Integrated Ocean Drilling Program
Expedition 337 Preliminary Report

Deep Coalbed Biosphere off Shimokita

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

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Fumio Inagaki, Kai-Uwe Hinrichs, Yu'suke Kubo,
and the Expedition 337 Scientists

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Expedition 337 participants

Expedition 337 scientists

Kai-Uwe Hinrichs
Co-Chief Scientist
Center for Marine Environmental Sciences (MARUM)
University of Bremen
Leobener Strasse
D-28359 Bremen
Germany
khinrichs@uni-bremen.de

Fumio Inagaki
Co-Chief Scientist
Kochi Institute for Core Sample Research
Japan Agency for Marine-Earth Science and Technology
200 Monobe Otsu
Nankoku City
Kochi 783-8502
Japan
inagaki@jamstec.go.jp

Yu'suke Kubo
Expedition Project Manager
Center for Deep Earth Exploration
Japan Agency for Marine-Earth Science and Technology
3173-25 Showa-machi
Kanazawa-ku, Yokohama
Kanagawa 236-0001
Japan
kuboy@jamstec.go.jp

Yoshinori Sanada
Logging Staff Scientist
Center for Deep Earth Exploration
Japan Agency for Marine-Earth Science and Technology
3173-25 Showa-machi
Kanazawa-ku, Yokohama
Kanagawa 236-0001
Japan
sanada@jamstec.go.jp

Stephen Bowden
Organic Geochemist
Department of Petroleum Geology
University of Aberdeen
Aberdeen AB2A 3UE
United Kingdom
s.a.bowden@abdn.ac.uk

Marshall Bowles
Organic Geochemist
Center for Marine Environmental Sciences (MARUM)
University of Bremen
Leobener Strasse
D-28359 Bremen
Germany
bowlesmw@uni-bremen.de

Clemens Glombitza
Microbiologist
Institute for Earth and Environmental Sciences
University of Potsdam
Karl-Liebknecht-Strasse 24
D-14476 Potsdam
Germany

Present address (October 2012):
Center for Geomicrobiology
Department of Bioscience
Aarhus University
Ny Munkegade 114
DK-8000 Aarhus C
Denmark
clemens.glombitza@biology.au.dk

Guy Harrington
Micropaleontologist
Earth Sciences
School of Geography, Earth, and Environmental Sciences
University of Birmingham
Birmingham B15 2TT
United Kingdom
g.j.harrington@bham.ac.uk

Verena Heuer
Organic Geochemist
Center for Marine Environmental Sciences (MARUM)
University of Bremen
Leobener Strasse
D-28359 Bremen
Germany
vheuer@uni-bremen.de

Tomoyuki Hori
Microbiologist
Research Institute for Environmental Management Technology
National Institute of Advanced Industrial Science and Technology (AIST)
16-1 Onogawa
Tsukuba
Ibaraki 305-8569
Japan
hori-tomo@aist.go.jp

Tatsuhiko Hoshino
Microbiologist
Kochi Institute for Core Sample Research
Japan Agency for Marine-Earth Science and Technology
200 Monobe Otsu
Nankoku City
Kochi 783-8502
Japan
hoshinot@jamstec.go.jp

Akira Ijiri
Organic Geochemist
Kochi Institute for Core Sample Research
Japan Agency for Marine-Earth Science and Technology
200 Monobe Otsu
Nankoku City
Kochi 783-8502
Japan
ijiri@jamstec.go.jp

Mark Alexander Lever
Microbiologist
Center for Geomicrobiology
Aarhus University
Ny Munkegade 114
Building 1535-40
DK-8000 Aarhus C
Denmark
mark.lever@biology.au.dk

David Limmer
Sedimentologist
Department of Petroleum Geology
University of Aberdeen
Aberdeen AB2A 3UE
United Kingdom
dlimmer2012@gmail.com

Yu-Shih Lin
Organic Geochemist
Center for Marine Environmental Sciences (MARUM)
University of Bremen
Leobener Strasse
D-28359 Bremen
Germany
yushih@uni-bremen.de

