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YK06-05 CRUISE REPORT

Exploration of the cold seep ecosystem at the Japan Trench

May 27 (Yokosuka) –June 12 (Miyako), 2006

YK06-05 Shipboard Scientific Party

Shipboard Scientific Party

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Introduction

Microbial communities in marine sediments play important roles for biogeochemical cycles on the Earth. Recent progress of molecular ecological and biogeochemical analyses has greatly expanded our knowledge on the deep-sea microbial ecosystems. In particular, the cold seep ecosystem is of great interest for broader researchers such as biologists, microbiologists, and geochemists because the steep redox-gradient between seawater and seep sediments harbors unique benthic chemosynthetic community. The remarkable abundance of specialized invertebrates such as giant tube worms or bivalves is one of the most striking features of seep communities and one of the best "indicators" of fluid emission at the seafloor. The biogeochemical processes fueled by the seepage of methane and sulfide provide the habitat for rich and complex ecosystems. They provide the carbon and energy sources for abundant chemosynthesis-based communities, like bacterial mats but also epifaunal assemblages of clams that derive their nutrition from symbiotic relationships with sulfide- or methane-oxidizing bacteria.

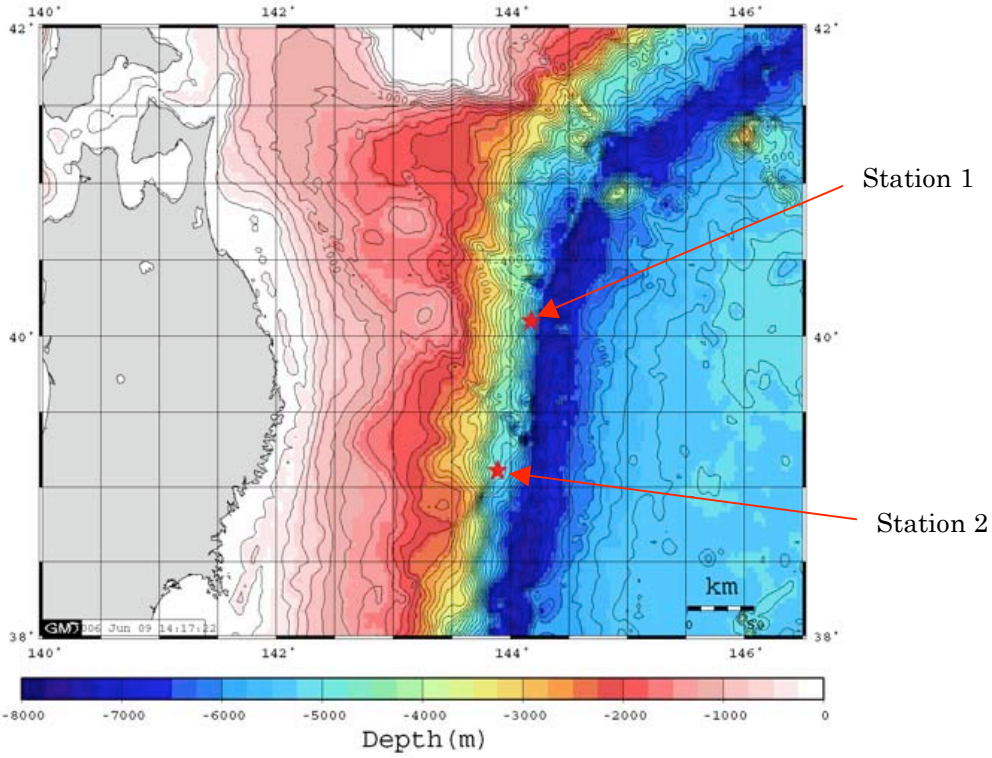
The free living and symbiotic microbial communities associated with some invertebrates (*vesicomyid* clams, *vestimentiferan* tubeworms, *mytilid* mussels) are thereby nourished by the chemical energy rising from the basis of cold seep ecosystems (Sibuet & Olu, 1998). These often take the form of dense and endemic benthic communities, in which the high production of organic carbon sustains large size or typical animals and very high biomasses. In high methane flux areas, the benthic biomass produced through chemosynthetic processes can be 1,000 to 50,000 times greater than the deep-sea biomass resulting indirectly from photosynthetic production.

At the oxic-anoxic interfaces but also in the anoxic zone a variety of biogeochemical processes are relevant for the turnover of organic and inorganic molecules. Microbial catalyzed anaerobic transformations, e.g. AOM, hydrogenotrophic methanogenesis, acetoclastic methanogenesis, homoacetogenesis, sulfate reduction, aerobic methane oxidation may contribute significantly to the overall reaction depending on the environmental conditions.

The work carried out during YK-06-05 aims at the following questions by applying a wide range of methods and tools:

- How is the anaerobic oxidation of methane (AOM) to CO_2 linked to the reduction of sulfate to sulfide is the dominant process in and above methane-rich marine sediments.
- How important is the subsequent process of aerobic sulfide oxidation due to free-living and symbiotic bacteria because it re-channels sulfur into the stable marine sulfate pool.
- To understand the influence of abiotic, i.e. physical and chemical factors (concentrations of reactants and products, pH, etc.) on the rate of the anaerobic oxidation of methane (AOM) and growth of methanotrophic microorganisms.
- Analyze the diversity and habitat-specific dominance of methanotrophic microorganisms in the context of the biogeochemical factors

We measured *ex situ* as well as *in situ* fluxes and substrate turnovers by focusing on oxygen consumption, sulfate reduction as well as methane oxidation and production. We also we took samples to link geochemical processes and gradients to the distribution of microorganism. Therefore, several methods are used to examine the diversity and function of microbes in this ecosystem.


140°
142°
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146°
42°
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38°
Station 1
Station 2
km
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-7000
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-5000
-4000
-3000
-2000
-1000
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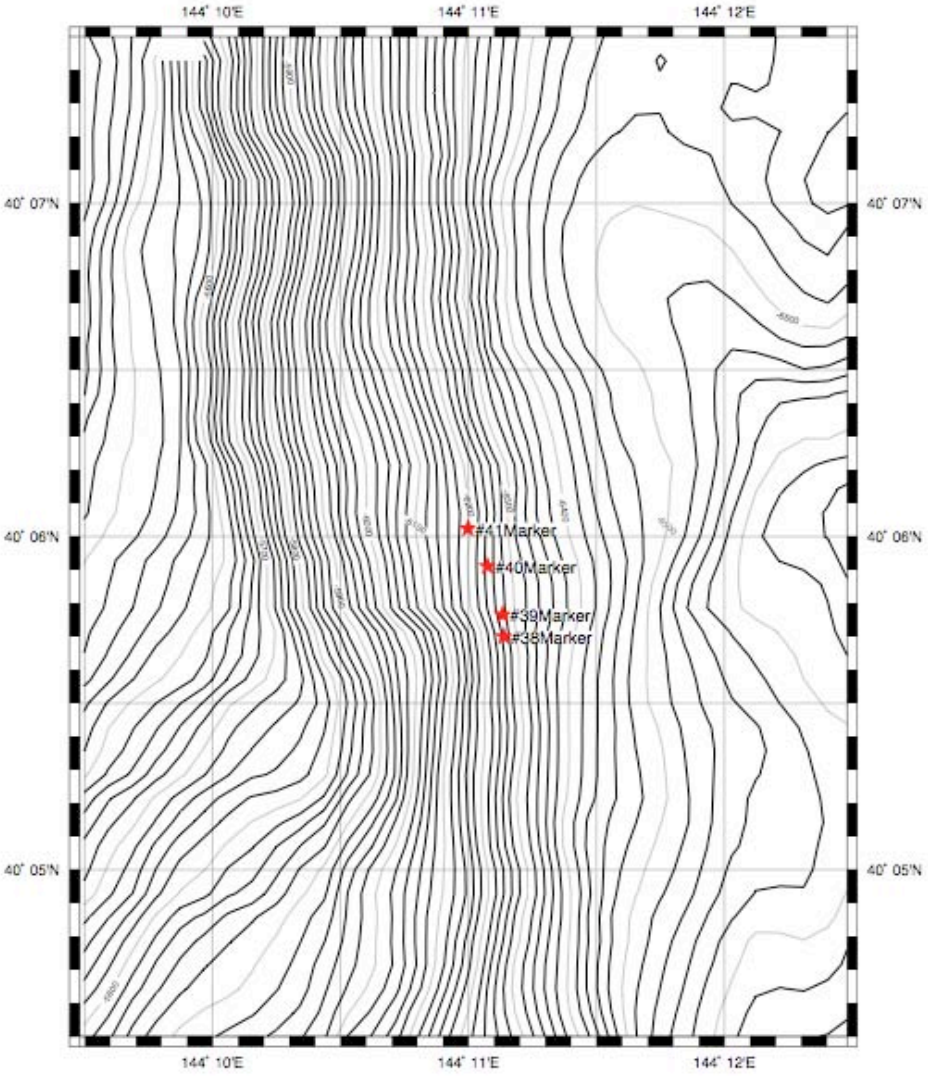
Depth (m)

Japan Trench Site1



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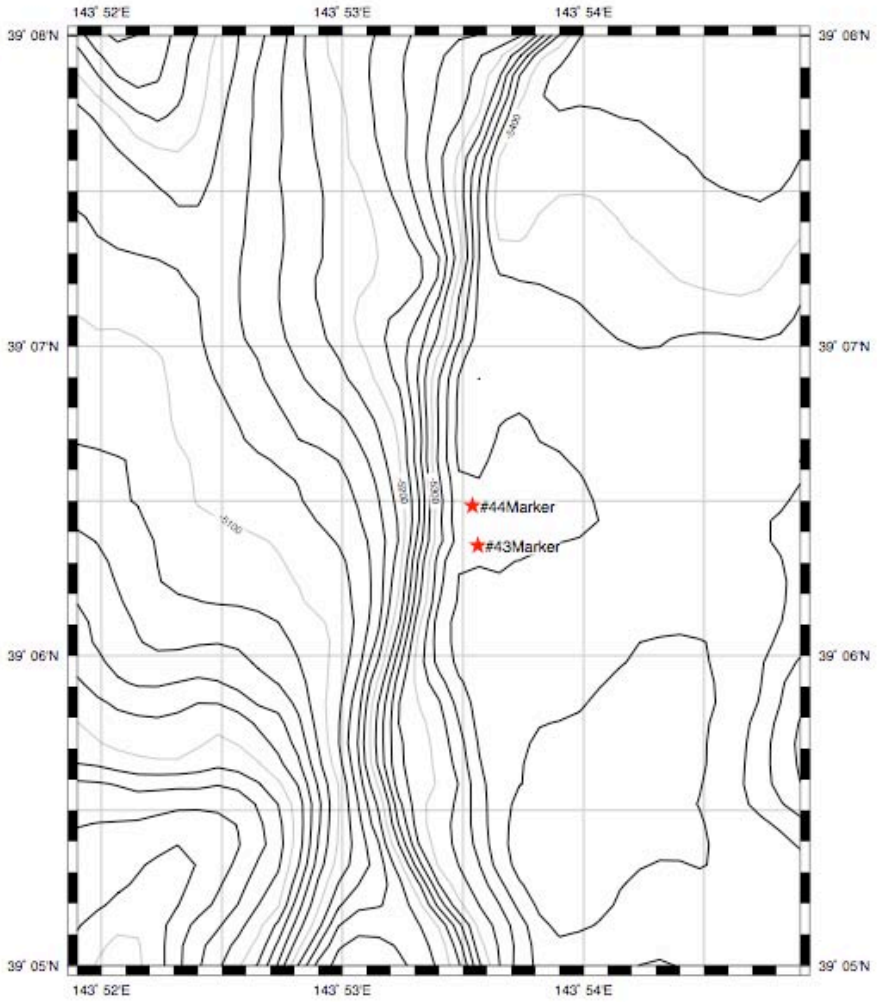
Japan Trench Site2



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Materials and Methods

In-Situ measurements

Profiler-Module and Benthic Chamber

To investigate in situ biogeochemical processes two benthic modules, a profiler and a flux chamber, have been deployed and operated by Shinkai. The autonomous profiler module (Wenzhofer *et al.*, 2000) hosted 3 O_2, 1 temperature (Pt100, UST Umweltsensortechnik GmbH, Geschwenda, Germany), 3 pH and 2 H_2S microsensors (Fig. 1). Except from the temperature sensor all electrodes have been constructed in our microsensor laboratory at the MPI in Bremen. Positioned at the sediment surface the profiler gradually moves the sensors downwards in increments of 100 µm for a total distance of 15 - 20 cm and the sensor recordings were stored internally. Afterwards the sensors were moved back to the starting position. Sensors were calibrated prior to deployment and for the O_2 sensors the readings in the bottom water of know O_2 concentration and in the anoxic sediment was used to cross-check the calibration curves.

Fig. 1 Profiler module for in situ high-resolution microprofile measurements of O_2, pH, H_2S and temperature.

The benthic chamber module is a modified version of the free-falling chamber lander previously used to study benthic processes in the deep-sea (Wenzhofer & Glud, 2004). This small benthic module consists of a circular chamber, an electronic cylinder, a water sampling system and a battery which can be operated by the submersible (Fig. 2). The chamber encloses an area of ca. 283 cm^2 together with 4 -6 l of overlying bottom water. Two oxygen microelectrodes mounted in the chamber lid monitor the concentration change in the enclosed water body while at preprogrammed time intervals 5 water samples (each 50 ml) were retrieved for later analyses of O_2, DIC, H_2S and nutrients.

Fig. 2 Benthic chamber module for in situ total exchange rate measurements.

During this cruise a new *in situ* tool was used to investigate the N-cycle of deep sea sediments under *in situ* conditions. The tracer injection system INSINC works like a push core. The core liner with the injection needle is pushed into the sediment by the Shinkai 6500 manipulator. The injection system is then activated by pulling two security bars by which the tracer is injected every 0.7 mm over 20 cm. The Insinc cores where left at the seafloor for 2 days before they were recovered by Shinkai6500. Back on board the sediment was fixed for later analyses. For more details on the process studied see section 1.2.

