Integrated Ocean Drilling Program Expedition 337 Scientific Prospectus

Deep Coalbed Biosphere off Shimokita

Microbial processes and hydrocarbon system associated with deeply buried coalbed in the ocean

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This IODP *Scientific Prospectus* is based on precruise Science Advisory Structure panel discussions and scientific input from the designated Co-Chief Scientists on behalf of the drilling proponents. During the course of the cruise, actual site operations may indicate to the Co-Chief Scientists, the Expedition Project Manager, and the Operations Superintendent that it would be scientifically or operationally advantageous to amend the plan detailed in this prospectus. It should be understood that any proposed changes to the science deliverables outlined in the plan presented here are contingent upon the approval of the CDEX Science Operator Science Manager in consultation with IODP-MI.

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Abstract

Among the least characterized geobiological systems on Earth that can be accessed by scientific ocean drilling are deeply buried hydrocarbon reservoirs in sedimentary formations along continental margins. In particular, the microbiological and abiotic processes associated with a deeply buried coalbed in the ocean remain poorly understood and pose the following questions: (1) what role does subsurface microbial life play in the formation of hydrocarbon reservoirs? (2) Do deeply buried hydrocarbon reservoirs act as geobiological reactors that sustain microbial life by releasing nutrients and energy sources? (3) Do the conversion and transport of hydrocarbons and other reduced compounds influence biomass, diversity, activity, and functionality of deep subseafloor microbial populations? (4) What are the fluxes of both thermogenically and biologically produced organic compounds and how important are these for the carbon budgets in the shallower subsurface and the ocean? To address these important scientific questions, Integrated Ocean Drilling Program (IODP) Expedition 337 will drill and study the deep coalbed biosphere off the Shimokita Peninsula in the northwestern Pacific using the D/V *Chikyu*. This expedition aims to extend the maximum penetration of scientific ocean drilling to over 2200 meters below seafloor (mbsf) and will explore the microbial ecosystem associated with a deeply buried coalbed (i.e., a habitat that had never been accessed by previous scientific ocean drilling). The riser drilling and operational strategy will involve spot coring in representative intervals throughout the hole, continuous mud gas logging (gas composition and stable isotope), continuous geochemical and sedimentological analysis of cuttings retrieved during riser drilling, wireline logging from 647 to 2200 mbsf including sampling of pristine formation fluids, and pressure core sampling in methane hydrate–bearing strata in an adjacent hole under the in situ pressure condition. The cored intervals in the deep hole will be subject to intense sampling for shipboard and shore-based investigations. In addition, we will address scientific problems related to CO_2 sequestration in offshore coalbed formations through ex situ experiments using the recovered core materials. The latter experiments will investigate how supercritical CO_2 interacts with minerals, organic matter, and life in the deep subsurface and examine how CO_2 storage may affect biogeochemical carbon cycling. Ultimately, Expedition 337 will greatly advance our knowledge of the coalbed subseafloor hydrocarbon system and the deep biosphere, including its CO_2 sequestration potential.

Schedule for Expedition 337

Integrated Ocean Drilling Program (IODP) Expedition 337 is based on drilling Proposal 745-CPP (available at **www.iodp.org/700/**). Following ranking by the IODP Scientific Advisory Structure, the expedition was scheduled for the D/V *Chikyu,* operating under contract with the Japanese Implementing Organization, Center for Deep Earth Exploration (CDEX). At the time of publication of this *Scientific Prospectus,* the expedition is scheduled to depart Hachinohe, Japan, on 15 March 2011 and return to the same port on 21 May 2011. A total of 68 days will be available for the drilling, coring, and downhole measurements described in this prospectus (for the current detailed schedule, see **www.iodp.org/expeditions/**). Further details on the facilities aboard the *Chikyu* and CDEX can be found at **www.jamstec.go.jp/chikyu/eng/.** Supporting site survey data for Expedition 337 are archived at the **IODP Site Survey Data Bank.**

Expedition 337 is the first expedition in IODP history to originate from a Complimentary Project Proposal (CPP). Platform operational cost for this expedition, as well as the development of sampling and analytical facilities on the *Chikyu* such as hybrid-pressure coring system (Hybrid-PCS) (Aumann & Associates, Inc.), radioisotope, and mud gas monitoring container laboratories, is covered by the Strategic Fund for Strengthening Leading-edge Research and Development from the Japan Society for the Promotion of Science (JSPS), Ministry of Education, Culture, Sports, Science, and Technology (MEXT), Japan (FY2010–2011, Principal Investigator: Fumio Inagaki, Japan Agency for Marine-Earth Science and Technology [JAMSTEC]).

Introduction

Marine subsurface hydrocarbon reservoirs and the associated microbial life in continental margin sediments are among the least characterized Earth systems that can be accessed by scientific ocean drilling. Our knowledge of the biological and abiotic processes associated with hydrocarbon production is limited because of the highly limited opportunities to conduct scientific ocean drilling initiatives using deep-riser coring in natural gas and oil fields. A number of fundamental questions regarding deep subseafloor hydrocarbon systems remain unanswered. For example:

- What role does subsurface microbial life play for the formation of hydrocarbon reservoirs?

- Do the deeply buried hydrocarbon reservoirs such as methane hydrates and terrestrial coalbeds act as geobiological reactors that sustain life by releasing nutrients and carbon substrates?
- Do the conversion and transport of hydrocarbons and other reduced compounds influence biomass, diversity, activity, and functionality of deep subseafloor microbial populations?
- What are the fluxes of both thermogenically and biologically produced organic compounds, and how important are these for the carbon budgets in the shallower subsurface and the ocean?

To address these important scientific questions, Expedition 337 will drill and study the hydrocarbon system associated with a deeply buried coalbed off the Shimokita Peninsula in the northwestern Pacific by coring to 2200 meters below seafloor (mbsf) using the riser drilling system of the *Chikyu* (Fig. **F1**). Based on the existing seismic profiles as well as previous natural gas drilling surveys, it has been demonstrated that thick Eocene to Cretaceous lignite layers (~60% total organic carbon [TOC], ~100 m in thickness) are present under an Oligocene unconformity layer, hosting large quantities of coalbed methane (CBM) with a production rate of ~210×10^3 m^3/day (Osawa et al., 2002). During the *Chikyu* shakedown cruise (Expedition CK06-06) in 2006, methane hydrates were observed in porous ash and sand layers of shallow subsurface sediments to 350 mbsf. In the recovered sediments, counts of living microbes exceeded 10^7 cells/cm^3 and were thus highly abundant compared to other continental margin locations (Morono et al., 2009) (Fig. **F2**). This project aims to extend the drilling depth to 2200 mbsf and explore the microbial ecosystem associated with the deep coalbed in oceanic sediments, a habitat that has not been accessed by previous scientific ocean drilling. Hence, the study area is an ideal natural subsurface laboratory to study the deep carbon cycle and the deep biosphere.

Deep subseafloor life in continental margin sediments

Subseafloor sediments harbor a remarkably sized microbial biosphere that constitutes ~10%–30% of the total living biomass on our planet (Parkes et al., 1994, 2000; Whitman et al., 1998; Lipp et al., 2008; D'Hondt et al., 2009). To date, microbial cells have been observed in sediments ranging in age to the Cretaceous and in subsurface depth to 1626 mbsf (Newfoundland Margin, Ocean Drilling Program [ODP] Leg 210; Roussel et al., 2008). Diagenetic models of pore water chemical constituents as well as ^{14}C- and ^{35}S-radiotracer incubation experiments showed that metabolic activities of deep

subseafloor microbes are extremely low because of the low supply of energy-rich substrates (D'Hondt et al., 2002, 2004) but are often stimulated at geochemical and/or lithologic interfaces such as porous ash layers and sulfate-methane transition (SMT) zones (Inagaki et al., 2003; Parkes et al., 2005; Biddle et al., 2006; Sørensen and Teske 2006). The metabolic activities of subseafloor microbial communities are controlled by the flux of bio-available electron donors and/or acceptors, some of which are derived either from the overlying seawater by photosynthetic primary productions (D'Hondt et al., 2004, 2009; Lipp et al., 2008) or from crustal fluids underlying sedimentary habitat (Cowen et al., 2003; Nakagawa et al., 2006; Engelen et al., 2008). Fluid flow regimes in the subseafloor environment control availability of energy to microbial life. Hence, the geologic and sedimentological characteristics represent crucial factors controlling habitability of the deep subsurface. Culture-independent molecular ecological surveys of 16S ribosomal ribonucleic acid (rRNA) gene fragments reveal that the microbial communities in continental margin sediments are predominantly composed of species lacking cultivated relatives, such as the bacterial members within the candidate division JS1, Chloroflexi, and Planctomycetes, as well as the archaeal members within the Deep-Sea Archaeal Group, the Miscellaneous Crenarchaeotic Group, and the South African Gold Mine Euryarchaeotic Group (e.g., Inagaki et al., 2003, 2006b; Inagaki and Nakagawa, 2008; Inagaki, 2010). The carbon isotopic analysis of intact polar lipids (IPLs) and fluorescence in situ hybridization (FISH)-stained cells suggest that sizeable populations of heterotrophic Archaea significantly contribute to microbial biomass in organic-rich sediments, even at the SMT zone where the occurrence of anaerobic oxidation of methane (AOM) mediated by methanotrophic archaea and sulfate-reducing bacteria takes place (Biddle et al., 2006). Quantitative analysis of the IPLs extracted from sediments (>1 mbsf) at a variety of oceanographic settings reveal that ~87% of IPLs are archaeal, suggesting that the previous deoxyribonucleic acid (DNA)-based polymerase chain reaction (PCR) experiments had significant biases affecting the extraction and quantification (Lipp et al., 2008; Teske and Sørensen, 2008). Despite the significance of heterotrophic microbes in biogeochemical cycling within the continental margin sediments, the metabolic characteristics of organic matter degradation and fluxes of secondary metabolites remain largely unknown (e.g., Hinrichs et al., 2006).

Coal diagenesis: microbiological significance for biogeochemical cycles

Within the generally energy-starved deep subseafloor biosphere, lignite coal is a conceivable source of nutrients and energy for deep microbial communities. Microbio-

logical and geochemical studies of terrestrial coal deposits and subsurface aquifers suggest that microorganisms play important ecological roles in coal diagenesis, resulting in substantial quantities of CBM as a terminal product (Brown et al., 1999; Detmers et al., 2001; Fry et al., 2009; Krüger et al., 2008; Shimizu et al., 2007; Strapoc et al., 2008) (Fig. F3). The microbial communities in terrestrial coaly habitats are phylogenetically highly diverse with relatively low cell density of $<10^6$ cells/cm^3. For example, methane-producing archaea (i.e., methanogens) such as the genera *Methanoculleus, Methanobacterium, Methanolobus,* and *Methanosarcina,* as well as some potential acetate-producing bacteria (i.e., acetogens), such as *Acetobacterium,* were dominant in a deep borehole aquifer directly connected to coal deposits in Hokkaido Island, Japan (Shimizu et al., 2007). Using incubation tracer experiments and the FISH technique, active methanogenesis was found to occur even in a highly altered graphite deposit (Krüger et al., 2008). Very recently, Fry et al. (2009) reported sizable cultivatable populations of potential sulfate-reducing bacteria, methanogens, acetogens, and lignite-utilizing heterotrophs in the uplifted coaly sediments of northern New Zealand based on results from the most probable number cultivation method. Metabolic activities were stimulated at the geologic interfaces between coal and sand/silt layers as reported from other terrestrial deep subsurface black shales (e.g., Krumholz et al., 1997), and the concentrations of organic acids in the coal layers were higher than in normal deposits, consistent with the co-occurrence of coal diagenesis and microbial processes.

Despite the microbiological and (bio)geochemical significance of coaly deposits for the global carbon cycle, there have been no studies of coal layers that are deeply buried in the subseafloor, mainly because of the safety regulations applied to hydrocarbon gas–related hazards during riserless drilling. In continental margin sediments, large quantities of gaseous hydrocarbons, as well as H_2, CO, CO_2, organic acids, aromatic compounds, NH_4, N_2, sulfur compounds, etc., are potentially generated by thermogenic and/or biogenic degradation processes from the coaly deposit. All of these compounds are potential nutrient and energy sources that support redox reactions mediated by the deep subseafloor microbial communities. Hence, coalbeds and their active microbial life may influence the upward transport of dissolved gases and organic matter in geofluids as well as the accumulation of gas hydrates in overlying sediments. In this regard, the connectivity between deep subsurface microbial activity and the formation and deposition of gas hydrates is a frontier research theme in geobiology and biogeochemistry that can only be studied by a dedicated initiative as Expedition 337.

Exploring the feasibility of CO_2 sequestration in deep offshore geological repositories

To date, CO_2 capture and sequestration (CCS) into deep subsurface environments such as oil, gas, and porous aquifers is considered as a solution for reducing the emission of substantial amounts of anthropogenic CO_2 and preventing dangerous consequences of the anticipated future climate change. CCS offshore deep subseafloor environments has a number of advantages, including a positive risk assessment compared to shallow-water CCS (Schrag, 2009; House et al., 2006). It has been predicted that CCS can potentially reduce future world emissions from fuel energy by 20% (Dooley et al., 2006). However, the behavior and stability of CO_2 as well as its chemical reactions in deep subsurface repositories are still largely uncertain.

In this project, we will address the multiple scientific issues of the geological CO_2 sequestration through ex situ experimentations using cored materials, tackling the following fundamental questions:

- How does liquid or supercritical CO_2 spatially penetrate into the various lithostratigraphic settings?
- How does CO_2 react with minerals, organic matter, and life in the deep subsurface?
- What are the impacts of long-term CO_2 storage on biogeochemical carbon cycling and the subsurface biosphere on different time scales?

Conducting various multidisciplinary ex situ experimental studies using cored materials as well as in situ logging characterizations of the deep riser hole, Expedition 337 will significantly expand our knowledge of the coalbed subseafloor hydrocarbon system, including the physicochemical and biological factors that determine the potential for CO_2 sequestration.

Background

Drilling site off Shimokita Peninsula

In 2002 and 2003, two-dimensional (2-D) seismic surveys off Shimokita Peninsula were carried out in a 15 km (north–south) × 30 km (east–west) area by the R/V *Polar Duke* and *Polar Princess*. During the NT04-01 cruise using the R/V *Natsushima* in 2003, the detailed bathymetry mapping was performed using SeaBat 8160 Multibeam Echosounder with a frequency of 50 kHz (Taira and Curewitz, 2005) (Fig. F4). Site C9001

is located on the cross point of seismic Lines ODSR03-BS and ODSRW03-H81. During the *Chikyu* shakedown cruise (Expedition CK06-06) in 2006, 365 m of sediment cores were recovered from the upper sedimentary section at Site C9001 (41°10.5983′N, 142°12.0328′E, 1180 m water depth), ~80 km off the coast of Shimokita Peninsula, Japan (Figs. **F1, F4**) (Aoike, 2007). Riser drilling was also tested to 647 mbsf without coring at Site C9001, 20 inch casings were installed to 511 mbsf, and the riser hole was suspended for the future riser drilling opportunity. Given those pilot surveys, the geologic resistance and potential safety hazards for the riser drilling operations at Site C9001 have already been evaluated as feasible.

Geological setting

Site C9001 is located in a forearc basin formed by the subduction of the Pacific plate (~8 cm/y, west-northwest plate motion vector, Seno et al., 1996) beneath northeastern Honshu, Japan (Fig. **F1**). The Hidaka Trough, a sedimentary basin formed by subsidence in the drilling area, originates just offshore southwest of Hokkaido and extends to the Japan Trench. Along the coastal area of the Shimokita Peninsula, both sedimentary and volcanic rocks younger than Late Cretaceous lie scattered on Triassic to Early Cretaceous sedimentary rocks or Cretaceous granites.

Several scientific drilling expeditions have been carried out off Shimokita Peninsula: International Phase of Ocean Drilling (IPOD) Legs 56 and 57 in 1977, IPOD Leg 87 in 1982, and ODP Leg 186 in 1999. In addition, well data are available from hydrocarbon drilling explorations carried out between 1977 and 1999 (Japan Natural Gas Association and Japan Offshore Petroleum Development Association, 1992; Osawa et al., 2002). Seismic profiles around Site C9001 show pull-up blanking reflections below bottom-simulating reflectors (BSRs) at ~360 mbsf, suggesting the occurrence of methane hydrates and a strong upward flux of free hydrocarbon gases (Fig. **F5**). A thick and prominent Quaternary sedimentary unit onlaps a Pliocene unit and is thought to be composed mainly of alternating beds of mud and sand with intercalations of thin volcanic tephras and locally developed gravel/sand layers. The Pliocene unit consists primarily of alternating beds of mudstone and sandstone. Below these relatively recent formations, sedimentary deposits range from Cretaceous to Miocene in age and are cut by many landward-dipping normal faults. The presence of thick coal layers was confirmed by natural gas drilling exploration at Site MITI Sanriku-Oki, ~50 km southward of Site C9001 (Fig. **F1**) (Osawa et al., 2002). Sonic logging data in the MITI Sanriku-Oki well showed that three major tuff layers involving coal layers with 30, 45, and 80 m thickness (40%–60% TOC in lignite coal layer and 0.5%–2% TOC in tuffs)

are present in Eocene and Pliocene–Upper Cretaceous horizons in which vitrinite reflection values (R_o) ranged between 0.5 and 0.7, indicating relatively immature coal (Osawa et al., 2002). In situ temperatures are well within the range of the habitable zone of microbes, based on the reported thermal gradient of 22.5°C/km (Osawa et al., 2002).