Changhong Liu
Microbiologist
Nanjing University
22 Hankou Road
Nanjing, Jiangsu 210093
China
chliu@nju.edu.cn

Sumito Morita
Physical Properties Specialist
Institute for Geo-Resources and Environment (GREEN)
Geological Survey of Japan
National Institute of Advanced Industrial Science and Technology (AIST)
1-1-1 Higashi
Tsukuba
Ibaraki 305-8567
Japan
morita-s@aist.go.jp

Yuki Morono
Microbiologist
Kochi Institute for Core Sample Research
Japan Agency for Marine-Earth Science and Technology
200 Monobe Otsu
Nankoku City
Kochi 783-8502
Japan
morono@jamstec.go.jp

Masafumi Murayama
Sedimentologist
Kochi University
200 Monobe Otsu
Nankoku City
Kochi 783-8502
Japan
murayama@kochi-u.ac.jp

Natascha Riedinger
Inorganic Geochemist
Department of Earth Sciences
University of California–Riverside
900 University Avenue
Riverside CA 92521
USA
natascha.riedinger@ucr.edu

Young-Soo Park
Sedimentologist
Petroleum and Marine Resources Research Division
Korea Institute of Geoscience & Mineral Resources (KIGAM)
30 Gajeong-dong
Yuseong-gu, Daejeon 305-350
Korea
pysoo@kigam.re.kr

Stephen Phillips
Sedimentologist
Department of Earth Sciences
University of New Hampshire
56 College Road, James Hall
Durham NH 03824
USA
phillips.stephen.c@gmail.com

Marcella Purkey
Micropaleontologist
University of Nebraska–Lincoln
214 Bessey Hall
Lincoln NE 68588
USA
marcella.purkey@huskers.unl.edu

Doris Reischenbacher
Sedimentologist
Chair of Petroleum Geology
Montanuniversitaet Leoben
Peter-Tunner-Strasse 5
8700 Leoben
Austria
doris.reischenbacher@unileoben.ac.at

Justine Sauvage
Inorganic Geochemist
University of Rhode Island
Oceanography 215
South Ferry Road
Narragansett RI 02882
USA
justine_sauvage@my.uri.edu

Glen Snyder
Inorganic Geochemist
Department of Earth Science
Rice University
MS-126, 6100 Main Street
Houston TX 77005
USA
gsnyder@rice.edu

Rita Susilawati
Sedimentologist
School of Earth Science
University of Queensland
St. Lucia, Brisbane QLD 4072
Australia
rita.susilawati@uqconnect.edu.aucn

Wataru Tanikawa
Physical Properties Specialist
Kochi Institute for Core Sample Research
Japan Agency for Marine-Earth Science and Technology
200 Monobe Otsu
Nankoku City
Kochi 783-8502
Japan
tanikawa@jamstec.go.jp

Elizabeth Trembath-Reichert
Microbiologist
Geological and Planetary Sciences
California Institute of Technology
1200 East California Boulevard MC 100-23
Pasadena CA 91106
USA
e.t.r@caltech.edu

Wei-Li Hong
Organic Geochemist
College of Earth, Ocean, and Atmospheric Sciences
Oregon State University
104 CEOAS Administration Building
Corvallis OR 97331
USA
whong@coas.oregonstate.edu

Yasuhiro Yamada
Logging Scientist
Kyoto University
Katsura, Nishikyo
Kyoto 615-8540
Japan
yamada@earth.kumst.kyoto-u.ac.jp

Shipboard personnel and technical representatives

Captains (Mantle Quest Japan)
Yasushi Minoura
Yuji Onda

Offshore Installation Managers (Mantle Quest Japan)
Peter Hetherington
Masayuki Kawasaki

Operations Superintendents (CDEX)
Tomokazu Saruhashi
Ikuo Sawada

Drilling Engineers (CDEX)
Daiji Ikenomoto
Sho Kataoka
Tadashi Otani
Takahiro Yokoyama