Ex-Situ microprofile measuremenets
High resolution microprofiles of O_2, pH and H_2S were measured on retrieved sediment cores at in situ temperature in the laboratory. Directly after retrieval the cores were placed in an aquarium and profiles were measured using an automated micromanipulator set up (Fig. 3) with a vertical resolution of 100 µm.

Fig. 3 Laboratory set up for microprofile measurements.

Sulfate reduction and methane oxidation rates
Sediment samples from various push cores were investigated for microbially mediated sulfate reduction rates and methane oxidation. From the retrieved push cores sub cores (up to 3 sub cores ø2.5 cm) were taken and radio tracer labeled substrate was injected in 1cm intervals through small, silicon sealed holes. Sediments were incubated with either $^{14}CH_4$ or $^{35}SO_4^{2-}$ for 1-3 days at *in situ* temperature under anaerobic conditions and then fixed in NaOH and Zn-Ac, respectively, for further measurements of remaining substrate ($^{14}CH_4$, $^{35}SO_4^{2-}$) and product ($^{14}CO_2$, $H_2{}^{35}S$) activity. The ratio of product to substrate activity multiplied with substrate concentrations yields then actual rates. Up to three biological and one abiological control were incubated per site and sediment horizon. All samples will be analyzed in the home laboratory.

Cell counts and FISH
2.5 ml of sediment volume were fixed in 9 ml of 2% formalin in seawater for 2 – 4h. 2 ml of the sediment-formalin suspension were centrifuged and supernatant was discarded. The pellet was washed two times in 3 ml 1*PBS-buffer (resuspension, centrifugation, discarding of supernatant). Finally, the pellet was fixed in 2 ml of a 1:1 (v:v) solution of Et-OH:1*PBS (50% final concentrations) and kept at –20°C until further analyses.

DNA analysis
Fresh sediment (ca. 4g) was immediately frozen at –20°C prior to laboratory use.

Anaerobic turnover rates under high pressure conditions
After sample retrieval sediment slurries from pushcores were prepared anaerobically. Sediment dilution with artifical sea water medium / sulfate reducer medium was ~ 1:5 (v/v). 0.8 ml of sediment slurries were transferred into 5 ml Hungate tubes prepared for pressure incubation. Tubes were completely filled with prepared anaerobic media with different substrate amendments respectively. Tracers were injected to each tube separately. After tracer injection tubes were pressurized in high pressure stainless steel vessels and incubated for 24 h to 36 h. Samples were either stored in 2.5% NaOH or 20% ZnAc for later analysis.

Incubation of (pressure adapted) microorganisms which are involved in catalyzing AOM

The objective for onboard experiments was to estimate the potential rates and metabolic capacities of anaerobic microorganisms from sediment under high pressure conditions and different concentrations of potential substrates i.e. methane, hydrogen, acetate and oxygen. To measure the potential turnover rates four different tracer molecules ^{35}S-sulfate, ^{14}C-acetate, ^{14}C-Bicarbonate or ^{14}C-methane were used. The basic experimental scheme was according figure 4 assuming a central reaction network. Turnover rates of molecules indicated yellow or orange boxes will be measured by tracer analysis, molecules indicated in green boxes will be amended specifically. Other rates will be estimated after calculation of different tracer turnover rates. Immediately after retrieval of the push cores sediment samples from different horizons were transferred into artificial sea water medium / sulfate reducer medium using anaerobic techniques. When necessary media were additionally reduced with sodium sulfide to adjust redox potential appropriate for anaerobic microorganisms. Sample slurries were either kept in DURAN bottles with methane headspace under atmospheric pressure or in small serum bottles under in situ pressure. Both containments are incubated at 4°C and will be transported to MPI, Germany for further incubation and isolation. Experimental work at MPI will be strongly focusing on AOM activity under high-pressure conditions.

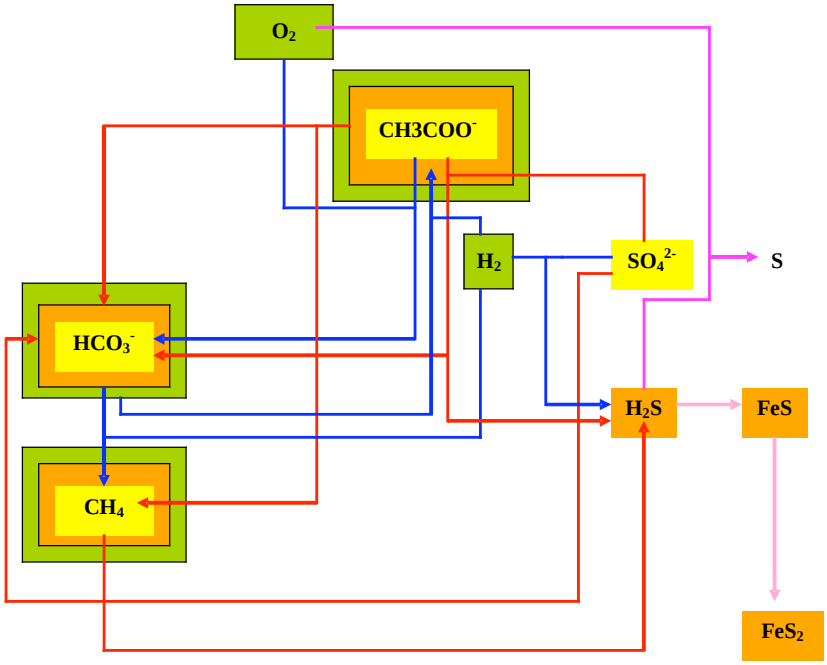


Fig. 4 Hypothesized metabolic network. Red arrow: measured. Blue arrow: branch net. Yellow box: Ratio-isotope tracers. Orange box: Isotope measurement. Green Box: Variable.

Pore water chemical measurements

The objective of the pore water geochemistry program was to characterize the geochemistry of near surface sediments around the *Calyptogena* colonies. Geochemical pore water analysis was performed on sediment taken by push cores. After sample retrieval the cores were sliced into 1-4 cm sections and pore water was squeezed through 0.45 µm regenerated cellulose membrane filters using a mechanical polypropylene press and stored either cold or frozen. Pore water concentrations of nutrients (e.g. NO_3, NO_2, NH_4^+, SO_4^{2-}), sulfide and DIC will be analyzed back home.

Gas and biomarker investigations

In addition to on-board analysis of methane, five types of samples were collected during the cruise for shore-based analysis by the organic geochemistry group at the University of Bremen. (1) Pore water was sampled in collaboration with Daniela Franzke (MPI –

Nutrients Group) for qualitative, quantitative and carbon-isotopic analysis of water soluble volatile fatty acids (VFA) that are produced and consumed by live microorganisms. In this context, we are particularly interested in acetate since it is an important precursor for methanogenesis. We expect the carbon isotopic composition of acetate to be indicative for the dominant biogeochemical processes and reaction networks in natural environments. With our investigations we intend to contribute to a better understanding of the carbon flow in methane laden sediments. Subsamples for δ^{13}C-DIC analysis were taken from the pore water. (2) In order to relate the carbon isotopic composition of acetate to its precursors, solid phase residues (squeeze cakes) were collected after pore water squeezing for carbon isotopic analysis of total organic carbon. (3) Furthermore, for carbon isotopic analysis of methane, solid phase samples were taken at the same depth as the samples in which methane was measured on-bard. Samples were stored with 1 N NaOH in headspace vials. In addition, we plan to use these samples to investigate the amount of methane adsorbed to the solid phase of the sediment. (4) In order to relate the pool of adsorbed methane to the composition of the solid phase, additional solid phase samples were taken for clay mineralogical analysis. (5) At selected sites, additional solid phase samples were taken for analysis of intact polar lipids (IPLs), which are characteristic biomarkers for live microorganisms. With our qualitative and carbon isotopic investigations of IPLs and additional molecular investigations that focus on microbial ecology and diversity, we intend to elucidate the composition of microbial communities in methane laden sediments as well as their carbon source.

It was also intended to perform on-board hydrogen analysis during the cruise. Though, due to technical problems with the hydrogen gas chromatograph, these analyses were not possible. Some samples were frozen for future analysis at the University of Bremen.

Samples were taken with several instruments deployed to the ocean seafloor by the JAMSTEC submersible Shinkai 6500. Sampling stations were chosen after a first Shinkai survey dive. Sediment samples were taken from 50 cm and 35 cm long push corers (C). Bottom water samples were subsampled from supernatant water in the push cores (C), from Niskin bottles (N) and bag samplers (B).

The liners from the push corers were inspected visually before subsampling. The sediment from the liner was pushed up with the help of a piston and sampled continuously in form of 1-4 cm slices. Pore water was squeezed from the sediment through 0.45 µm regenerated cellulose membrane filters at up to 3 bar pressure applying argon gas with a mechanical polypropylene press. In general, sampling was completed in less than 3 hours after core retrieval. For analysis of VFAs, subsamples were collected in pre-combusted 7 mL glass vials and stored at -20°C. Samples for δ^{13}C-DIC were collected in 2 mL vials with as less air as possible and quickly stored at -20°C. After pore water squeezing, the solid phase residue (squeeze cake) was taken for carbon isotopic analysis of total organic carbon, packed and stored at -80°C.

For quantitative and isotopic analysis of dissolved and adsorbed methane, 3 cc of sediment were taken by syringe and transferred to a gas-tight headspace vial. Samples for on-board analysis of methane were immediately sealed and heated at 60°C for 20 minutes and then analysed with a gas chromatograph with a FID detector. In the samples for on-shore isotopic analysis of methane 5 mL of 1 N NaOH were added, air was removed by blowing nitrogen on the samples and then the vials were quickly sealed and stored at -20°C. From the same depth intervals, 5 cm^3 subsamples were taken by syringe for clay mineralogical analysis and stored at -20°C.

Samples for IPL analysis were taken at the same depth as for pore water. When enough material was left after pore water sampling, non-squeezed sediment was frozen and stored at -80°C. Eventually samples from other core sediments were also collected in polypropylene vials and stored at -80°C. Finally, bottom water samples were taken from the supernatant water inside the push corers, from Niskin bottles or from the water bag-sampler.

Preliminary Results

In situ measurements

Profiler-module:
The profiler module was deployed at both sites at the rim of a *Calyptogena* colony. After placing the module at the seafloor the measurement was started by the Shinkai pilot by pressing a magnetic switch on the module. Due to battery capacity problems during the deployment at site 1 the profiler program stopped after 2/3 of the measurement with the sensor still in the water column. At site 2 a full set of in situ microprofiles could be measured. As an example the O_2 microprofiles are shown in Fig. 5. The average oxygen penetration depth was 17 mm with a maximum penetration of 20 mm and a minimum of 10 mm. The pH and H_2S profiles remain to be analysed.

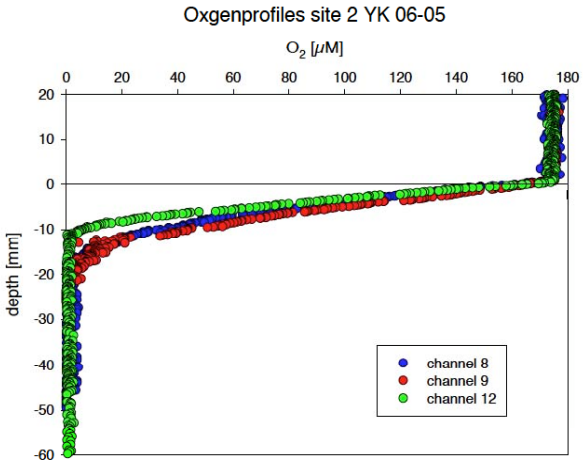
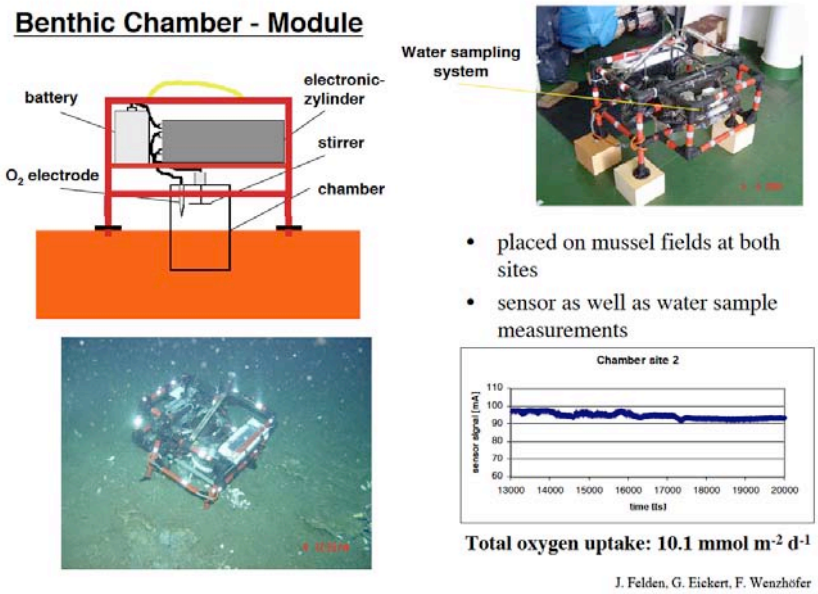


Fig. 5 An oxygen penetration depth was 5mm with decreasing pH of oxygen consumption. Free H2S was not detected.

Benthic Chamber:
As for the profiler two deployments, one at each site, was performed. At both sites the chamber was placed on a clam filed to measure the exchange of solutes over time. The oxygen electrodes monitored a decrease of the oxygen concentration with time indicating oxygen consumption – flux rates have to be calculated. Up to 5 syringes were taken during the incubation which still has to be analyzed.



Ex situ measurements

All measurements of microbial activity (e.g. sulfate reduction and anaerobic methane oxidation) investigated under atmospheric and in situ pressure as well as identification and distribution of microbes and pore water and gas concentrations will be done in the home lab. An overview of the samples taken and experiments performed is given in Table 1.

Tab.1: Sample list for microbial rate measurements (SRR – sulfate reduction rates, AOM – anaerobic methane oxidation) and microbial analysis (AODC – Acridine Orange Direct Counts, FISH – Fluorescence *in-situ* Hybridization, DNA – Deoxyribonucleic Acid).