Preliminary scientific results from shallow sedimentary column at Site C9001

The cored sediments taken from Site C9001 during the *Chikyu* shakedown cruises were composed primarily of diatom-rich hemipelagic silty clay intercalated with volcanic tephras and sand layers (Fig. **F6**). Preliminary biostratigraphic age models indicate very high sedimentation rates, ranging from 54 to 95 cm/k.y., and an approximate core-bottom age of 640 ka (Aoike, 2007; Aoike et al., 2010; Domitsu et al., 2010). During the Expedition CK06-06 *Chikyu* shakedown cruise, core temperature anomalies were monitored immediately after recovery by ThermoView infrared camera in order to identify and locate methane hydrates. We observed methane hydrate formations (Fig. **F7**) as well as microbial aggregates (Fig. **F8**) in porous ash and sandy layers. Geochemical analyses of interstitial waters consistently showed that the chloride concentrations (and other sea salts) were notably depleted within the porous layers as a result of hydrate dissociation (Tomaru et al., 2009). Iodine concentrations and radio-isotopic compositions ($^{129}I/I$) of deep pore waters suggest that the iodine and oldest hydrocarbon sources could be as old as 40 Ma. Acetate concentrations in pore waters were >100 µmol/L throughout the sediment column (maximum 313 µmol/L), which is presumably related to coal diagenesis in the deeper zone (H. Yoshioka et al., unpubl. data).

A newly developed cell counting technique using a computer image showed that the cored sediments contain abundant microbial cells with counts $>10^7$ cells/cm^3 down to 365 mbsf (Morono et al., 2009); these counts were approximately two orders of magnitude higher than those in sediments from the Nankai Trough seismogenic zone (i.e., microbiological samples from IODP Expeditions 315 and 316; see Fig. **F2**). The abundance of Bacteria and Archaea was studied by quantitative real-time PCR and slot-blot hybridization techniques, suggesting a significant contribution of Archaea to the subseafloor microbial biomass (average = 40% at DNA level) (Lipp et al., 2008).

The metabolic activity of organiclastic sulfate reduction, sulfate reduction coupled with AOM, aceticlastic methanogenesis, and autotrophic (CO_2 reducing) methano-

genesis rates were investigated using ^{35}S and ^{14}C radiotracers, showing high AOM activity below the SMT zone and relatively low methanogenic activity throughout the core column (F. Inagaki and H. Yoshioka, unpubl. data). Using a sediment sample from Site C9001, the carbon and nitrogen incorporation rate of deep subseafloor microbes was studied at single-cell level using nano-scaled secondary-ion mass spectrometry (NanoSIMS) (Y. Morono, unpubl. data). A large fraction of subseafloor microbes was found to incorporate ^{13}C- and ^{15}N-labeled substrates into the biomass, and their metabolic rates provide in vitro evidence for energy starvation.

Cultivation of aerobic and anaerobic microorganisms has been conducted and a variety of microbes and their enzymatic activities were observed in the core sediments (Kobayashi et al., 2008). Using a continuous downflow bioreactor system, the cored sediments were anaerobically incubated at 10°C. Synthetic seawater containing glucose, yeast extract, acetate, and propionate as energy sources was supplemented into the bioreactor. After 289 days of operation, significant methane production was observed (Imachi et al., submitted). The $\delta^{13}C_{CH4}$ value was approximately –80‰, strongly suggesting the occurrence of microbial methanogenesis. 16S rRNA and its gene-based clone analyses of the bioreactor enrichment culture revealed that phylogenetically diverse microbes were cultivated in the bioreactor system and the dominating phylotypes were closely related to the typical environmental phylotypes that have been frequently observed in various subseafloor zones. Predominant archaeal members enriched in the reactor were affiliated with methanogens, such as the genera *Methanobacterium, Methanoccoides,* and *Methanosarcina* and the uncultured archaeal lineages (Fig. **F9**). Several attempts at transferring into traditional batch-type cultivations successfully led to the isolation of several methanogens and anaerobic microbes. These provide direct evidence for the presence of metabolically active and cultivatable microbial populations in subseafloor habitats off Shimokita Peninsula.

Scientific objectives and hypotheses

During Expedition 337, extending the riser drilling/coring depth at Site C9001 is planned to 2200 mbsf, where the terrigenous Eocene coalbed (lignite) is situated beneath the overlying marine sedimentary realm (Fig. **F5**). In addition, using Hybrid-PCS on the *Chikyu,* the shallow sedimentary sections to the maximum depth of 365 mbsf will be retrieved under in situ pressure condition, including methane hydrate–bearing sediments (Fig. **F6**).

The proposed drilling exploration of the deep hydrocarbon system off Shimokita provides the unique opportunity to examine geobiological and diagenetic processes at interfaces between marine and terrigenous sediment and coal formation in deeply buried strata. No microbial life or its activities have been documented to date at the targeted burial depths and environments. Expedition 337 will be driven by three overarching testable hypotheses:

1. The deeply buried Eocene coalbed acts as a geobiological reactor that releases dissolved organic compounds such as methane, acetate, and other substances.
2. The conversion and transport of coalbed-derived organic substances influence microbial and diagenetic processes in the overlying, shallower strata.
3. The subsurface coalbed has the potential to serve as a cap rock for potential CO_2 sequestration and can support biological conversion of CO_2 into biomass and organic compounds even at high CO_2 concentrations.

The following operational objectives, to be addressed during Expedition 337, will be tied to the above hypotheses and guide our research strategy:

- Constrain the impact of a thermally immature coalbed on the diagenetic and microbial processes at great burial depths;
- Quantify the upward fluxes of dissolved organic compounds, such as gaseous hydrocarbons and volatile fatty acids, out of the coalbed and evaluate their impact on microbial processes in shallower strata; and
- Test whether distinct active microbial communities inhabit the deeply buried coalbed, the overlying sediments of terrigenous origin, and the even shallower marine sediments and how they respond to high CO_2 concentrations and low pH.

We will address the following set of specific research questions:

- What is the ecological and biogeochemical relevance of deeply buried lignite in the natural hydrocarbon system offshore the Shimokita Peninsula?
- What are the fluxes of both thermogenically and biologically produced methane and other diagenetic products, such as organic acids, into shallower strata and how important are these for the carbon budget?
- How does coal diagenesis affect subseafloor microbial biomass, diversity, and metabolic activities?
- Does the presence of the low-maturity coalbed stimulate heterotrophic and autotrophic microbial communities?

- What is the natural flux of CO_2 and CH_4 from the coalbed hydrocarbon system, and what is the potential for CO_2 sequestration in the Shimokita system?
- How does excess CO_2 react with minerals and organic matter in the drilled formation, how will this change the physical and chemical characteristics, and how will it affect the microbial communities?
- What is the paleoenvironmental information recorded at Site C9001?
- What is the extent of subseafloor life and the biosphere?

During Expedition 337, we will meet these objectives by: (1) spot coring marine (Pliocene to Oligocene) and terrestrial (Eocene) sediments, which include unconformity layers as well as coal-tuff-sand layers; (2) wireline logging of various geophysical and geochemical properties in situ; (3) sampling of in situ pristine formation fluids using wireline sampling tool; and (4) undertaking extensive microbiological, biogeochemical, geological, and geophysical analyses of the cores and borehole logging data.

This project will expand our knowledge of geobiological and biogeochemical properties in the coalbed hydrocarbon system. Similar coaly environments are widely distributed along the western coast of the Pacific Ocean, and hence our results will be of great societal relevance. Because the effect of high CO_2 concentrations and the associated decrease in pH under conditions of CO_2 sequestration into the deep coal/sand-layers is one of the primary objectives to be addressed, the shore-based laboratory experiments will include quantitative evaluation and modeling of fluid flow and biological systems in the subseafloor environment, including their response to high CO_2 concentrations and low pH. These applied scientific aspects will add an important new component to IODP.

Drilling and coring plan

We will depart the port of Hachinohe and move to Site C9001 (41°10.5983′N, 142°12.0328′E). After the seafloor survey by remotely operated vehicle, we will deploy transponders on the seabed, and then spud in the suspended hole, where 20 inch casing pipes were previously installed to 511 mbsf. The corrosion cap will be retrieved, and a blowout preventer (BOP) and riser pipes will be connected to the borehole. We will conduct riser drilling to 1220 mbsf with spot coring using rotary core barrel (RCB). The spot coring is planned every 150 m, at the following four depth intervals: 670–679.5, 820–829.5, 970–979.5, and 1120–1129.5 mbsf (Tables **T1**, **T2**). Then the first series of wireline logging runs will be performed for the depth interval between

647 and 1220 mbsf before installing 13⅜ inch casing pipes (see **"Logging, downhole measurements, and in situ sampling plan"**).

From 1220 mbsf to the target depth of 2200 mbsf, we will continue riser drilling to take cores at the following depths: 1270–1279.5, 1370–1379.5, 1470–1479.5, 1581.5–1648, 1648–1675, 1770–1789, 1870–1889, 1933–1990, 1990–2044, 2140–2149.5, and 2190.5–2200 mbsf. The coring at 1648–1675 and 1990–2044 mbsf will utilize large diameter coring (LDC) systems (Baker Hughes INTEQ, Inc.) (Table **T1**). The LDC systems provide core material that is 10 cm (4 inch) in diameter and up to 27 m long. The LDC system is equipped with either a Hydrolift or Jam-Buster system, which improves core recovery, particularly in problematic lithologies such as brittle lignite. Core retrieval by the LDC system requires pipe trip, not wireline trip, and is contained in an aluminum inner tube. The LDC system will be used for two critical intervals: one corresponding to the Eocene–Oligocene unconformity and the other for the central part of the Eocene unit that contains the lignite layers. After reaching the target depth of 2200 mbsf, we will conduct the second series of wireline logging, including in situ sampling of the formation fluids. The hole will be suspended by cementing without additional casing pipe installation.

After completion of the riser hole, we will conduct coring at Site C9001 by Hybrid-PCS. Hybrid-PCS contains a bearing and a nitrogen-charged pressure regulator section, enabling recovery of a 54 mm diameter × 3.5 m long core under in situ pressure conditions. Using the Hybrid-PCS, we will collect spot-core samples at several representative depth intervals (0–3.5, 10–13.5, 100–103.5, and 206.5–231 mbsf) (Table **T1**). During riserless Hybrid-PCS operations, formation temperature is measured with the advanced piston corer temperature tool (APCT-3) at selected depth horizons. Retrieved cores are transferred without pressure loss into an aluminum chamber in the Pressure Core Analysis and Transfer System (PCATS) (GeoTek, Ltd., UK). The PCATS enables us to measure *P*-wave velocity and gamma density, as well as nondestructive 2-D and three-dimensional (3-D) X-ray computed tomography (CT) images (Schultheiss et al., 2009).

The currently projected depth intervals for coring are shown in Table **T1.** We intend to recover sediments from all representative lithologies. At the same time, relatively regular spacing of spot coring is useful to obtain reliable variation of physical properties with depth. Actual coring intervals are subject to change on board the ship based on initial results of coring and observation of cuttings and logging data. Analysis of

cuttings, cored sediments, log data, and seismic integration will be an important task of the science party.

Logging, downhole measurements, and in situ sampling plan

In order to fill the gap of information between spot coring intervals, the logging program is essential to document geophysical, geochemical, hydrogeological, and geobiological properties in the Shimokita coalbed hydrocarbon system. In addition, in situ sampling of formation fluids as well as in situ measurement of pH, hydrocarbon composition, and pCO_2 in the formation fluid using logging tools will provide information that is important for the understanding of the coalbed hydrocarbon system.

During Expedition 337, we will conduct wireline logging operations at two depth intervals (647–1220 and 1220–2200 mbsf). The current logging plan consists of four runs in the first shallow interval and seven runs in the second interval. Tool sketches and configuration figures are shown in Figure **F10.** The following tools will be used during Expedition 337:

- High-Resolution Laterolog Array (HRLA) tool for lateroresistivity.
- Platform Express (PEX) for density, porosity, 1-arm caliper, microresistivity, and photo electric factor and Hostile Environment Natural Gamma Ray Sonde (HNGS) for gamma ray spectroscopy.
- Fullbore Formation MicroImager (FMI) for electrical borehole image and Dipole Sonic Imager (DSI) for *P* and *S* sonic velocity.
- Versatile Seismic Imager (VSI) for vertical seismic velocity profile by check shot.
- Combinable magnetic resonance (CMR) tool for Nuclear Magnetic Resonance (NMR) porosity, permeability, and pore-size distribution.
- Modular Formation Dynamics Tester (MDT) for collecting in situ formation fluid samples using Quicksilver Probe and measurement of resistivity, pressure, temperature, hydrocarbon composition (C_1–C_6), pH, and pCO_2, using InSitu Fluid Analyzer (IFA) at the sampling line.
- Mechanical Sidewall Coring Tool (MSCT) for retrieval of multiple minicores from the borehole sidewall.

The first two runs will provide basic physical property data, such as natural gamma ray, resistivity, density, photoelectric factor, and porosity, providing useful data for characterizing high-resolution stratigraphy within the formation. The caliper log will

allow us to assess the hole conditions and chances of success of subsequent logging runs.

The high-resolution FMI images in the third run will provide fine-scale stratigraphy and the best information about the extent of deformation and brecciation. DSI will provide the first measurements of in situ formation sonic velocity, allowing the generation of synthetic seismograms for detailed seismic log correlations and characterization of the petrophysical properties.

The fourth run will be a check shot using vertical seismic profile to tie the well data to the seismic survey data. Data will be recorded with a VSI containing a three-axis geophone.

The subsequent three runs will only be used in the second interval. High-resolution NMR measurement provides the porosity and the distribution of pore size within the formation. These data are processed to estimate the permeability and pore throat size, which is essential to evaluate the chance of success and operation plan of the subsequent formation fluid sampling.

MDT using Quicksilver Probe, which is an in situ fluid sampling module, will provide formation fluid samples with minimal contamination. IFA measures fluid resistivity, temperature, pressure, pH, pCO_2, and hydrocarbon concentrations in the sampling port line of the MDT. Prior to opening the valve for bottle sampling, the real-time monitoring of the quality of sampled fluids will be performed with IFA, allowing the best timing for sampling of pristine formation fluids. These in situ fluid measurement and sampling operations will be carried out at intervals of representative lithology, including intervals across the unconformity and coal-bearing (or organic-rich) permeable layers. The actual sampling strategy, however, will depend on borehole conditions, and measurement target depths will be selected after observations of FMI images, Environmental Measurement Sonde (EMS) calipers and other logs from previous runs.

If time and budget allow, the MSCT will be attempted to retrieve minicore samples (0.92 inch in diameter) from the scientifically important horizons (e.g., unconformity layers, coalbed, coal-associated sands, and breccia) and/or the RCB spot coring gaps. The sidewall minicore samples will be used for the study of sedimentological, geochemical, and microbiological characteristics. Deployment of the MSCT is still uncertain, however, depending on further review of operational constraints together with scientific priorities.

All logging data will processed on board the ship to interpret sedimentary facies and to integrate the data with those from core, mud logging, and seismic profiles to construct geological and hydrogeological models. These models will be fundamental for constraining the flux of substrates from the coalbed into shallower strata and the surrounding area.

Details of logging operations are subject to change for operational reasons.

Analytical research plan

Core flow for onboard measurements and storage

Despite the multiple coring strategies and disconnected intervals, we will follow IODP standard measurements in the shipboard laboratory. All sections (1.5 m in length) go through X-ray CT scan and further analysis/sampling strategies will be determined based on the CT observation. Intervals for whole-round core sampling will be determined at this point for microbiological analysis/sampling and interstitial water extraction. The fluid samples will be subjected to standard analyses of interstitial water chemistry.

Cores will be split into working and archive halves after physical property measurements by multisensor core logger. Archive halves will be used for visual core descriptions and image/color scan. Working halves will be used for physical property measurements, such as thermal conductivity and shear strength. Discrete samples will be used for measurements of moisture and density, *P*-wave velocity, and bulk chemistry. Bulk carbon and sulfur analyses and organic matter maturity measurements by Rock-Eval will provide constraints on chemical processes in and around the coalbeds.

The cryogenic magnetometer will be unavailable through the expedition because of current upgrading of the equipment. Considering the sparse coring, we will have to omit shipboard measurements of remnant magnetism.

After shipboard processing, all cores will be packed anaerobically in oxygen-impermeable bags filled with N_2 and stored at 4°C. The anaerobic storage at 4°C will maintain subseafloor microbial activities; hence, these sediments can still be used for a variety of additional postcruise microbiological and biogeochemical studies.

Cuttings analysis

In addition to core samples, cuttings material recovered from circulating drilling mud will also be available for scientific analysis. Because of the sparse coring, we will rely on micropaleontological observation of cuttings for age determination. Lithology observation will also be important for decisions regarding the drilling/coring strategy on board the ship.