Operation Geologists (CDEX)
Kan Aoike
Ryotaro Emori (JGI)
Masanori Kishima (JGI)
Takamitsu Sugihara

Technical Advisors (CDEX)
Masanoro Kyo
Eigo Miyazaki
Yasuyuki Yamazaki

Assistant Expedition Project Manager (CDEX)
Lena Maeda

Associate Logging Staff Scientists (CDEX)
Yukari Kido
Yasuyuki Nakamura
Moe Kyaw Thu

Tool Pusher/Coring Supervisor (Mantle Quest Japan)
Geoff Cook
Teruyuki Koyama
Atsushi Oikawa
Paul Thornton

Laboratory Officers (Marine Works Japan)
Satoshi Hirano
Hiroaki Muraki
Tomoyuki Tanaka

Assistant Laboratory Officers (Marine Works Japan)
Toru Fujiki
Shunsuke Miyabe
Soichi Moriya
Masahiro Nishimura
Masumi Sakaguchi

Curators (Marine Works Japan)
Yohei Arakawa
Masaru Yasunaga
Toshikuni Yabuki

Laboratory Technicians (Marine Works Japan)
Nobuhiro Anraku
Akihiko Fujihara
Yuji Fuwa
Kazuki Harumoto
Kentaro Hatakeda
Takehiro Higashi
Naotsugu Higashimaru
Yuya Hitomi
Hiroshi Hoshino
Tatsuya Kawai
Sayaka Kawamura
Daiki Kawata
Yoshiki Kido
Tetsuya Matsunaka
Yoshitomo Mochizuki
Atsushi Ohashi
Satomi Saito
Kimiko Serizawa
Hiroyoshi Shimizu
Kiyoshi Shiono
Ryuichi Shitahaku
Hiromichi Soejima
Kei Suminaga
Takahiro Suzuki
Miki Tawata
Takahiro Yabuki
Nagisa Yamamoto
Wataru Yoshioka

Publications Assistants
Rhonda Kappler (USIO)
Yoko Okamoto (Marine Works Japan)

Abstract

Integrated ocean Drilling Program (IODP) Expedition 337 was the first expedition dedicated to subseafloor microbiology that used riser drilling technology. IODP drill Site C0020 is located in a forearc basin formed by the subduction of the Pacific plate off the Shimokita Peninsula at a water depth of 1180 m. Seismic profiles strongly suggested the presence of deep, coal-bearing horizons at ~2 km subseafloor depth. Our primary objectives during Expedition 337 were to study the relationship between the deep microbial biosphere and the subseafloor coalbed and to explore the limits of life in horizons deeper than ever probed before by scientific ocean drilling. Among the questions that guided our research strategy was: Do deeply buried hydrocarbon reservoirs such as coalbeds act as geobiological reactors that sustain subsurface life by releasing nutrients and carbon substrates? To address this question and other objectives, we penetrated a 2466 m deep sedimentary sequence with a series of coal layers at ~2 km below the seafloor. Hole C0020A is currently the deepest hole in the history of scientific ocean drilling. Drilling at this site extended the previous maximum penetration depth in scientific ocean drilling by 355 m and provided the chance that our postcruise research will extend the current evidence of deepest subseafloor life by more than 800 m. Riser drilling at Site C0020 provided an unprecedented record of dynamically changing depositional environments in the former forearc basin off the Shimokita Peninsula during the late Oligocene and Miocene. This record comprises a rich diversity of lithologic facies reflecting environments ranging from warm-temperate coastal backswamps to cool-water continental shelf. The use of riser drilling technology in very deep sediment created both unique opportunities and new challenges in the study of subseafloor life. The use of drilling mud during riser drilling required implementation of a rigorous program dedicated to quality assessment and quality control of the sampled materials and data. We successfully added chemical tracers to monitor the levels of drilling mud contamination of samples and quantified levels of mud-derived solutes in interstitial fluid. These data provide the framework for differentiating signals of indigenous microbes from those of contaminants. For the first time in scientific ocean drilling, we conducted downhole fluid analysis and sampling, and logging operations yielded data of unprecedented quality that provide a comprehensive view of sediment properties at Site C0020. The estimated temperature gradient was 24.0°C/km or slightly lower; estimated temperatures in coal-bearing horizons are ~50°C and thus provide comfortable conditions, temperature-wise, for many microbes. We conducted gas analysis using a newly installed mud-gas monitoring laboratory. Gas chemistry and isotopic compo-