Station	devices	color	SRR	AOM	AODC	FISH	DNA
952	PC	red			x	x	
955	PC	green			x	x	x
955	PC	white			x	x	x
955	subcores	blue	x				
955	subcores	yellow	x				
956	PC	black			x	x	x
956	PC	yellow	x		x	x	x
956	PC	red-white	x	x	x	x	x
956	PC	red	x	x	x	x	
956	PC	white	x	x	x	x	x
957	PC	white	x		x	x	
957	PC	blue	x		x	x	
957	PC	red			x	x	x
958	PC	black			x	x	x

High resolution concentration profiles of O_2, H_2S and pH were performed in the laboratory at in situ temperature. Mircroprofiles on a retrieved sediment core from site 2 (5380 m) at the rim of the *Calyptogena* colony revealed an oxygen penetration depth of 5 mm together with an decrease of pH in the zone of oxygen consumption (Fig. 6). Free H_2S was not detected down to a depth of 4 cm.

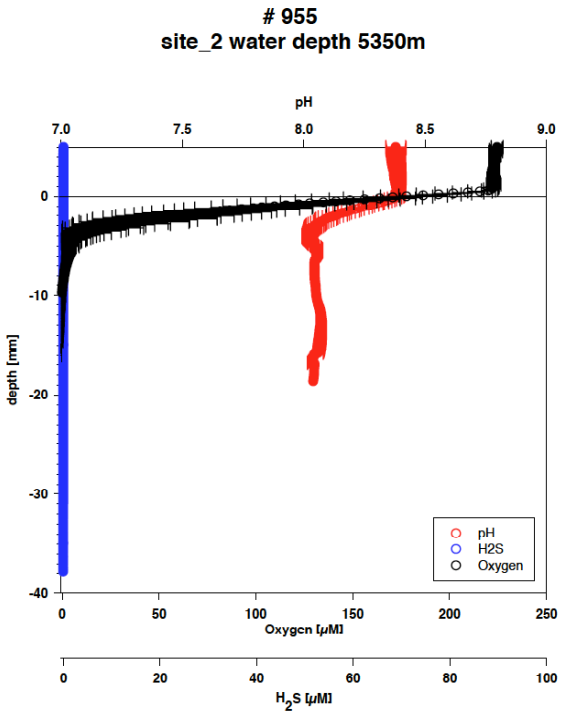


Fig. 6 Laboratory microprofiles of O_2, pH and H_2S on a retrieved sediments from Site 2.

For gas chemistry and biomarker analyisis a total number of mote then 318 samples were taken (see Table 2 for detailed information about the samples collected during the YK06-05 cruise). Most of these samples are to be analysed on-shore at the University of Bremen. On-board analysis of methane was performed in samples from 4 different Sinkai dives (#953, #954, #955 and #957). Dives 953 and 954 took place in the first sampling station (water depth: 6268m) and the other dives took place in the second station (water depth: 5347m). Results obtained are presented in Tab. 3.

Tab. 2: Type and number of samples taken. Planned on-shore analysis by the organic geochemistry group. Colours refer to the colour code for the different push core liners. (BW: bottom water in the push corer; Nis: Niskin bottle.

Dive Nr.	Sample type							
	Water Samples			Sediment samples				
	Pore Water		Bottom Water	Squeeze cakes	Non-pressed IPLs	Headspace		Clay Minerals
	δ^{13}C-VFAs	δ^{13}C-DIC		δ^{13}C-TOC		On-board	δ^{13}C-CH_4	
#950	7 (red)	8 (red)	2 (BW red) 1 (250mL Nis)	7 (red)	7 (red)	--	--	--
#951	Shinkai dive aborted due to bad weather conditions !!!							
#952	14 (black)	13 (black)	1 (BW red) 1 (BW black)	14 (black)	8 (black)	--	--	--
#953	--	--	2 (BW blue) 2 (BW yellow)	--	2 (blue) 1 (yellow)	--	--	--
#954	--	--	2 (BW red) 2 (BW green)	--	--	2 (red) 2 (green)	1 (red) 1 (green)	1 (red) 1 (green)
#955	15 (black)	15 (black)	2 (BW black) 1 (250mL BW)	15 (black)	15 (black) 4 (red)	5 (red)	3 (red)	3 (red)
#956	14 (green)	13 (green)	2 (BW black) 2 (bag sampler)	14 (green)	11 (green) 4 (yellow)	--	--	--
#957	15 (green)	14 (green)	2 (green)	15 (green)	15 (green) 5 (white)	3 (H_2 yellow) 3 (CH_4 yell.)	3 (yellow)	3 (yellow)
#958								

Tab.3:: Results of the on-board methane analysis.

Dive Nr.	Sampling station	Water depth (m)	Sample code	Sediment depth (cm)	[CH_4] (µM)	Observations
#953	1	6268	6K953CBlue	Bottom water	0,25 (*)	
#954	1	6268 m	6K954CR	Bottom water	0,10 (*)	Core was not retained in the liner. The whole sediment was collected in a bag.
				Sediment	0,08	
			6K954CG	Bottom water	0,12 (*)	
				Sediment	0,06	
#955	2	5347 m	6K955CR	Bottom water	0,31 (*)	*Calyptogena* blood in water
				0 – 1	10,28	Oxic layer
				2-10	122,99	Black sulfidic layer
				approx. 15	31,82	Interface
				approx. 20	65,45	Grey sediment
#957	2	5347 m	6K957CY	0-10	96,63	Black layer
				15-20	302,74	Interface
				25-30	220,98	Grey layer
#958	2					

(*) Results from bottom water samples are not reliable. The method used is adequate for analysis of methane in sediment samples. In the case of water samples, some methane could remain in the water phase. On contrary, when having sediment samples, after heating at 60ºC for 20 minutes, all the methane is in the headspace.

Nitrogen cycling processes in the Japan Trench cold seep environments

In global oceans, nitrogen is often regarded as the limiting nutrient for biological production. The current paradigm of marine nitrogen cycling indicates that fixed, or bioavailable, nitrogen is supplied to the surface ocean via either nitrogen fixation, or the upward flux of nitrate from the deep sea as upwelling and diapycnal mixing; while nitrogen losses are attributed to heterotrophic denitrification in sediments and the oxygen minimum zones (OMZs) of the water column, or as recently reported, also to anaerobic ammonium oxidation with nitrite (anammox) that produces dinitrogen gas (N_2) (Kuypers *et al.*, 2005). Benthic denitrification (and anammox) is by far the major contributor to total marine nitrogen loss (180-300 Tg N y^{-1}, >60% total N loss) (Codispoti *et al.*, 2001; Gruber, 2004), with the majority believed to occur along continental margins (Christensen *et al.*, 1987; Devol, 1991). However, actual rate measurements have been limited to less than 1000 m deep. Meanwhile, although ammonium content has been reported relatively high in hydrocarbon seepage fluids, little is known about the fate of this ammonium or nitrogen cycling in such environments. Hence, at the two deep cold seeps sites in the Japan Trench visited on this cruise – Station 1 (40°5.5529'N, 144°11.1745'E, 6280 m) and Station 2 (39°6.3560'N, 143°53.5619'E, 6300 m 5300 m), we investigated the nitrogen cycling processes in the sediments, the seepage water and the immediately overlying water column.

The seepage water was collected using a bag sampler system (Fig. 7) attached to an impecular pump with a nozzle maneuvered by the Shinkai6500 that enabled water collection at a point-source directly above an active *Calyptogena* colony (Fig. 8). Four bag samplers were used, and they have been pre-injected with 15N-stable isotope tracers for *in situ* incubation experiments to measure simultaneously denitrification and anammox rates by the isotope pairing technique (Dalsgaard & Thamdrup, 2002; Kuypers *et al.*, 2005) as well as nitrification rates: (1) $^{15}NH_4^+$, (2) $^{15}NH_4^+$ and $^{14}NO_2^-$, (3)$^{15}NO_2^-$ and $^{14}NH_4^+$, (4) $^{15}NO_3^-$, $^{15}NH_4^+$ and thiosulfate. All samplers were also amended with $H^{13}CO_3^-$ to measure organic carbon production. The bag samplers were left on the seafloor for 1-2 days, and then retrieved in the following submersible dive. Samples were collected afterwards for stable isotopic analyses of N_2, NH_4^+ , NO_2^- ,NO_3^-, particulate nitrogen, particulate organic carbon, dissolved inorganic carbon, as well as nutrient concentrations and molecular biological analyses (microbial community analyses with FISH and qPCR targeting 16S rRNA genes, functional gene expression analyses related to nitrogen cycling processes). Meanwhile, a single reference bag sampler was also collected at the same site and the sample was brought back to the surface on the same day for reference measurements, along with a time series incubation experiment onboard, performed at both 1 atmospheric pressure and *in situ* pressure to test for pressure effects on such reactions. Similar experiments were also performed with background deep-sea water collected via Niskin water samplers on board the Shinkai 6500.

To study N cycling in sediments, samples collected with push-cores were used to make slurries with degassed low-nitrate seawater for the isotope pairing experiments as briefly described above for water samples. *In situ* incubations were also performed using the *In Situ* Incubation corer (InSInc) to measure anammox and denitrification rates. The corers were pre-treated with: (1) $^{15}NH_4^+$ and $^{14}NO_2^-$, (2)$^{15}NO_3^-$ and $^{14}NH_4^+$, which were then left on the seafloor for two days before retrieval. Samples were collected for various stable isotopic analyses as well as molecular biological analyses as for water samples.

Fig. 7: Four bag samplers next to an active calyptogena colony.

Fig. 8: Water collection for bag samplers through the nozzle placed above the calyptogena colony.

Dive Reports

#950 Dive Report

Date: 31 May, 2006
Area: Sanriku Escarpment in the Japan Trench
Depth: 6300 m
Landing: 40°5.6732'N, 144°11.1379'E, 6265 m
Leave Bottom: 40°6.1163'N, 144°11.0018'E, 6177 m

Diver: Chiaki Kato (Extremobiosphere Research Center, JAMSTEC)
Pilots: Ohno and Komuku

Theme: *Survey of chemosynthetic biological community associated with cold seep at a depth of around 6300m in the Japan Trench*

Major objectives:

1. To observe the distribution and number of *Calyptogena* communities along the thrush fault at around 6300 depth,
2. To find a suitable working station for subsequent dives, and
3. To collect seawater samples at 6300m and sediment cores from the working station.

Payload Equipments:

1. Niskin bottles (2.5L) x 3
2. MBAR-type push corer (50cm) x 6
3. ROV Homer
4. Marker x 6
5. Bio-box
6. Scoop

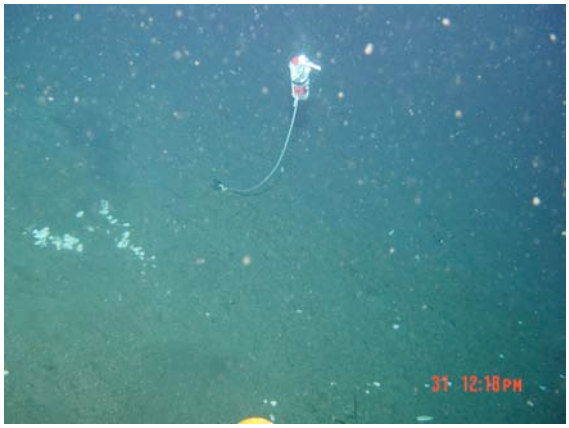
Sampling Points and Markers:

(1) Site 1 *Calyptogena* communities, Marker #38. At just 10 m above the site 1, three Niskin samplings were performed.
(2) Small *Calyptogena* communities between site 1 and 2, Marker #39.
(3) Site 2 *Calyptogena* communities, Marker #40.
(4) Site 3 *Calyptogena* communities, Marker #41. Target site was found at a flat *Calyptogena* community. Four MBARI core sediment samplings were completed at just beside the target community. *Calyptogena* samples (10 *C. phaseoliformis*, 20 *C. fossajaponica*) were also obtained from another small community.

The photographs of the sampling points and markers were shown below:

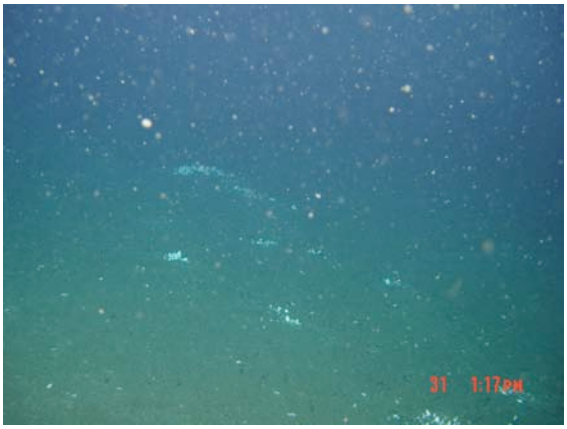
Site 1. #38 Marker site

#39 Marker



Site 2, #40 Marker site

Site 3, #41 Marker site



During #950 dive, we found a number of *Calyptogena* communities at depth of 6200~6300 m line at the almost same diving point of 6K#552, that had performed on six years ago. Three Niskin sea water samples were obtained just 10 m above the Calyptogena communities. We identified suitable *Calyptogena* community site (Site 3) for the following *in situ* measurement experimental dives, where would be a flat area. Four MBARI core sediment samples were completed beside the community, and two different Calyptogena species (*C. phaseoliformis* and *C. fossajaponica*) were also sampled from another community.

<Survey of the diving point>
We have identified three major *Calyptogena* communities as follows;
Site 1, Several large communities were observed, but slope was so hard, so it might be so hard to stand the *in situ* measurement systems safety.
Site 2, A few middle size of communities were observed, but slope was also hard.
Site 3, Several middle size of communities were observed at flat area, so we decided here could be suitable point for *in situ* measurement systems.
Those sites were shown above.