Gas monitoring

During the riser drilling operation, we will continuously monitor the chemical composition (C_1/C_{2+}) of mud gas with gas chromatography deployed in a newly constructed mud-gas container lab on the *Chikyu*. Circulating drilling mud will be sampled as soon as possible, and the resulting gas sample will be transferred to the mud-gas container lab via the shipboard flow-through pipeline. Carbon isotopic composition of methane ($\delta^{13}C_{CH4}$) will be continuously monitored using an automated wavelength-scanned cavity ring-down spectrometer. These mud-gas measurements will fill the gap of coring intervals, and be useful in monitoring how biological and nonbiological diagenetic processes affect the vertical transition of the coalbed hydrocarbon system as well as the relationship between gaseous components and lithostratigraphy. Mud-gas samples will be also available for more detailed shore-based analysis, such as hydrogen isotopic composition of methane.

High-pressure core

During riserless operations using Hybrid-PCS and PCATS, a 3.5 m length high-pressure core will be transferred to an aluminum high-pressure chamber with PCATS in the cooled container. During the transfer operation, *P*-wave velocity and gamma-density data as well as X-ray CT scan images will be simultaneously obtained by nondestructive measurement in PCATS. Using the high-pressure chamber system, we will measure in situ chemical composition and concentration of free hydrocarbon gas by conducting a gas-production test using PCATS.

Sampling strategy

Shipboard and shore-based researchers should refer to the IODP Sample, Data, and Obligations policy posted on the Web at **www.iodp.org/.** This document outlines the policy for distributing IODP samples and data to research scientists, curators, and ed-

ucators. The document also defines the obligations that sample and data recipients incur. The Sample Allocation Committee (SAC) (composed of Co-Chief Scientists, Staff Scientist, the IODP curator on shore, and the curatorial representative on board the ship) will work with the entire scientific party to formulate a formal expedition-specific sampling plan for shipboard and postcruise sampling.

Shipboard scientists are expected to submit sample requests (at **smcs.iodp.org/**) 2 months before the beginning of the expedition. Based on sample requests (shore-based and shipboard) submitted by this deadline, the SAC will prepare a tentative sampling plan that will be revised on the ship as dictated by recovery and expedition objectives. The sampling plan will be subject to modification depending on the actual material recovered and collaborations that may evolve between scientists during the expedition. Modification of the strategy during the expedition must be approved by the Co-Chief Scientists, Staff Scientist, and curatorial representative on board the ship.

The minimum permanent archive will be the standard archive half of each core. All sample frequencies and sizes must be justified on a scientific basis and will depend on core recovery, the full spectrum of other requests, and the cruise objectives. Some redundancy of measurement is unavoidable, but minimizing the duplication of measurements among the shipboard party and identified shore-based collaborators will be a factor in evaluating sample requests.

If some critical intervals are recovered, there may be considerable demand for samples from a limited amount of cored material. These intervals may require special handling, a higher sampling density, reduced sample size, or continuous core sampling by a single investigator. A sampling plan coordinated by the SAC may be required before critical intervals are sampled.

Routine microbiology samples will be reserved for archiving purposes. A 10 cm whole-round core sample is taken at every 10 m of core and is kept at –80°C. These samples will be available for future microbiological studies on shore.

Contamination assessment for riser coring

For geochemistry and microbiology, determining a sample's degree of contamination with alkaline (~pH 10) mud circulation fluids will be crucial because some facultative anaerobic halophilic or halo-tolerant microbes such as *Halomonas* may grow in the

circulation mud tank (Masui et al., 2008). Also, the pore waters squeezed from consolidated sedimentary rocks will be highly sensitive to contaminating chemicals. During Expedition 337, we will test the use of perfluorocarbon tracers (PFTs) for all riser drilling cores. In the circulation mud tank, PFT concentrations will be kept at 1 part per million (approximately half the concentration of saturation in seawater). During the mud circulation, 1 kg of PFT will be supplemented with 100 m^3 of mud in the tank. The tracer permeation in core sections will be evaluated with a gas chromatograph in the microbiology lab on the *Chikyu* according to previously established protocols (Smith et al., 2000; House et al., 2003; Lever et al., 2006).

Subseafloor biomass profiling with multiple methods to 2200 mbsf

Expedition 337 will provide an unprecedented opportunity to study deep subseafloor microbial communities inhabiting organic-rich, gassy sediments to 2200 mbsf. The targeted depth of maximum penetration is similar to or even extends the previous depth record of scientific ocean drilling, which is currently 2111 mbsf and held by the R/V *JOIDES Resolution* during ODP Leg 148 in Hole 504B off Costa Rica (Alt, Kinoshita, Stokking, et al., 1993). Because the current depth record of the existence of subseafloor life is at 1626 mbsf at the Newfoundland margin (Roussel et al., 2008), our study of vertical distribution of microbial biomass will significantly extend our understanding of the extent of subseafloor life and the biosphere on Earth. In addition, the analysis of the distribution and quantity of subseafloor biomass will provide primary information on how microbial populations are sustained by flux of nutrients and electron donors and acceptors, sediment porosity and fluid flow regimes along the unconformity layers, and other lithostratigraphic interfaces (e.g., coal/sand interface).

To detect and quantify the biomass of deep biosphere microbial populations, we will use a newly developed computer-based image analysis (Morono et al., 2009). To detach the cells effectively, the sediments will be washed with hydrofluoric acid and the microbial cells will be separated from solid sediment particles using the Nicodenz gradient method (Kallmeyer et al., 2008). Using SYBR-stained cells, the cell detection and enumeration will be performed with an automated slide-leader system equipped with autofocused fluorescent microscopy (Morono and Inagaki, 2010). We will also compare results using the new high-throughput cell counting technique for geological habitats, based on high-specification flowcytometry (Y. Morono, J. Kallmeyer, and F. Inagaki, pers. comm., 2010) (Fig. **F11**). In addition, we will use established protocols involving the analysis of intact polar membrane lipids (e.g., Lipp et al., 2008).

Microbial community composition across the marine/terrestrial interface

We will study microbial diversities and community structures using molecular ecological approaches. Most microorganisms are expected to be uncultured heterotrophs, as previously observed at other organic-rich subseafloor sedimentary environments like ODP Sites 1229 and 1230 off Peru (e.g., Parkes et al., 2005; Biddle et al., 2006; Inagaki et al., 2006b). For deep biosphere communities, the relevance of paleoenvironmental conditions during sediment deposition in structuring the microbial community composition, diversity richness, and evenness remains unknown. What are the chemical or geophysical constraints on the microbial community structures? How do lithostratigraphic variations play a role for the migration or stratification of microbial communities? To address these fundamental questions related to the diversity and community structure, we will study samples from all recovered lithostratigraphic units corresponding to marine and terrestrial deposits and coal layers.

With regard to molecular techniques, DNA (and/or ribonucleic acid [RNA]) will be extracted from sediment core samples by newly developed extraction techniques that minimize bias (Y. Morono et al., unpubl. data). Cell lysis efficiency will be determined for all samples by checking the number of SYBR-stainable cell particles using an automated fluorescent image analysis (Morono et al., 2009; Morono and Inagaki, 2010). 16S rRNA gene and other functional gene fragments will be amplified with tagged primer sets, sequenced with 454 pyrosequencing and/or other high-throughput sequencing technologies, and then statistically and phylogentically analyzed. In addition, some specific phylotypes of biogeochemically relevant key players (e.g., methanogens and acetogens) will be visualized by FISH-based techniques using specifically designed probes.

Geobiological studies of autotrophic microbes

To understand the potential carbon flow patterns in the coalbed subseafloor microbial ecosystem, understanding of distribution, diversity and functioning of autotrophic microbial communities (i.e., CO_2-assimilating microbes) is important because of their capability to convert inorganic substrates into organic matter. Autotrophs such as homoacetogens may play an important role by converting CO_2 to acetate and biomass in an artificial CO_2 deposit. Both reactions, CO_2-reduction to methane and to acetate, are likely exergonic in pore water with elevated dissolved H_2 concentrations as a result of lignite diagenesis. Therefore, we will pay special attention to the

population and activity of methanogens and other autotrophic communities using multiple cultivation and culture-independent approaches, including an analysis of their sensitivity toward high CO_2 and low pH.

To understand the methanogenic archaeal populations, a key gene for methanogenesis pathway, methyl coenzyme-M reductase (MCR) genes will be phylogenetically characterized and quantified by PCR-based molecular ecological techniques (e.g., Colwell et al., 2008). We will also study the coenzyme F430, the specific nickel-containing prosthetic group by multiple structural analyses, such as spectroscopic analysis, high-resolution X-ray structure analysis, and time-of-flight mass spectrometry (TOF-MS) analysis. In addition to these culture-independent analyses on shore, we will cultivate methanogens and other deep subseafloor microbes using a flow-through bioreactor system under the in situ high pressure and temperature. A variety of methanogens have already been successfully retrieved from the shallow subsurface sediments at the same site (Imachi et al., submitted). However, we still do not know what kinds of methanogens and associated communities are mainly fueling the significant accumulation of biogenic methane above the coal layer. Using the high-pressure flow-through reactor system, we will also study carbon- and hydrogen-isotopic fractionation of methanogens in the subseafloor sedimentary microbial ecosystem. The expected cultivation-based evidence will contribute to the understanding of the hydrocarbon system and the microbial potential for CO_2 conversion.

Inorganic and organic geochemistry: understanding the deep carbon cycle

During the *Chikyu* shakedown cruise (Expedition CK06-06) in 2006, the shallow sedimentary unit above the BSR at 365 mbsf contained methane hydrate in porous ash and sandy layers. Given the moderately low concentrations of organic carbon (0.8%–1.8%) in the strata, it is conceivable that the methane originates from greater depths, presumably the deeply buried coalbed. Diffusion of biologically produced methane from organic-rich sediments into overlying organic-lean sediments has been demonstrated at other deep drilling sites (e.g., from deeply buried Cretaceous black shales drilled during ODP Leg 207 at Demerara Rise [Arndt et al., 2006]). Using cored materials, we will conduct detailed analysis of numerous geochemical parameters that will enable us to quantify the fluxes of various compounds from and into the Eocene coalbed. Concentrations of major anions and cations (e.g., sulfate, chloride, alkalinity, sulfide, phosphate, ammonium, magnesium, potassium, and calcium) in pore water samples and formation fluids will be determined. We will determine concentrations

and stable carbon isotopic compositions of various carbon-bearing compounds (i.e., CH_4 and C_{2+} hydrocarbons, volatile fatty acids, dissolved organic carbon, and dissolved inorganic carbon [DIC] [e.g., Heuer et al., 2009: Lever et al., 2010]). We will determine δD values of CH_4 in order to distinguish between different pathways of methanogenesis (cf. Whiticar, 1999). The dissolved organic matter in the pore and formation water and its structural link to lignite-derived organic compounds will be studied by Fourier transform ion cyclotron mass spectrometry (FT-ICR-MS) (e.g., Schmidt et al., 2009). We will seek to apply new assays based on solid-phase microextraction for the quantification and isotopic analysis of methylotrophic substrates such as methylamines and methanol in pore fluids. This set of analyses will provide information pertinent to the geobiological carbon cycling and will enable us to model fluxes of carbon-bearing compounds in and out of the coalbed (e.g., Sivan et al., 2007; Wang et al., 2008). We will determine concentrations and stable isotopic compositions of organic carbon, carbonate, nitrogen, and sulfur and hydrogen indexes by Rock-Eval pyrolysis to further characterize the diagenetic setting and broadly distinguish sources of organic material. Some of these techniques will be applied to selected, cleaned core cuttings in order to supplement geochemical data with continuous information. In addition, we will measure $^{129}I/^{127}I$ ratios of pore waters to examine the age distribution of pore water enriched in iodine and methane in gas-rich strata (e.g., Tomaru et al., 2009).

Biogeochemical and geobiological experiments: activities and fluxes

We will use large-volume samples of live sediments that are taken in regular intervals of 1 per recovered core (i.e., whole-round core) to conduct laboratory incubation to monitor the production potential for methane and methanogenic substrates such as methyl-compounds, acetate, and H_2. To better assess fluxes of compounds in organic-rich strata and in the adjacent sediment horizons, we will quantify microbial respiration processes by high-sensitivity methods such as radiotracer turnover based on ^{14}C-, ^{35}S-, and deuterium-labeled compounds. These ex situ experiments will inform us on the potential activity of the coalbed microbial communities and provide information on the reactivity of the lignite. These experiments will be anaerobically conducted at near in situ temperature in the newly developed radioisotope container lab on the *Chikyu*. Rate measurements directly related to methanogenesis are the priority, such as acetate turnover, methanol turnover, methane turnover, and CO_2 turnover. In addition, bulk community growth can be studied by thymidine incorporation. As microbial communities in these experiments, we will use both the

natural indigenous microbial population and microbial inoculates that have been tested in coal-to-methane degradation (e.g., Jones et al., 2008a, 2008b; Orem et al., 2010). The inocula also include mesophilic to thermophilic methanogens isolated from subseafloor sediments in shallow zones of the same drilling site (C9001) and from high-CO_2 hydrothermal fluids in the Okinawa Trough hydrothermal fields. In selected experiments, we will use stable isotope–labeled substrates in order to establish reactant-product relationships (e.g., ^{13}C-methanol, ^{13}C-DIC, etc.) and quantify the relative importance of the various pathways metabolizing C_1 compounds (e.g., Wegener et al., 2008). The rate of ^{13}C-labeled substrate incorporations will be determined at single-cell levels using a combined FISH-NanoSIMS approach (Musat et al., 2008) as well as on the basis of microbial biomarker analysis (e.g., Wegener et al., 2008). Overall, these experiments will provide information crucial to the assessment of the lignite's potential to generate methane and other dissolved organic species and will inform the design of experiments under in situ pressure and temperature.

Shore-based study of offshore CO_2 sequestration potentials: does a deeply buried coalbed act as a "Subsea Forest?"

One of the great concerns regarding geological CO_2 storage is the behavior of CO_2 and its impact on ecological balance of carbon cycling. To store substantial quantities of CO_2 in the deep underground or marine subsurface, the captured CO_2 is condensed as liquid. At the high pressure and high temperature in the deep subsurface, the CO_2 will be present in supercritical state. Where liquid or supercritical CO_2 is in contact with a water phase, the CO_2 levels will be in equilibrium with the liquid CO_2, thus be saturated. The pH of pore water or solvent water for CO_2 sequestration will be significantly decreased; hence, the mineral trap through carbonation is extremely low. A diffusion of liquid CO_2 through the surrounding pore spaces is expected. However, the dense liquid or supercritical CO_2 is hydrophobic and thus would barely mix with pore water. These physical and chemical characteristics of CO_2 suggest that once liquid and/or supercritical CO_2 is injected in deep subsurface repositories, CO_2 may be retained and these unusual artificial environments may remain stable over geologic timescales. Biological conversion of the injected CO_2 in the subsurface to organic compounds such as methane will depend on how microorganisms will respond to the chemical perturbation and on the intrinsic reducing power of the subsurface environment. In fact, C_1-metabolizing microbial life has been observed in the natural deep-sea CO_2-seep and hydrothermal environments (Inagaki et al., 2006a).

What are the drivers in microbial succession in the deep subseafloor biosphere? Previous deep biosphere studies using ^{14}C tracers demonstrated that potential rates of CO_2-reducing methanogenesis in typical coastal organic-rich marine sediments are a few tens of picomols per cubic centimeter per day (as reviewed by Parkes et al., 2000). In contrast, the activity of batch-cultured thermophilic methanogens is ~0.1 to 1 mmol/cm^3/day, that is, roughly eight to nine orders of magnitude higher than subseafloor methanogenesis activity. As a consequence of hypothetical carbon storage, CO_2 concentration could approach 1 mol/L, the conversion of CO_2 to current ambient levels by the indigenous subseafloor microbial ecosystem would take over hundreds of millions of years. However, if there are substantial sources of energy and reducing power, such as H_2 or acetate generated by the diagenesis of organic matter at elevated temperatures (Parkes et al., 2007), the CO_2 turnover time may be significantly reduced based on the potential activity of methanogens. We suggest that the potentially active and abundant microbial communities associated with the deeply buried coalbeds—the so called "Subsea Forest"—constitute a highly interesting target for testing CO_2 and pH effects on subsurface life, including effects on C_1-metabolisms, heterotrophic consumption, lipid and DNA formation, and carbon assimilation.

To produce such energy and nutrient sources for microbes, the coal needs to be relatively immature, because then it still contains not only hydrogen but also N- and P-bearing compounds and the porosity of lignite is generally higher than that of more mature graphite coal. Indeed, microbiological studies of terrestrial coal environments revealed the presence of hydrogenotrophic methanogens (Shimizu et al., 2007; Krüger et al., 2008: Strapoc et al., 2008). The coals in the Shimokita gas field are mostly composed of lignite and are intercalated with porous sandy layers, like lens structure (Osawa et al., 2002). Along with the migrating CO_2, it is possible that dissolved organics or reduced chemical compounds may be advected by the CO_2 conveyor and fuel heterotrophic respiration (Onstott, 2005). Especially, energy sources co-migrating with liquid CO_2 such as sulfide, methane, or H_2 may be oxidized by the subsurface microbiota driving autotrophic growth, or—if physiological functions are repressed by low pH—will not be utilized and transported further. Using high-pressure reactors, sediments samples from Expedition 337 will be incubated under high CO_2 and compared to in situ conditions to assess whether the communities can adapt to high CO_2 and, if so, over which timescale.

