During the dive HPD#1359, four flashes were used and it was found that the flashes were much brighter than the laser line, so that no camera gain could be found that was suitable for both laser line imaging and flash photography. Also, the lens used was not focus correctly during this dive resulting in blurred images as can be seen in fig. 9. In this image, the sensitivity of the camera was set to its minimum value to prevent saturation of the image. The image was recorded from an altitude of 6m. It can be seen that colour information and imaging of the seafloor can be obtained from high altitude in relatively turbid waters. During HPD#1362, only 2 flashes were used and semi-transparent plastic sheets were wrapped around them to decrease their brightness. Good images of the laser line projection could be taken such as in fig. 10 (a), but this time, it turned out that the flash photos were too dark, as shown in fig. 10 (b). As a remedy, Hyper-Dolphin's lights were pointed downwards, illuminating part of the image and leading to clear photos of the seafloor, as shown in fig. 11, which was taken from 5m altitude. Although the first operation of the long range mapping system during this cruise was riddled with problems due to human error during setup, the images show that colour imaging from 5~6m is possible even in turbid waters, and it is considered that mapping from distances of greater than 8m should be possible with the developed system, provided that the camera and lighting setup is correct.

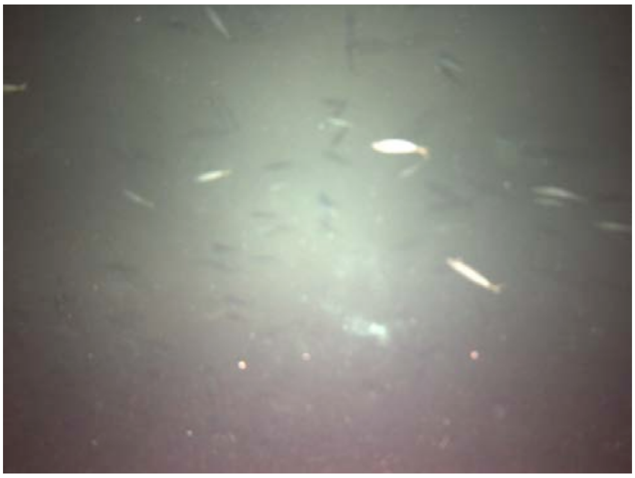

Fig. 9: Flash photo (blurred due to an unfocused lens) taken during HPD#1359 showing fish and their shadows on the seafloor. White discoloration of the seafloor is visible in the image. This image was taken from an altitude of 6m.

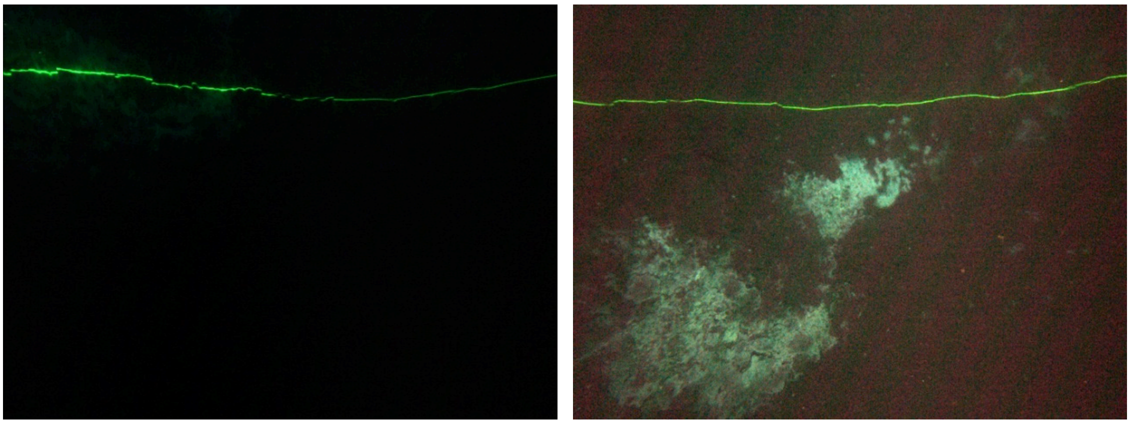

Fig. 10 (a): Photo of laser line projection; (b): Flash photo taken during HPD#1362 from an altitude of 5m. Too little light from the flashes reaches the seafloor due to over enthused efforts to avoid image saturation, leading to a dark image even with high software gain.

