



NATSUSHIMA Cruise Report
NT10-05 Leg2

Wakamiko Caldera
Kagoshima Bay, Japan

March 18, 2010 – March 30, 2010

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in collaboration with:
Central Research Institute of Electric Power Industry,
Kyushu University,
&
Japan Agency for Marine-Earth Science and Technology
(JAMSTEC)

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Preface

This report describes preliminary information on the data taken during NT10-05 Leg2 cruise by R/V Natsushima with ROV HyperDolphin from March 18 to 30, 2010.

NT10-05 Leg2 cruise was conducted based on the proposal #S09-76 titled “A study on adaptive *in situ* measurement method based on Mapping-by-AUV” (representative: Teruo Fujii / University of Tokyo) and #S09-75 titled “Geochemical studies on gas-fluid-sediment interactions within modern sediment at the Wakamiko submarine crater, Kagoshima Bay, south Kyushu” (representative: Junichiro Ishibashi / Kyushu University).

As for #S09-76, the purpose of the cruise is to examine the feasibility of a new method of adaptive measurement by ROV based on the results of mapping by AUV, combining small-sized *in situ* measurement systems and mapping technologies to understand the complex spatio-temporal behavior of hydrothermal plumes.

As for #S09-75, the purpose of the cruise is to study variety of geochemical processes in a marine shallow-water hydrothermal system located in the Wakamiko submarine crater. The working hypothesis is involvement of submarine fumarole into the hydrothermal fluid modifies fluid chemistry such as pH, and controls hydrothermal interactions and precipitations occurred in the sediment layer.

In this cruise, the research works described in the following have been conducted mostly focusing on the hydrothermal activity in Wakamiko caldera. The adaptive measurement using AUV and ROV has been done in the area of “Haorimushi site” at the east end of the caldera considering the maximum operating depth 100m of AUV REMUS. Two prototypes of *in situ* measurement systems (IISA-gene and IISA-AMISpH) were deployed into the deep-sea environment on ROV HyperDolphin, and the analysis and measurement have been successfully carried out. Although the detailed analyses are needed to validate the data, a plenty of useful information to further improve the systems could be collected. As for the plume mapping technology, eight horizontal layers were measured at two different depths and in four different tidal phases. Using the data obtained, we are building the numerical model taking into account of the effect of tidal currents that will lead to 4D pictures of hydrothermal plumes. In addition to the mapping study, sampling of fluid, sediment, gas and seawater were conducted. The purpose of the sampling study is to reveal various geochemical processes associated with hydrothermal fluid venting in a marine shallow-water hydrothermal system. Also some test operations of instruments newly developed for

functioning on the seafloor were conducted. Geochemical and mineralogical analyses will further be conducted with the collected samples for the detailed understanding of the hydrothermal system in the area.

Finally, I would like to thank all the members of the HyperDolphin operation team (Chief: Yoshio Ohno), and the crews of R/V Natsushima (Captain: Tomotugu Susami) for their dedicated efforts to make this cruise so successful.

March 2010

Teruo Fujii (NT10-05 Leg 2 Chief Scientist)

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1. Cruise Information

(1) Cruise number/ship name: NT10-05 Leg2/ Natsushima

(2) Title of the cruise:

FY2009 Deep Sea Survey by ROV Hyper Dolphin in Kagoshima Bay

(3) Proposal number/Title of the proposal:

S09-76 (representative: Teruo Fujii, University of Tokyo)

“A study on adaptive in situ measurement method based on Mapping-by-AUV”

S09-75 (representative: Junichiro Ishibashi, Kyushu University)

“Geochemical studies on gas-fluid-sediment interactions within modern sediment at the Wakamiko submarine crater, Kagoshima Bay, south Kyushu”

(4) Period of the cruise: 13 days from March 18 to March 30, 2010

(5) Port calls: dep. Kagoshima, ret. Kagoshima

(6) Research area: Wakamiko Caldera in Kagoshima Bay (Fig.1)

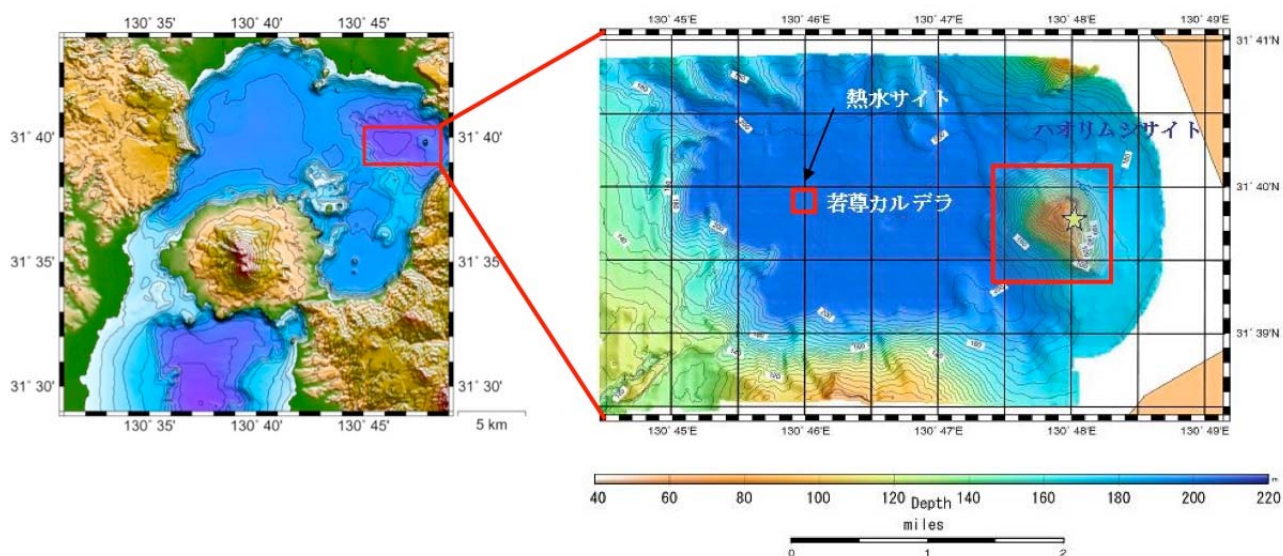


Fig.1 Research Area (Wakamiko Caldera in Kagoshima Bay)

(7) Dive list (Fig.2)

#1088: ROCS deployment, MEDUSA deployment, SAHF, Sampling (water)

#1089: ADCP deployment, Mapping Trial, Sampling (mud)

#1090: GAMOS deployment, ROCS recovery, Sampling (water)

#1091: Marker deployment, SAHF relocation, Sampling (gas, water, mud, rocks)

#1092: Verification of the measurement by AUV, Sampling (water, mud)

#1093: GAMOS recovery, SAHF, ROCS, Sampling (water, mud)

#1094: iPow deployment, SAHF, ROCS, Sampling (water, mud)

- #1095: iPow recovery, ROCS, Subsurface-pH, Sampling (water, mud)
- #1096: Plume Mapping, MEDUSA deployment, Sampling (water, rocks)
- #1097: ROCS, Sampling (gas, water, mud)
- #1098: Plume Mapping, Sampling (water)
- #1099: Plume Mapping
- #1100: SAHF, ROCS, Sampling (gas, water, mud)
- #1101: Mapping, ROCS, MEDUSA recovery, Subsurface-pH, Sampling (gas, water, mud)
- #1102: SAHF, ADCP recovery, Sampling (water, mud)
- #1103: ROCS, Marker deployment, Sampling (water, mud)

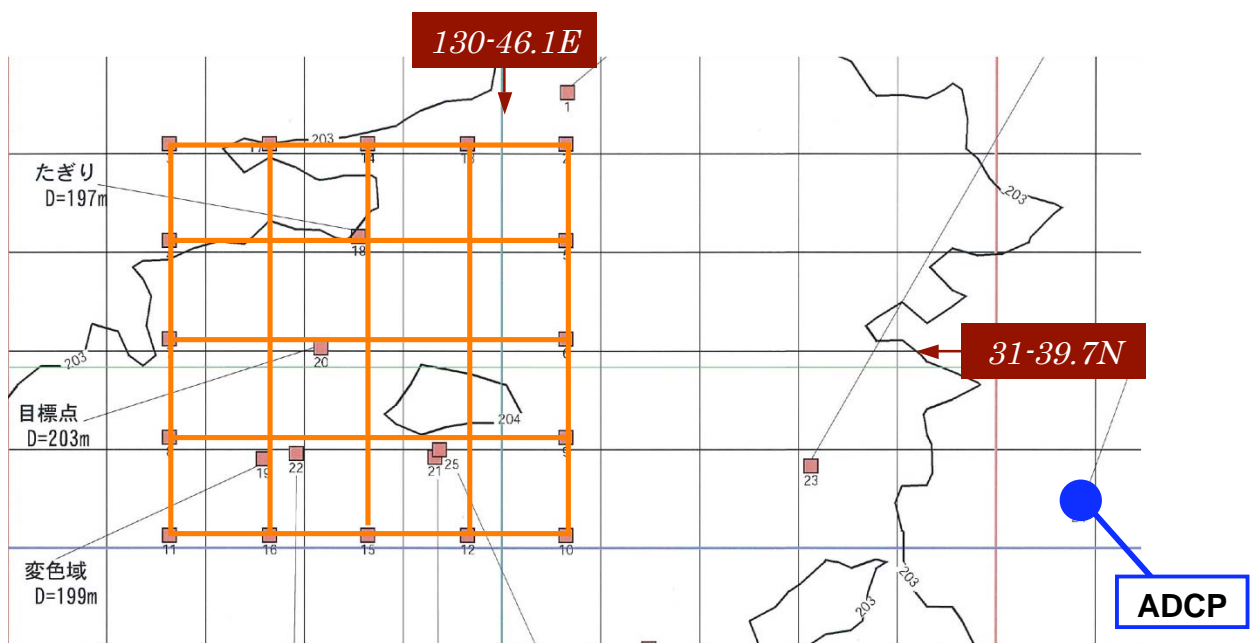


Fig. 2 Measurement Lines for Plume Mapping and Locations of ADCP

2. Researchers

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Junichiro ISHIBASHI [Kyushu University]

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3.1 Adaptive Measurement Method Based on Mapping-by-AUV

Y. Maeda (CERES), T. Fujii (IIS, University of Tokyo)

M. Kyo (JAMSTEC), and K. Shitashima (CRIEPI)

(1) Introduction

The development of relatively low-cost, portable autonomous underwater vehicles (AUVs) in the past few years has made a new class of ocean measurements possible. For example, the REMUS (Remote Environmental Monitoring UnitS) AUV built by Hydroid, Inc. has been used to make a variety of biological, chemical, and physical measurements in the near-shore environment. REMUS has been used to measure properties including scale-dependent dispersion on the continental shelf, bioluminescence, and turbulence microstructure, and also to successfully track chemical plumes using an adaptive mission planner. More recently, investigators have used the vehicle to deduce bottom roughness characteristics, quantify sand ripple structure, and measure chlorophyll-*a* concentrations. In each of these example applications, the REMUS AUV has provided a platform much more stable than typical ship-deployed/mounted instrumentation as well as greater maneuverability and more accurate positioning. In several instances, instrumentation installed on the REMUS AUV has been used as a substitute for traditional ship measurement techniques and, in some cases, has allowed for the collection of data unattainable by traditional ship-based techniques. In this cruise, the research works described in the following has been conducted mostly focusing on the hydrothermal activity in Wakamiko caldera. The adaptive measurement using AUV and ROV has been done in the area of “Haorimushi site and Tagiri site” at the east end of the caldera considering the maximum operating depth 100m of AUV REMUS (Fig.3).