sitions provide the first indication of the existence of a subseafloor biosphere in deep horizons associated with the coalbed. Last but not least, this expedition also provided a testing ground for the use of riser drilling technology to address geobiological and biogeochemical objectives and was therefore a crucial step toward the next phase of deep scientific ocean drilling. Potential benefits of deep riser drilling for the scientific communities are enormous. Its implementation will require the adaptation of this technology to the needs of basic sciences.

Introduction

Marine subsurface hydrocarbon reservoirs and the associated microbial life in continental margin sediment are among the least characterized systems on Earth that can be accessed by scientific ocean drilling. Our scientific 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 drilling in natural gas and oil fields. A number of fundamentally important questions regarding deep subseafloor hydrocarbon systems have remained unanswered. For example

- What role does subsurface microbial activity play in the formation of hydrocarbon reservoirs?
- Do the deeply buried hydrocarbon reservoirs such as natural gas and coalbeds act as geobiological reactors that sustain subsurface 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, Integrated Ocean Drilling Program (IODP) Expedition 337 aimed to drill and study a hydrocarbon system associated with deeply buried coalbeds off the Shimokita Peninsula, Japan, in the northwestern Pacific using the riser drilling system of the D/V *Chikyu*.

Deep subseafloor biosphere

Subseafloor sediment harbors a remarkably sized microbial biosphere on our planet (Parkes et al., 1994, 2000; Whitman et al., 1998; Lipp et al., 2008; Kallmeyer et al., 2012; Hinrichs and Inagaki, 2012). To date, microbial cells have been observed in sediment ranging in age to the Cretaceous and in subsurface depth to 1626 meters below seafloor (mbsf) (Newfoundland margin, Ocean Drilling Program [ODP] Leg 210; Roussel et al., 2008). Diagenetic models of pore water chemical constituents as well as radioactive and stable isotope tracer 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), whereas most deeply buried microbial cells are physiologically active (Morono et al., 2009) or quiescent as the dormant phase or spore (Lomstein et al., 2012); however, the activity of microbial communities is 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 bioavailable electron donors and/or acceptors, some of which are derived either from the overlying seawater by photosynthetic primary production (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 marine subsurface environment. Culture-independent molecular ecological surveys of 16S rRNA gene fragments reveal that the microbial communities in continental margin sediment 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, 2006; Inagaki and Nakagawa, 2008). 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 sediment (Lipp et al., 2008), 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). Despite the significance of an organic-fueled microbial ecosystem in biogeochemical cycling within continental margin sed-

iment, 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, the deeply buried immature coal (e.g., lignite) is a potential source of nutrients and energy for microbial communities. Previous studies of terrestrial coal deposits suggest that microorganisms play important ecological roles in coal diagenesis, resulting in substantial quantities of coalbed methane and secondary products of microbial activities (e.g., Brown et al., 1999; Detmers et al., 2001; Fry et al., 2009; Krüger et al., 2008; Shimizu et al., 2007; Strapoc et al., 2008; Jones et al., 2008; Glombitza et al., 2009; Orem et al., 2010; Ünal et al., 2012). 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 predominant in a deep borehole aquifer directly connected to the coal deposits in Hokkaido Island, Japan (Shimizu et al., 2007). Using incubation tracer experiments and the FISH technique, active aceticlastic methanogenesis was found to occur even in a highly altered graphite deposit (Krüger et al., 2008). Fry et al. (2009) reported sizable cultivable populations of potential sulfate-reducing bacteria, methanogens, acetogens, and lignite-utilizing heterotrophs in the uplifted coaly sediment of northern New Zealand based on results from the most probable number (MPN) 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 sediment, large quantities of gaseous hydrocarbons and its derivatives (e.g., H_2, organic acids) are potentially generated by thermogenic and/or biogenic degradation processes of deeply buried organic matter like lignite coal. All of these diagenetic compounds are potential nutrient and energy sources that support energy-retrieving redox chemical