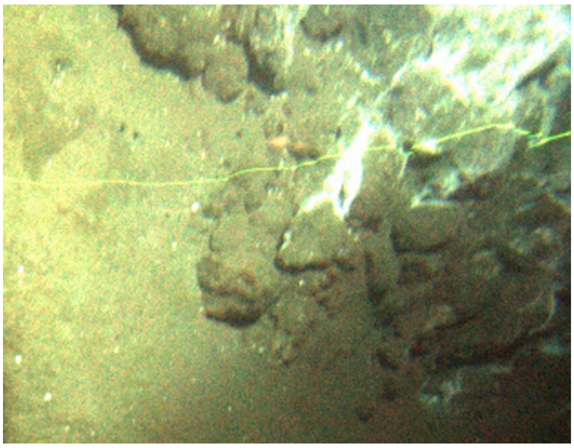

Fig. 11: Illuminated section of a colour photo taken from an altitude of 5m using Hyper-Dolphins lights

3.3 GB-2 gamma radiation sensor

The GB-2 is a sensor developed to perform in situ measurements of gamma radiation underwater and is capable of measuring an energy spectrum with 1024 channel resolution. GB-2 has a diameter of 110mm and is 500mm long, weighing 9.6kg in air and 5.6kg in water. During this cruise a 76.2 by 76.2mm BGO scintillator was used as a detector. Due to the attenuation of gamma radiation in water, the scintillator must be placed within 10cm of its target to make measurements underwater. GB-2 was mounted on the payload box of Hyper-Dolphin so that when the ROV lands on the seafloor, its detector is located within 5cm of the seafloor.

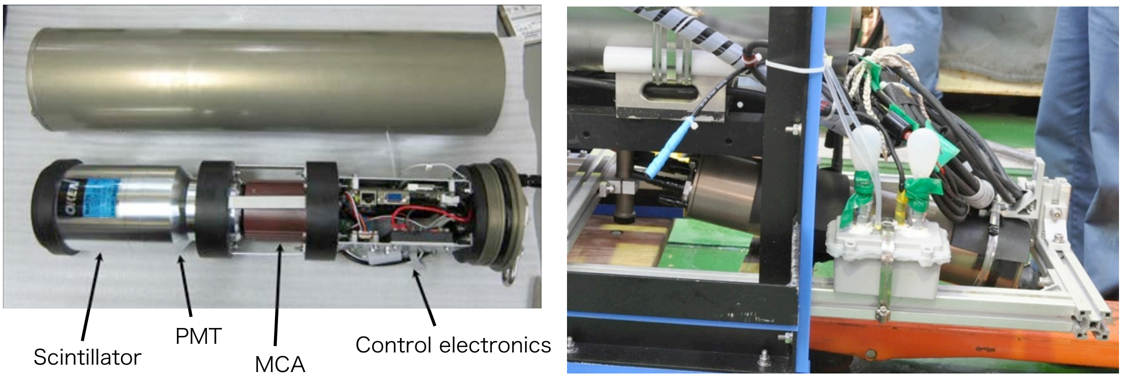

Fig. 12: Main housing of GB-2 (left) and GB-2 mounted on the payload box of Hyper-Dolphin (right)

During dives HPD#1359 and HPD#1362, GB-2 was used to perform measurements of seafloor radioactivity around the hydrothermal site. Figure 13 shows the measurements made by the sensor during HPD#1359.

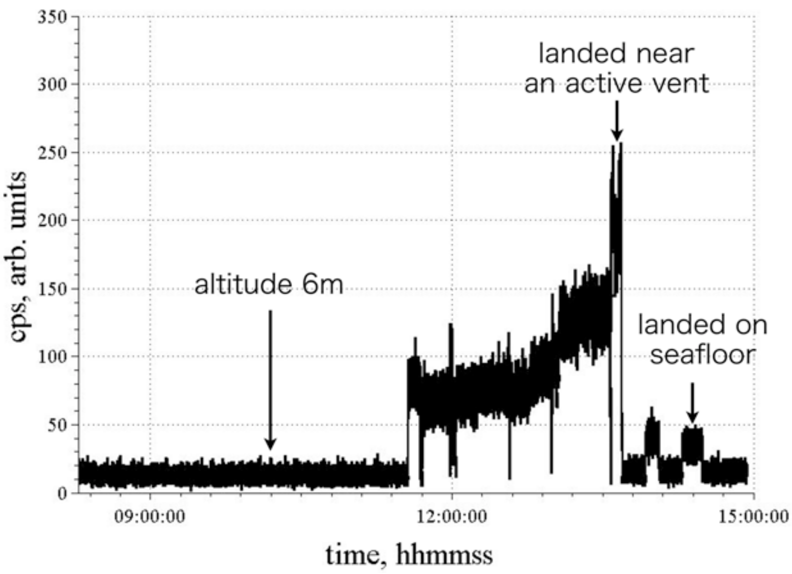

Fig. 13: Measurements of GB-2 during HPD#1359

3.4 ISFET-pH

Two pH sensors were attached to front left and right sides of Hyper-Dolphin where seawater flow is unobstructed. An ISFET (ion-sensitive field-effect transistor) is used for these devices to achieve high resolution measurements of seawater pH. Calibration with two kinds of buffer solutions, AMP and TRIS, was conducted before and after each of the dives to achieve high accuracy. These sensors were used during HPD#1359 and HPD#1362. Profiles of the seawater characteristics were successfully obtained along the submarine caldera from the seawater surface to 200m depth.