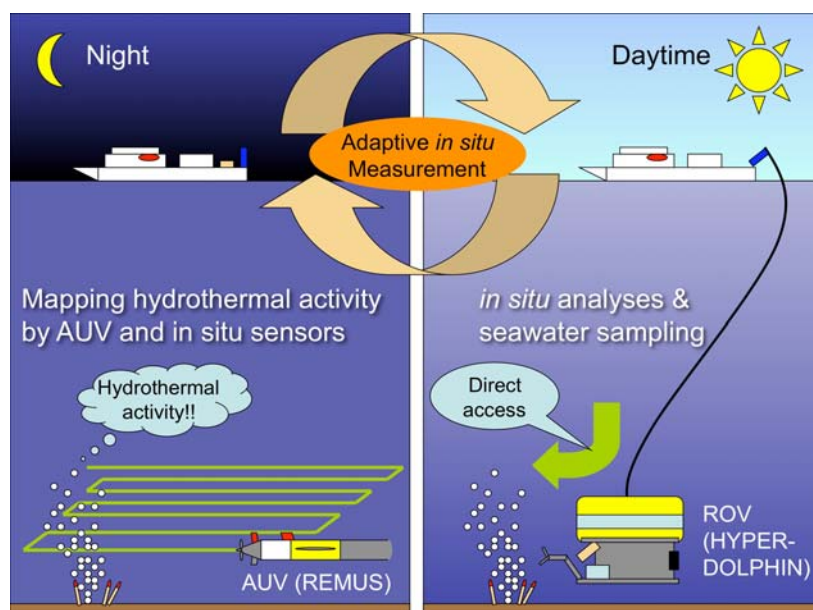


Fig. 3 Concept of adaptive measurement method

In order to develop a new method of adaptive measurement by ROV based on mapping-by-AUV, the operation of AUV REMUS from R/V Natushima was attempted. The deployment and recovery of mooring systems for REMUS transponders were carried out from R/V Natsushima, and the transponder installations and the deployment and recovery of the vehicle were conducted using a small boat launched from R/V Natsushima.

(2) Specifications and payload sensor of REMUS AUV

The REMUS-100 AUV size is 19cm in diameter and the standard vehicle is nominally 158cm long. It is a compact AUV easy to operate. The REMUS-100 has a special nose-corn to load various chemical sensors and is 200cm long. Payload sensors of REMUS is ADCP CT-sensor, Eco-puck and Oxygen optode sensor (See Fig. 4 and Table 1).

Table 1 Specifications and payload sensor of REMUS AUV

Navigation system	Dead reckoning and LBL by DVL and D-GPS
Bathymetric	Side scan sonar (900KHz and 1800KHz)
Current	ADCP/DVL(Top and Bottom)
Oxygen	Oxygen Optode
Chlorophyll-a and Turbidity.	Eco Puck
Temperature and Salinity	G-CTD9
pH/ORP and pCO ₂	CRIEPI's sensor
Communication	ACOMS / Iridium satellite communications (surfaces)



Fig.4 Deployment of AUV REMUS and Transponder mooring

(3) Deployment of AUV REMUS

The REMUS (Remote Environmental Measuring UnitS) Autonomous underwater vehicle (AUV) is an observation instrument, which is considerably effective for hydrothermal plume mapping. Bathymetric, current, salinity, temperature, turbidity, DO and pH/pCO₂ and ORP data are collected while the vehicle performs a ladder type search across and downstream of the hydrothermal plume source. These data are used to validate and refine an analytical plume behavior.

Wakamiko caldera (31°39'N-31°41'N and 130°45'E-130°40'E) located in KAGOSHIMA BAY, has caldera-shaped topography (Fig.5). In the East of this caldera, “Haori-mushi site”, the west are called “Tagiri site”. We deployed the REMUS AUV in this caldera. REMUS carried out the mission to dive around the mountain in “Haori-mushi site”, on April 20 and to dive depth 100m in “Tagiri site” on April 22 and 23rd (See Table 2 and the trajectories shown in Fig. 6 and Fig.7).

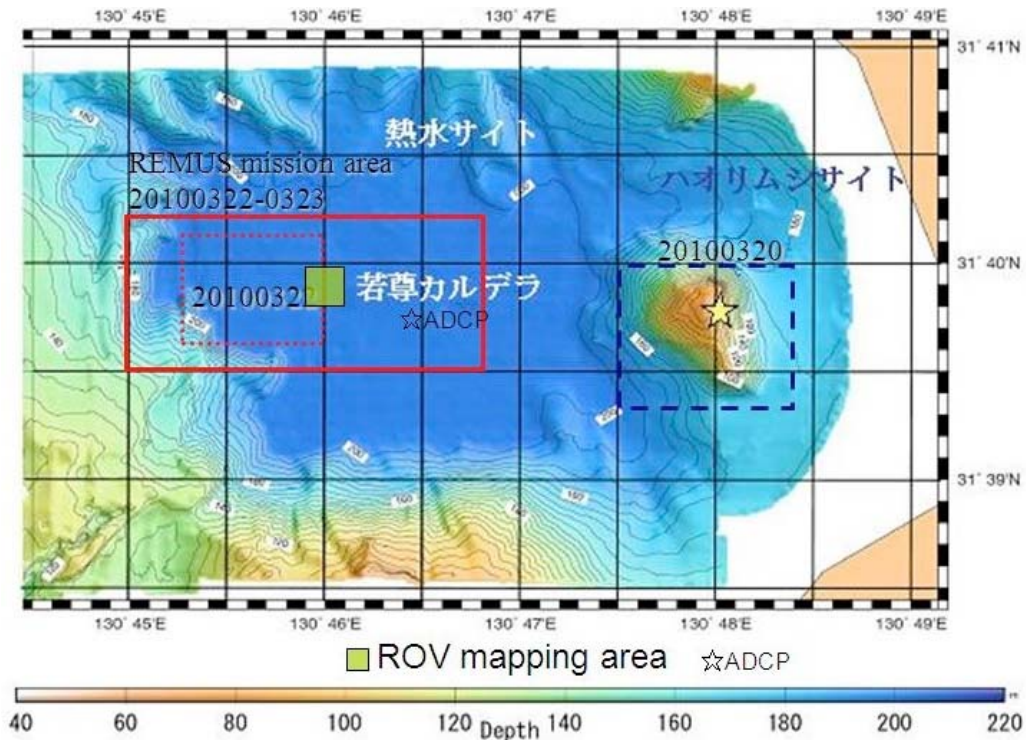


Fig. 5 Wakamiko caldera located in KAGOSHIMA BAY

Table 2 Deployment of AUV Schedule

Date&Time	Mission site	Area and depth
20March 8:30-9:50 (Mission failure)	Haori-mushi site	Depth around 100m
22March 8:10-11:30	Tagiri site	1.2km × 1.0km depth 100m
22March 17:00-07:00(23March)	Tagiri site	2.7km × 1.4km depth 100m

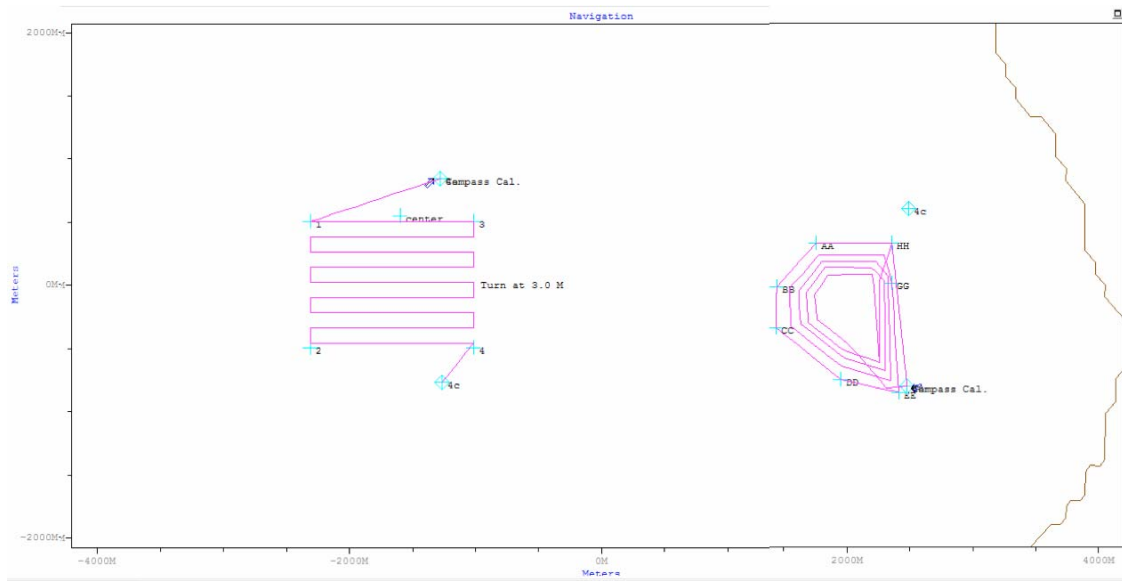


Fig.6 Deployment of AUV Mission (4/20 and 4/22 mission)

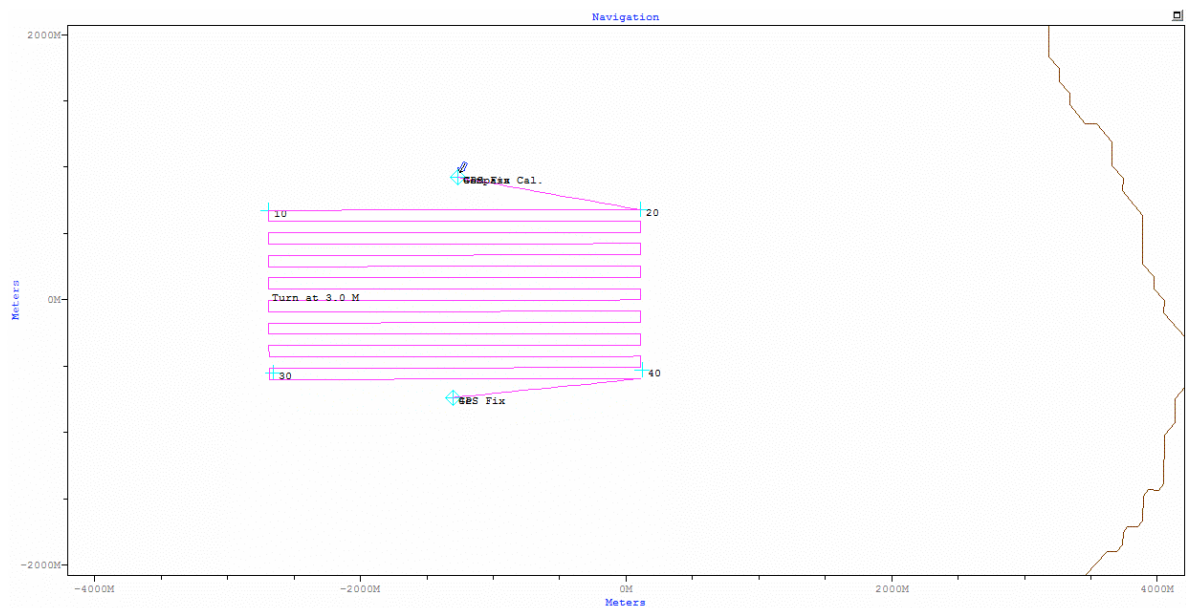


Fig.7 Deployment of AUV Mission (from 4/22 to 4/23 overnight mission)

(4) Results of REMUS Operation

(4-1) Haori-mushi site

We planned a mission to run at 100m deep, 90m and 80m at first around a mountain. But REMUS hit the seabed in mission halfway, and urgently surfaced. The measured distribution of water temperature, turbidity and pH are shown in Fig. 8. As the result of pH distribution, detected pH anomaly with two points around the Haori-mushi site. We used ROV and were to confirm this point, but it was impossible for the ROV to dive due to many fishing boats playing around this point.