Geophysical implications for CO_2 sequestration potentials

During the proposed hydrocarbon expedition, a variety of logging and experimental data will be obtained from the borehole and drilled cores. In particular, the high-pressure immersion experiments using representative core materials and CO_2-rich fluids will provide critical information for simulating the behavior of CO_2 in the deep subseafloor environment. As the significant geophysical and sedimentologial parameters, permeability, porosity and capacity of CO_2 storage will be experimentally determined using fluid flow reaction chambers ex situ and under varied temperature and pressure conditions. During and after the incubation, we will evaluate how the CO_2 fluid-rock reaction changes mineral compositions and physical properties of sediments. Using a supercomputer device (e.g., Earth Simulator at JAMSTEC), the analysis of these in situ and ex situ data, including the seismic data set, will constitute regional 3-D models of CO_2 dispersal with time, migration behaviors, and environmental changes (e.g., porosity, pH, and pCO_2). The modeling will include rates of biological CO_2 turnover to CH_4 or other hydrocarbons, representing the feedback velocity of CO_2 disposal and the enhanced gas production rates. Also, these computational simulations will provide significant information to plan a scientifically sound set of experiments for the large-scale active experimentation in the future and will contribute to the preparation of large-scale carbon storage in similar environmental settings around the western coast of Pacific Ocean.

Science party

To complete the onboard sampling, analyses, and logging as described above, Expedition 337 will invite a shipboard scientific party that consists of 25–26 scientists, including the two Co-Chief Scientists, sedimentologists, organic and inorganic geochemists, (geo-)microbiologists, physical property specialists, micropaleontologists, and structural geologists. In addition to typical requirements in each of these specialties, particular needs in Expedition 337 include, but are not limited to, the following specific expertise:

- Petroleum geologists and sedimentologists with strong interest in biogeochemical diagenetic processes on the deeply buried coalbed and/or shore-based CO_2 sequestration into a subseafloor hydrocarbon system;
- Biogeochemists and geochemists with experience/background in pore water analysis of biologically relevant chemical species, in rate measurements using radioactive tracers, in hydrogen and hydrocarbon gas analysis, in mud gas monitoring includ-

ing isotope analysis, in geochemical modeling of transport and reaction of dissolved constituents, and in quantitative and compositional analysis of dissolved organic matter;

- Microbial ecologists and molecular biologists with experience in shipboard cell enumeration using flow cytometry and/or image-based microscopic analysis, in shipboard anaerobic and aseptic sampling of sediment or rock, in shore-based anaerobic cultivation techniques using high-pressure chamber and/or flow-through reactor systems, in shipboard contamination assessment using tracers such as fluorescent microbeads and/or PFT, in DNA/RNA-based functional gene analyses, in RNA-based molecular analyses of community structure and/or gene expression, and/or in single cell biology and (meta)genomics;
- Physical property specialists with experience/background in measurement of porosity, permeability, and determination of fluid migration in cored materials, sidewall minicores, and wireline logging data and/or in reactions between liquid and supercritical CO_2-containing fluids and sedimentary rock/minerals;
- Biostratigraphers with experience in micro- and nannofossil age determination in the northeastern Pacific and terrestrial/coastal deposits and/or in isotopic analyses using beryllium, rhenium-osmium, and iodine for depositional and fluid age; and
- Logging specialists with experience in in situ geophysical and geochemical measurements, in borehole sampling of formation fluids and sidewall minicores, and/or in handling and data processing of multiple logging tools as planned in Expedition 337.

Operational risk

Hydrocarbons

The drilling site is located near commercial gas production wells, but no evidence of overpressure was found at the offset well. The previous expedition showed that C_1/C_2 is >1000 without detection of $>C_3$ gas in the piston cores to 350 mbsf. The site is positioned within a basement syncline in an area where shallow hazards were considered minimal. Even if hydrocarbons would be encountered, mud weights can be increased to control possible flow from the formation during riser drilling.

Weather

Expedition 337 has been scheduled to take place during spring, when no typhoon or seasonal severe weather is expected. The drilling site is free from strong ocean current. In the case of extreme weather, we would evacuate after disconnecting the BOP. Re-entry would be possible.

Contingency and alternate plan

Unforeseen circumstances could result in insufficient time being available to complete the entire operations plan. Examples include collapse of a borehole because of difficult formation conditions (unstable sands), hazardous weather, and hardware failures. In anticipation of challenging and fluctuating environmental conditions, we have included 8 days of contingency for the entire expedition in the operations plan and time estimate. In case further delay in operation takes place, we will adjust the time by reducing the number of spot cores.

References

Alt, J.C., Kinoshita, H., Stokking, L.B., et al., 1993. *Proc. ODP, Init. Repts.,* 148: College Station, TX (Ocean Drilling Program). **doi:10.2973/odp.proc.ir.148.1993**

Aoike, K. (Ed.), 2007. *CDEX Laboratory Operation Report: CK06-06 D/V* Chikyu *shakedown cruise offshore Shimokita:* Yokohama (CDEX-JAMSTEC). **http://sio7.jamstec.go.jp/JAMSTEC-exp-report/902/CK06-06_CR.pdf**

Aoike, K., Nishi, H., Sakamoto, T., Iijima, K., Tsuchiya, M., Taira, A., Kuramoto, S., Masago, H., and the Shimokita Core Research Group, 2010. Paleoceanographic history of offshore Shimokita Peninsula for the past 800,000 years based on primary analyses on cores recovered by D/V *Chikyu* during the shakedown cruises. *Fossils,* 87:65–81. (in Japanese, with English abstract and figures)

Arndt, S., Brumsack, H.-J., and Wirtz, K.W., 2006. Cretaceous black shales as active bioreactors: a biogeochemical model for the deep biosphere encountered during ODP Leg 207 (Demerara Rise). *Geochim. Cosmochim. Acta,* 70(2):408–425. **doi:10.1016/j.gca.2005.09.010**

Biddle, J.F., Lipp, J.S., Lever, M.A., Lloyd, K.G., Sørensen, K.B., Anderson, R., Fredricks, H.F., Elvert, M., Kelly, T.J., Schrag, D.P., Sogin, M.L., Brenchley, J.E., Teske, A., House, C.H., and Hinrichs, K.-U., 2006. Heterotrophic Archaea dominate sedimentary subsurface ecosystems off Peru. *Proc. Natl. Acad. Sci. U. S. A.,* 103(10):3846–3851. **doi:10.1073/pnas.0600035103**

Brown, C.J., Coates, J.D., and Schoonen, M.A.A., 1999. Localized sulfate-reducing zones in a coastal plain aquifer. *Ground Water*, 37(4):505–516. **doi:10.1111/j.1745-6584.1999.tb01136.x**

Colwell, F.S., Boyd, S., Delwiche, M.E., Reed, D.W., Phelps, T.J., and Newby, D.T., 2008. Estimates of biogenic methane production rates in deep marine sediments at Hydrate Ridge, Cascadia margin. *Appl. Environ. Microbiol.,* 74(11):3444–3452. **doi:10.1128/AEM.02114-07**

Cowen, J.P., Giovannoni, S.J., Kenig, F., Johnson, H.P., Butterfield, D., Rappé, M.S., Hutnak, M., and Lam, P., 2003. Fluids from aging ocean crust that support microbial life. *Science,* 299(5603):120–123. **doi:10.1126/science.1075653**

Detmers, J., Schulte, U., Strauss, H., and Kuever, J., 2001. Sulfate reduction at a lignite seam: microbial abundance and activity. *Microb. Ecol.,* **42(3)**:238–247. **doi:10.1007/s00248-001-1014-8**

D'Hondt, S., Jørgensen, B.B., Miller, D.J., Batzke, A., Blake, R., Cragg, B.A., Cypionka, H., Dickens, G.R., Ferdelman, T., Hinrichs, K.-U., Holm, N.G., Mitterer, R., Spivack, A., Wang, G., Bekins, B., Engelen, B., Ford, K., Gettemy, G., Rutherford, S.D., Sass, H., Skilbeck, C.G., Aiello, I.W., Guerin, G., House, C.H., Inagaki, F., Meister, P., Naehr, T., Niitsuma, S., Parkes, R.J., Schippers, A., Smith, D.C., Teske, A., Wiegel, J., Naranjo Padillo, C., and Solis Acosta, J.L., 2004. Distributions of microbial activities in deep subseafloor sediments. *Science,* 306(5705):2216–2221. **doi:10.1126/science.1101155**

D'Hondt, S., Rutherford, S., and Spivack., A.J., 2002. Metabolic activity of the subsurface life in deep-sea sediments. *Science,* 295(5562):2067–2070. **doi:10.1126/science.1064878**

D'Hondt, S., Spivack, A.J., Pockalny, R., Ferdelman, T.G., Fischer, J.P., Kallmeyer, J., Abrams, L.J., Smith, D.C., Graham, D., Hasiuk, F., Schrum, H., and Stancine, A.M., 2009. Subseafloor sedimentary life in the South Pacific Gyre. *Proc. Natl. Acad. Sci. U. S. A.,* 106(28):11651–11656. **doi:10.1073/pnas.0811793106**

Domitsu, H., Nishi, H., Uchida, J., Oda, M., Ogane, K., Taira, A., Aoike, K., and the Shimokita Microfossil Research Group, 2010. Age model of core sediments taken by D/V *Chikyu* during the shakedown cruises off Shimokita Peninsula. *Fossils,* **87**:47–64. (in Japanese, with English abstract and figures)

Dooley, J.J., Dahowski, R.T., Davidson, C.L., Wise, M.A., Gupta, N., Kim, S.H., and Malone, E.L., 2006. *Carbon Dioxide Capture and Geological Storage: a Core Element of a Global Energy Technology Strategy to Address Climate Change.* Technol. Rep.–Global Energy Technol. Strategy Program. **http://tsa.pnl.gov/publications/pdf/pnwd_3602_gtsp_css_report_2006.pdf**

Engelen, B., Ziegelmüller, K., Wolf, L., Köpke, B., Gittel, A., Cypionka, H., Treude, T., Nakagawa, S., Inagaki, F., Lever, M.A., and Steinsbu, B.O., 2008. Fluids from the ocean crust support microbial activities within the deep biosphere. *Geomicrobiol. J.,* 25(1):56–66. **doi:10.1080/01490450701829006**

Fry, J., Horsfield, B., Sykes, R., Cragg, B.A., Heywood, C., Kim, G.T., Mangelsdorf, K., Mildenhall, D.C., Rinna, J., Vieth, A., Zink, K.-G., Sass, H., Weightman, A.J., and Parkes, R.J., 2009. Prokaryotic populations and activities in an interbedded coal deposit, including a previously deeply buried section (1.6–2.3 km) above ~150 Ma basement rock. *Geomicrobiol. J.,* 26(3):163–178. **doi:10.1080/01490450902724832**

Heuer, V.B., Pohlman, J.W., Torres, M.E., Elvert, M., and Hinrichs, K.-U., 2009. The stable carbon isotope biogeochemistry of acetate and other dissolved carbon species in deep subseafloor sediments at the northern Cascadia Margin. *Geochim. Cosmochim. Acta,* 73(11):3323–3336. **doi:10.1016/j.gca.2009.03.001**

Hinrichs, K.-U., Hayes, J.M., Bach, W., Spivack, A.J., Hmelo, L.R., Holm, N.G., Johnson, C.G., and Sylva, S.P., 2006. Biological formation of ethane and propane in the deep marine subsurface. *Proc. Nat. Acad. Sci., U. S. A.,* 103(40):14684–14689. **doi:10.1073/pnas.0606535103**

House, C.H., Cragg, B.A., Teske, A., and the Leg 201 Scientific Party, 2003. Drilling contamination tests during ODP Leg 201 using chemical and particulate tracers. *In* D'Hondt, S.L., Jørgensen, B.B., Miller, D.J., et al., *Proc. ODP, Init. Repts.,* 201: College Station, TX (Ocean Drilling Program), 1–19. **doi:10.2973/odp.proc.ir.201.102.2003**

House, K.Z., Schrag, D.P., Harvery, C.F., and Lackner, K.S., 2006. Permanent carbon dioxide storage in deep-sea sediments. *Proc. Natl. Acad. Sci. U. S. A.,* 103(33):12291–12295. **doi:10.1073/pnas.0605318103**

Imachi, H., Aoi, K., Tasumi, E., Saito, Y., Yamanaka, Y., Saito, Y., Yamaguchi, T., Tomaru, H., Takeuchi, R., Morono, Y., Inagaki, F., and Takai, K., submitted. Cultivation of methanogenic community from subseafloor sediments using a continuous-flow bioreactor. *ISME J.*

Inagaki, F., 2010. Deep subseafloor microbial communities. *Encycl. Life Sci.* **doi:10.1002/9780470015902.a0021894**

Inagaki, F., Kuypers, M.M.M., Tsunogai, U., Ishibashi, J.-I., Nakamura, K.-I., Treude, T., Ohkubo, S., Nakaseama, M., Gena, K., Chiba, H., Hirayama, H., Nunoura, T., Takai, K., Jørgensen, B.B., Horikoshi, K., and Boetius, A., 2006a. Microbial community in a sediment-hosted CO_2 lake of the southern Okinawa Trough hydrothermal system. *Proc. Natl. Acad. Sci. U.S.A.,* 103(38):14164–14169. **doi:10.1073/pnas.0606083103**

Inagaki, F., and Nakagawa, S., 2008. Spatial distribution of the subseafloor life: diversity and biogeography. *In* Dilek, Y., Furnes, H., and Muehlenbachs, K. (Eds.), *Links Between Geological Processes, Microbial Activities and Evolution of Life Microbes and Geology.* Mod. Approaches Solid Earth Sci., 4:135–158. **doi:10.1007/978-1-4020-8306-8_4**

Inagaki, F., Nunoura, T., Nakagawa, S., Teske, A., Lever, M., Lauer, A., Suzuki, M., Takai, K., Delwiche, M., Colwell, F.S., Nealson, K.H., Horikoshi, K., D'Hondt, S., and Jørgensen, B.B., 2006b. Biogeographical distribution and diversity of microbes in methane hydrate–bearing deep marine sediments on the Pacific Ocean margin. *Proc. Natl. Acad. Sci. U. S. A.,* 103(8):2815–2820. **doi:10.1073/pnas.0511033103**

Inagaki, F., Suzuki, M., Takai, K., Oida, H., Sakamoto, T., Aoki, K., Nealson, K.H., and Horikoshi, K., 2003. Microbial communities associated with geological horizons in coastal subseafloor sediments from the Sea of Okhotsk. *Appl. Environ. Microbiol.,* 69(12):7224–7235. **doi:10.1128/AEM.69.12.7224-7235.2003**

Japan Natural Gas Association and Japan Offshore Petroleum Development Association (Eds.), 1992. Oil and Gas Resources in Japan (revised ed.). Tokyo. (in Japanese)

Jones, D.M., Head, I.M., Gray, N.D., Adams, J.J., Rowan, A.K., Aitken, C.M., Bennett, B., Huang, H., Brown, A., Bowler, B.F.J., Oldenburg, T., Erdmann, M., and Larter, S.R., 2008a. Crude-oil biodegradation via methanogenesis in subsurface petroleum reservoirs. *Nature, (London, U. K.),* 451(7175):176–180. **doi:10.1038/nature06484**

Jones, E.J.P., Voytek, M.A., Warwick, P.D., Corum, M.D., Cohn, A., Bunnell, J.E., Clark, A.C., and Orem W.H., 2008b. Bioassay for estimating the biogenic methane-generating potential of coal samples. *Int. J. Coal Geol.,* 76(1–2):138–150. **doi:10.1016/j.coal.2008.05.011**

Kallmeyer, J., Smith, D.C., Spivack, A.J., and D'Hondt, S., 2008. New cell extraction procedure applied to deep subsurface sediments. *Limnol. Oceanogr.: Methods,* 6:236–245. **http://www.aslo.org/lomethods/free/2008/0236.pdf**

Kobayashi, T., Koide, O., Mori, K., Shimamura, S., Matsuura, T., Miura, T., Takaki, Y., Morono, Y., Nunoura, T., Imachi, H., Inagaki, F., Takai, K., and Horikoshi, K., 2008. Phylogenetic and enzymatic diversity of deep subseafloor aerobic microorganisms in organics- and methane-rich sediments off Shimokita Peninsula. *Extremophiles,* 12(4):519–527. **doi:10.1007/s00792-008-0157-7**

Krüger, M., Beckmann, S., Engelen, B., Thielemann, T., Cramer, B., Schippers, A., and Cypionka, H., 2008. Microbial methane formation from hard coal and timber in an abandoned coal mine. *Geomicrobiol. J.,* 25(6):315–321. **doi:10.1080/01490450802258402**

Krumholz, L.R., McKinley, J.P., Ulrich, G.A., and Suflita, J.M., 1997. Confined subsurface microbial communities in Cretaceous rock. *Nature (London, U. K.),* 386(6620):64–66. **doi:10.1038/386064a0**

Lever, M.A., Alperin, M., Engelen, B., Inagaki, F., Nakagawa, S., Steinsbu, B.O., Teske, A., and IODP Expedition Scientists, 2006. Trends in basalt and sediment core contamination during IODP Expedition 301. *Geomicrobiol. J.,* 23(7):517–530. **doi:10.1080/01490450600897245**

Lever, M.A., Heuer, V.B., Morono, Y., Masui, N., Schmidt, F., Alperin, M.J., Inagaki, F., Hinrichs, K.-U., and Teske, A., 2010. Acetogenesis in deep subseafloor sediments of the Juan de Fuca Ridge flank: a synthesis of geochemical, thermodynamic, and gene-based evidence. *Geomicrobiol. J.,* 27(2):183–211. **doi:10.1080/01490450903456681**

Lipp, J.S., Morono, Y., Inagaki, F., and Hinrichs K.-U., 2008. Significant contribution of Archaea to extant biomass in marine subsurface sediments. *Nature (London, U. K.),* 454(7207):991–994. **doi:10.1038/nature07174**

Masui, N., Morono, Y., and Inagaki, F., 2008. Microbiological assessment of circulation mud fluids during the first operation of riser drilling by the deep-earth research vessel *Chikyu*. *Geomicrobiol. J.,* 25(6):274–282. **doi:10.1080/01490450802258154**

Morono, Y., and Inagaki, F., 2010. Automatic slide-loader fluorescent microscope for discriminative enumeration of subseafloor life. *Sci. Drill.,* 9:32–36. **doi:10.2204/iodp.sd.9.06.2010**

Morono, Y., Terada, T., Masui, N., and Inagaki, F., 2009. Discriminative detection and enumeration of microbial life in marine subsurface sediments. *ISME J.,* 3(5):503–511. **doi:10.1038/ismej.2009.1**

Musat, N., Halm, H., Winterholler, B., Hoppe, P., Peduzzi, S., Hillion, F., Horreard, F., Amann, R., Jørgensen, B.B., and Kuypers, M.M.M., 2008. A single-cell view on the ecophysiology of anaerobic phototrophic bacteria. *Proc. Nat. Acad. Sci. U. S. A.,* 105(46):17861–17866. **doi:10.1073/pnas.0809329105**

Nakagawa, S., Inagaki, F., Suzuki, Y., Steinsbu, B.O., Lever, M.A., Takai, K., Engelen, B., Sako, Y., Wheat, C.G., Horikoshi, K., and Integrated Ocean Drilling Program Expedition 301 Scientists, 2006. Microbial community in black rust exposed to hot ridge-flank crustal fluids. *Appl. Environ. Microbiol.,* 72(10):6789–6799. **doi:10.1128/AEM.01238-06**

Onstott, T.C., 2005. Impact of CO_2 injections on deep subsurface microbial ecosystems and potential ramifications for the surface biosphere. *In* Thomas, D.C., and Benson, S.M. (Eds.), *Carbon Dioxide Capture for Storage in Deep Geological Formations: the CO_2 Capture Project,* Vol. 2: London (Elsevier Ltd.), 1207–1239.