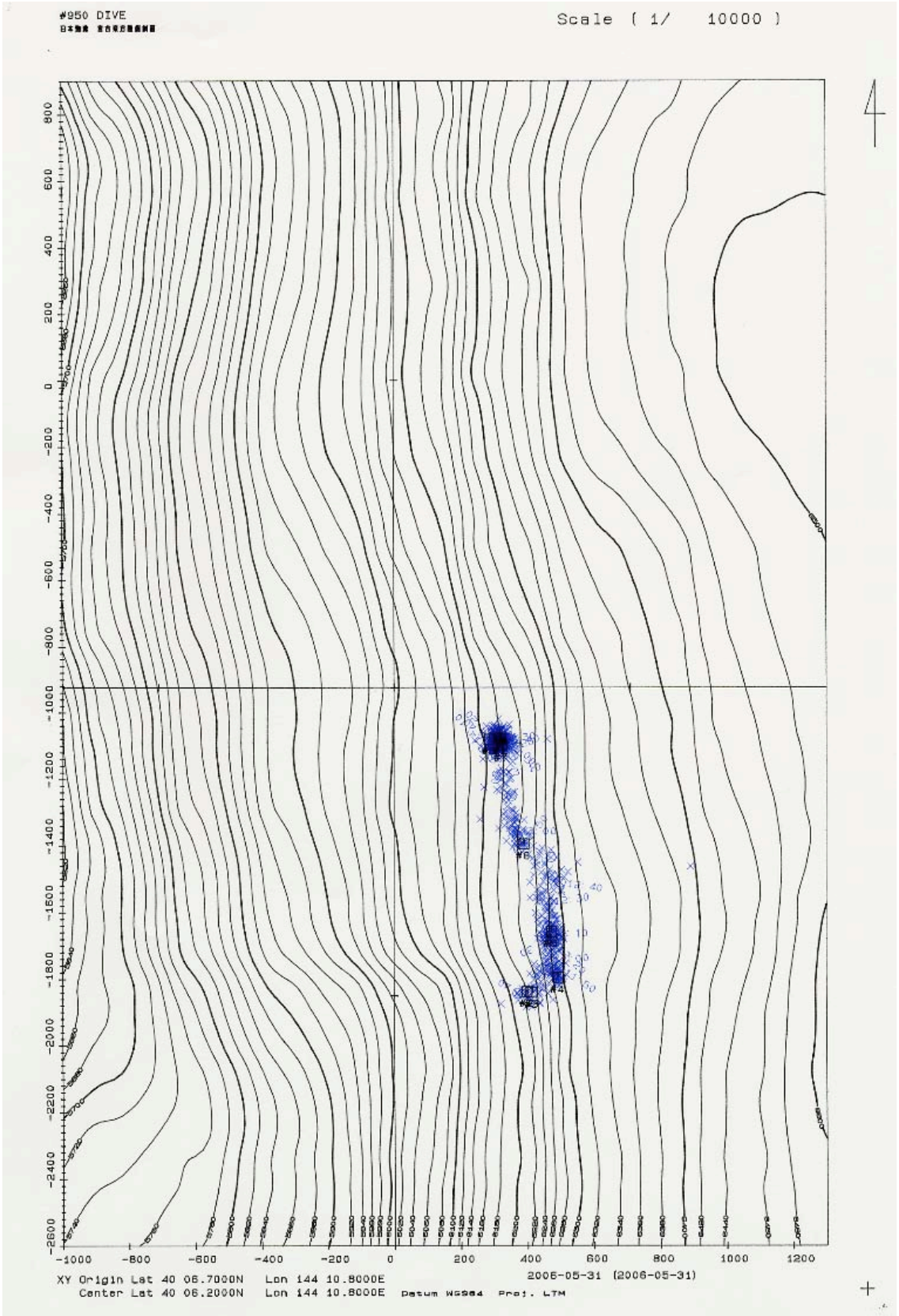
<Sampling>
Three Niskin water samples were obtained from at just 10 m above of site 1. Four MBARI core sediment samples were completed beside the target community at site 3. Calyptogena community was also sampled from another community at site 3.

<Sample list>

Sample name	Sampling points	Depth	Material	Sample distribution	Others
6K950-N1	40°5.7067'N, 144°11.0815'E (Site 1)	6255 m	Niskin sea water samples just above site 1.	Phyllis	
6K950-N2				Chiaki, Hiro	
6K950-N3				Chiaki, Hiro	
6K950-C1	40°6.0245'N, 144°10.9971'E (Site 3)	6181 m	MBARI core (50cm) beside the community at site 3.	Frank G,	
6K950-C2					
6K950-C3					
6K950-C4				Fumio, Chiaki	
6K950-M1			*Calyptogena phaseoliformis* 10	Tad, Chiaki, Fumio	
6K950-M2			*Calyptogena fossajaponica* 20		

<Video Topics>

Time	Topics	Others
11:34:37~11:35:06	Niskin sea water sampling	
11:50:47~11:58:21	Site 1, *Calyptogena* community & #38 Marker	
12:18:03~12:18:18	#39 Marker	
12:19:10~12:19:36	Macroid fish	
12:46:18~12:50:45	Site 2, *Calyptogena* communities & #40 Marker	
13:16:57~13:28:12	Site 3, *Calyptogena* communites	
13:44:12~14:12:52	MBARI core sediment sampling	
14:14:28~14:16:04	*Calyptogena phaseoliformis* colony	
14:26:52~14:27:08	#41 Marker & ROV homer	
14:29:43~14:32:39	Clams sampling	
14:43:17~14:43:28	*Calyptogena fossajapponica* colony	
14:46:03~14:46:05	Leaving from bottom	


#950 DIVE
Scale (1/ 10000)
XY Origin Lat 40 06.7000N Lon 144 10.8000E
Center Lat 40 06.2000N Lon 144 10.8000E
2006-05-31 (2006-05-31)

#951 Dive Report

Date: 1 June 2006
Area: Sanriku Escarpment in the Japan Trench
Depth: 5300 m
Landing Target: 39°6.4690'N, 143°53. 4925'E

Diver: Hiroyuki Imachi (Extremobiosphere Research Center, JAMSTEC)
Pilots: Kawama and Ueki

Theme: *Survey of chemosynthetic biological community associated with cold seep at a depth of around 5300m in the Japan Trench*

Major objectives:

1. To observe the distribution and number of *Calyptogena* communities along the thrush fault at around 5,300 depth,
2. To find a suitable working station for subsequent dives, and
3. To collect seawater samples at 5,300m and sediment cores from the working station.

Payload Equipments:

1. Niskin bottles (2.5L) x 3
2. MBARI-type push corer (50cm) x 6
3. Janine-type push corer
4. Marker x 6
5. Bio-box
6. Scoop

Unfortunately, we came back the mother ship without watching the sea bottom in the Dive#951, because wind was getting stronger (we reached at depth of around 2,500m!). We, therefore, did not have any pictures and video regarding this dive. On the way to the mother ship, we collected seawater by Niskin bottles at depth of around 80-100 m for measurement of nitrate concentration.

<Sample list>

Sample name	Sampling points	Depth	Material	Sample distribution	Others
6K951-N1	39°6. 5407'N, 143°52.9745'E	80-100 m	Niskin sea water samples	Phyillis Lam	

#952 Dive Report

Date: 02 June, 2006
Area: Sanriku Escarpment in the Japan Trench
Depth: 6200 m
Landing: 40°6.1059'N, 144°11.0281'E, 6180 m
Leave Bottom: 40°6.1347'N, 144°11.0234'E, 6182 m

Diver: Frank Wenzhöfer (Microbial habitat Group, Max Planck Institute for Marine Microbiology, Bremen, Germay)

Pilots: Sasaki and Matsumoto

Theme: *In situ measurements and sampling around a Calyptogena colony at a depth of around 6300m in the Japan Trench*

Major objectives:

1. Place the microprofiler module at a suitable site close a *Calyptogena* community,
2. Take push cores in the vicinity of the microprofiler module and close to the clams, and
3. To collect clams

Payload Equipments:

1. Microprofiler module (3 Oxygen -,
2. H_2S -, 3 pH-, 1 Resistivity -,
   1 Temperature - Microsensor)
3. MBARI-type push corer (50cm) 3x
4. MPI_triple_subcore corer 1x
5. Marker x 2
6. Bio-box
7. Scoop

Sampling Points and Markers:

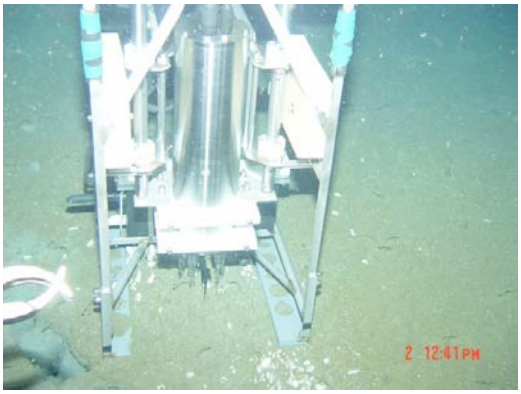
(ア) Site_3 *Calyptogena* communities, Marker #41. Target site for in situ measurements and core and clam sampling

(イ) Site_4 *Calyptogena* communities, Marker #42 (marker got tied up to Shinkai and was accidentally recovered during ascent)

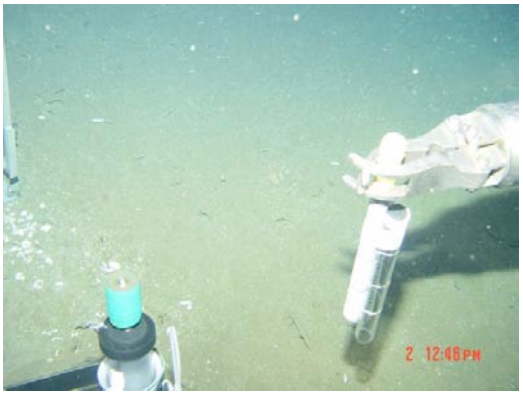
Selected pictures of the dive:

Targeted Site 3. Marker #41:

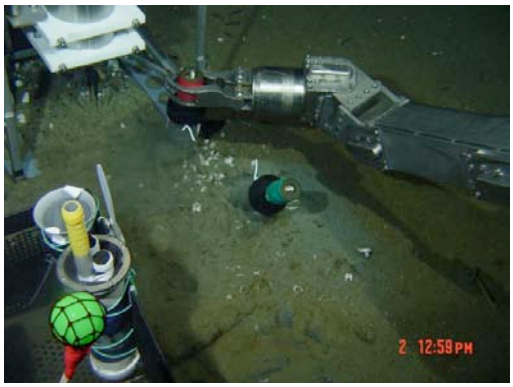
In situ microprofiler module placed at the clam rim between the two communities centers:

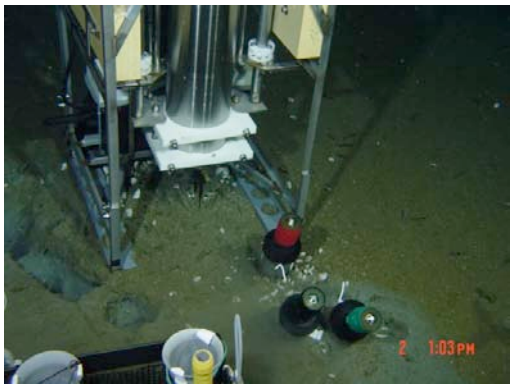


Sampling with the MPI Subcore Corer:

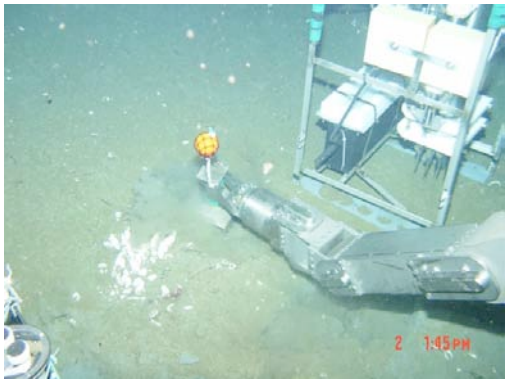


Push core sampling close to the microprofiler:





Calyptogena sampling:



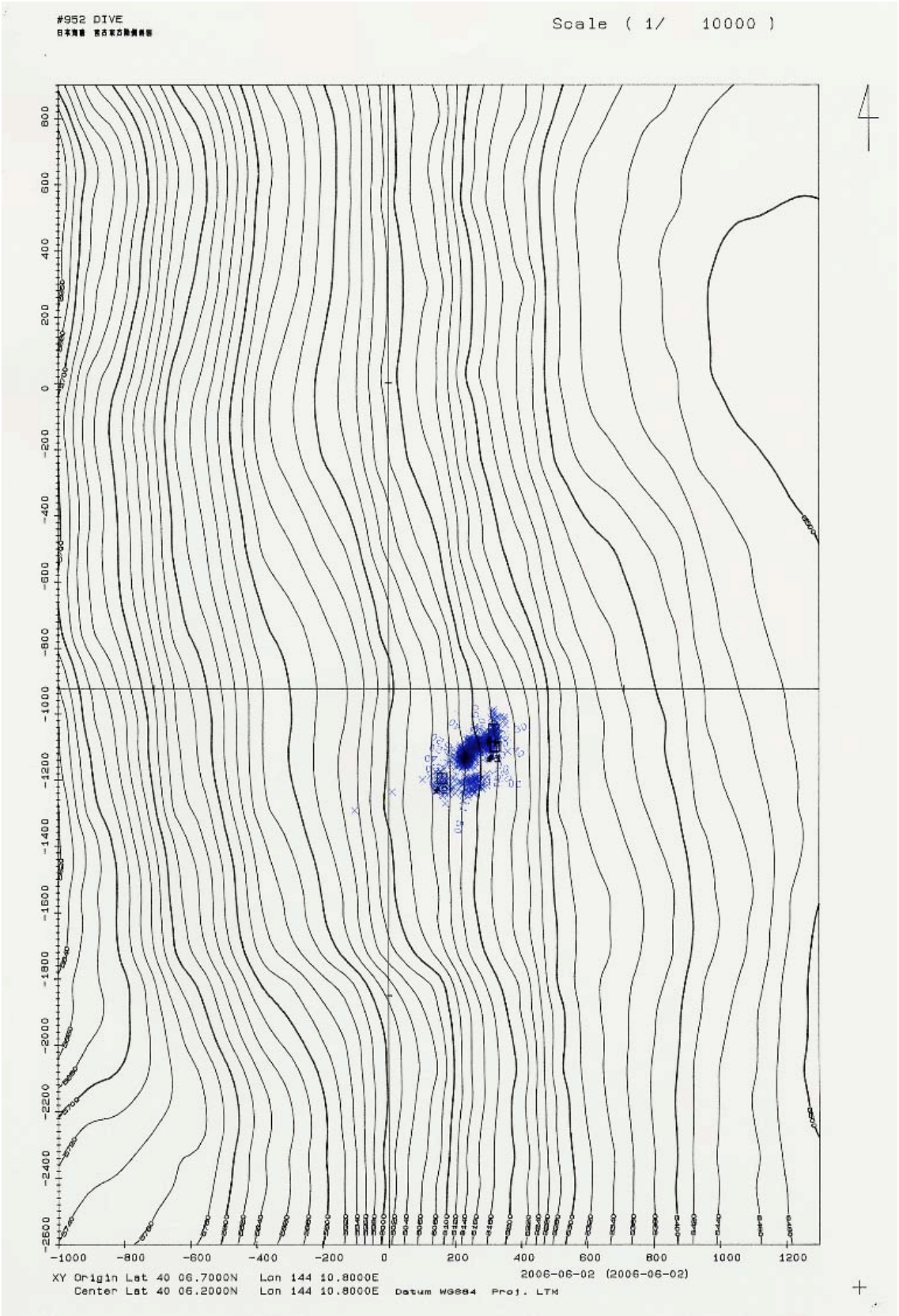
During dive #952 the *Calyptogena* community at site_3 (Marker 41) was visited do perform in situ microsensor measurements and to sample sediment cores as well as clams. After finishing the sampling a short survey was done and Marker 42 placed at another *Calyptogena* colony.