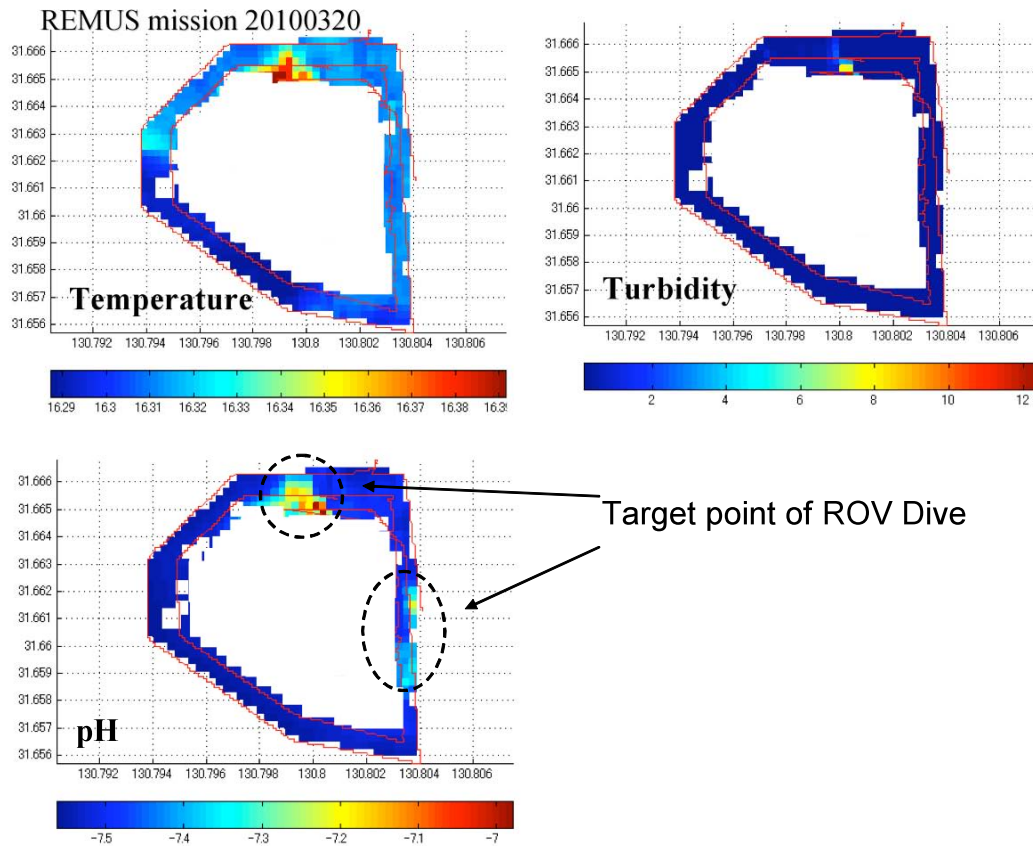


Fig.8 Distribution of temperature, turbidity and pH by REMUS

(4-2) TAGIRI site

At TAGIRI site, the 1st REMUS mission of March 22 was 1.2km×1.0km wide at 120 m spacing, diving depth is 100m and the speed is 2 knots. Mission time is about 3 hours. Temperature anomaly was detected in a wide area as shown in Fig.9. It is suggested that Plume of this temperature anomaly moves southwest from the northeast.

The 2nd REMUS mission of from March 22 to 23 was 2.7km×1.4km wide at 80m spacing, diving depth is 100m and the speed is 2 knots. Mission time is about 13 hours. In this mission, REMUS covered all half areas of a WAKAMIKO Caldera.

A total of 3 plume search missions were conducted in 3 days. A number of different operations in different areas were performed. Various data was collected on missions. Analysis of the data served to demonstrate the utility of using the REMUS to collect plume information.

NT10-05 AUV mapping at Kagoshima WAKAMIKO Caldera
 REMUS mission 20100322

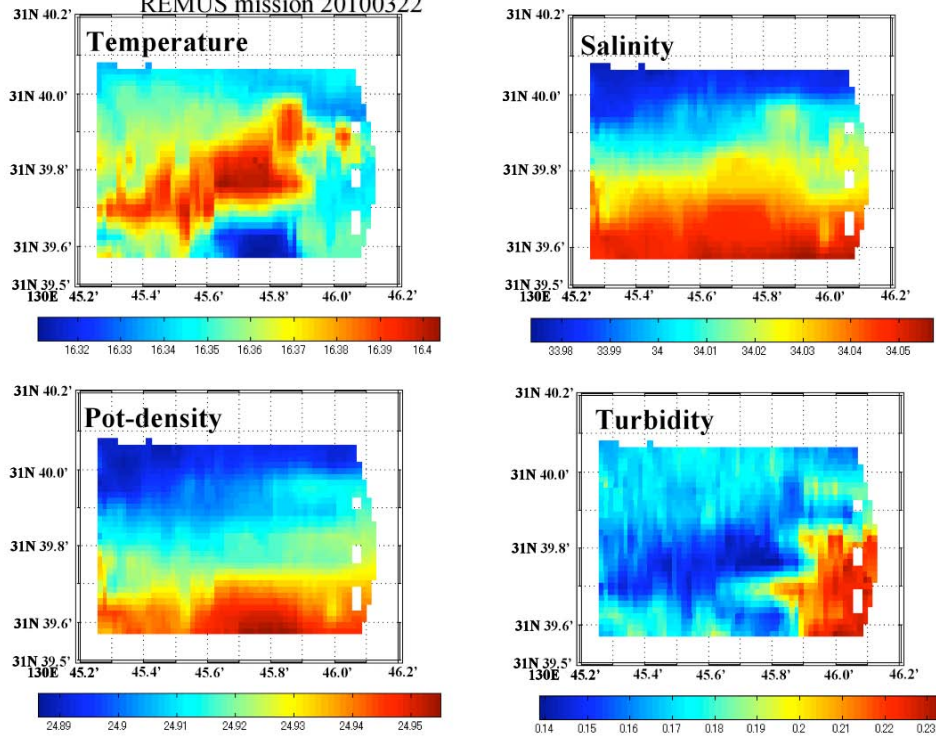


Fig.9 Distribution of temperature, turbidity and pH by REMUS (March 22)

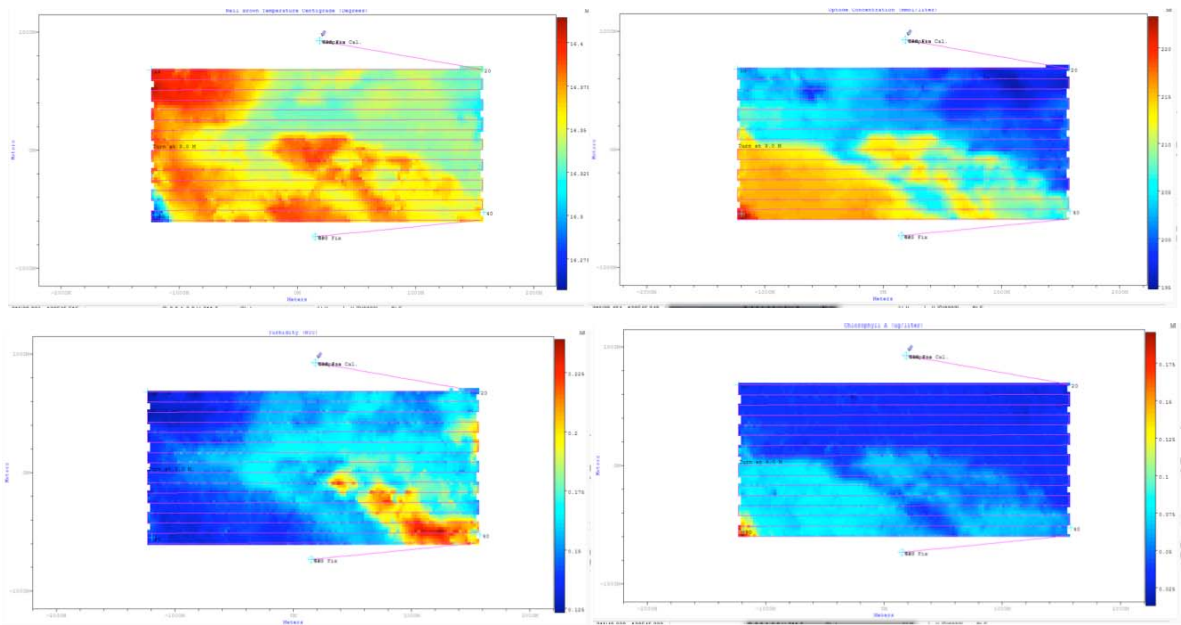


Fig.10 Distribution of temperature, turbidity and pH by REMUS (March 22 to 23)

(5) Observation by ROV following AUV Operation

The adaptive measurement using AUV and ROV has been done in the area of “Haori-mushi site” at the east end of the caldera and “Tagiri site”, considering the maximum operating depth 100m of AUV REMUS.

The horizontal distribution of temperature taken by the AUV on March 23 is shown in Fig.11. The Red marks (+) show the points where ROV dived after the AUV mission. There was a plume source on the seabed of the area where the temperature anomaly found by AUV and the spot of high water temperature was discovered in ROV Dive on March 24 or later. We will try to understand behavior of hydrothermal plume in this area considering this temperature distribution and the seawater current.

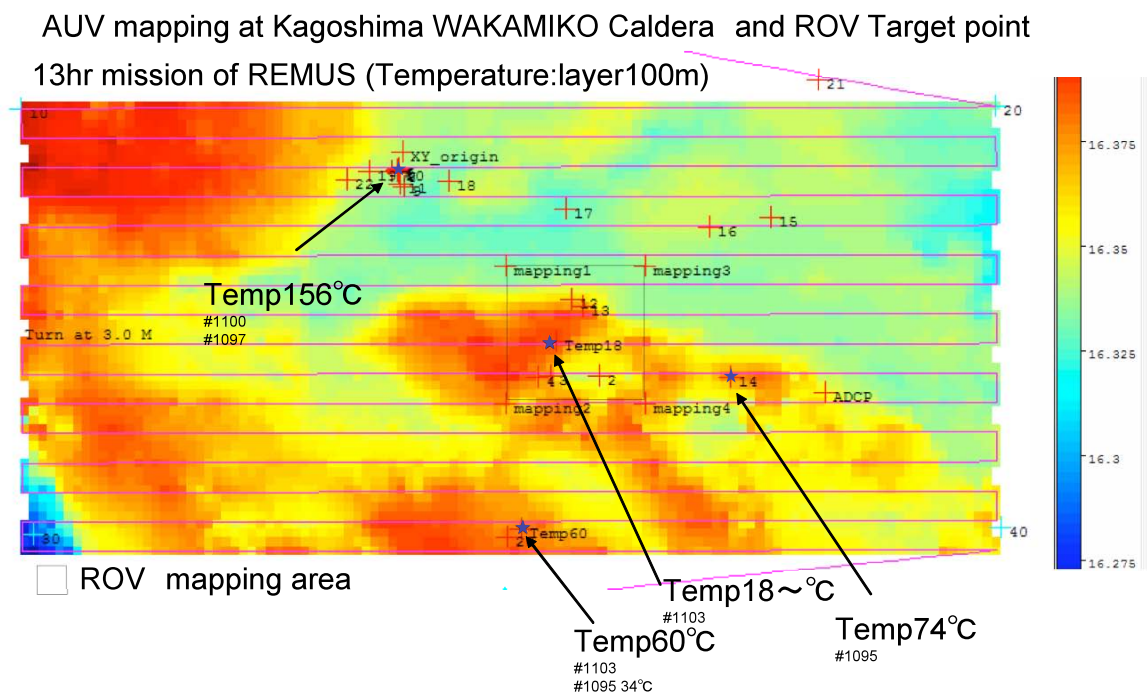


Fig.11 Temperature distribution measured by REMUS on March 23, and the dive points by ROV on March 24 or later

3.2 Field Trials of IISAs (Integrated in situ Analyzers)

T. Fukuba, T. Kusunoki, and T. Fujii (IIS, University of Tokyo)

M. Kyo (JAMSTEC), K. Shitashima (CRIEPI)

(1) Introduction

Making use of microfluidic technologies, we've been developing *in situ* analysis systems named "IISA (Integrated in situ Analyzer)". In this cruise, we operated two systems, an IISA-Gene and an IISA-AMISpH for field evaluation. Development and operation of functionally integrated and miniaturized devices are one of the most challenging and pioneering works in the field of microTAS (Micro Total Analysis Systems) as well as in oceanography.