Orem, W.H., Voytek, M.A., Jones, E.J., Lerch, H.E., Bates, A.L., Corum, M.D., Warwick, P.D., and Clark, A.C., 2010. Organic intermediates in the anaerobic biodegradation of coal to methane under laboratory conditions. *Org. Geochem.,* 41(9):997–1000. **doi:10.1016/j.orggeochem.2010.03.005**

Osawa, M., Nakanishi, S., Tanahashi, M., Oda, H., and Sasaki, A., 2002. Structure, tectonic evolution and gas exploration potential of offshore Sanriku and Hidaka provinces, Pacific Ocean, off northern Honshu and Hokkaido, Japan. *J. Jpn. Assoc. Pet. Technol.,* 67(1):38–51. (in Japanese, with English abstract and figures)

Parkes, R.J., Cragg, B.A., Bale, S.J., Getliff, J.M., Goodman, K., Rochelle, P.A., Fry, J.C., Weightman, A.J., and Harvey, S.M., 1994. Deep bacterial biosphere in Pacific Ocean sediments. *Nature (London, U. K.),* 371(6496):410–413. **doi:10.1038/371410a0**

Parkes, R.J., Cragg, B.A., and Wellsbury, P., 2000. Recent studies on bacterial populations and processes in subseafloor sediments: a review. *Hydrogeol. J.,* 8(1):11–28. **doi:10.1007/PL00010971**

Parkes, R.J., Webster, G., Cragg, B.A., Weightman, A.J., Newberry, C.J., Ferdelman, T.G., Kallmeyer, J., Jørgensen, B.B., Aiello, I.W., and Fry, J.C., 2005. Deep sub-seafloor prokaryotes stimulated at interfaces over geological time. *Nature (London, U. K.),* 436(7049):390–394. **doi:10.1038/nature03796**

Parkes, R.J., Wellsbury, P., Mather, I.D., Cobb, S.J., Cragg, B.A., Hornibrook, E.R.C., and Horsfield, B., 2007. Temperature activation of organic matter and minerals during burial has the potential to sustain the deep biosphere over geological timescales. *Org. Geochem.,* 38(6):845–852. **doi:10.1016/j.orggeochem.2006.12.011**

Roussel, E.G., Cambon Bonavita, M.-A., Querellou, J., Cragg, B.A., Webster, G., Prieur, D., and Parkes, J.R., 2008. Extending the sub-sea-floor biosphere. *Science,* 320(5879):1046. **doi:10.1126/science.1154545**

Seno, T., Sakurai, T., and Stein, S., 1996. Can the Okhotsk plate be discriminated from the North American plate? *J. Geophys. Res., [Solid Earth],* 101(B5):11305–11315. **doi:10.1029/96JB00532**

Schmidt, F., Elvert, M., Koch, B.P., Witt, M., and Hinrichs, K.-U., 2009. Molecular characterization of dissolved organic matter in pore water in continental shelf sediments. *Geochim. Cosmochim. Acta,* 73(11):3337–3358. **doi:10.1016/j.gca.2009.03.008**

Schrag, D.P., 2009. Storage of carbon dioxide in offshore sediments. *Science,* 325(5948):1658–1659. **doi:10.1126/science.1175750**

Schultheiss, P., Holland, M., and Humphrey, G., 2009. Wireline coring and analysis under pressure: recent use and future developments of the HYACINTH system. *Sci. Drill.,* 7:44–50. **doi:10.2204/iodp.sd.7.07.2009**

Shimizu, S., Akiyama, M., Naganuma, T., Fujioka, M., Nako, M., and Ishijima, Y., 2007. Molecular characterization of microbial communities in deep coal seam groundwater of northern Japan. *Geobiology,* 5(4):423–433. **doi:10.1111/j.1472-4669.2007.00123.x**

Sivan, O., Schrag, D.P., and Murray, R.W., 2007. Rates of methanogenesis and methanotrophy in deep-sea sediments. *Geobiology,* 5(2):141–151. **doi:10.1111/j.1472-4669.2007.00098.x**

Smith, D.C., Spivack, A.J., Fisk, M.R., Haveman, S.A., and Staudigel, H., 2000. Tracer-based estimates of drilling-induced microbial contamination of deep sea crust. *Geomicrobiol. J.,* 17(3):207–219. **doi:10.1080/01490450050121170**

Sørensen, K.B., and Teske, A., 2006. Stratified communities of active archaea in deep marine subsurface sediments. *Appl. Environ. Microbiol.,* 72(7):4596–4603. **doi:10.1128/AEM.00562-06**

Strapoc, D., Picardal, F.W., Turich, C., Schaperdoth, I., Macalady, J.L., Lipp, J.S., Lin, Y.-S., Ertefai, T.F., Schubotz, F., Hinrichs, K.-U., Mastalerz, M., and Schimmelmann, A., 2008. Methane-producing microbial community in a coal bed of the Illinois basin. *Appl. Environ. Microbiol.,* 74(8):2424–2432. **doi:10.1128/AEM.02341-07**

Taira, A., and Curewitz, D. (Eds.), 2005. *CDEX Technical Report* (Vol. 2): *Shimokita Area Site Survey: Northern Japan Trench Seismic Survey, Northern Honshu, Japan:* Yokohama (CDEX-JAMSTEC)

Teske, A., and Sørensen, K.B., 2008. Uncultured archaea in deep marine subsurface sediments: have we caught them all? *ISME J.,* 2:3–18. **doi:10.1038/ismej.2007.90**

Tomaru, H., Fehn, U., Lu, Z., Takeuchi, R., Inagaki, F., Imachi, H., Kotani, R., Matsumoto, R., and Aoike, K., 2009. Dating of dissolved iodine in pore waters from the gas hydrate occurrence offshore Shimokita Peninsula, Japan: ^{129}I results from D/V *Chikyu* shakedown cruise. *Resour. Geol.,* 59(4):359–373. **doi:10.1111/j.1751-3928.2009.00103.x**

Wang, G., Spivack, A.J., Rutherford, S., Manor, U., and D'Hondt, S., 2008. Quantification of co-occurring reaction rates in deep subseafloor sediments. *Geochim. Cosmochim. Acta,* 72(14):3479–3488. **doi:10.1016/j.gca.2008.04.024**

Wegener, G., Niemann, H., Elvert, M., Hinrichs, K.-U., and Boetius, A., 2008. Assimilation of methane and inorganic carbon by microbial communities mediating the anaerobic oxidation of methane. *Environ. Microbiol.,* 10(9):2287–2298. **doi:10.1111/j.1462-2920.2008.01653.x**

Whiticar, M.J., 1999. Carbon and hydrogen isotope systematics of bacterial formation and oxidation of methane. *Chem. Geol.,* 161(1–3):291–314. **doi:10.1016/S0009-2541(99)00092-3**

Whitman, W.B., Coleman, D.C., and Wiebe, W.J., 1998. Prokaryotes: the unseen majority. *Proc. Natl. Acad. Sci. U. S. A.,* 95(12):6578–6583. **doi:10.1073/pnas.95.12.6578**

Table T1. Projected coring depth intervals, Expedition 337. (See table note.)

Hole	Depth (mbsf) Start	Depth (mbsf) End	Length (m)	Drilling method	Coring method	Expected sedimentary age and lithologic characteristics
A	670.0	679.5	9.5	Riser	RCB	Upper Pliocene hemipelagic mud
	820.0	829.5	9.5	Riser	RCB	Middle Pliocene hemipelagic mud
	970.0	979.5	9.5	Riser	RCB	Lower Pliocene hemipelagic mud
	1120.0	1129.5	9.5	Riser	RCB	Pliocene–Miocene unconformity
	1270.0	1279.5	9.5	Riser	RCB	Miocene–Oligocene hemipelagic mud
	1370.0	1379.5	9.5	Riser	RCB	Oligocene hemipelagic mud
	1470.0	1479.5	9.5	Riser	RCB	Oligocene sandstone
	1581.5	1648.0	9.5 (×7)	Riser	RCB	Oligocene sandstone
	1648.0	1675.0	27.0	Riser	LDC	Oligocene–Eocene unconformity
	1770.0	1789.0	9.5 (×2)	Riser	RCB	Eocene lacustrine mud
	1870.0	1889.0	9.5 (×2)	Riser	RCB	Eocene lacustrine mud
	1933.0	1990.0	9.5 (×6)	Riser	RCB	Eocene lignite
	1990.0	2044.0	27.0 (×2)	Riser	LDC	Eocene lignite
	2140.0	2149.5	9.5	Riser	RCB	Eocene lacustrine mud
	2190.5	2200.0	9.5	Riser	RCB	Eocene lacustrine mud
B	0	3.5	3.5	Riserless	Hybrid-PCS or HPCS	Mudline sample
	10.0	13.5	3.5	Riserless	Hybrid-PCS or HPCS	Quaternary sediment
	100	103.5	3.5	Riserless	Hybrid-PCS	Quaternary sediment
	206.5	231.0	3.5 (×7)	Riserless	Hybrid-PCS	Hydrate-bearing sediment

Note: RCB = rotary core barrel, LDC = large diameter coring, Hybrid-PCS = hybrid-pressure coring system, HPCS = hydraulic piston coring system.

Table T2. Planned operations and schedule, Expedition 337. (**See table notes.**)

Shimokita Deep Biosphere

Water Depth : 1,180m
Total Depth : 2,200mbsf (3,380mMSL)

Re-entry to the Shimokita-West hole, which wss drilled and suspended in 2006.

* 36"Conductor at 55.5mbsf.
* 20"CSG at 511mbsf.
* 17-1/2"hole was drilled to 647mbsf below 20"CSG shoe

Lithology (mbsf)	(mMSL) W.D 1,180m	Casing (Depth : mbsf)	Coring	Operation	Days	Sub Total (days)	Cummulative days	
				Move to Shimokita from Hachinohe				1.0
0	1,180	36" 55.5m; Plug Back CMT 455m 541m		1) Preparation for Spud ROV Seafloor Survey, Deploy Transponders, Retrieve the Corrosion cap by ROV (Meanwhile preparation for running BOP & Riser)	3.0	3.0	3.0	
500	1,680	20" 511m		2) Run BOP & Riser Run BOP & Riser Land & Test BOP	4.0 1.0	5.0	8.0	
1,000	2,180	12-1/4" hole 647mbsf		3) Set 13-3/8" CSG M/up & run 17-1/2"DOC ass'y, Displace hole with mud, DOC to 647m (TD), POOH Drill w/center bit & Cut RCB core from 647m to 1,220m (38 m of RCB core). W-Log #1 (include Wiper Trip before logging, VSP) Run 17-1/2"Open hole Ass'y, Open hole to 17-1/2" until 1,220m Wiper Trip, Run & Cement 13-3/8"CSG	2.0 3.5 3.0 2.0 4.0	14.5	22.5	66.0
1,500	2,680	13-3/8" 1220m		4) Drill to 2200mbsf (TD) M/up & run 12-1/4"drilling ass'y, DOC, Run LOT Drill & Cut RCB core from 1,220m to 1,648m (95m of RCB core). Change BHA to industrial coring ass'y, Cut Industry core from 1,648m to 1,675m (27m) Change to RCB ass'y, Drill & cut core from 1,675m to 1,990m (95 m of RCB core) Cut 8-1/2"Indudtrial core from 1,990m to 2,044 m (54m) Open hole to 10-5/8"hole, Drill & Cut RCB core from 2,044m to 2,200m (TD)(19m of RCB core) W-Log #2 (include Wiper Trip, VSP) Plug and abondon hole	1.5 4.0 3.0 3.5 3.5 1.0 6.0 3.0	25.5	48.0	
2,000	3,180			5) Suspend Hole Retrieve BOP & Riser Set Corrosion Cap	5.0 1.0	6.0	54.0	
2,500	3,680	10-5/8" Open Hole 2200 m		6) Riserless Pressure Core Hole Move to core hole site, Seafloor survey Run pressure core or HPCS w/ APCT-3 ass'y, Drill and cut core to max. 365mbsf（Pressure Core 10 cores） Spot kill mud and POOH、Retrieve Transponders	4.0	4.0	58.0	
				7) *Contingency* *Contingency Break down* *Equipment Downtime 4.0 8% of operation time* *Wait on Weather 4.0 8% of operation time* *Total 8.0 days*	8.0	8.0	66.0	
				Move to Hachinohe				1.0
						Total days		**68.0**

Unit F: Quaternary; BSR (?) 370 mbsf
610 mbsf
Unit E: Pliocene
1,110 mbsf
Unit D: Miocene - Oligocene
1,660 mbsf
Intercalations of lignite 1,930 -2,100
Unit C: Eocene
TD 2,200mbsf (3,380mMSL)
clay/claystone
silt/siltstone
sand/sandstone
ash/tuff
lignite/coal

Notes: CSG = casing. ROV = remotely operated vehicle. BOP = blowout preventer. DOC = drill out cement. TD = total depth. POOH = pull out of hole. RCB = rotary core barrel, BHA = bottom-hole assembly. VSP = vertical seismic profile. LOT = leak-off test. HPCS = hydraulic piston coring system, APCT-3 = advanced piston corer temperature tool.

Figure F1. Index map of off Shimokita drilling test area with bathymetry, seismic survey track lines, and locations of existing drill holes. Hydraulic piston coring system coring locations (Sites C9001 and C9002) drilled in late November 2005 are also indicated. Inset map exhibits plate configuration around Japanese Islands and the location of the index map (red square).