reactions mediated by the deep subseafloor microbial communities. Hence, coalbeds and microbial life may influence characteristics of dissolved gases and organic matter along with depths, as well as influencing the accumulation of gas hydrates in the shallow sedimentary sequence. In this regard, the connectivity between deep subseafloor microbial ecosystem and dynamics of deep subseafloor hydrocarbon system is a frontier research theme in geobiology and geochemistry that can only be studied by a dedicated initiative such 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 (House et al., 2006; Schrag, 2009). It has been predicted that CCS can potentially reduce future world emissions from fuel energy by 20% (Dooley et al., 2006). In a terrestrial saline aquifer located in Germany, CO_2 injection has stimulated deep microbial population and activities, indicating that deep microbial communities may adapt to the drastic environmental change and play some roles in biogeochemical cycles (Morozova et al., 2010; Krüger et al., 2011). However, the behavior and stability of CO_2 as well as its geochemical and biological reactions in deep marine subsurface repositories are still almost completely uncertain (Onstott, 2005).

Using cored samples from Expedition 337, multiple scientific issues regarding the geological CO_2 sequestration will be addressed through shore-based ex situ experimentations. For example

- How does liquid or supercritical CO_2 spatially penetrate into 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 timescales?

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 physiochemical and biological factors that determine the potential for CO_2 sequestration.

Background

Geological setting

IODP Site C0020 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: Deep Sea Drilling Project–International Phase of Ocean Drilling (DSDP-IPOD) Legs 56 and 57 in 1977, DSDP-IPOD Leg 87 in 1982, and ODP Leg 186 in 1999. In addition, well data are available from hydrocarbon drilling exploration 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 C0020 show pull-up blanking reflections below bottom-simulating reflectors (BSRs) at ~360 mbsf, suggesting the occurrence of methane hydrates in shallow sedimentary realms and a strong upward flux of free hydrocarbon gases from deep hydrocarbon reservoirs (Fig. **F2**). 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 coal formations has been confirmed by natural gas drilling exploration at Site MITI Sanriku-Oki, ~50 km southward of Site C0020 (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% total organic carbon [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 coals are buried in the ocean (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).

In 2002 and 2003, 2-D seismic surveys off Shimokita Peninsula were carried out by the Japan Agency for Marine-Earth Science and Technology (JAMSTEC) in a 15 km (north–south) × 30 km (east–west) area using the R/V *Polar Duke* and *Polar Princess*. During the NT04-01 cruise using R/V *Natsushima* in 2003, detailed bathymetry mapping was performed using a SeaBat 8160 multibeam echosounder with a frequency of 50 kHz (Taira and Curewitz, 2005) (Fig. **F2**). Site C0020, which is alternatively designated as Site C9001 by JAMSTEC, 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 core was 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 (Fig. **F1**) (Aoike, 2007). During the same cruise, riser drilling was also tested to 647 mbsf without coring at Site C9001, 20 inch casing was installed to 511 mbsf, and then the riser hole was suspended for a future riser drilling opportunity (i.e., Expedition 337).

Pilot studies of shallow subsurface sediment at Site C0020

The cored sediment from JAMSTEC Site C9001 during the *Chikyu* shakedown cruises was composed primarily of diatom-rich hemipelagic silty clay intercalated with volcanic tephra and sand layers. 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 by Thermo-View infrared camera after recovery in order to identify and locate methane hydrates. We observed methane hydrate formations as well as metabolically active microbial aggregates, which could be visualized by FISH technique, in porous ash and sandy layers. Geochemical analyses of interstitial water consistently showed that the chloride concentrations (and other sea salts) were notably depleted within the porous layers as a result of hydrate dissociation. Iodine concentrations and radioisotopic compositions ($^{129}I/I$) of deep pore waters suggest that the iodine and oldest hydrocarbon sources could be as old as 40 Ma (Tomaru et al., 2009). 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).