<In situ measurements>
The micropofiler module was placed between the two *Calyptogena* patches to measure microprofiles of O_2, pH, H_2S and T at a vertical resolution of 100 µm within the sediment. The measuring routine was started by pressing a switch on the module. Unfortunately the program stopped after 2/3 of the profiling distance due to battery capacity problems and the microsensors did not penetrate into the sediment.

<Sampling>
Three MBARI push cores were take in close vicinity to the microprofiler unit between the two *Calyptogena* communities. Additionally two Subcore Corer were taken at the same spot. The sediment samples are used for analyses of pore water concentrations of nutrients and gases, process rate measurements and microbiological investigations. After sediment sampling clams were collected from the patch with smaller clams and after removing the microprofiler module also from the big patch with bigger clams.

<Sample list>

Sample name	**Sampling point**	**Depth**	**Material**	**Sample distribution**	**Others**
6K952-MP	40°6.1055'N, 144°11.0252'E	6182m	In situ microprofiles (O_2, H_2S, pH, T)	Janine, Frank, Gaby	
6K952-SC	40°6.1055'N, 144°11.0252'E	6182m	Sub core sediment samples for SR	Janine, Frank	
6K952-C Green	40°6.1055'N, 144°11.0252'E	6182m	Sediment samples between clam communities	Fumio, Chiaki, Hiro, Tad, Christian, Phyllis, Xavier, Gaby, Daniela, Frank	
6K952-C red	40°6.1055'N, 144°11.0252'E	6182m			
6K952-C black	40°6.1055'N, 144°11.0252'E	6182m			
6K952-M	40°6.1055'N, 144°11.0252'E	6182m	Clams form both *Calyptogena* patches	Fumio, Tad, Hiro	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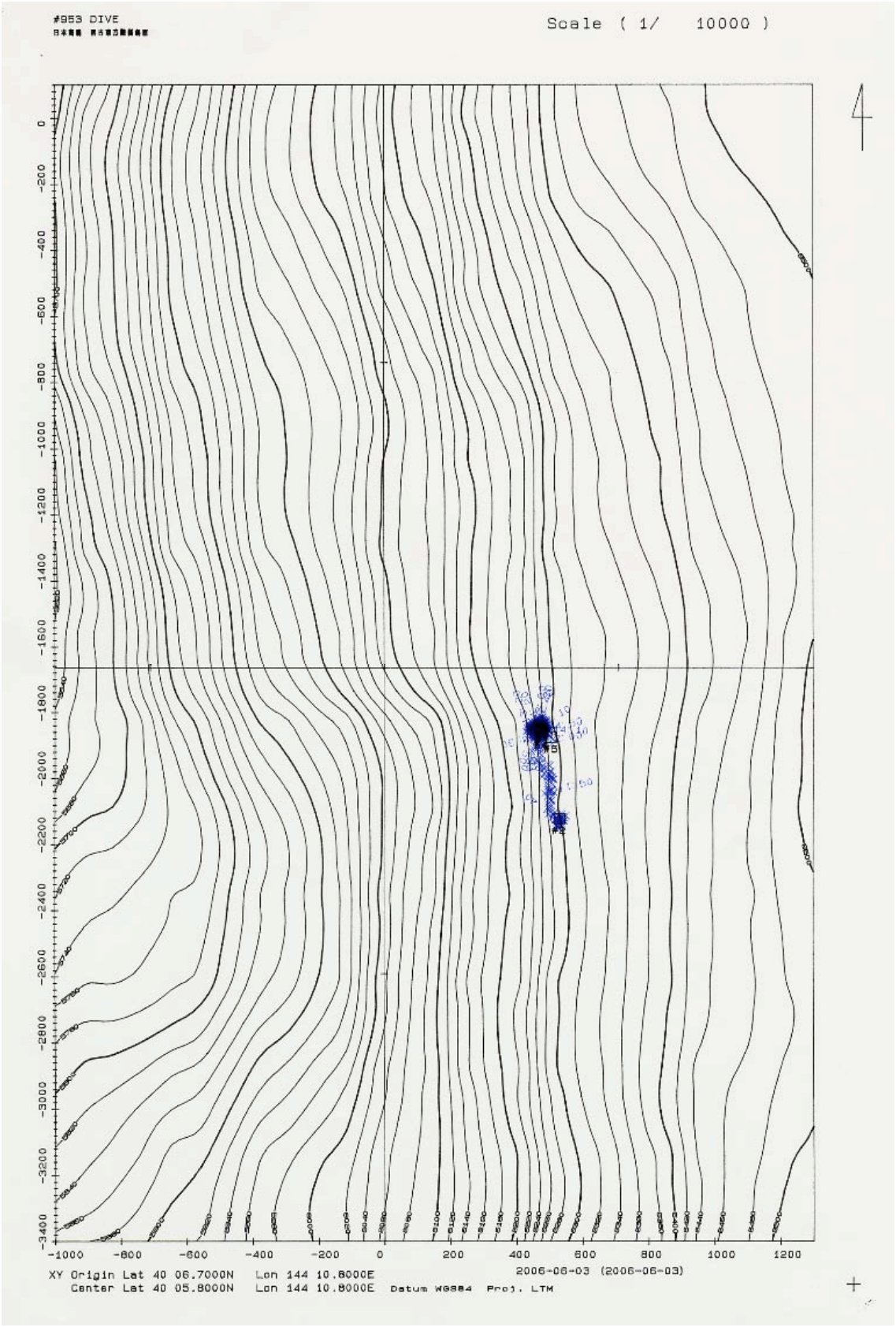
Scale (1/ 10000)
XY Origin Lat 40 06.7000N Lon 144 10.8000E
Center Lat 40 06.2000N Lon 144 10.8000E Datum WGS84 Proj. LTM
2006-06-02 (2006-06-02)

#953Dive plan

潜航者：Phyllis Lam
潜航地点：S1 サイト、イベントマーク３８
潜航目的：日本海溝冷水湧出域における微生物代謝活性の現場測定
ペイロード：
① ４連バック採水器
② ニスキン採水器× 3
③ MBARI コアラー（50cm）・サブコアラー×できるだけ
④ バイオボックス
⑤ くまで
作業予定：
① イベントマーク３８に着底。着底前にニスキン採水３本。
② ４連バック採水：一番大きなコロニーのシロウリ直上（貝の隙間）から位置を変えながら４発。４発とも時間を合わせる（水量をある程度一定にするため）。
③ ４連バック採水の設置：ホースカット後海底に放置。採水場所から多少動いても可。（次のダイブで回収予定）
④ 採水を行ったコロニーにマーカーを設置（次のダイブで作業をするため）。
⑤ MBARI コア・サブコア採取。（コロニー内側か際：黒っぽいのが欲しい）
⑥ 貝採取。
⑦ 浮上

今後の予定

#954(6/4)でバックを回収。採水した場所で、単発バック採水・MPI コアラー２本。EM41 に向かい、チャンバー設置（一日放置）。#955(6/5)でチャンバー回収とコア・生物取り作業。潜航予定者：#954 丸山正、#955Christian Deusner。S1 での作業を終了し、後半戦４ダイブで S2 を調査する


#953 DIVE
Scale (1/ 10000)
XY Origin Lat 40 06.7000N Lon 144 10.8000E
Center Lat 40 05.8000N Lon 144 10.8000E Datum WGS84 Proj. LTM
2006-06-03 (2006-06-03)

#954 Dive Report

Date: 3 June, 2006
Area: Sanriku Escarpment in the Japan Trench
Depth: 6300 m
Landing: 40°5.5457'N, 144°11.1946'E, 6291 m (11:35)
Leave Bottom: 40°5.6916'N, 144°11.1074'E, 6258 m (15:00)

Diver: Tadashi Maruyama (Extremobiosphere Research Center, JAMSTEC)
Pilots: Y. Ohno and H. Ueki

Theme: *Survey of chemosynthetic biological community associated with cold seep at a depth of around 6300m in the Japan Trench*

Major objectives:

1. To deploy the chamber module profiler at a suitable place and leave it for 2 hours for measuring the changes of the environmental factors.
2. To insert two MBARI push corers in sediments with active *Calyptogena* clams and leave them for some period.
3. To collect water near the Calyptogena clams with the one-bag water sampler.
4. To retrieve the two MBARI push corers described above (#2).
5. To retrieve the 4 bag water sampler, which was deployed on the dive 6K-953.
6. To retrieve the deployed chamber module profiler described above (#1).

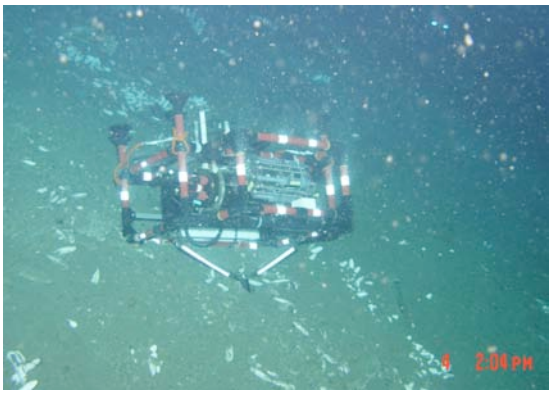
Payload Equipments:

1. Chamber module profiler
2. MBAR-type push corer x 2
3. One-bag water sampler
4. A space for the 4-bag water sampler for the retrieval

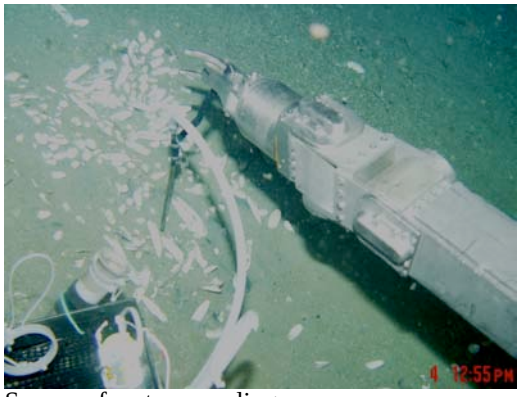
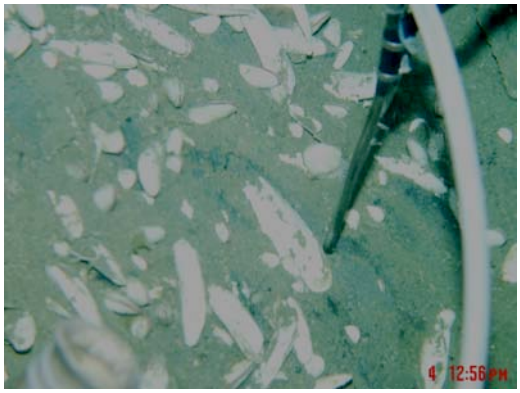
Sample collections:

(ア) Deplyoment of the chamber module profiler at 40º 5.6898' N 144º 11.1355'E at depth of 6268 m (12:22).
(イ) Deployment of two MBARI corers (red and blue) side by side in the sediments at 40º 5.6898' N 144º 11.1355'E at depth of 6268 m.
(ウ) Water sampling with the one-bag water sampler at 40º 5.6898'N 144º 11.1355'E at depth of 6269 m (13:14).
(エ) Retrieval of the 4-bag water sampler at 40º 5.6898 N 144º11.1355'E at depth of 6269 m (13:44)
(オ) Retrieval of the chamber module profiler at 40º 5.6898'N 144º 1355'E at depth of 6269 m (14:18). Around the time of 13:57, the chamber module profiler was turned up-side-down on the slope.
(カ) Retrieval of two MBARI corers at 40º5.6898'N 144º 11.1355'N at depth of 6268m (14:46).

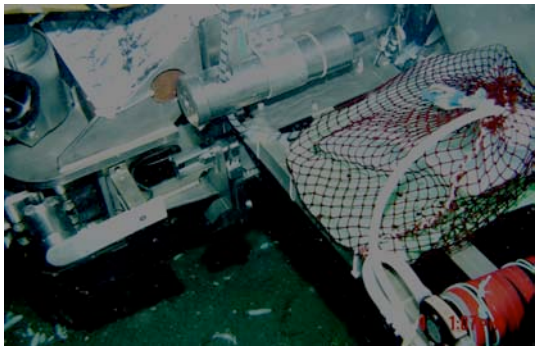
The photographs of the samples and equipments;
1. Deployed chamber module profiler.
After the deployment, it stood on their foots, but later it turned on its back down.