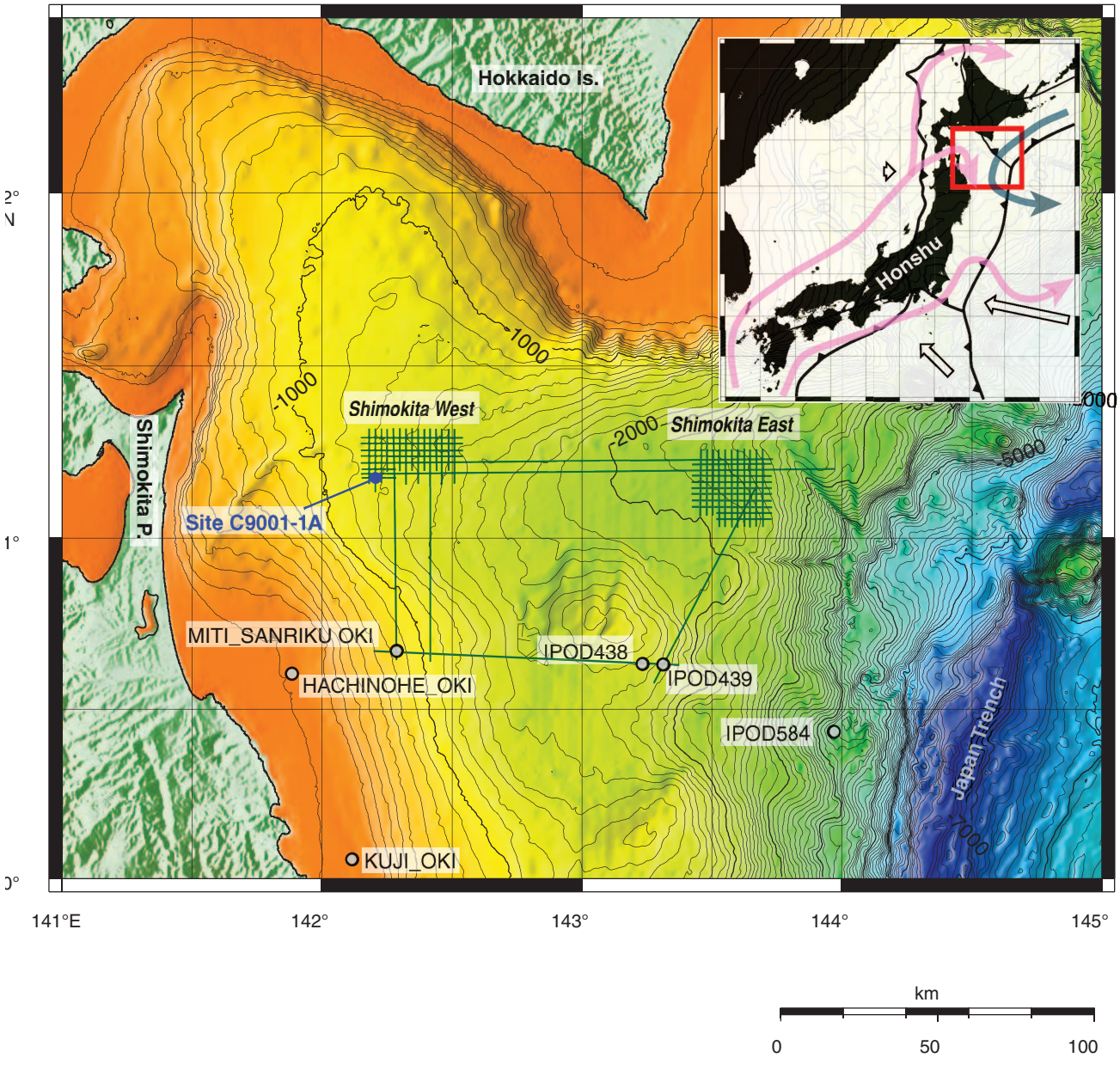


Figure F2. Microbial cell abundance in marine subsurface sediments. Red = cell abundance in sediments cored from offshore Shimokita Peninsula, blue = cell abundance in sediments cored from offshore Nankai Trough seismogenic zone. Plot data were obtained by the image-based cell enumeration technique (Morono et al., 2009).

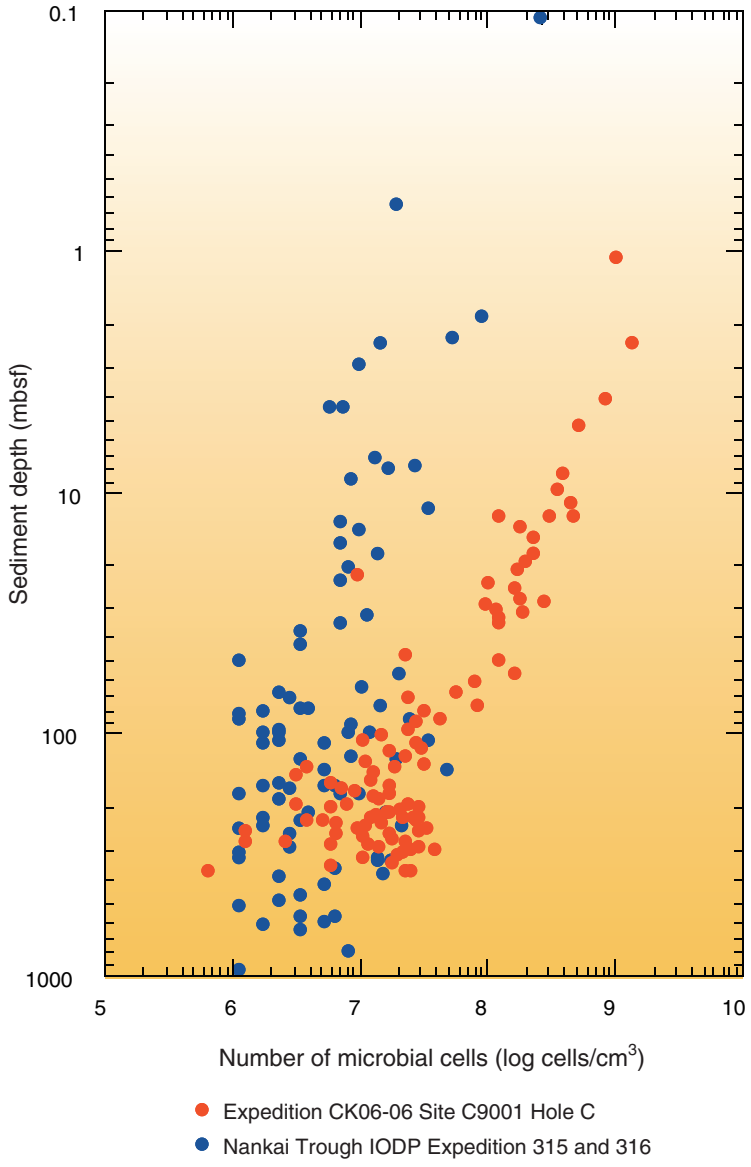
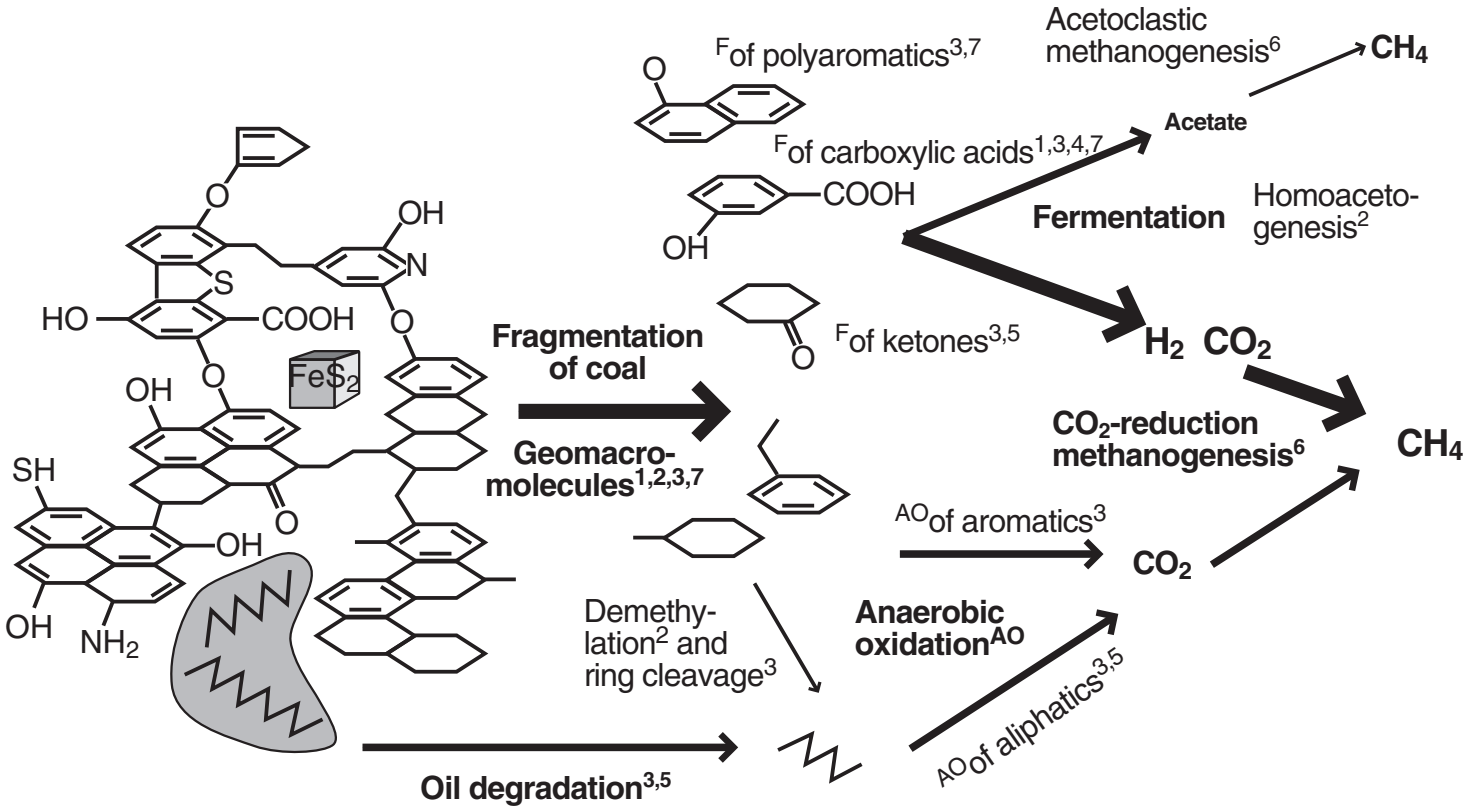


Figure F3. Schematic view of coal-to-methane biodegradation based on observation from a subsurface coalbed in the Illinois Basin. The degradation sequence from complex macromolecules to low molecular–weight substrates and finally methane is proposed to be carried out by microbes that have either been isolated or detected by a genetic survey (numbers associated with processes). Reproduced from Strapoc et al. (2008).



[1]Spirochaeta, [2]Sporomusa, [3]Cytophaga, [4]Acidoaminococcus, [5]Flavobacterium, [6]Methanocorpusculum, [7]Rhodobacter(?)

Figure F4. Location map of seismic survey tracklines in (**A**) 2002 and (**B**) 2003 and existing drill holes. Red squares = areas for high resolution two-dimensional (2-D) seismic survey. Bottom-simulating reflector (BSR) is recognized at ~600 mbsf in the western area and 640 mbsf in the eastern area. In addition, existence of free gas is anticipated in the western area, adjacent to the BSR-developed zone.

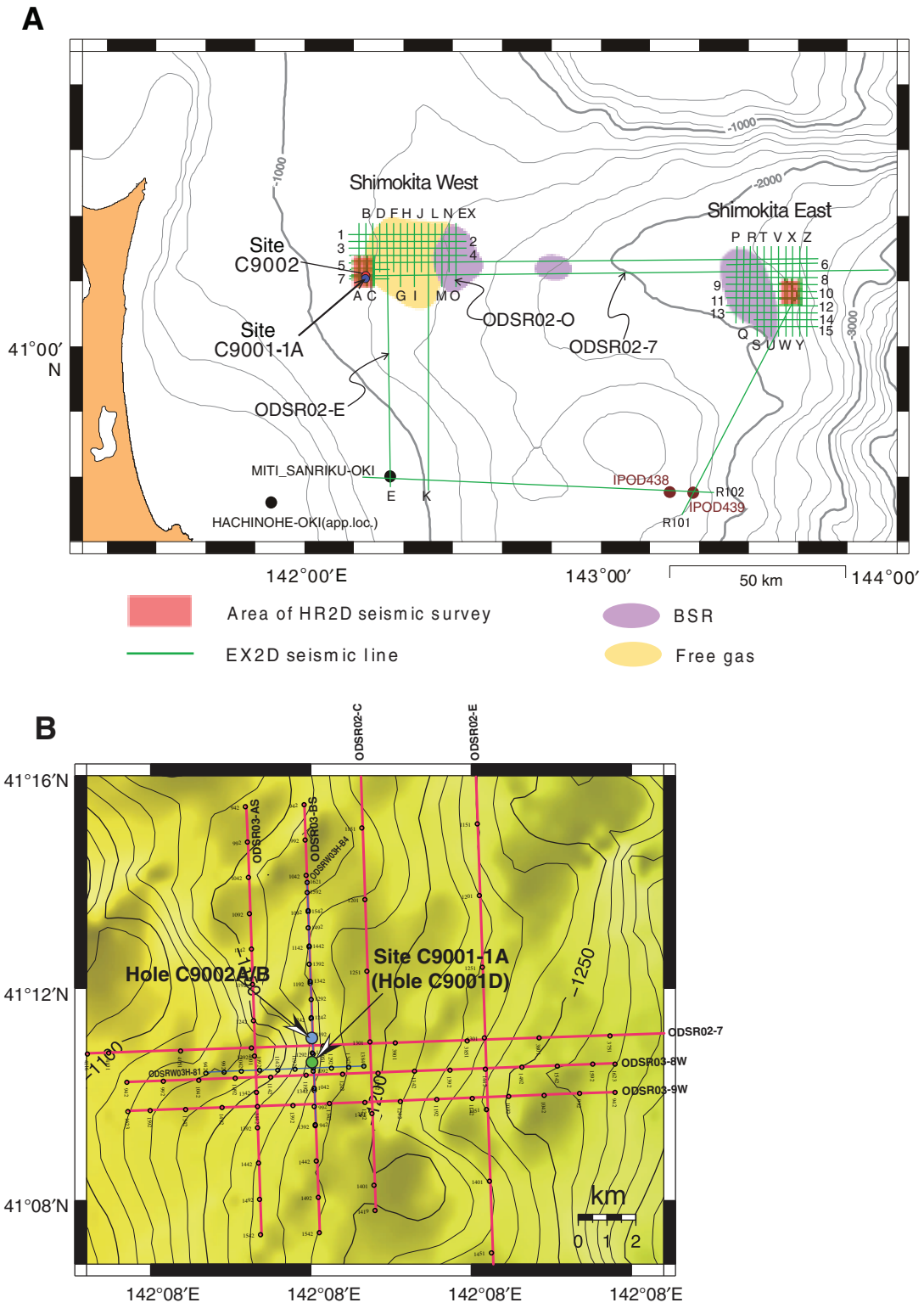


Figure F5. Seismic profile in the vicinity of Site C9001 off Shimokita Peninsula. Gray zones = potential coal-bearing layers, green dotted lines with arrows = fluid and gas plumes. Picked lines = predicted age of stratigraphic boundaries including the Oligocene unconformity. WD = water depth.

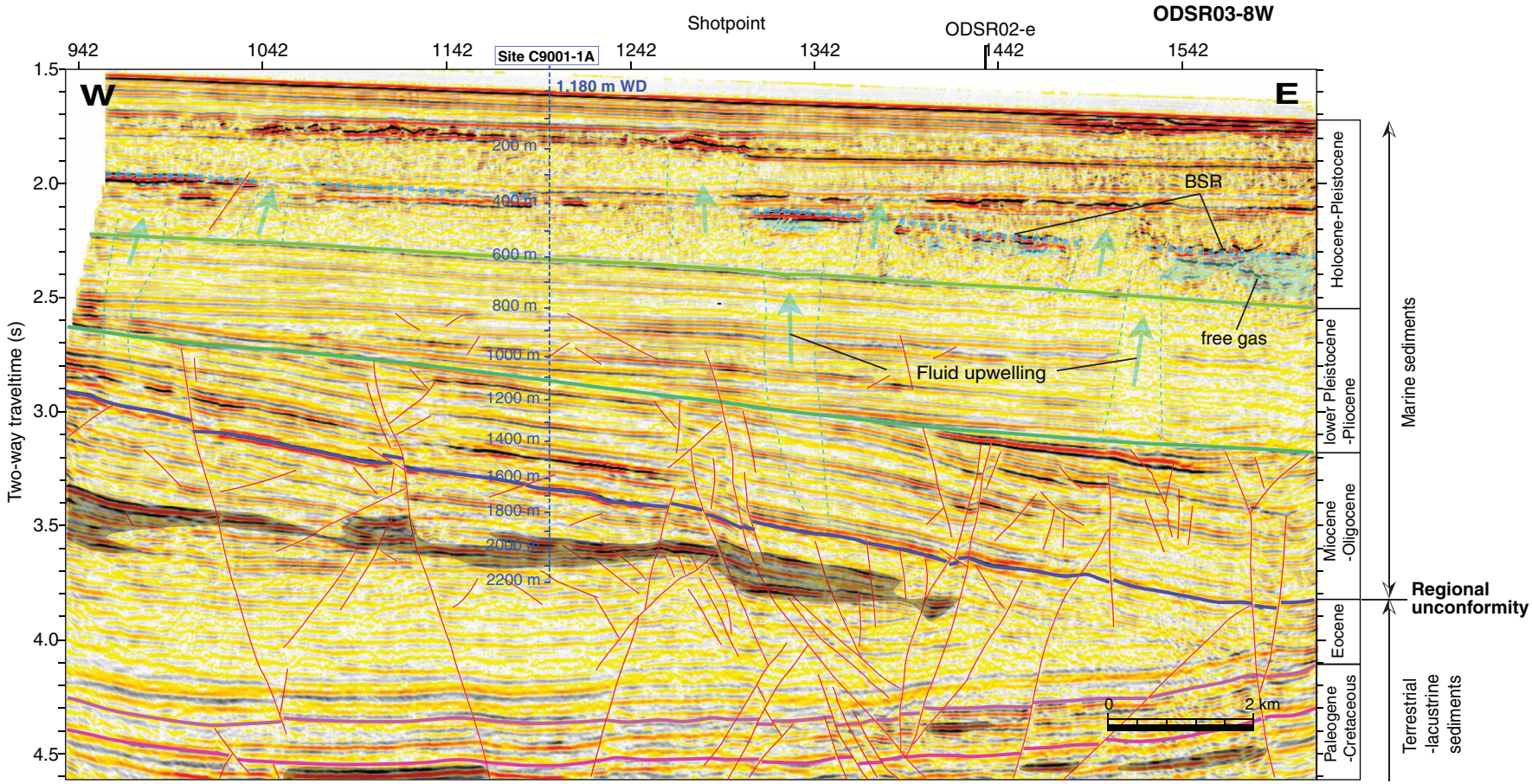


Figure F6. Lithostratigraphy of 365 m sediment core from Hole C9001C, recovered during the 2006 D/V *Chikyu* shakedown expedition.