Microbial cell numbers in sediment from Site C9001 were evaluated by the fluorescent image-based automated cell count system, which showed that the sediments contain abundant microbial cells with counts >10^7 cells/cm^3 down to 365 mbsf (Morono et al., 2009). The abundance of bacteria and Archaea was studied by quantitative real-time polymerase chain reaction (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 organoclastic sulfate reduction, sulfate reduction coupled with AOM, aceticlastic methanogenesis, and autotrophic (CO_2 reducing) methanogenesis rates were investigated using ^{35}S and ^{14}C radiotracers, which showed high AOM activity below the SMT zone and relatively low methanogenic activity throughout the core column (F. Inagaki et al., unpubl. data). Using a sediment sample from Site C9001, the carbon and nitrogen incorporation rate of deep subseafloor microbes was studied at the single-cell level using nanoscale secondary-ion mass spectrometry (NanoSIMS) (Morono et al., 2011). A large fraction of subseafloor microbes was found to incorporate ^{13}C- and ^{15}N-labeled substrates into the biomass, indicating evidence that deeply buried microbial cells are physiologically alive.

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, phylogenetically diverse anaerobic microbes were successfully activated, including methanogens such as the genera *Methanobacterium, Methanoccoides,* and *Methanosarcina* and uncultured archaeal and bacterial lineages (Imachi et al., 2011). Several attempts at traditional batch-type cultivations also successfully led to the isolation of several subseafloor anaerobic microbes, including new genus species of *Geofilum rubicundum* (Miyazaki et al., 2012) and *Spinavirga faexivivus* (Takai et al., in press) within the order Bacterioidales. These provide direct evidence for the presence of metabolically and/or physiologically active microbial populations in deep subseafloor sedimentary habitats off Shimokita Peninsula.

In addition, during the first operation of riser drilling during the *Chikyu* shakedown Expedition CK06-06, the shift of microbial communities in the riser drilling mud tank and circulated mud fluid was examined through cultivation and cultivation-independent molecular ecological studies (Masui et al., 2008). Despite the high alkalinity of the mud circulation fluid (~pH 10), the predominance of *Xanthomonas* DNA as well as the potential growth of facultatively anaerobic and halophilic bacteria *Halomonas*

suggest the potential use of these molecular and microbiological signatures as tracers of contamination from the riser drilling mud during Expedition 337.

Scientific objectives and hypotheses

During Expedition 337, extending the riser drilling/coring depth at Site C0020 (JAMSTEC Site C9001) was originally planned to 2200 mbsf (maximum penetration depth at 2500 mbsf), where the terrigenous to shallow-marine coalbed is situated beneath the overlying marine sedimentary realm. The riser drilling exploration of the deep hydrocarbon reservoir off Shimokita provided the unique opportunity to examine geobiological and diagenetic processes associated with coal formation in deeply buried strata. No microbial life or its activities have been documented to date at the maximum targeted burial depths in any marine environment. Expedition 337 was driven by three overarching testable hypotheses:

1. The deeply buried coalbeds act as geobiological reactors that release dissolved organic compounds such as methane, acetate, and other substances.
2. The conversion and transport of the 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 future activities of 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 addressed during Expedition 337 are 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;
- Assess the limits of life and potential geochemical and geophysical constraints to microbial population, diversity, metabolic activity, and functioning in the deep biosphere; 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.

We also 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?
- How do minerals and organic matter react in the coalbed formation, how will this change the physical and chemical characteristics, and how will it affect the microbial communities?
- What paleoenvironmental information and sedimentary regimes are recorded at Site C0020?
- 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?

In order to address these scientific objectives, we performed (1) spot coring of marine sediments and coalbed layers, (2) wireline logging of various geophysical and geochemical properties in situ, and (3) sampling of in situ formation fluids associated with coalbeds using a wireline fluid sampling tool. These materials and data are used for extensive microbiological, biogeochemical, geological, and geophysical analyses on board the ship and in shore-based laboratories. Achieving these scientific objectives 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. These applied scientific aspects will add an important new component to IODP.