The IISA-Gene system can perform PCR-based gene analysis in deep-sea environments. Microbial cells are introduced into the device and their genomic DNA is purified. To amplify targeted sequence from the genomic DNA, PCR is performed and the PCR products are detected optically.

The IISA-AMISpH is a system to measure pH accurately by using accumulation method ion sensor (AMIS) type pH ISFET (Bio-X, Japan). An AMIS sensor head is enclosed in an oil-filled box. A DC motor pump is equipped to pump seawater to the AMIS sensor head. The system has its own power supply (Li-ion battery), and PC in a pressure resistant housing. In addition, we also deployed a conventional pH-ISFET to compare the results.

During the IISA-Gene deployment, seawater samples were collected by Niskin bottles to analyze DNA contents onboard. From the water samples, we will try to detect total bacteria and methane oxidizers by using the IISA-Gene for system evaluation.

(2) Deployment of the IISA systems

The IISA-Gene system was connected to an ROV "HYPER DOLPHIN" for power supply (24VDC) and communication. Researchers could control the system by using a laptop PC via a RS-232C communication line.

Operation of the IISA-AMISpH was autonomously performed by embedded PC control. Temperature in an oil-filled box of the IISA-AMISpH sensor head was monitored using a RTD to compensate a temperature effect afterwards. The IISA-AMISpH and the pH-ISFET were calibrated using pH standards (AMP, TRIS) before and after each dive. To avoid air bubble clogging in a fluidic line, seawater inlet was protected by a plastic box that was filled with pH standard solution until the dive starts. The deployment list is shown in Table 3.

Table 3 IISA deployment list (O: deployed, -: Not deployed)

Date	Dive #	IISA-Gene	IISA-AMISpH	ISFET
19 Mar	#1088	-	-	-
	#1089	-	-	O
21 Mar	#1090	-	O	O
	#1091	O	-	O
23 Mar	#1092	-	O	O
24 Mar	#1093	-	O	O
	#1094	-	O	O
25 Mar	#1095	-	-	-
	#1096	-	O	O
26 Mar	#1097	-	-	-
	#1098	-	O	O
27 Mar	#1099	-	O	O
	#1100	-	-	-
28 Mar	#1101	O	O	O
29 Mar	#1102	-	-	-
	#1103	O	O	O



Fig.12 the IISA-Gene Mounted on HYPER-DOLPHIN (Dive #1091)

(3) The IISA-Gene deployment results

The IISA-Gene was operated in 3 dives (#1091, #1101 and #1103) (Fig.12). Targeted genes were bacterial 16S rRNA gene and pMMO (particulate methane monooxygenase) gene. A procedure for gene analysis operation is;

- 1) Sea water sample collection step (500 μ l, 2-3 min).
- 2) Cell lysis step (40 min)
- 3) Washing step (12 min)
- 4) PCR step (30-60 min)
- 5) Wash step (30 min)

The steps 5) is optional for multiple PCR operations.

During dive #1091 and #1101 and #1103, the IISA-Gene worked well and amplification of 16S rRNA gene and pMMO gene was attempted subsequent to seawater sample collection at near hydrothermal plumes. Immediately after the sample collection, all procedure for gene analysis was performed.

During the dive #1091, 16S rRNA gene was chosen as a target gene that will be amplified by PCR. For the first sample, we observed very high background signal before start the PCR (data not shown). During the PCR process, the signal kept high level constantly. It is because of unexpected intrusion of oil for PCR product segmentation into the microfluidic device. After washing out the oil from the microfluidic device by conducting an additional washing procedure, we could attempt a second PCR operation using a newly collected sample. In 20 min, there was a sharp increase of a signal (Fig.13). After the PCR for 16S rRNA gene, the PCR products were transferred to a PCR product collection coil for further onboard analysis.

During the dive #1101, 16S rRNA gene and pMMO gene was analyzed using the IISA-Gene. Subsequent to 16S rRNA amplification (Fig. 14, black), pMMO gene analysis was conducted. However, fluorescence intensity was not increased drastically as shown in Fig. 14 (gray line). After each PCR operation, PCR products were transferred to the PCR product collection coil for further onboard analysis.

During the dive #1103, the IISA-Gene was mounted and operated without any electrical troubles and 16S rRNA gene and pMMO gene was analyzed *in situ*. However, there was no signal increase during the analysis (data not shown). At that time, the microfluidic device had a same trouble with the dive #1091 and the microchannel was totally stopped by the oil.

Seawater samples were collected using Niskin water sampler when the IISA-Gene was operated. The collected water samples will be manually processed to extract microbial genomic DNA in laboratory to compare the gene analysis results with

in situ analysis data obtained by IISA-Gene.

Here, we conclude that the practical data from the real environment were successfully obtained by the IISA-Gene system. We also succeeded to figure out some improvement points on electronics and a fluidic component to realize more reliable system to operate *in situ*.

(4) IISA-AMISpH and ISFET pH sensor deployment results

The IISA-AMIS pH was mounted on HPD in 9 dives (#1090, #1092, #1093, #1094, #1096, #1098, #1099, #1101, and #1103) in total (Fig. 15). The pH-ISFET was also operated simultaneously to compare the data.

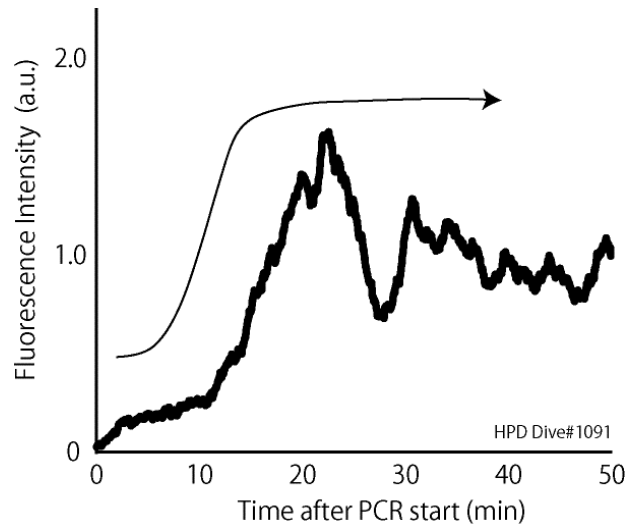


Fig. 13 Result of IISA-Gene operation in dive #1091

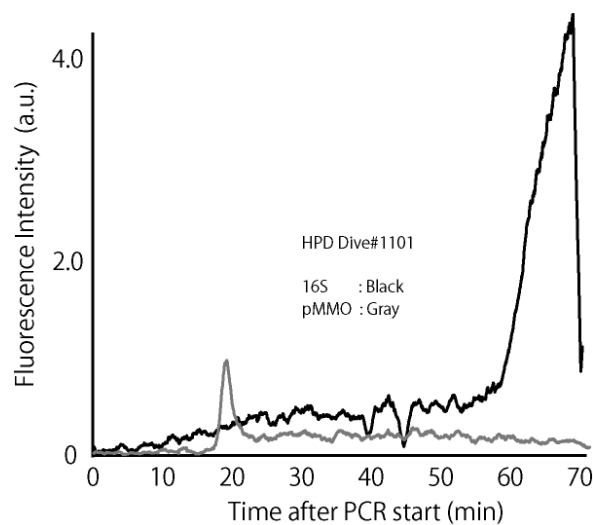


Fig. 14 Result of IISA-Gene operation in dive #1101

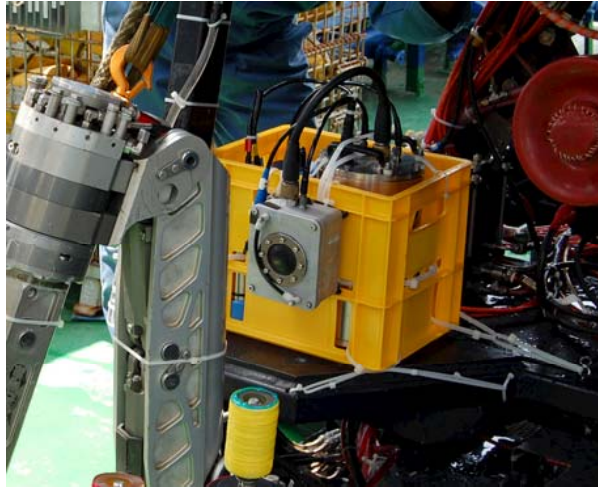


Fig. 15 IISA-AMISpH and ISFET pH sensor mounted on HYPER-DOLPHIN

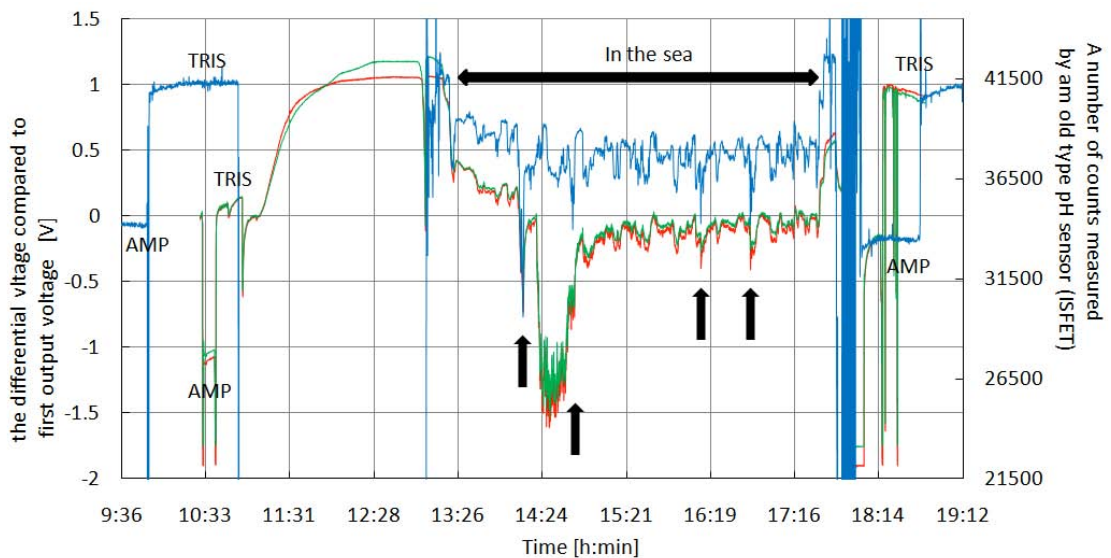


Fig. 16 Result of calibration and field operation of the IISA-AMISpH during dive #1098: Red and green lines show differential output voltage compared to the initial output voltage of each IISA-AMISpH ion sensitive membrane. Blue line is a number of counts measured by the ISFET-pH sensor.