2. Water collection with the one-bag water sampler.

Scenes of water sampling



Showing the water bag during sampling.

3. Retrieval of the 4-bag water sampler

The 4-bag water sampler was sitting up-side-down.

4. Sediment core sampling.

Two MBARI corers. A clam In the right corer was destroyed during the core insertion and red blood oozed out from the clam.

Other organisms oberved.

A ctenophore(?) on the sediment.

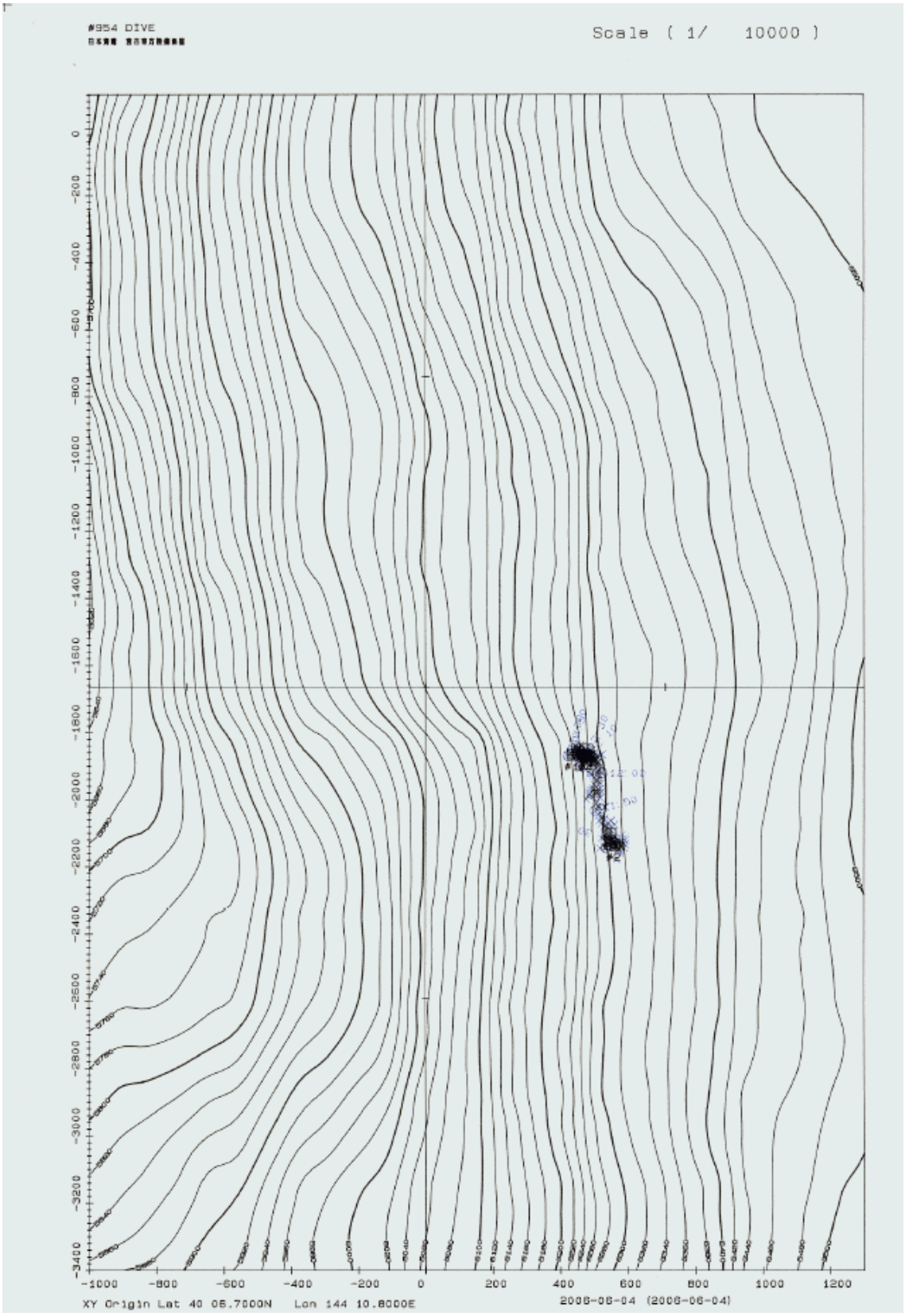
Two sea-anemones on a *Calyptogena* clam shell.

Pogonophora tubeworms.

Calyptonega clams with extending foot.

In the 6K-#954 dive, we collected two MBRI cores of sedemnt samples, environmental data measured with the chamber module profiler, a water sample of the one-bag water sampler, and the one day in situ Incubated water sample of 4-bags water sampler.

<Sediment sample>
The sediment at depth of 6268 m was collected with two MBARI corers.
<Water samples>
We retrieved the 4-bag water sampler, which was deployed yesterday on the dave 6K-953. One bag of water sample was collected with the one-bag water sampler.
<In situ measurement of environmental factors>
We deployed the chamber module profiler and left it for approximately one hour and a half. Although it was turned up-side-down (means the termination of the incubation of the sediment enclosed with the chamber), it was safely retrieved.


#954 DIVE
Scale (1/ 10000)
0
-200
-400
-600
-800
-1000
-1200
-1400
-1600
-1800
-2000
-2200
-2400
-2600
-2800
-3000
-3200
-3400
-1000
-800
-600
-400
-200
0
200
400
600
800
1000
1200
XY Origin Lat 40 08.7000N Lon 144 10.8000E
2006-06-04 (2006-06-04)

#955 Dive Report

Date: 5 June 2006
Area: Sanriku Escarpment in the Japan Trench
Depth: 5346m
Landing Target: 39°6.4690'N, 143°53. 4925'E

Diver: Fumio Inagaki (Extremobiosphere Research Center, JAMSTEC)
Pilots: Kawama and Komuku

Theme: *In situ measurement of chemosynthetic (micro-)biological activities at the station 2 cold seep site in the Japan Trench*

Major objectives:

This is the first dive to visit station 2 at a depth of 5300m during this expedition. Major goals of the dive are:

1. To find *Calyptogena* community for the working station ,
2. To deploy multiple bag sampler for in situ incubation,
3. To deploy MPI cores for in situ incubation,
4. To collect sediment cores from the Calyptogena colony, and
5. To collect *Calyptogena* clams for the endosymbiont study under highpressure.

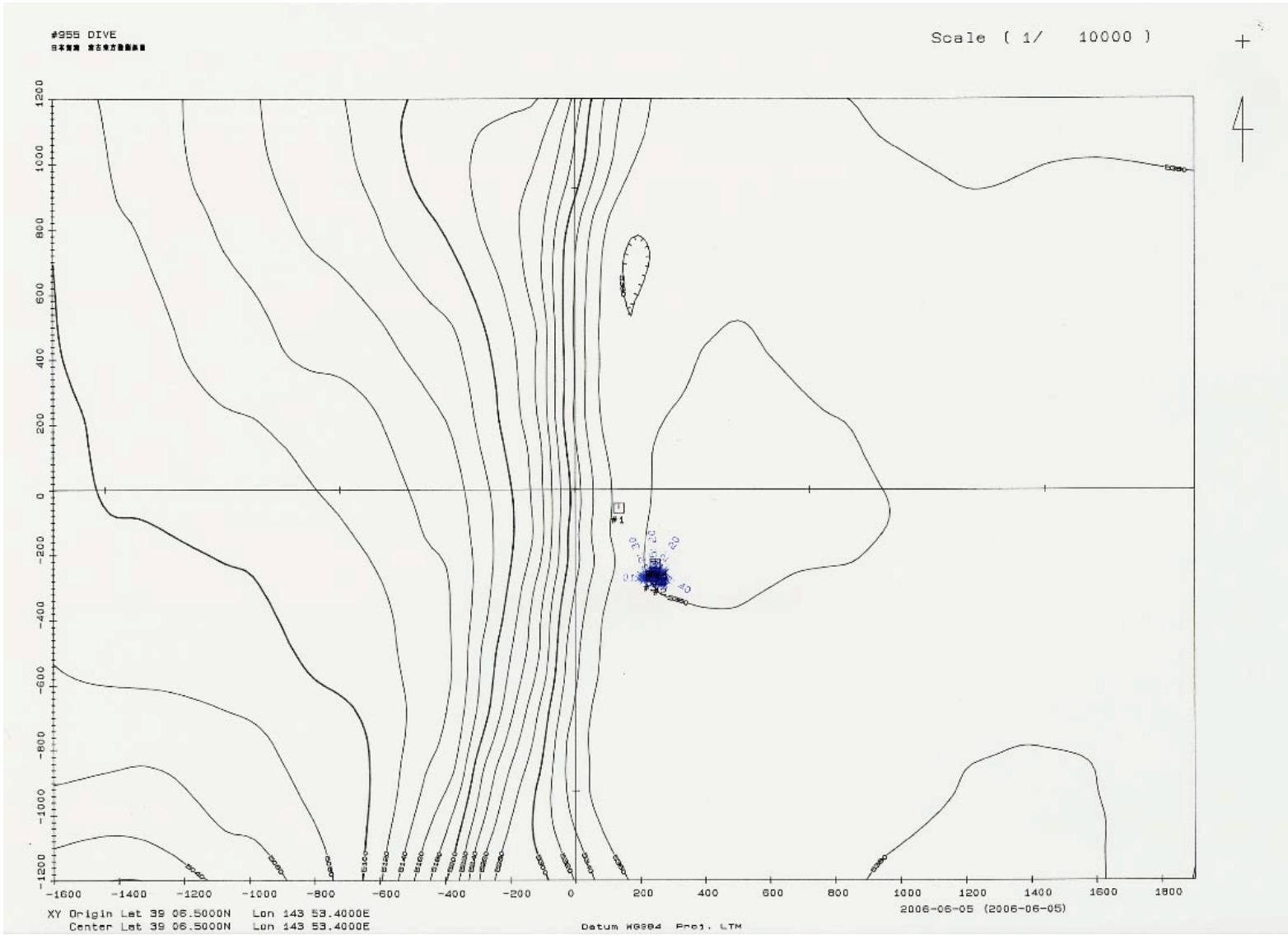
Payload Equipments:

1. Niskin bottles (2.5L) x 3
2. Multiple Bag Sampler x 1 (4x20L Bag)
3. MBARI-type push corer (50cm) x 3, (30cm)x3
4. Mini-corerx2
5. Marker
6. Bio-box
7. Scoop

During the Shinkai going down, we changed the landing point slightly southeast since the flat area is necessary for following operations. We landed at the flat area, where we can see many benthic organisms such as sponges and sea anemones. I observed somewhat whitish patch from the left window, which was found to be big *Calyptogena* colony. Amazingly, almost no movement after landing. When we close to the colony, we took seawater just above the colony with three Niskin bottles. Next, we checked the quality of sediment by taking a MBARI push core. The sediment looked like very soft, sulfidic, and mud, so we decided here to be the working station. We took seawater samples for multiple bag sampler from 1cm above *Calyptogena* colony with continuously changing the sampling position. Each bag takes 2 min 30 sec. After the seawater sample collection, we cut off the inlet tube of the multiple bag sampler, and then leave it beside the colony. Nest, we took MBARI-type corers and mini-corers from the rim of colony. The cores contained black sulfide layers overlaying a few cm of oxic layer. After the coring, we deploy two MPI-type corers into the sediment, and remove two triggers. Both multiple bag sampler and MPI-corer were left in situ for a few days to monitor the microbial activities of nitrogen cycle. We collected 10-15 clams for endosymbiont study. At the end, we set Marker 43, and left the bottom. Overall, this dive was very successful because we could find a nice working station very soon and achieve all operations that we expected.

<Sample list>

Sample name	Sampling points	Depth	Material	Sample distribution	Others
6K955N	39°6. 3560'N, 143° 53.5619'E	5347 m	Niskin sea water samples	JAMSTEC MPI	
6K955CG, CY, CW, CR, CB, CYB	39°6. 3560'N, 143° 53.5620'E	5348 m	Sediment core from the rim of Calyptogena colony	JAMSTEC, MPI, Univ. Bremen	
6K956M-Calyptogena	39°6. 3560'N, 143° 53.5621'E	5349 m	Calyptogena spp.	JAMSTEC	


#955 DIVE
Scale (1/ 10000)
XY Origin Lat 39 06.5000N Lon 143 53.4000E
Center Lat 39 06.5000N Lon 143 53.4000E
Datum WGS84 Proj. LTM
2006-06-05 (2006-06-05)

#956 Dive Report

Date: 7th June, 2006
Area: Sanriku Escarpment in the Japan Trench
Depth: 5347 m
Landing: 39º 6.3532' N, 143º 53.5841' E, depth: 5347 m (12:25 h)
Leave Bottom: 39º 6.4670' N, 143º 53.5412' E, depth: 5347 m (15:05 h)

Diver: Christian Deusner (Max Planck Institute, Bremen)
Pilots: K. Matsumoto and Y. Sasaki

Theme: *Survey of chemosynthetic biological community associated with cold seep at a depth of around 5350m in the Japan Trench*

Major objectives:

1. To retrieve the 4-bag water sampler, which was deployed on dive 6K-955 at marker #43
2. To collect reference water sample from above Calyptogena colony using 1-bag water sampler
3. To retrieve 2 MPI-Corers (Insinc Modules) which were deployed on dive 6K-955 at marker #43
4. To collect 8x MBAR type push cores
5. To survey area N from Marker #43
6. To collect clams from separate colony

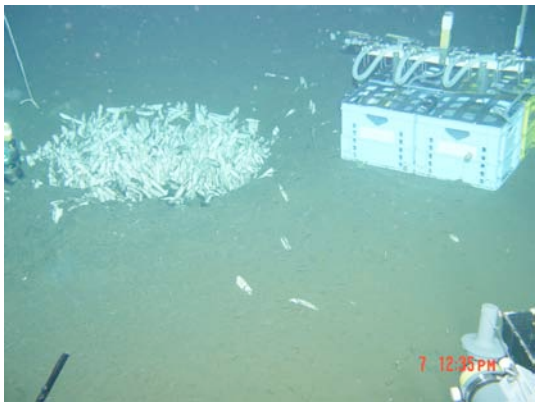
Payload Equipments:

1. MBAR-type push corer (4 x 35cm, 3 x 50cm)
2. Niskin bottles (2.5L) x 3
3. One-bag water sampler
4. Marker x 3
5. Bio-box
6. Scoop

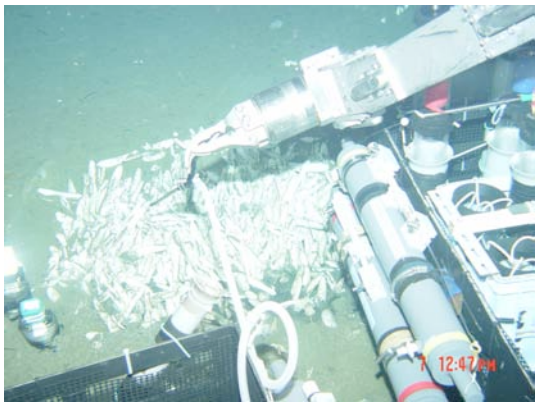
Sample collection:

(5) 3 Niskin Water Samples at 39º 6.3560' N, 143º 53.5619' E at a depth of 5347 m (12:44 h).
(6) Water sampling with the one-bag water sampler at 39º 6.3560' N, 143º 53.5619' E at a depth of 5347 m (12:57 h).
(7) Retrieval of 2 MPI type corers at 39º 6.3560' N, 143º 53.5619' E at a depth of 5347 m (13:04 h).
(8) Retrieval of 4-bag water sampler at 39º 6.3560' N, 143º 53.5619' E at a depth of 5347 m (13:12 h).
(9) Sediment samples with MBAR cores (8) at 39º 6.3560' N, 143º 53.5619' E at a depth of 5347 m (14:00 h).
(10) Sampling of Calyptogena clams, deployment of marker #44 at 39º 6.4836' N, 143º 53.5401' E at a depth of 5347 m (15:01 h).