Operations

The *Chikyu* left Hachinohe Port at 1200 h on 26 July 2012 and arrived at Site C0020 after 7.5 h of transit. The corrosion cap of Hole C9001D, which was drilled to 647 m drilling depth below seafloor (DSF) and cased to 511 m DSF during the *Chikyu* shakedown cruise in 2006, was retrieved at the surface by 2400 h on 27 July. Ten transponders were installed on the seafloor by remotely operated vehicle on 28 July, and failure mode effect analysis for the Acoustic Position Reference System was completed on 29 July.

While conducting a surface pressure test of the blowout preventer (BOP), the science party was shuttled aboard by helicopter flights on 31 July. Technical problems were found during the BOP function test, and troubleshooting continued until 8 August. A successful BOP landing was confirmed on 11 August. Extensive BOP tests were carried out until completion of the pressure test on 14 August. Because of the delay in operations, planned spot coring in the 647–1220 mbsf interval was cancelled and a 17½ inch hole was started to drill out cement and reach 1220 mbsf.

On 16 August, we encountered total mud loss at 2375 m drilling depth below rig floor (DRF). After spotting lost circulation material twice, the mud loss rate decreased to 0.8 m^3/h. A decision to continue drilling with seawater gel mud was made.

Drilling resumed on 18 August and reached 2471.5 m DRF (1263 m DSF); 13⅜ inch casing was installed to this depth. Coring operations started on 25 August.

Riser drilling with spot cores continued to Core 337-C0020A-7R, which hit a low rate of penetration (ROP) interval at 2812.5 m DRF (1604.0 m DSF). The core included gravels of volcanic origin and showed different lithology from previous cores. The Co-Chief Scientists decided to take an industry-type large diameter core (LDC) at this depth.

LDC operations were stopped at 2834 m DRF before reaching the 27 m of drilling advance in the original plan, as core jamming was suspected when an increase in pump pressure and no penetration were observed. The LDC was recovered on deck on 30 August and 10.0 m of core was recovered from 21.5 m of advance. The core was cut into 1.0 m long sections at the middle pipe rack and transferred to the laboratories.

Rotary core barrel (RCB) coring resumed and Cores 337-C0020A-9R to 14R were taken before continuous coring through the coal-bearing horizons started at 1919 m DSF.

After four consecutive RCB cores were recovered, the drill bit appeared to be worn out. Consequently, it was decided to change the drill bit.

After coring of Core 337-C0020A-18R, it was also decided that we continue RCB coring and cancel planned LDC operations. The new drill bit was installed and coring operations resumed on 4 September. Another seven consecutive RCB cores were taken from 3158.5 to 3212.0 m DRF (1950–2003.5 m DSF). Core recovery was high, and coal-bearing sequences were obtained. As we were able to sample various lithologies within and around the coal-bearing formation, the Co-Chief Scientists concluded that we fulfilled our operational mission in this interval. They decided to drill deeper to investigate the broader range of the hydrocarbon system and to explore the limits of life.

We deepened the hole while spot coring in 100 m intervals and reached the terminal depth of Hole C0020A at 2466 m DSF on 9 September. After pulling out of the hole, wireline logging operations started.

Logging Run 1 (Platform Express [PEX]) started on 10 September, followed by Runs 2 (Formation MicroImager [FMI]) and 3 (combinable magnetic resonance [CMR]). High-permeability layers were selected for the Modular Formation Dynamics Tester (MDT) based on the results from the first three runs. After a wiper trip, Run 4 (MDT-gamma ray [GR]) started on 12 September. Pretests for fluid mobility and formation pressure measurements were carried out at 31 horizons. Formation fluid samples were taken at 6 horizons of high mobility, and the 6 bottles were recovered on deck on 14 September. The sample bottles were delivered to the laboratory during the following Run 5 (vertical seismic profile [VSP]). The last run was completed on 15 September, ending scientific operations on the rig floor.