Both pH sensors worked successfully during grid mapping operation and sampling operation at sea bottom. In some position, low-pH anomalies were detected when HPD approached to hydrothermal active area.

In dive #1090, a pH profile obtained by the IISA-AMISpH and the pH-ISFET were not consistent. It may be caused by troubles in a pumping system on the IISA-AMISpH (i.e. air bubble clogging). Same trouble occurred also in dive #1092.

In dive #1096, pH profiles obtained by the IISA-AMISpH and the pH-ISFET were consistent. However, pH variation measured by the IISA-AMISpH was smaller than that of the pH-ISFET. It is because the IISA-AMISpH measures pH values of the seawater samples that were pumped in the system. On the other hand, ion sensitive membrane of pH-ISFET is directly exposed to ambient seawater. So, the pH-ISFET shows sharp pH fluctuation caused by local pH heterogeneities. During the dives from #1098 to #1102, pH values were successfully obtained. Especially in dive #1098, pH anomalies were clearly observed using the IISA-AMISpH when the HYPER DOLPHIN approached to hydrothermal plumes with air bubbles (Fig. 16).

(5) Sample collection and onboard processing

Seawater samples were collected using Niskin for gene analysis and CTD Pylon water samplers for ATP quantification. The water was collected on the occasions when 1) the IISA-Gene was operated (Niskin), 2) the vehicle got closer to hydrothermal fluid (Pylon), 3) during the grid survey (Pylon).

50 ml portions of the Niskin samples collected when IISA-Gene was operated were filtrated by disposable syringes with 0.22 μm pore size filter (Sterivex-GV, Millipore Co., USA) for immediate onboard DNA analysis. Microbial genomic DNA was extracted from microbes collected on a filter and analyzed by PCR. 16S rRNA gene was successfully amplified for all the samples analyzed. pMMO was also amplified in some samples.

CTDT Pylon containing twelve Niskin bottles was mounted onto the vehicle to obtain water sample for plume mapping operations. Total (dissolved and particulate) ATP was quantified for all pylon seawater samples collected during the dive #1098 and 1099. Calibration curves were obtained by using 0 to 2×10^{-9} M of ATP standards prepared by filtrated and sterilized artificial seawater. All measurements were performed in RT condition. Chemicals for ATP quantification were purchased from Kikkoman Co. Japan. Bioluminescence was measured by Lumitester C-100N (Kikkoman Co., Japan).

The results of total ATP quantification are shown in Figs. 17 and 18. Average total ATP quantity is in the order of 100 pM (10^{-10} M) for most of the sample. The reasons of variation of ATP contents between the samples analyzed here are under investigation. The data will be integrated with the temperature, turbidity and pH data that obtained during this cruise to understand distributions and dynamic behaviors of hydrothermal plumes.

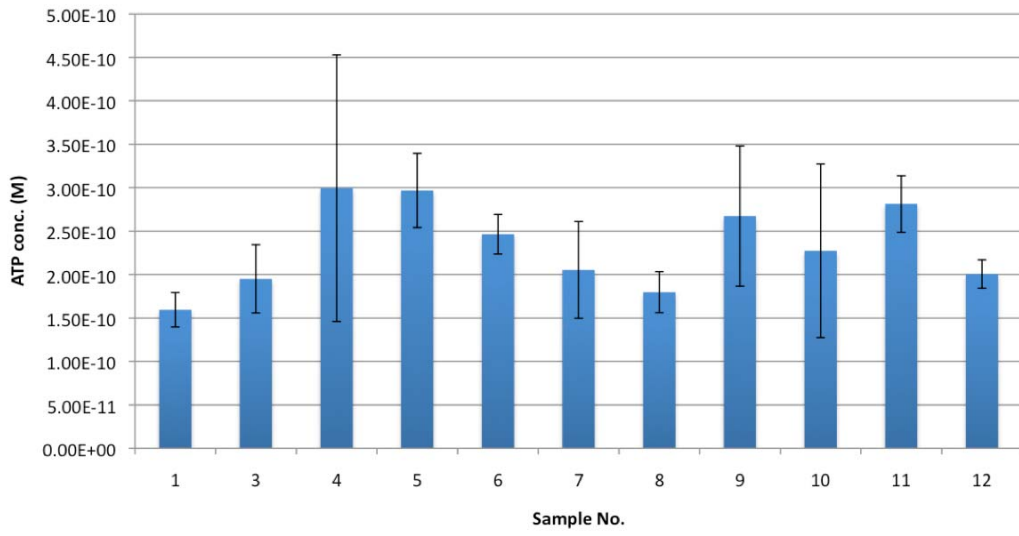


Fig. 17 Results of ATP Quantification (#1098)

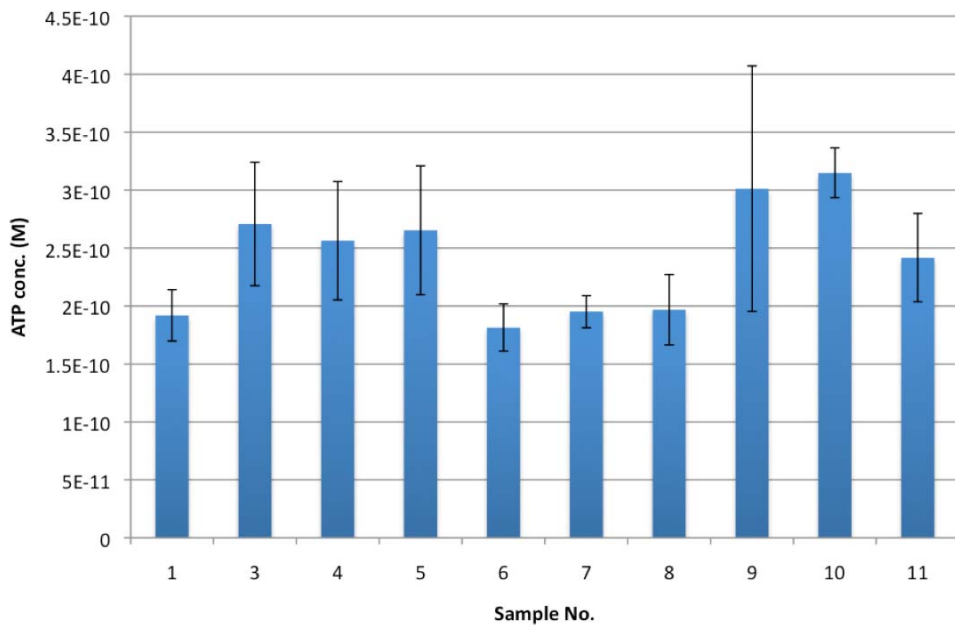


Fig. 18 Results of ATP Quantification (#1099)

3.3 4D Mapping of Hydrothermal Plumes

Y. Maeda (CERES), T. Fukuba, T. Fujii (IIS, University of Tokyo)
M. Kyo (JAMSTEC), and K. Shitashima (CRIEPI)

(1) Introduction

Hydrothermal plumes are created and sustained by the heat of volcanic processes along the Mid-Ocean Ridge system that circles the globe. Hydrothermal systems consist of circulation zones where seawater interacts with rock, thereby changing chemical and physical characteristics of both the seawater and the rock. The altered seawater, called hydrothermal fluid, is injected back into the ocean at hydrothermal vent fields and forms hydrothermal plumes. Hydrothermal fluids are circulated (convected) upwards until they're cooled down and reach density equilibrium, at which point their advection occurs laterally with the current. The neutrally buoyant plume layer can have length scales on the order of several kilometers, and it therefore provides the best means to detect the presence of vent fields on the seafloor. Hydrothermal plumes can be detected in seawater overlying vent fields, and beyond, because they have a distinctly different physical and chemical signature from the surrounding seawater. The heat and particle content of hydrothermal plumes are two readily measurable parameters. These parameters are usually elevated relative to unaffected seawater, and measurable differences can be detected as far as tens to hundreds of kilometers away from the vent fields. But, the distribution and intensity of hydrothermal plumes is variable in both space and time. And it is still so difficult to acquire a complete data set of on the behavior of hydrothermal plumes in space and time. Thus, it is necessary to develop a certain technique to compensate the lack of data and to predict the overall behavior of the plumes based on the partial data that can be obtained by existing observation and measurement methods and limited ship-time.

The primary purpose of this work is to establish a new technique to capture the overall behavior of hydrothermal plumes in 4D, i.e., in space and time, by integrating numerical simulation with measured data. In this cruise, we measured physicochemical data around a hydrothermal area in Wakamiko Caldera in Kagoshima Bay, Japan, in two horizontal planes and in four different tidal phases (sixteen planes in total) as one of the data sets that can be used for the development of the new technique.

(2) Survey site

Wakamiko caldera (31°39'N-31°41'N and 130°45'E-130°40'E) located in KAGOSHIMA BAY has a caldera-shaped topography (Fig.19). The caldera is about

several km × several km wide. The hydrothermal field comprises high temperature vents and biological communities in many places in caldera. The deepest point of the caldera is 200m. These plumes are bubbling of gas from the seabed that precipitates rapidly as hot hydrothermal fluids (with temperatures as high as 150°C) mix with cold seawater (usually about 16°C) at or just below the vent orifice.

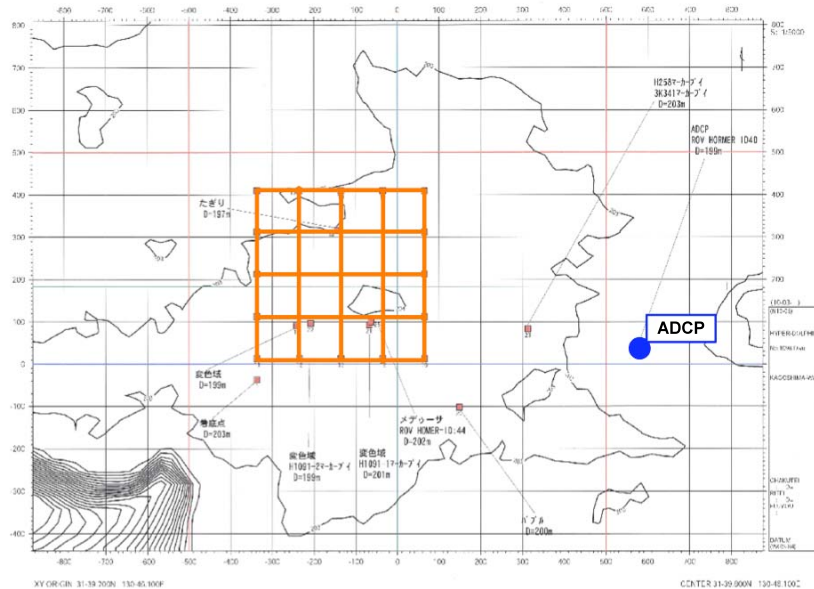


Fig.19 ROV mapping area (see the green square in Fig.5)

(3) Instruments and methods

(3-1) Current measurement

RDI Acoustic Doppler Current Profiler (ADCP 300kHz) (Fig.20) uses the so-called Doppler principle to measure velocities in water. The ADCP uses four transducers. This introduces higher degree of accuracy than using only the three beams strictly necessary for resolving three dimensions current. Vertical velocities in particular are measured with better resolution. In addition, the four beam system allows for better analysis of the relationship between instrument signal strength, ambient noise levels and the return signal received by the instrument indicators of measurement accuracy. Further instrument specifications as supplied by RD Instruments are shown in Table 4.