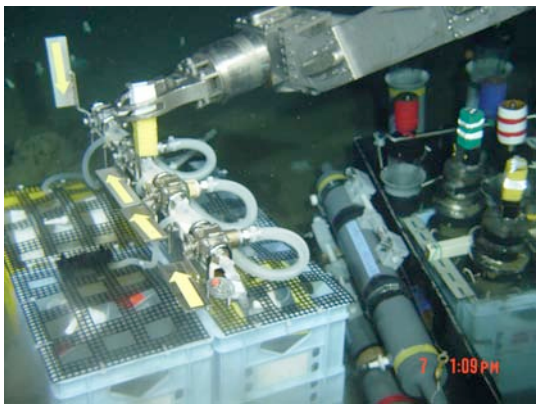
All instruments for in situ analysis deployed on dive 6K955 were successfully retrieved on dive 6K956. Sampling of MBAR pushcores (3x50 cm, 5x35cm) was carried out just at the rim of Calyptogena colony at marker #43. Water sampling was carried out above the colony. Sediment sampling and water sampling was carried out to further complete sample needs for detailed biogeochemical and microbial analysis of Calyptogena colony at marker #43. The subsequent survey was showing that Calyptogena colonies were widespread but the colony at marker #43 was exceptionally large.



Calyptogena colony at marker #43



Water sampling at marker #43

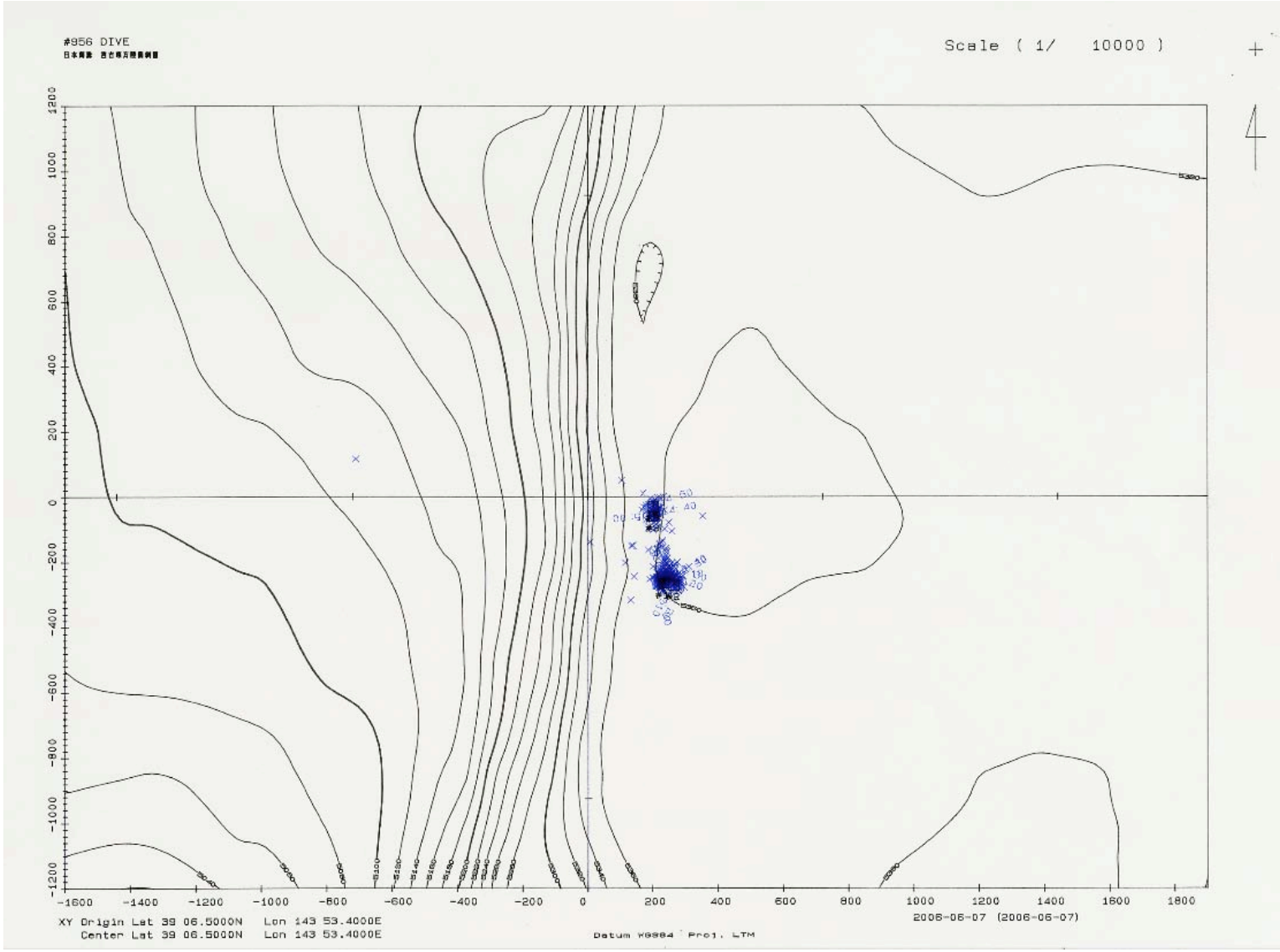


Retrieval of 4-bag water sampler

MBAR type core sampling

Retrieval of pushcores

Calyptogena colony #44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Datum WGS84 Proj. LTM
2006-06-07 (2006-06-07)

#957 Dive Report

Date: 8th June, 2006
Area: Sanriku Escarpment in the Japan Trench
Depth: 5347 m
Landing: 39º 6.3560' N, 143º 53.5524' E, depth: 5347 m (12:15 h)
Leave Bottom: 39º 6.3658' N, 143º 53.5436' E, depth: 5347 m (15:21 h)

Diver: Xavier Prieto (Research Center Ocean Margins, Bremen)
Pilots: T. Sakurai and H. Ueki

Theme: *Survey of chemosynthetic biological community associated with cold seep at a depth of around 5350m in the Japan Trench*

Major objectives:

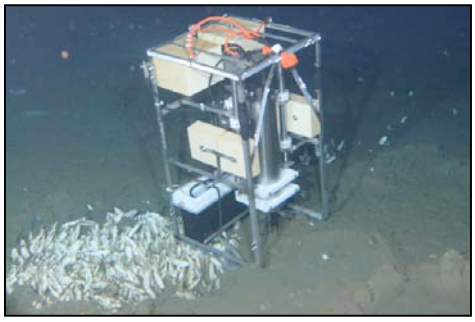
1. To deploy the profiler module as close as possible to the *Calyptogena* community (Marker 43), start the program and leave it for 2 hours measuring several chemical parameters (temperature, pH, S^{2-} and O_2) within the depth inside the sediment.
2. To collect water with the one-bag water sampler as close as possible to the place where the sensors from the profiler module have started their measurements.
3. To do some survey SW from Marker 43 while profiler module is working. To retrieve one 35cm long MBARI push corer during this survey, as a reference from a non active site.
4. To come back to Marker 43 and to insert two 35cm long MBARI push corers in the sediment next to the profiler module.
5. To collect some clams from the middle of the *Calyptogena* community.
6. To insert four 50cm long MBARI push corers in the sediment where the clams have been collected.
7. To retrieve all MBARI push corers described above (#4 and #6).
8. To retrieve the deployed profiler module described above (#1).

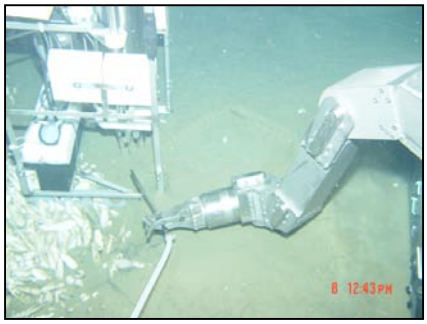
Payload Equipments:

1. Profiler module
2. MBARI-type push corer (2 x 35cm, 4 x 50cm)
3. One-bag water sampler
4. Scrub for clam collection

Sample collection:

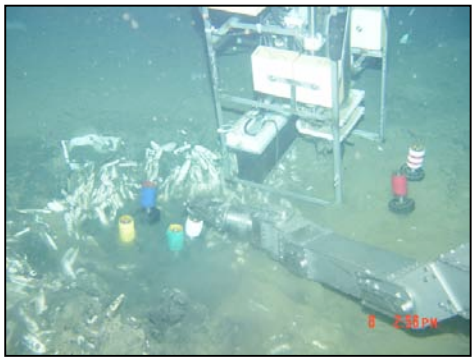
① Deployment of the profiler module at 39º 6.3560' N, 143º 53.5619' E at a depth of 5347 m (12:33 h).
② Water sampling with the one-bag water sampler at 39º 6.3560' N, 143º 53.5619' E at a depth of 5347 m (12:56 h).





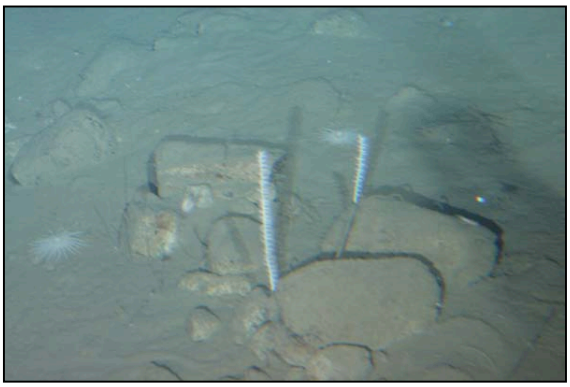
③ Deployment and retrieval of one 35cm MBARI corer (yellow/black) at a reference site at 39º 6.1669' N, 143º 53.4067' E at a depth of 5364 m (13:48 h).
④ Collection of some *Calyptogena* clams from the community at Marker 43 at 39º 6.3560' N, 143º 53.5619' E at a depth of 5347 m (14:39 h).
⑤ Deployment of two 35cm MBARI corer (red and white/red) in the sediment next to the profiler module at 39º 6.3560' N, 143º 53.5619' E at a depth of 5347 m.
⑥ Deployment of four 50cm MBARI push corer (blue, yellow, white and green) in the sediment where clams have been collected at 39º 6.3560' N, 143º 53.5619' E at a depth of 5347 m.
⑦ Retrieval of all MBARI corers at 39º 6.3560' N, 143º 53.5619' E at a depth of 5347 m. (15:06 h).

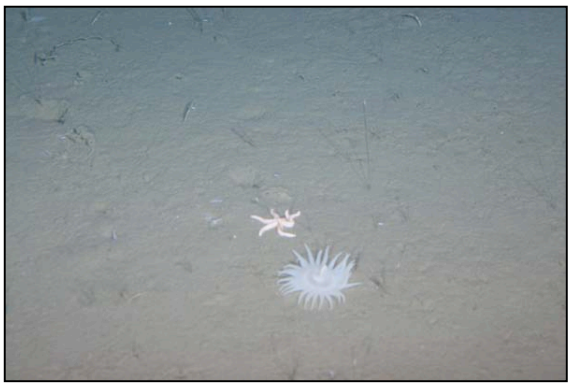
⑧ Retrieval of the profiler module at 39º 6.3560’ N, 143º 53.5619’ E at a depth of 5347 m (15:18 h).