3.5 Marine-biological survey methods

3.5.1 Towards the development of deep-sea organismal clocks

We deployed stainless steel frames (Namekujira_11) on which 24 individuals of *Scalpellum stearnsii* were attached on 13th April 2011 off Cape Noma-Misaki. They had been measured and stained using both calcein and strontium chloride for 1 day just before deployment. By recollecting them using Hyper-Dolphin, we could obtain the growth of individuals nearly 1 year later.

3.5.2 Study on reproductive and dispersal strategy of Osedax japonicus

We deployed the *Osedax* colonization device named "Namekujira_11" on April 13, 2011. It is composed of four frames with a cow bone and some pig bones, and expected to settle on the middle of two whale falls (No. 7 and No.11). We will recover some of frames (from KDM-9 to -12) by using Hyper-Dolphin. We will also visit and observe some of whale falls, and collect bones hopefully settled by *O. japonicus* to investigate the population diversity, male-female relationships, the mechanisms of sex-determination and host-symbiont relationships.

4. Dive summary

4.1 HPD#1359

LIBS measurements of seawater composition and visual mapping of a 100m by 100m area of the Wakamiko caldera at a depth of approximately 200m in Kagoshima Bay was performed during this dive. Gamma radiation and pH measurements were continuously performed. The LIBS setup without the fibre, where the beam is directly focused into the seawater was used. Measurements were performed using different levels of output power and using different gratings in the spectrometer.

At the beginning of the dive, the camera settings were adjusted. It became clear, that it was not possible to find a combination of camera gain and exposure that allows taking images of the laser line without saturating photos where the flash is triggered, and respectively allows taking properly illuminated flash photos and also images of just the (much darker) laser line. Therefore the camera settings were changed during the dive to take sets of photos with parameters adjusted for capturing the laser line and others with settings adjusted for flash images. Hyper-Dolphin performed a lawnmower pattern supported by a GUI developed by the research group, showing the pilot the ROV's position measured by the DVL and the path to be followed. Rock, sediment and water samples were taken throughout the dive.

Time	Depth	Remark
8:40	198m	Reach the seafloor, start of seawater measurements, seafloor mapping and sampling
11:56	198m	End seafloor mapping
12:26	198m	Deployment of long term temperature measurement device, Rock and sediment sampling
12:38	198m	Measurements near hydrothermal active vents
13:53	194m	Niskin bottle seawater sample 1
14:43	177m	Start of ascent
14:49	96m	Niskin bottle seawater sample 2
14:52	0m	Reaching surface

Table 3: Event log for HPD#1359

4.2 HPD#1360

Diving site: Off Cape Noma-Misaki, Kagoshima
Landing Point: 31°20.577'N, 129°59.294'E, 228 m deep
Leaving Point: 31°20.485'N, 129°59.330'E, 229 m deep
[Sampling/Information of research stations]
Osedax colonization device (Namekujira_11) was searched and discovered at the depth

of 229 m off Nomamisaki. Four frames (KDM-09 - 12) were recovered. Water was also sampled. Small individuals of *Osedax* sp. were found on at least pig bones in the frames. Only one of 24 individuals of *S. stearnsii* those were attached to the frames on Namekujira_11 and released on 13th April 2011. All other individuals were disappeared. We could collect several hundred individuals of *Heteralepas* sp., one individual of *Octolasmis* sp. and one individual of *Scalpellidae* sp. settled on the artificial substrata of Namekujira_11.

4.3 HPD#1361

Diving site: Off Cape Noma-Misaki, Kagoshima
Landing Point: 31°21.028'N, 129°59.161'E, 225 m deep
Leaving Point: 31°20.812'N, 129°59.238'E, 226 m deep
[Sampling/Information of research stations]
#6 whale fall was observed. After 10 years of deployment, the main purpose of this dive was to observe the change in the animal community living on whale bones, especially the bone-eating worm *Osedax japonicus*. We could find many individuals of *Osedax*, as well as sea anemones, crinoids, and fishes. There we collected four small pieces of whale bones, and two crinoids, some sea anemones, and water and sediment samples. Then the ROV moved towards #7 whale fall, and on the course we observed animal community on the sand bottom. We collected a fish, a ctenophoran (*Lyrocteis imperatoris*), a barnacle (*Scalpellum streansii*), and three individuals of octocorals.