The RDI ADCP is capable of measuring the current in up to 128 cells of 0.2 to 16 meter vertical extent, with a maximum measuring range of about 165m. Battery consumption depends on the number of individual acoustic pings the instrument sends out. The smaller the cells, the more pings are necessary per ensemble in order to reach an acceptable standard deviation level. The time interval between ensembles also

influences battery use. By choosing 4m cell height and 10minute ensemble interval, it was possible to cover a maximum total vertical range and still not to risk to run out energy before recovery of the mooring. Current was measured in a total of 27 depth cells. The water column from 200 m to 100 m depth was thus covered in the measurement series. Results of the deployment of ADCPs are shown in Table 5 and Fig.21. And the positions in WAKAMIKO caldera are indicated in Fig.19.



Fig.20 Photograph of ADCP before deployment, on ROV HYPER DOLPHIN

Table 4 ADCP Specifications

Parameter	Range:	Accuracy	Resolution
Temperature	-5°C ~ 45°C	±0.2°C	0.01°C
Tilt	±20°	±0.5	0.01
Compass	0° ~ 360°	±2°	0.01°
Pressure	6000m	0.25 % (Range)	

Table 5 Results of ADCP Deployment

	RDI ADCP
Instrument number	1230
Instrument depth	200m
Measurement depth	100-200m
Position Latitude	31 39.725N
Position Longitude	130 46.469E
Deployment time	14:52 March 19 2010
Recovery time	10:21 March 29 2010
Logging interval	10 minutes

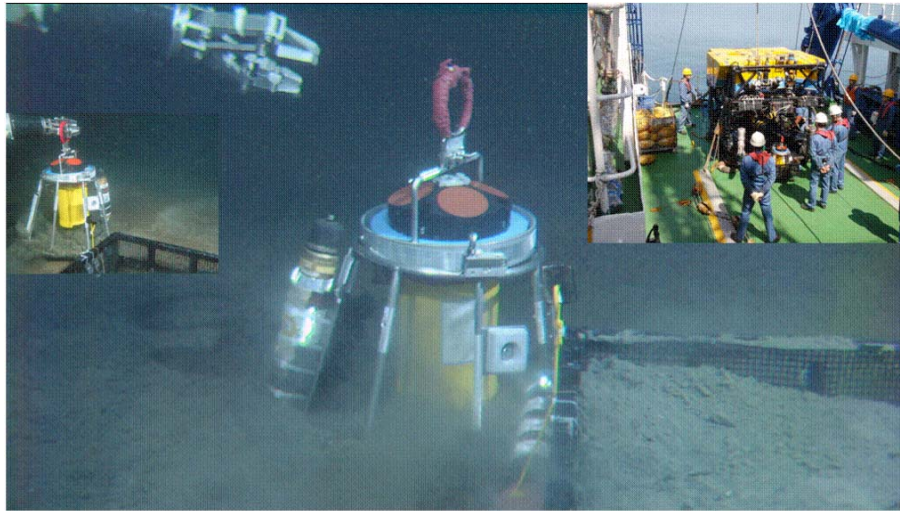


Fig.21 Photo of the deployed ADCP

(3-2) Geochemical measurement

(3-2-1) CTD-Pyron (12 series bottles)

CTDT-Pyron consists of CTD (FSI), Pyron (FSI), Turbidity sensor (SeaPoint), Sampling bottles (Ocean Equipment:2LNiskin). These systems are connected to a PC in a control room on a ship through a cable. Through this link, salt (C), temperature (T), depth (D), turbidity (T) can be monitored in real time. Specifications of the FSI CTD sensor are shown in Table 6 and the whole assembly of the system is shown in Fig.22.

Table 6 Specifications of CTD Sensor

Parameter (unit)	Conductivity (S/m)	Temperature (°C)	Pressure (dB)
Range	0~7.0	-2~35	0~7000 0~3000 0~1000
Accuracy	±0.0002	±0.002	±0.01% Full Scale
Stability / 1 month	±0.00005/month	±0.0002/month	±0.02% Full Scale
Resolution	0.00001	0.00005	±0.004% Full Scale
Response speed	5cm/sec or 1m/sec	150msec 20msec (Option)	25 msec



Fig.22 The Whole Assembly of CTD-Tytron System Mounted on the Vehicle

(3-2-2) SeaPoint Turbidity Meter

The SeaPoint Turbidity Meter detects light scattered by particles suspended in water, generating an output voltage proportional to turbidity or suspended solids. Specifications are shown in Table 7.

Table 7 Specifications of Turbidity Meter

Power Requirements	7-20 VDC, 3.5 mA avg., 6 mA pk.	
Output	0-5.0 VDC	
Output Time Constant	0.1 sec.	
RMS Noise	< 1 mV	
Power-up Transient Period	< 1 sec	
Light Source Wavelength	880 nm	
Sensing Distance (from windows)	< 5 cm (approx.)	
Linearity	< 2% deviation 0-750 FTU	
Sensitivity/Range Gain Sensitivity (mV/FTU)Range (FTU)		
100x	200	25
20x	40	125
5x	10	500
1x	2	** (** output is non-linear above 750 FTU)
Temperature Coefficient	< 0.05%/°C	
Depth Capability	6000 m (19,685 ft)	
Operating Temperature	0°C to 65°C (32°F to 149°F)	

(3-2-3) pH/pCO₂/ORP Sensor

We also used a new type pH sensor, developed by the group of Shitashima and Kyo, which uses an ion-sensitive field effect transistor (ISFET) as the pH electrode, and a chloride ion selective electrode (Cl-ISE) as the reference electrode. The devised in situ

pH sensor shows quick responses (within a few seconds) with high accuracy (0.005 pH) and a depth rating of 6000 m. The ISFET and Cl-ISE are connected to the pH amplifier in a pressure housing through an underwater cable connector. The pressure housing includes a pH amplifier, A/D converter, data logger, RS-232C interface and Li ion battery. The sampling time of the in situ pH/pCO₂ sensor was set at intervals of 10 s. Before and after deploying the ROV from the mother vessel, we made on-board corrections for electrical drift; the pH sensor was calibrated using two different standard buffer solutions, 2-aminopyridine (AMP; pH 6.787) and 2-amino-2-hydroxymethyl-1,3-propanediol (TRIS; pH 8.089).

The pCO₂ sensor was devised to incorporate the abovementioned newly developed pH sensor to measure the in situ pCO₂ in seawater. The principle of pCO₂ measurement is as follows. Both the ISFET-pH electrode and the Cl-ISE of the pH sensor are sealed in a unit with a gas permeable membrane whose inside is filled with inner electrolyte solution with 1.5% of NaCl. The pH sensor can detect pCO₂ change as inner solution pH change caused by permeation of carbon dioxide gas species through the membrane. An amorphous Teflon membrane (Teflon AFTM) manufactured by DuPont was used as the gas permeable membrane. The in situ (3000m depth, 1.8 8C) response time of the pCO₂ sensor was less than 60s. Since the calibration of in situ pCO₂ measurements was not conducted in our field application reported here, only raw data from the pCO₂ sensor output were obtained. Raw data showing small digit readings indicates pH depression of the inner solution, which reflects an increase in partial pressure of CO₂ in seawater. Fig.23 shows photographs of the whole assembly of the in situ pH/pCO₂/ORP sensor.

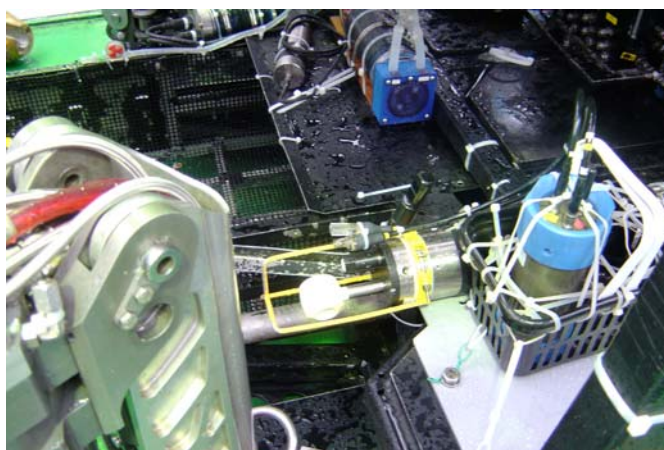


Fig.23 Photo of the Whole Assembly of in situ pH/pCO₂/ORP Sensor

(3-3) Mapping survey

The mapping survey of temperature, turbidity, and pH distribution was carried out on

the hydrothermal area at Wakamiko Caldera using the grid navigation of the Hyper-Dolphin equipped with CTD (Conductivity Temperature and Depth (including Turbidity sensor) and pH/pCO₂/ORP sensor. The schedule and mapping route are shown in Table 8. The mapping route and the tidal phase are shown in Fig.24 and Fig.25, respectively.

Table 8 Mapping Schedule and Routes

date	Item	route
19 March	Deployment of mooring and ADCP	
25 March	At high water (High Tide, 14:50) mapping layer:160m	①
26 March	Flood (From low to high water) mapping layer:185m	④
	At high water (high tide, 16:18) mapping layer:185m	①
27 March	Ebb (From high to low water) mapping layer:185m	②
	At low water (low tide, 11:30) mapping layer:185m	③
28 March	Ebb (From high to low water) mapping layer:160m	②
	At low water (low tide, 12:12) mapping layer:160m	③
	Flood (From low to high water) mapping layer:160m	④
29 March	Recovery mooring and ADCP	