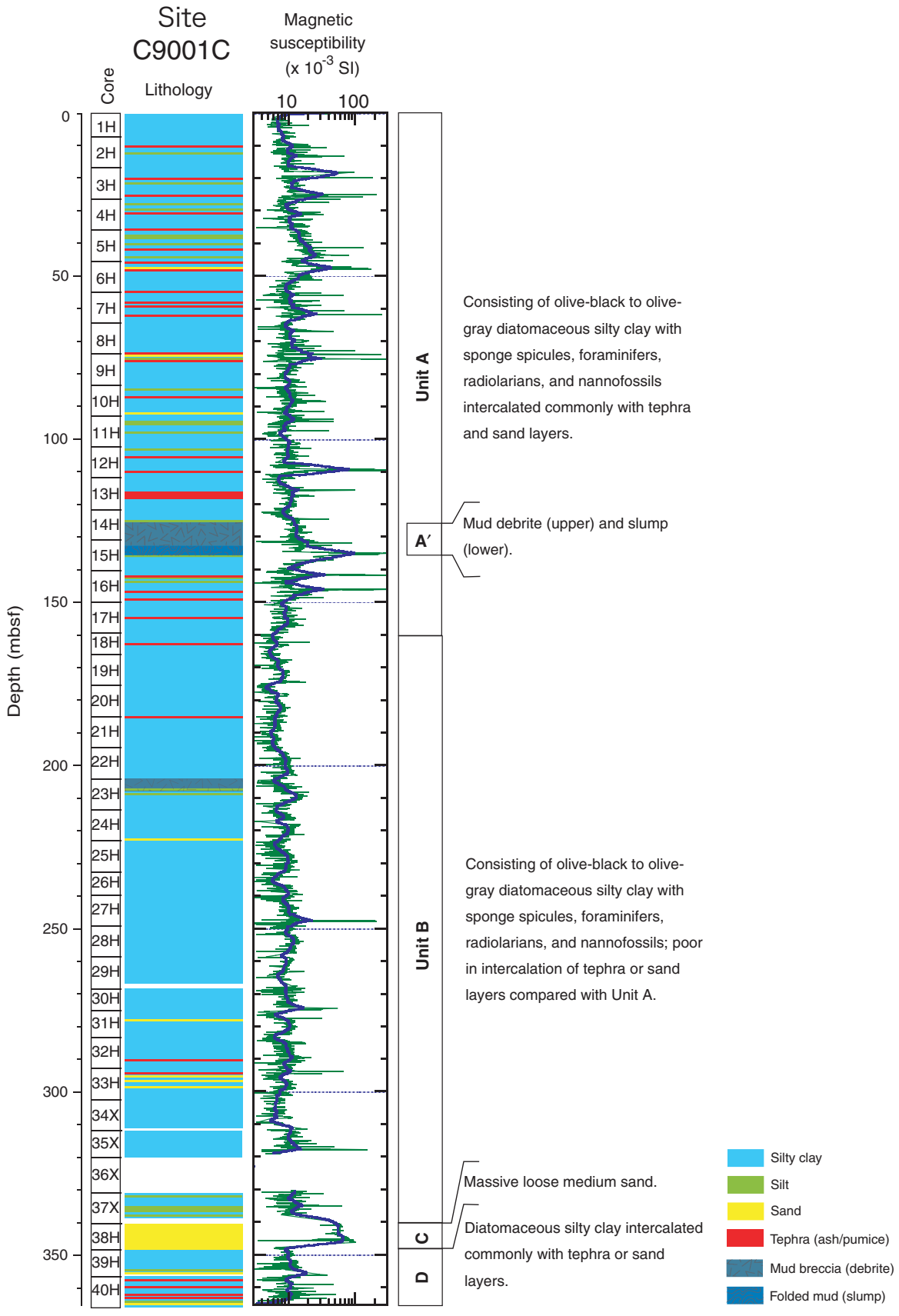


Figure F7. Detection of methane hydrate at Site C9001 by monitoring negative temperature anomalies using ThermoView camera.

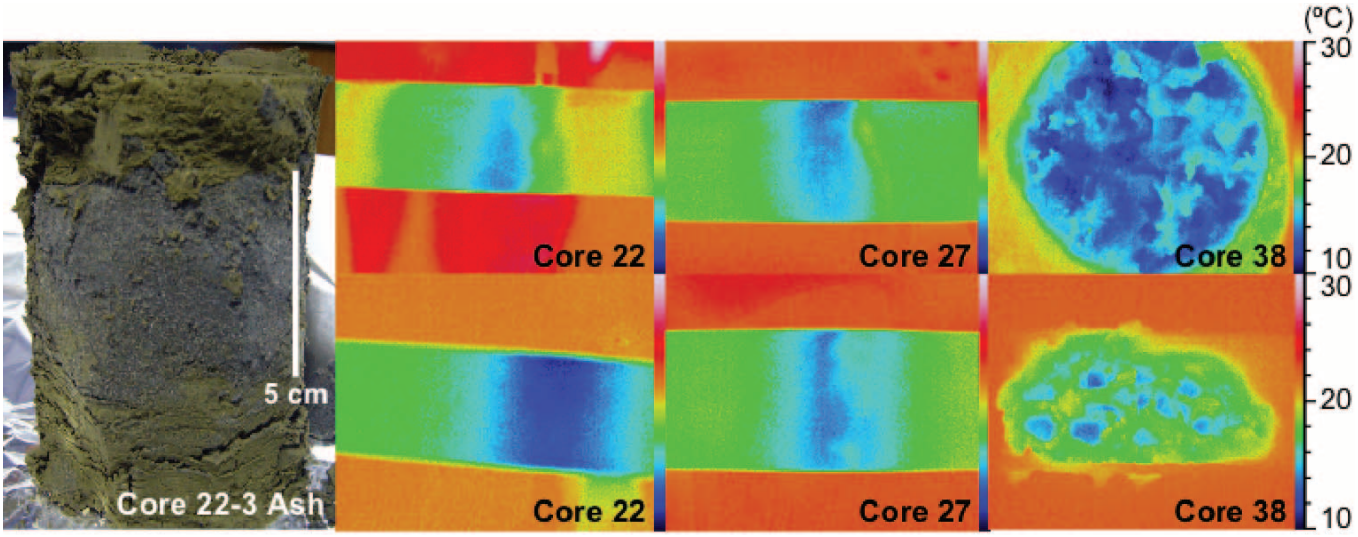


Figure F8. Microbial cells detected from methane hydrate–bearing sediments at 346 mbsf, Site C9001. **A.** Fluorescent microscopic image of SYBR Green I-stained cell aggregate. Scale bar = 2 µm. **B.** Scanning electron micrograph of cell aggregates. Scale bar = 200 nm.

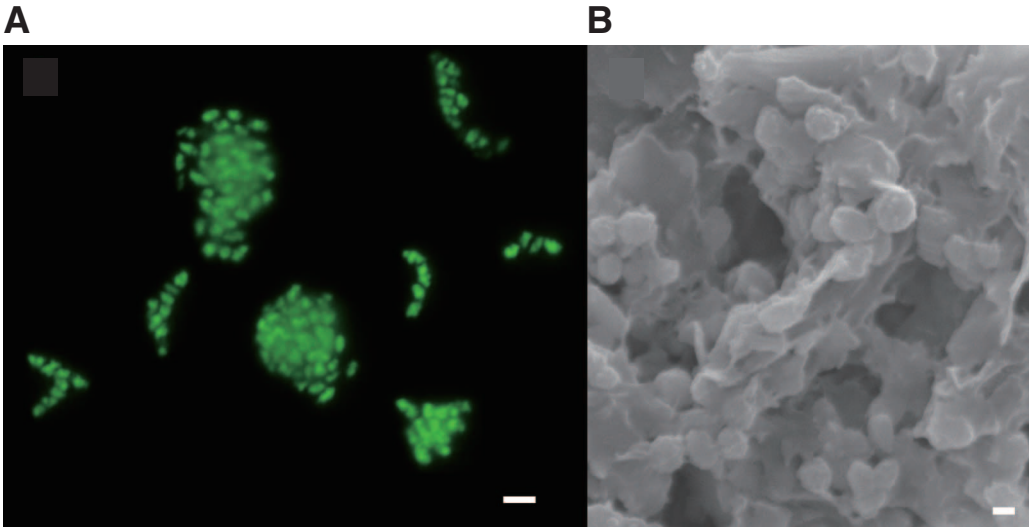


Figure F9. Methanogenic archaea isolated from sediments at Site C9001 using a flow-through bioreactor system. Photographs show microscopic image of isolated methanogens that produce autofluorescence from F420 under ultraviolet irradiation as shown in the bottom photographs. **A.** *Methanobacterium* sp. **B.** *Methanosarcina* sp.

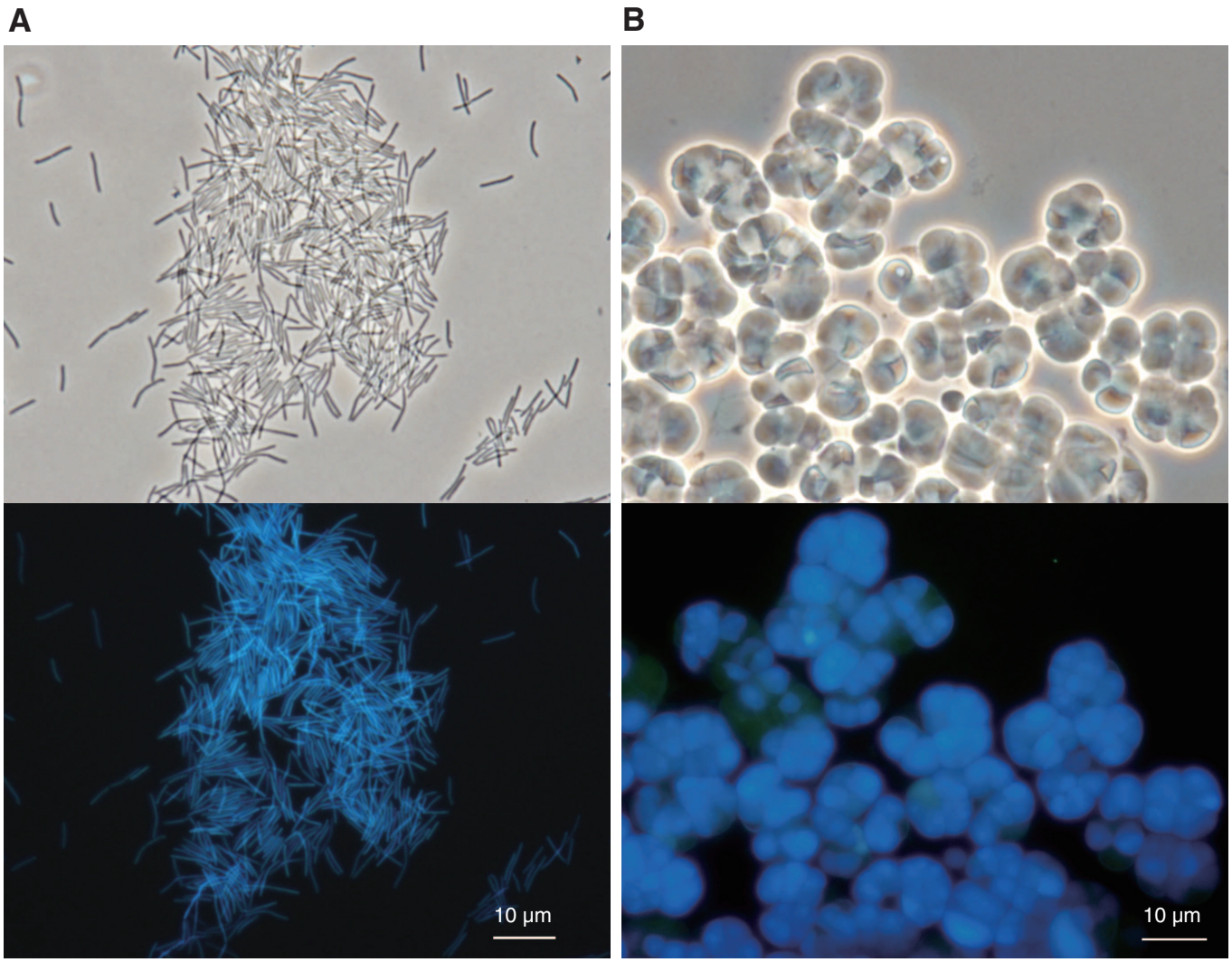


Figure F10. Sketch of wireline logging tools for (**A**) first three runs and (**B**) subsequent four runs. EMS = Environmental Measurement Sonde, HRLA = High-Resolution Laterolog Array, HNGS = Hostile Environment Natural Gamma Ray Sonde, HGNS = Highly Integrated Gamma Ray Neutron Sonde, HRMS = High-Resolution Mechanical Sonde, PEF = photoelectric effect, DSI = Dipole Sonic Imager, FMI = Formation MicroImager, MDT = Modular Formation Dynamics Tester, VSI = Versatile Seismic Imager, MSCT = Mechanical Sidewall Coring Tool, CMR = combinable magnetic resonance, NMR = Nuclear Magnetic Resonance. (**Continued on next page.**)

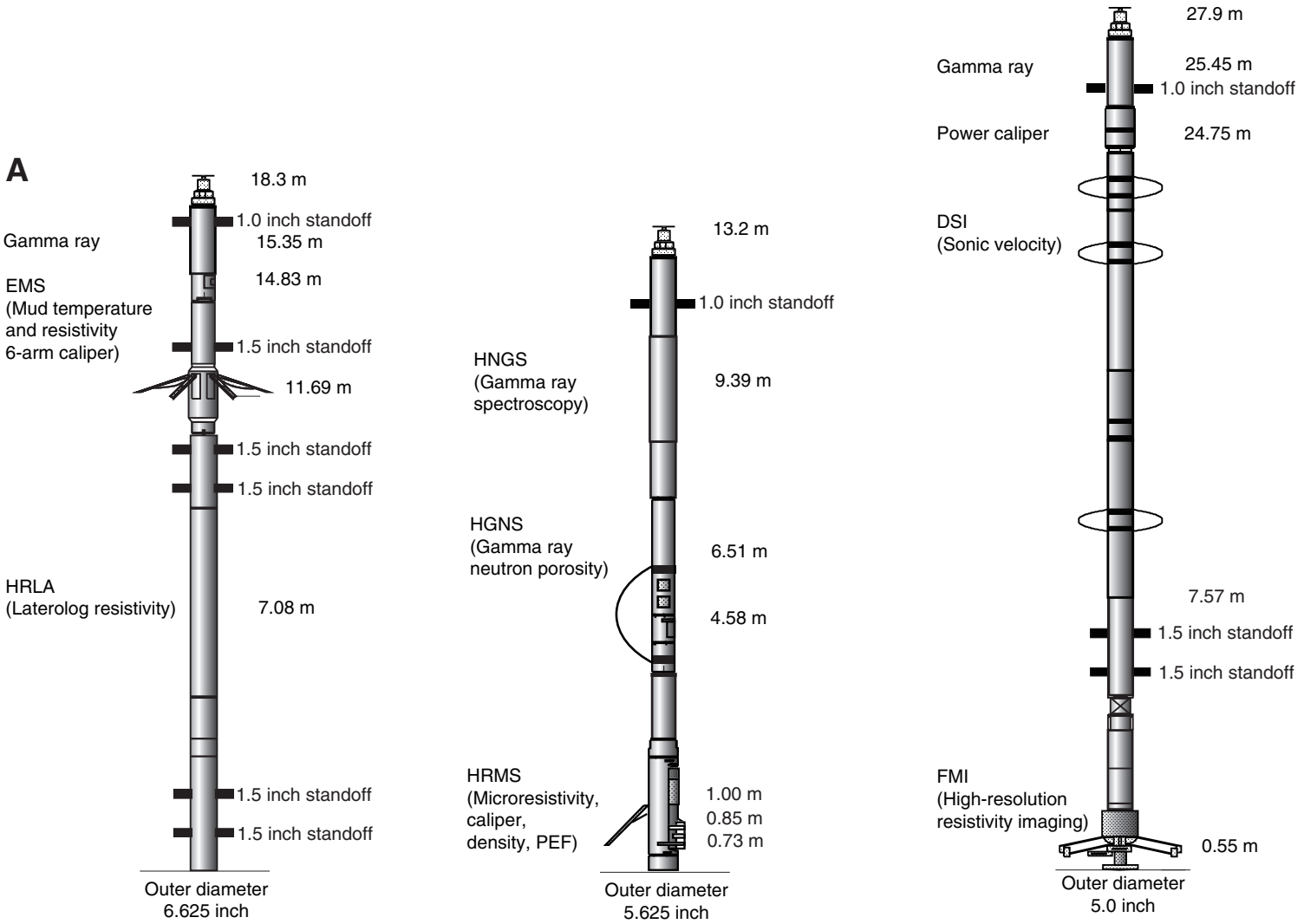


Figure F10 (continued).