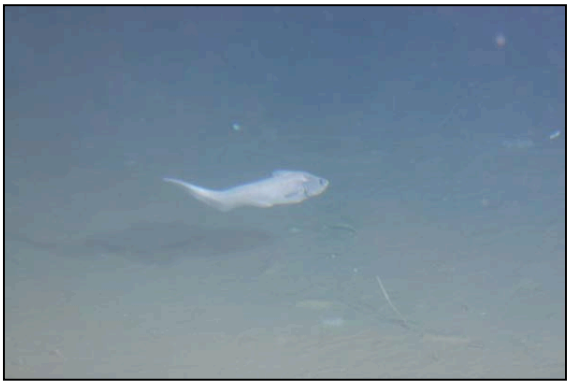


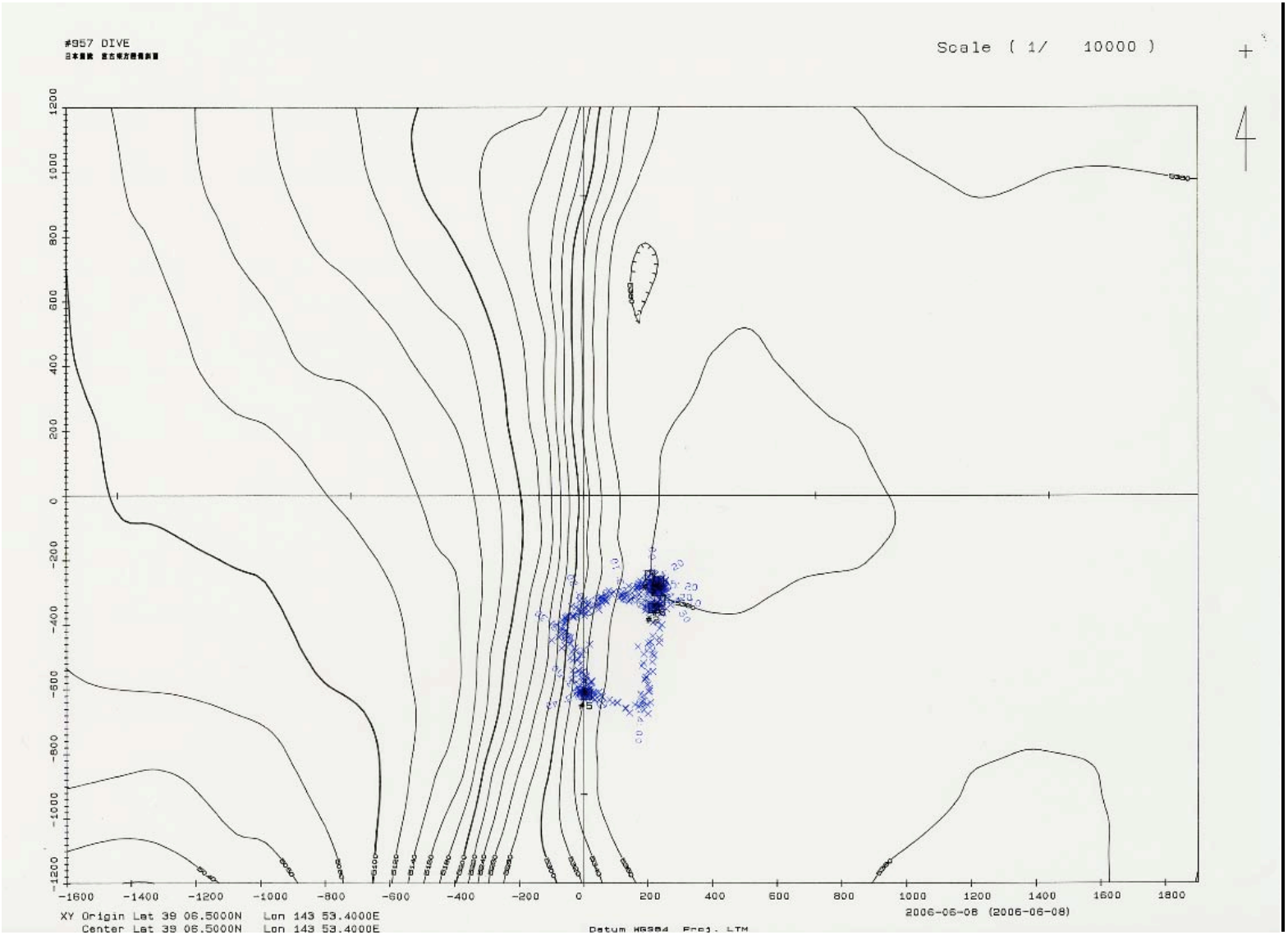
During the dive 6K957, seven MBRI cores of sediment samples were collected (3x35cm, 4x50cm), bottom water was sampled with the one-bag water sampler and several chemical parameters (temperature, pH, S^{2-} and O_2) in the sediment were successfully measured with the profiler module. Survey SW of marker 43 was also done. During the survey several organisms were observed.

Organisms observed during Dive#957:




#957 DIVE
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2006-06-08 (2006-06-08)

#958 Dive Report

Date: 11th June, 2006
Area: Sanriku Escarpment in the Japan Trench
Depth: 5347 m
Landing: 39º 6.2568' N, 143º 53.5249' E, depth: 5350 m (12:15 h)
Leave Bottom: 39º 6.3901' N, 143º 53.5466' E, depth: 5346 m (15:05 h)

Diver: Janine Felden (Max Planck Institute, Bremen)

Theme: *Survey of chemosynthetic biological community associated with cold seep at a depth of around 5350m in the Japan Trench*

Major objectives:

1. To collect water samples with Niskin bottle at 4 different water depths (500 m, 1000 m, 3000 m, 4000 m)
2. To deploy the chamber module on the mussel bed at marker # 43.
3. To collect 6x MBAR type push cores.
4. One bag sampler
5. To collect clams from a new mussel colony.
6. Survey at around the marker # 43.

Payload Equipments:

1. MBAR-type push corer (3 x 35cm, 3 x 50cm)
2. Niskin bottles (2.5L) x 4
3. Chamber Module
4. One-bag water sampler
5. Marker x 3
6. Bio-box

Sample collection:

(1) 4 Niskin Water Samples at 4 different water depths:
   i. 500 m: 9º 6.5007' N, 143º 53.5341' E (10:12 h)
   ii. 1000 m: 9º 6.5187' N, 143º 53.5018' E (10:23 h)
   iii. 3000 m: 9º 6.5396' N, 143º 53.5129' E (11:07 h)
   iv. 4000 m: 9º 6.5267' N, 143º 53.5166' E (11:30 h)
(2) Deployment of the chamber module on the mussel bed at the marker #43 (39º 6.3560' N, 143º 53.5619' E at a depth of 5347 m (12:47 h)).
(3) Retrieval of one-bag water sampler at 39º 6.3735' N, 143º 53.5642' E at depth of 5347 m (13:22 h).
(4) Sediment samples with MBAR cores (6) at 39º 6.3735' N, 143º 53.5641' E at depth of 5347 m (13:54 h).
(5) Sampling of Calyptogena clams, deployment of marker #45 at 39º 6.3735' N, 143º 53.5641' E at a depth of 5347 m (14:12 h).
(6) Retrieval of the chamber module at 39º 6.3560' N, 143º 53.5619' E at depth of 5347 m (14:59 h).

Water samples at 4 different water depths (500 m, 1000 m, 3000 m, 4000 m) were collected on the way to the sea floor. The chamber module were placed on the undisturbed part of the Calyptogena mussel bed at marker # 43 (39º 6.3560' N, 143º 53.5619' E at a depth of 5347 m) (Fig. 1, Fig. 2). The water bag sampler was collected at a new Calyptogena colony (39º 6.3735' N, 143º 53.5641' E at a depth of 5347 m). Afterwards, MBARI push cores were collected at the same site. Two (yellow; blue) of them were taken inside and the other four (red/white; green; black; red) at the rim of the Calyptogena mussel bed. Afterwards, mussels were collected from this colony. At the end, marker # 45 was placed at this Calyptogena mussel bed (Fig.3, Fig.4). The water as well as sediment samples are used for biogeochemical analyses to complete the data set of this sampling site.
The short exploration showed the abundance of many colonies in this area of the Japan Trench and occurrence of further invertebrates at this station. Several single mussels were observed that were moving on the seafloor due to the present of their track (Fig. 6). At the end of the dive the chamber module was retrieved, that measured oxygen consumption during the whole bottom time. Furthermore, water samples were taken in predefined time intervals by the module.

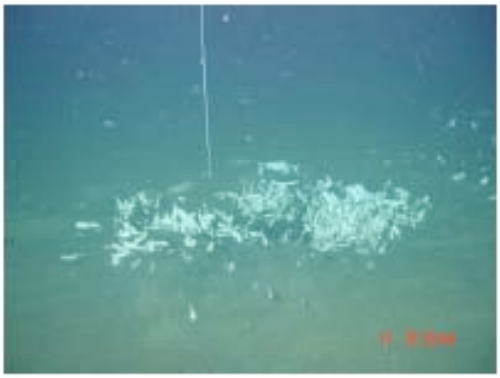


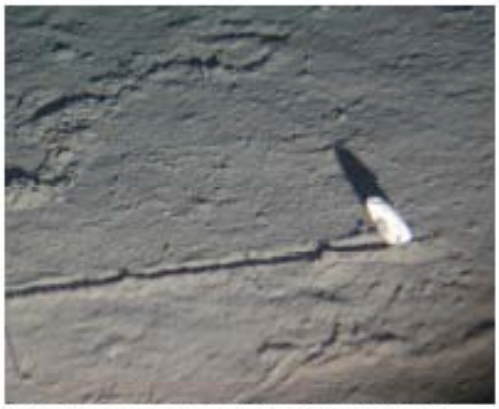
Figure 1: Calyptogena colony at marker #43

Figure 2: chamber module placed on the mussel bed at marker #43 with

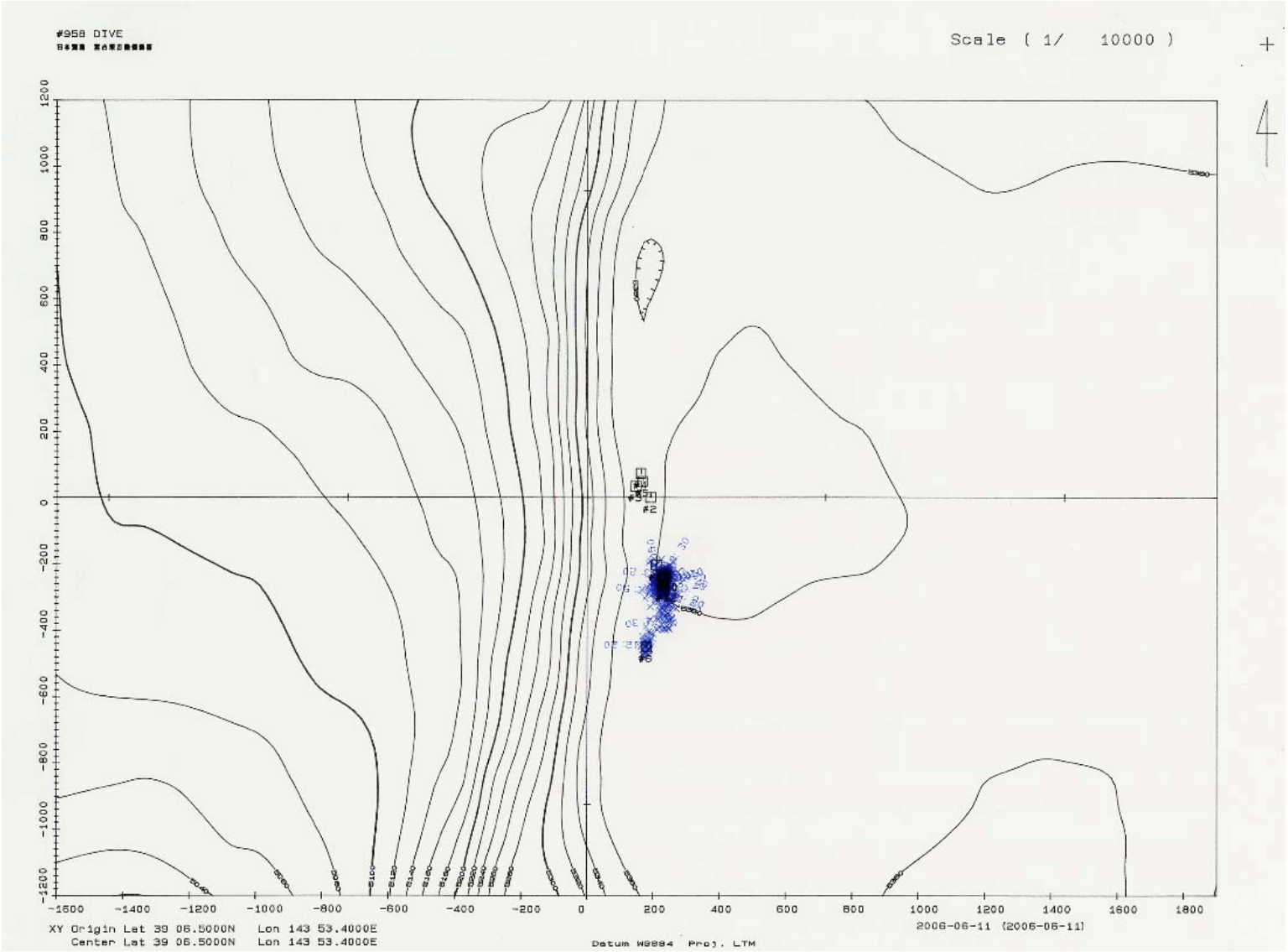
Figure 3: colony at marker #45

Figure 4: Calyptogena colony at marker #45 after sampling

Picture 5: Invertebrate next to the mussel bed at marker #43



Picture 6: the moving track of a Calyptogena mussel


#958 DIVE
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XY Origin Lat 39 06.5000N Lon 143 53.4000E
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Datum WGS84 Proj. LTM
2006-06-11 (2006-06-11)

Future plans

Chemical energy from subsurface processes (e.g. hydrothermal and gas seepage) supports rich and diverse chemosynthetic ecosystems at the sediment surfaces and the geochemical diversity within these systems strongly affects the biological diversity of surface communities. Microbial and faunal assemblages are thereby influenced by temporal and spatial variability at small scales within such an ecosystem. Unusually thick mats mixed with mineral precipitates have been discovered at sites of sulfur degassing e.g., Mariana arc seamounts, SuSu Knolls, Pacmanus, at iron rich sites as well as associated with CO_2 venting.

We would be very interested in a collaboration on such seep studies providing microsensor measurements and microsampling work on microbial mats and sediment patches which is required to determine geochemical gradients and calculate energy yields of redox reactions. Comparison of the composition, distribution and activity of microbial communities at different seep sites will allow us to address the question of interactions between the geological setting and the ecosystem. Physicochemical conditions and concentrations of key oxidants and reductants vary widely in different settings. This diversity in fluid composition reflects variations in the thermal, tectonic, and petrologic processes that create the lithosphere. It is the geochemical and microbial diversity within these seep systems that offers a great opportunity for new research directions in ocean sciences.

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