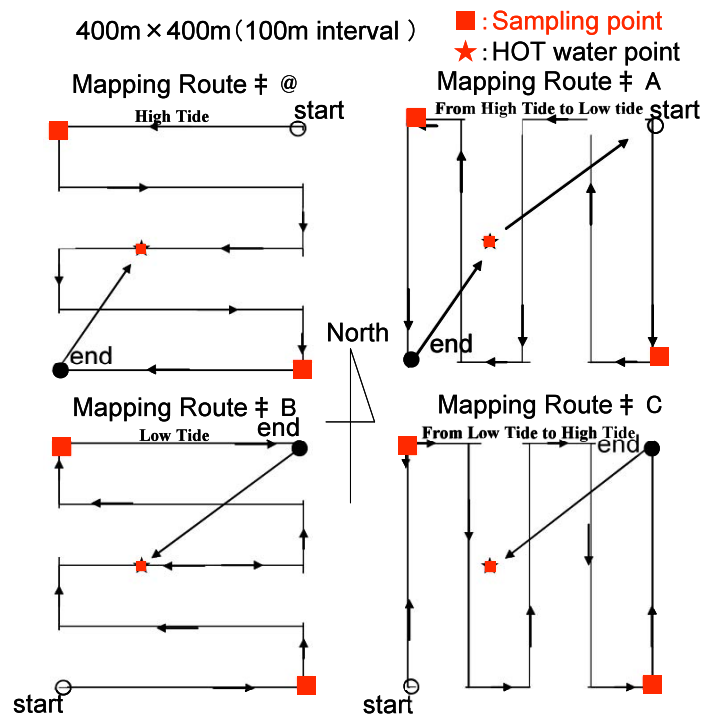


Fig.24 Mapping Routes

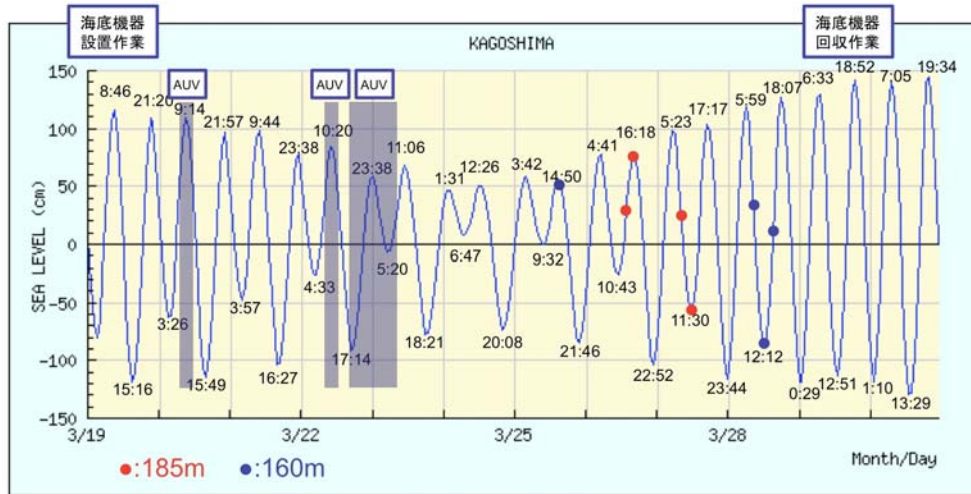


Fig. 25 Mapping Schedules in Tidal Phases

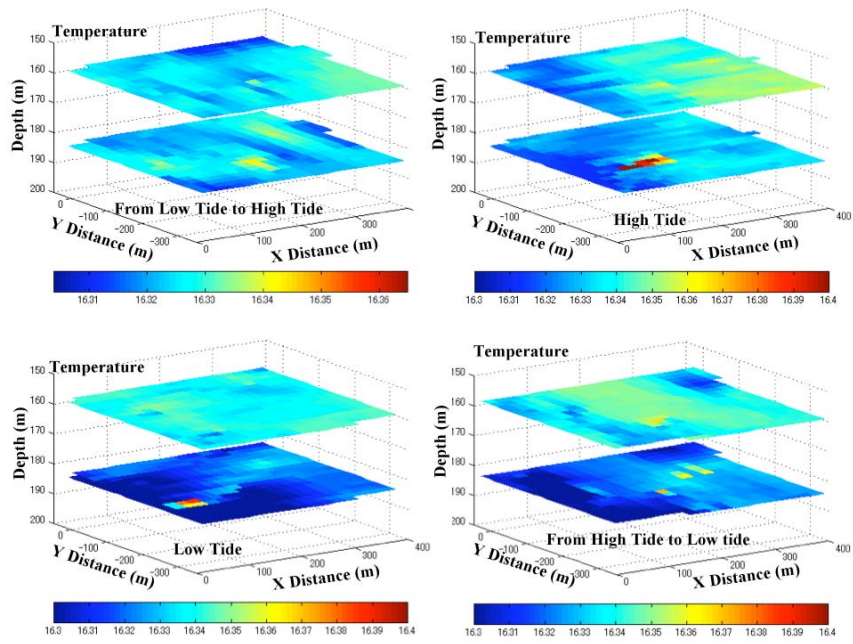
(4) Preliminary results

(4-1) Mapping survey in Wakamiko caldera

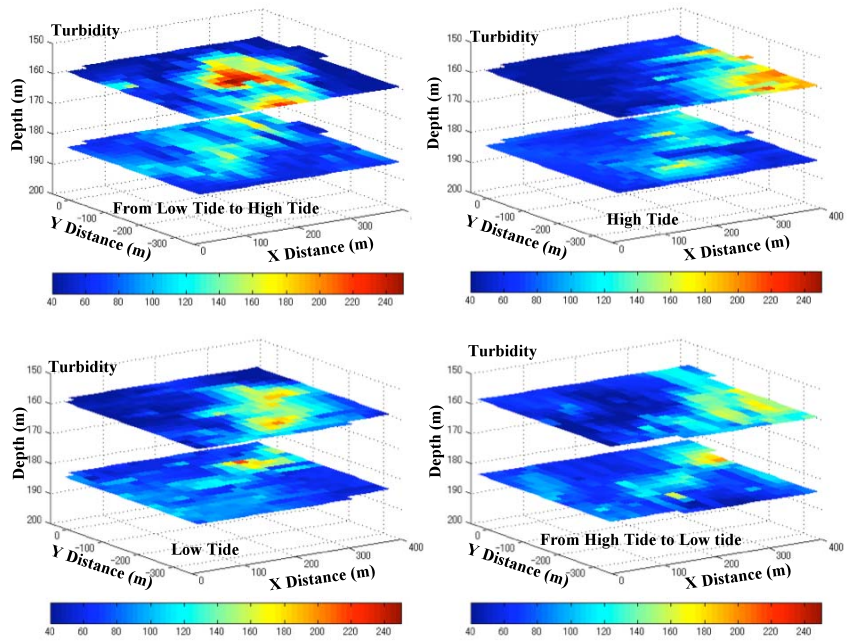
Examples of the results of temperature and Turbidity mapping in the cases of 4 tidal phases (high and low tides, etc.) are shown in Fig.26 (a) and (b). Contour maps of temperature are plotted over a 400m×400m area in the caldera for each of the depth layers at 160m and 185m. High temperature zones are distributed around the vents in the 185m depth layer especially in the case of high tide and low tide. This kind of results indicates that localization of high temperature zones can be properly measured and reconstructed in space and time.

(4-2) Tidal currents in Wakamiko caldera

The first valid data from RDI ADCP were collected at 11:00 March 19 and last good recording before retrieval was made at 11:00 March 29, giving a total of 1441 current ensembles for this instrument. Fig.27 gives an introductory view of these current measurements.



(a) Temperature



(b) Turbidity

Fig.26 Preliminary Results of Mapping (a :Temperature b:Turbidity)

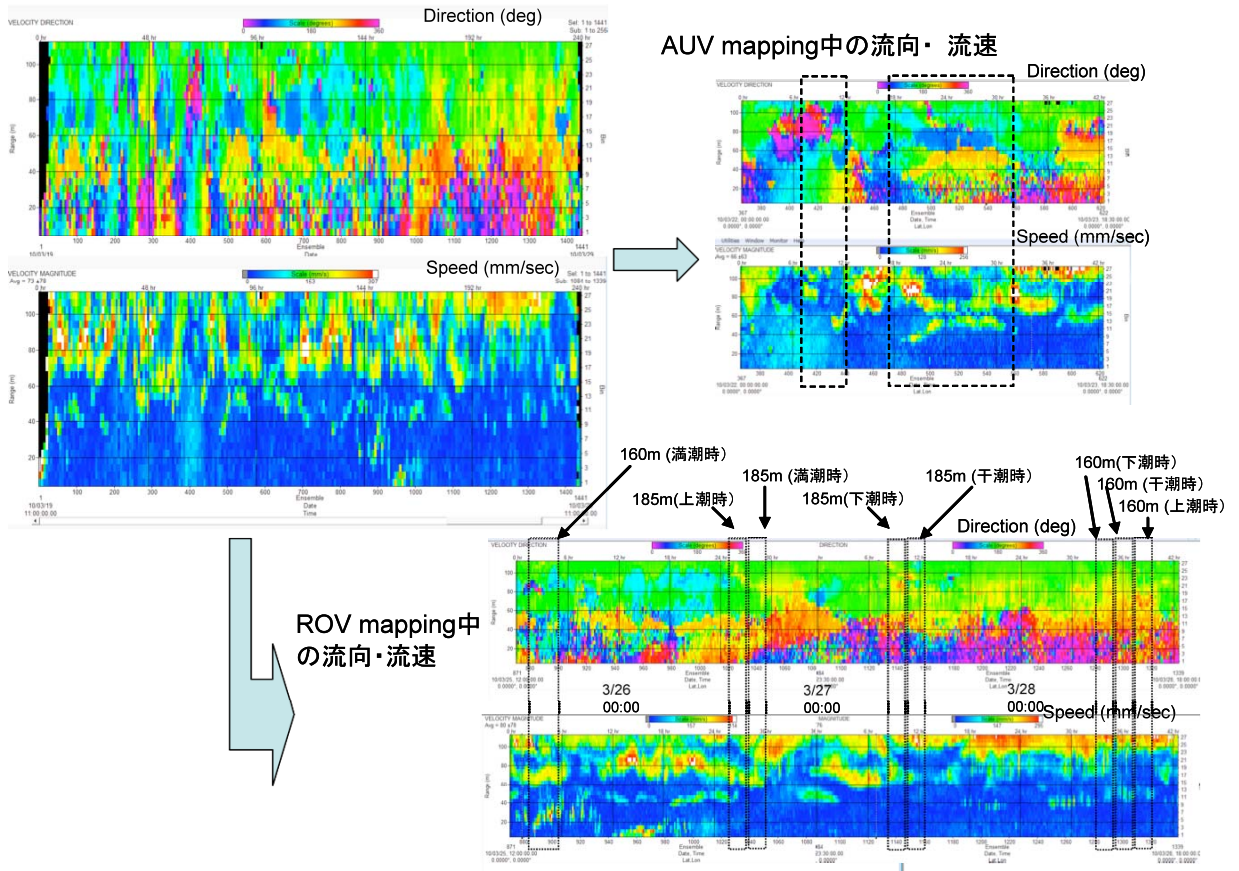


Fig.27 Horizontal velocity magnitude at ADCP
 (over the whole deployment period. The original 10 minute ensembles are shown)

3.4 Geochemical study of gas-fluid-sediment interactions

(1) Introduction

In a marine shallow-water hydrothermal system, diverse geochemical processes are often observed. The hydrothermal fluid venting from the seafloor is derived from various sources such as meteoric water, seawater and possibly magmatic water. The fluid chemistry is affected not only by water-rock interaction within a hydrothermal reservoir but significantly by secondary processes such as phase separation. In the case of a hydrothermal system associated with submarine volcano, volcanic gas and/or volcanoclastic sediment could play important role in these geochemical processes. In order to reveal these diverse processes, we extensively collected fluid, gas and sediment samples from the Wakamiko hydrothermal system to conduct geochemical and mineralogical studies, which would provide important constrains for discussion of the geochemical processes.

(2) Test operations

During the dive expedition of NT10-05 Leg2 cruise, we conducted test operation of following instruments those were developed for working on the seafloor. The ROCS *in situ* incubation system was developed by University of Tsukuba. The key aim of the test operation was to check the operation of the new ROCS system in accordance with the program under few hundred meters depth in water column and analysis of the *in situ* marine bacterial and archaeal activities.

Marine bacterial communities are widespread in ocean and its productivity is recognized to large energy force of marine material cycle. To investigate the marine bacterial productivity, it has been done incubation experiments that sea water was sampled with water sampler and cultured *in situ* condition on board or in laboratory. However, this method has some problem that sampling depth pressure and atmosphere pressure were greatly different, temperature and some environmental condition may also be varied during sample transfer. Therefore, we developed the rotary clean sea water sampler (ROCS) incubate system that can incubate discretional depth sea water under *in situ* environmental condition with adding some substrate for bacteria, and this system can fix a certain volume of incubating samples at discretional time interval.