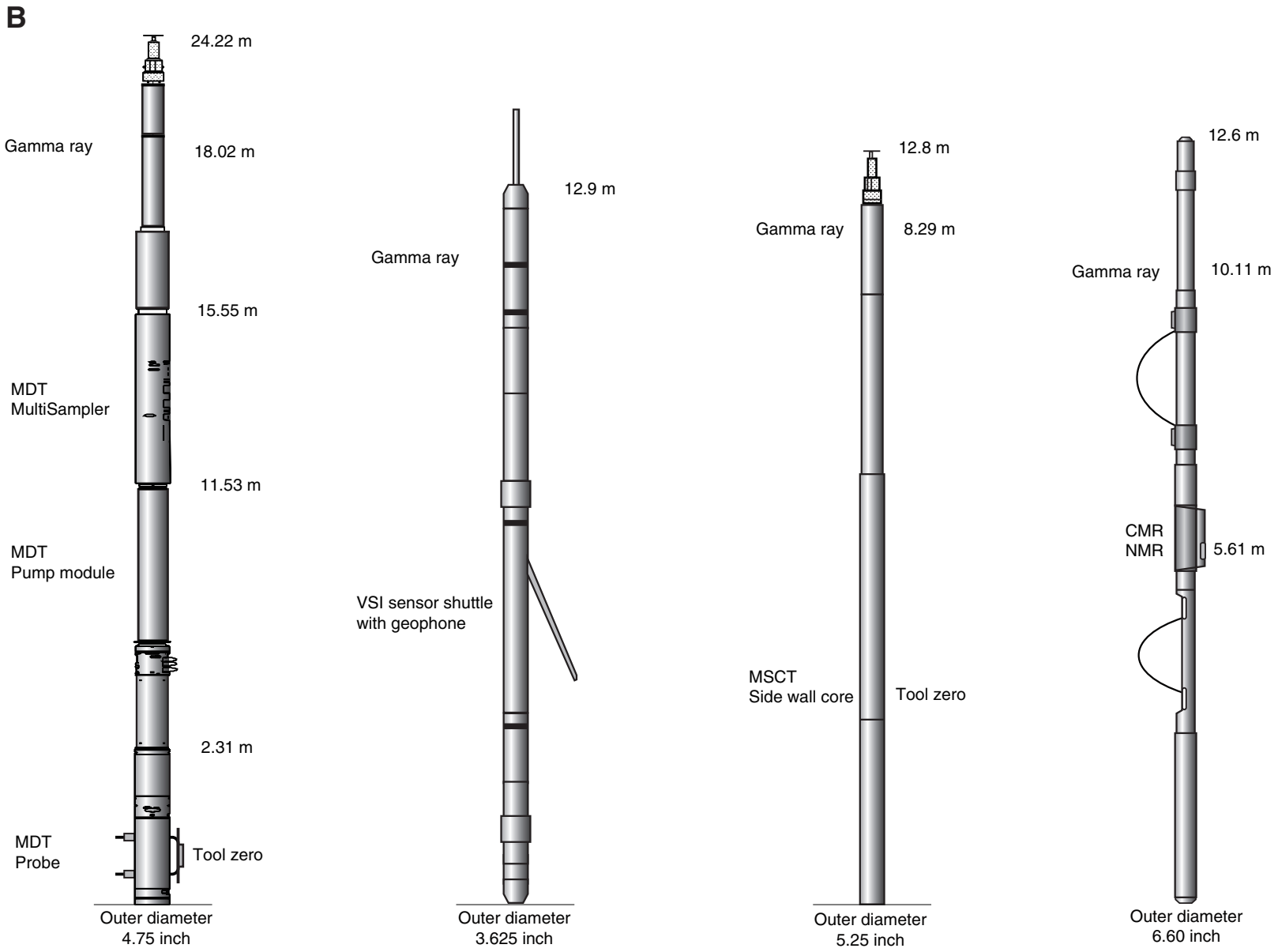
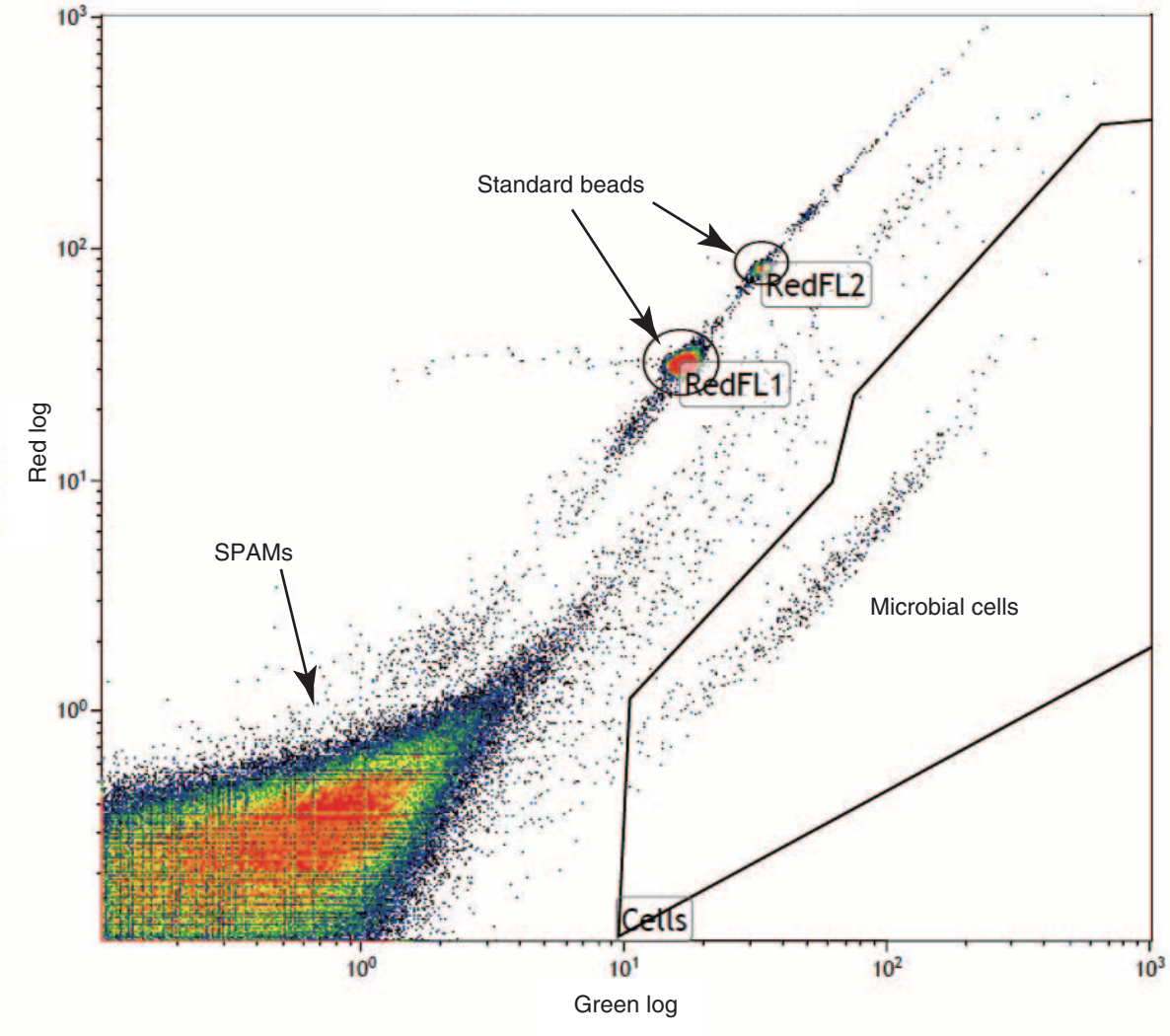


Figure F11. An image of spectrum separation of SYBR-stained microbial cells from background matrix of SYBR-stainable particulate matter (SYBR-SPAMs) using high-throughput flow cytometry.



Site summary

Site C9001

Priority:	Primary
Position:	41°10.5983′N, 142°12.0328′E
Water depth (m):	1180
Target drilling depth (mbsf):	2200
Approved maximum penetration (mbsf):	2500
Survey coverage (track map; seismic profile):	Primary lines: ODSRW03H-81~87 Crossing lines: ODSRW03H-B1~B7
Objective:	Exploration of coalbed-hydrocarbon system and deep biosphere
Drilling program:	Riserless: 0–365 mbsf Riser: 647–2200 mbsf
Logging program:	1. High-Resolution Laterolog Array (HRLA) 2. Platform Express (PEX) and Hostile Environment Natural Gamma Ray Sonde (HNGS) 3. Fullbore Formation MicroImager (FMI) and Dipole Sonic Imager (DSI) 4. Versatile Seismic Imager (VSI) for check shot 5. Combinable Magnetic Resonance (CMR) tool 6. Modular Formation Dynamics Tester (MDT) using Quicksilver Probe and InSitu Fluid Analyzer (IFA) 7. Mechanical Sidewall Coring Tool (MSCT) 650–1220 mbsf: Numbers 1–4 1220–2200 mbsf: Numbers 1–7
Nature of rock anticipated:	Hemipelagic silty clay, conglomerate, lacustrine sandstone, mudstone, and lignite coal

Figure AF1. Seismic Line ODSR03-8W at the drill site. The track line is shown in Figure F4. WD = water depth.

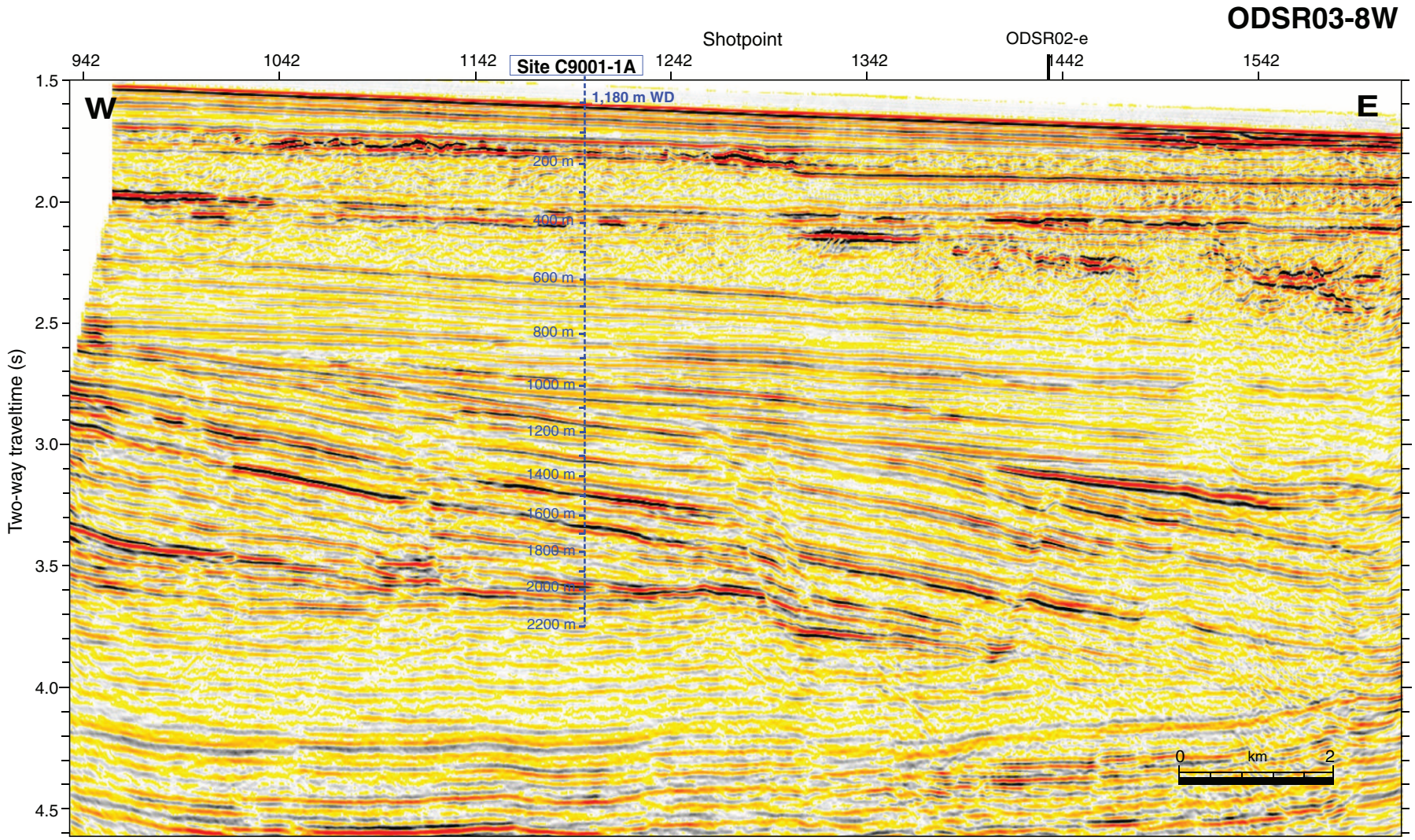
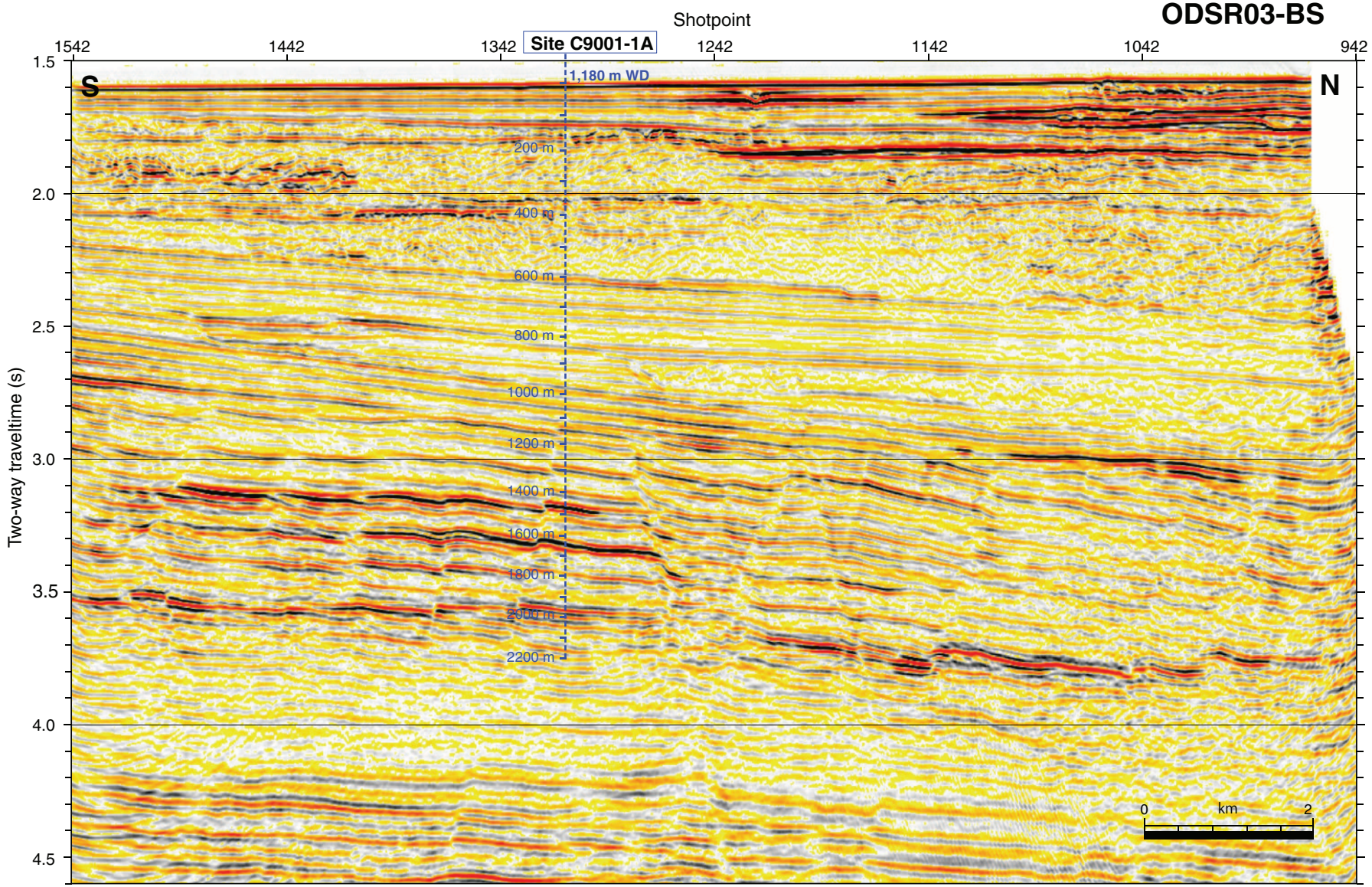


Figure AF2. Seismic Line ODSR03-BS at the drill site. The track line is shown in Figure F4. WD = water depth.



Expedition scientists and scientific participants

The current list of participants for Expedition 337 can be found at **www.jamstec.go.jp/chikyu/eng/Expedition/index.html.**