4.4 HPD#1362

Dive HPD#1362 took place again in the Wakamiko caldera of Kagoshima Bay. LIBS measurement of solids and seawater, visual mapping, gamma radiation measurements as well as sampling of sediments and rocks were performed. Mapping was performed during the first half of the dive, with Hyper-Dolphin following a lawnmower pattern covering an area of 70m by 70m. In the beginning the camera settings were adjusted and during mapping LIBS measurements of the water were taken as well. During the second half of the dive, LIBS measurements were performed on solids.

Time	Depth	Remark
8:32	193m	Reach the seafloor, Start mapping
10:34	197m	End mapping, Measure temperature White Cone vent fluids
10:38	197m	Measurements of solids and sampling
11:21	198m	Sediment sampling using MBARI sampler
11:22	198m	End of dive

Table 4: Event log for HPD#1362

5. Future Plans

5.1 Instrumentation

The deployment during this cruise was the first time a LIBS device has been taken to sea. Successful in situ multi-element analysis of seawater and solids were performed at a depth of 200m. The measurements made during this cruise are the first time plasma has been used as a mechanism for in situ multi-element analysis at sea. A number of points concerning the operation of the device were uncovered during this cruise and will be investigated in our future work. First, during some seawater measurements, signals exhibiting distinct spectral signatures were observed, and it is thought that these may originate from particulate matter suspended in the seawater, and future efforts should focus on this as a potential application for LIBS. Second, concerning the measurement of solids, although element specific spectra could be observed, focusing the laser onto solid targets with the ROV manipulator was more time consuming than expected. In the future, efforts are required to increase the depth of focus of the system and introduce mechanisms to focus the laser to allow for more efficient operation of the device. For the latter, it is suggested that the LIBS measurement probe can be mounted on a linear actuator to allow for fine adjustments of the distance to the target to be made by the user, or even auto-focusing to perform more efficient measurements. In addition to these practical developments, it is important to investigate the effects of the parameters of the laser on the sensitivity of the system and investigate methods to process the measured spectral data in a quantitative manner, using Calibration-Free LIBS analysis techniques. Testing of the data processing algorithms will be performed based on quantitative analysis of samples obtained during this cruise using techniques such as ICP-AES and ICP-MS. Also chemical analysis of fluid samples and sediments will be performed together with mineralogical studies of the hydrothermal deposits obtained during the cruise.

With regard to the mapping system, the luminosity of the sheet laser was relatively low compared to the flashes, and a more powerful sheet laser should be used in the future. We are also planning to improve the control program of the camera to allow changing gain and exposure settings between frames, so that flash photos and photos of the laser line can be taken using different settings even if they are taken in series.

5.2 Analysis of biological samples

On 28 March 2012 we recollected only one individual of *S. stearnsii* (No. 10) attached to the frames on Namekujira_11. This individual grew from 175.5 mm to 183.0 mm in total length, or from 57.65 mm to 58.85 mm in capitulum length, in almost 1 year. All other individuals disappeared, probably due to the damage of landing, staining or deployment. We could collect several hundred individuals of *Heteralepas* sp., one individual of *Octolasmis* sp. and one individual of *Scalpellidae* sp. settled on the artificial substrata of Namekujira_11. On HPD#1361, we could also collect an individual of wild *S. stearnsii* attached on a dead gastropod shell.

In our future work, for the one individual of *S. stearnsii* recollected, we will count the growth lines, and try to check the growth after staining. We fixed most individuals of *Heteralepas* sp. and will measure their sizes later. This will give us the information on their growth in maximum 1 year. In NT10-07, we also collected many individuals of the same species of *Heteralepas* that had been attached within 4 months. Thus, we have two calibrating time points for estimating their growth curves. In addition, we will rear the remaining (ca. 100) individuals of *Heteralepas* collected this time, to further observe their growth curves in the aquaria kept at 15 ^{o}C.

On HPD#1360 of 28 March 2012, we recovered four frames (from KDM-9 to -12) of Namekujira_11 and retrieved 2 cow bones and 12 pig bones from frames. We could find some individuals of *O. japonicas* on pig bones. On HPD#1361, we sampled a whale bone (upper jaw) from No. 6 whale and found some aggregations of collect an individual of *O. japonicus*. In our future work, *Osedax* will be cultured on a whale bone and pig bones in JAMSTEC and Nara women's university to observe their reproductive trait.

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