Ultrapure water was filled up in the titanium vessel (12 L) before the experiment start, and then the ROCS system was putted at 198 m depth for two day long (Fig. 1). The ROCS system replaced the ultrapure water to *in situ* depth sea water, and then started the *in situ* incubation in this closed system. After starting *in situ* incubation, sub-samples drag away from the vessel to the 500 mL cylinder in accordance with the program. Each sub-sample in the cylinders fixed with paraformaldehyde *in situ*. We

also sampled the same depth sea water with Niskin water sampler. For bacterial direct count, the water samples were filtrated with 0.2 μm pore size polycarbonate membrane filers (Whatman, 25 mm in diameter) on board after fixation with paraformaldehyde (final concentration in the sample was about 2%). And for extraction of bacterial DNA or RNA, the water samples were also filtrated with 0.2 μm pore size polycarbonate membrane filers (Whatman, 47 mm in diameter) as soon as possible on board. Then the filer samples were kept in -80°C during this cruise.

From the preliminary results, there might be much of Archaeal community, especially Euryarchaeota, near the bottom of the Wakamiko submarine crater Kagoshima bay using direct count with CARD-FISH technique.



Fig. 28 The ROCS system putted on the sea bottom at the Wakamiko submarine crater of the Kagoshima bay (Lat. 31-40.071N, Long. 130-45.689E, 198 m) on 19/03/2010 (D/M/Y), and took up 21/03/2010.

The GAMOS (Geochemical Anomaly MOnitoring System) is a flow injection analysis system working on the seafloor. This system The system was developed originally in ORI and has been modified at Kochi Univ. Center for Advanced Marine Core Research, in order to monitor time-series variations in the geochemical environment of a hydrothermal field (Okamura et al., 2004). During the NT10-05 Leg2 cruise, GAMOS was tuned for manganese concentration determination using chemo-luminescence and deployed together with CTD above a shimmering vent.

The *in situ* Pore water extraction system (iPow) was developed by Okayama Univ. in order to collect pore fluid sample directly from the surface sediment. Pore fluids are introduced slowly (at flow rate of 20ml/hour) into a sample syringe through a inline filter from a sampling port on the probe, one hour after a probe of 65 centimeters length penetrated into the sediment. The probe has four sampling ports (15 cm interval)

and two thermometers to reveal geochemical and thermal gradient in the sediment. During the NT10-05 Leg2 cruise, the iPow was deployed beside a tiny shimmering vent and successfully collected the pore fluid that contains the hydrothermal component.

The Medusa is a fluid current meter to monitor continuously venting hydrothermal fluid flux, by combination of two thermistors and a heater. During the NT10-05 Leg2 cruise, Medusa was deployed at two different hydrothermal fields.

(Reference)

Okamura K, Hatanaka H, Kimoto H, Suzuki M, Sohrin Y, Nakayama E, Gamo T, Ishibashi J (2004) Development of an in situ manganese analyzer using micro-diaphragm pumps and its application to time-series observations in a hydrothermal field at the Suiyo seamount. *Geochem. J.*, 38, 635-642.

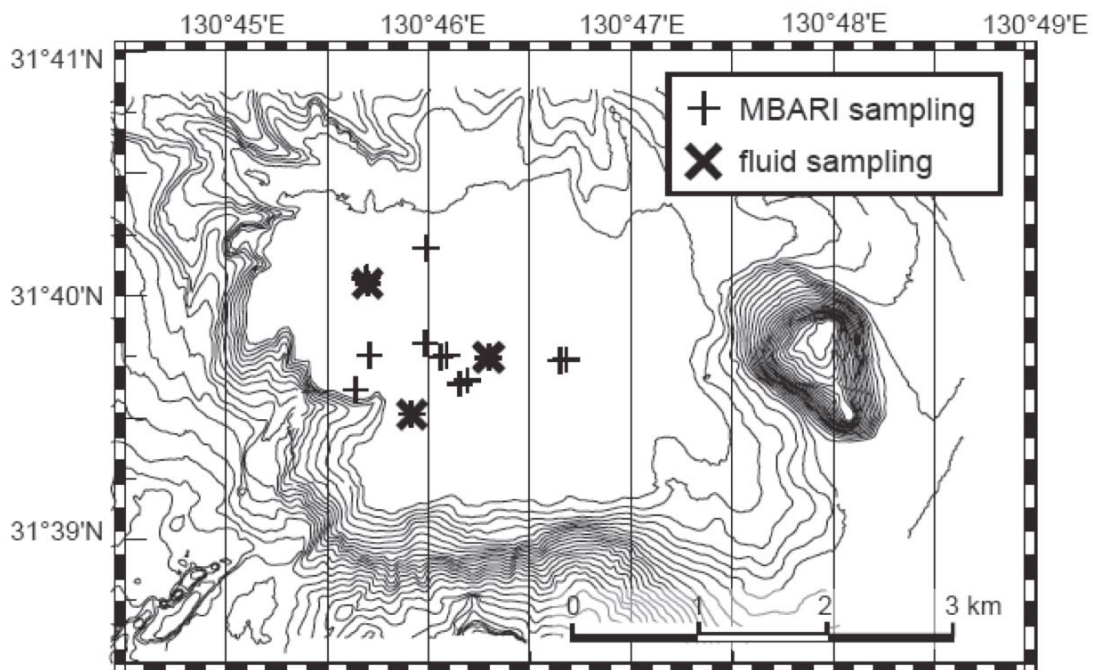


Fig. 29 Localities of fluid sampling and MBARI core sampling during NT10-05 Leg2 cruise

(3) Sampling and chemical analysis

Several numbers of samples were obtained during the NT10-05 Leg2 cruise; 27 fluid samples (mainly collected with the ROCS fluid sampler), 26 sediment samples (mainly collected with a MBARI corer), 5 gas samples. The sampling localities are

shown in Fig.29. Other than above, seawater samples in water column above the hydrothermal fields were collected by Niskin bottles.

Chemical analyses of major elements for the obtained fluid samples were conducted following conventional methods compiled by Gieskes et al. (1991). Onboard pH measurement was conducted as soon as possible after the sample recovery. Alkalinity was analyzed by acid titration, which end-point was determined according to the Gran plot. Silica and ammonia concentrations were determined onboard using colorimetry. Chloride concentration was determined by the Mohr titration method. Potassium and sulfate analysis was conducted using an ion chromatograph after 200-fold dilution. Concentration of other major cations (Na, Ca, Mg) were determined using an ICP-AES after 200-fold dilution of acidified samples. Analytical errors of these chemical analyses, estimated from replicate analysis, were within $\pm 5\%$ for instrumental analyses and within $\pm 1\%$ for the titrations. Chemical analysis of some trace elements such as mercury and arsenic, and isotopic analysis of boron will be conducted on-land facilities.

(4) Preliminary results of fluid chemistry studies

Preliminary results of analysis of the obtained vent fluid samples are presented in Fig.30 as Mg diagrams (relationship between a specific chemical property versus magnesium). In Cl vs Mg diagram, all the fluid samples follow the same relationship, which suggests four vent fluids (WHV, SWS, SEB and SES) originate from a common fluid reservoir. However, the fluid samples collected from the SES site showed somehow different trend in K vs Mg and Si vs Mg diagrams. This difference could be explained by modification of the fluid composition at lower temperature condition than that of the reservoir. Since vigorous gas emanation was observed at SES (and SEB) sites, involvement of volcanic gas during the fluid ascent could be one of possible factors.

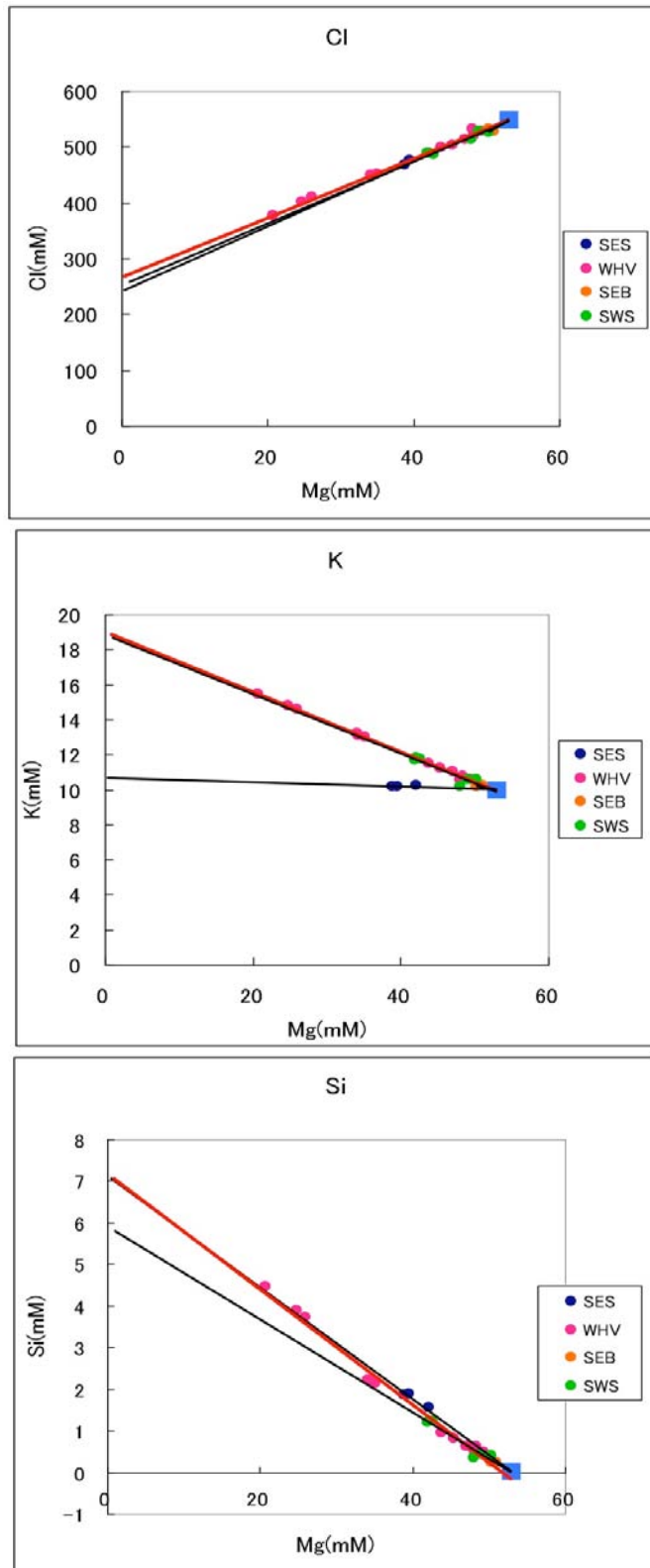


Fig. 30 Mg Diagrams (Cl, K, and Si vs Mg)

4. Summary

In this cruise, AUV REMUS was deployed from R/V Natsushima for the first time to develop a new method using both AUV and ROV and 13 hours mission was successfully conducted. The measured data of REMUS can be displayed only in 15 minutes after the mission, they can be used for the following ROV mission leading to 'adaptive' measurement. Two prototypes of *in situ* measurement systems (IISA-gene and IISA-AMISpH) were deployed and useful information to further improve the systems could be collected. Eight horizontal layers were measured for plume mapping using ROV. Based on the obtained data, a method to get 4D pictures of hydrothermal plumes will be developed. Moreover, a variety of samples in the Wakamiko area were collected, and *in situ* measurement machines were deployed. Geochemical processes in this area will be further discussed based on the analysis of these samples and measured data.