This expedition was originally scheduled for March–May 2011 but was postponed because of the Tohoku earthquake and the following tsunami hazard, which hit the eastern coast of Japan, including Hachinohe Port. The *Chikyu* suffered damage on the ship body and lost one of the thrusters during emergency evacuation from the port. Repair and reinstallation of the thruster were completed in June 2012, and this expedition was rescheduled for implementation in July 2012.

Site C0020 site summary

Expedition 337 was the first expedition dedicated to subseafloor microbiology using riser drilling technology. Hole C0020A is the deepest hole in the history of scientific ocean drilling. On 9 September 2012, we terminated drilling at a total drilling depth of 2466 mbsf. Site C0020 is the extension of JAMSTEC Hole C9001D drilled during the *Chikyu* shakedown cruise in 2006 (Aoike, 2007), when drilling pilot holes by both riserless and riser drilling systems was terminated at 647 mbsf and 36 and 20 inch casings were installed to 511 mbsf. During Expedition 337, we drilled from 647 mbsf to the final depth of 2466 mbsf (Table **T1**).

The use of riser drilling technology in very deep sediments created both unique opportunities and new challenges for the science party. Our experiences and results will be of great strategic value to future missions of deep scientific ocean drilling. Our scientific objectives focusing on the deeply buried coalbed strictly required the use of riser drilling technology. This technology enabled implementation of several operations of direct relevance to the Expedition 337 scientific objectives. For example, the use of a newly installed mud-gas logging container provided the opportunity to monitor a range of biogeochemically relevant gases, including real-time measurements of isotopic compositions of methane. For the first time in scientific ocean drilling, we conducted downhole fluid analysis and sampling, and logging operations yielded data of unprecedented quality. At the same time, the drilling technology, in particular the use of drilling mud, created substantial obstacles that posed considerable threats to the scientific success of Expedition 337. Riser drilling mud is saline, alkaline, contains a multitude of organic compounds, and, most importantly, also high microbe concentrations. Our strategies to detect, quantify, and minimize contamination as well as to deal with residual levels thereof have consequently played an important role for the shipboard scientific program and will undoubtedly require careful interpretation of all future data. A substantial portion of this preliminary report will therefore be dedicated to quality assessment (QA) and quality control (QC) of the sampled materials and data. Nevertheless, from an operational point of view this expedition was extremely successful: we carried out nearly all operations, we drilled and sampled several coalbed layers, and we were able to drill 266 m deeper than our initial target depth. The recovered samples hold the potential to extend the generally accepted evidence of deepest life below the seafloor (Roussel et al., 2008) by more than 800 m and provide the opportunities to address all major objectives related to the relationship of the deep coalbed and microbial life. In the following, we will provide principal

results according to discipline followed by a synthesis of currently available information as pertaining to our scientific objectives.

Lithostratigraphy and biostratigraphic age constraints

Riser drilling during Expedition 337 provided an unprecedented record of dynamically changing depositional environments in the former forearc basin off the Shimokita Peninsula during the late Oligocene and Miocene. This record is composed of a rich diversity of lithologic facies reflecting environments ranging from warm-temperate coastal backswamps to cool-water continental shelf. Four distinct lithologic units were identified in Hole C0020A on the basis of combined analysis of cuttings and cores and assisted by inspection of X-ray computed tomography (CT) scan images and wireline logging data (see Figs. **F3, F4, F5**).

Shipboard micropaleontology included diatoms, calcareous nannofossils, organic-walled dinoflagellate cysts (dinocysts), pollen, and spores. Micropaleontology was able to successfully secure an age of early Pliocene at the top of Hole C0020A at 647 mbsf and indicate a probable age of late Oligocene–early Miocene at the base of the hole at 2466 mbsf (Fig. **F3**). Diatoms were recovered from the upper parts of Hole C0020A but were absent, or very poorly preserved, throughout most of lithologic Units II, III, and IV (defined below). The samples could be loosely dated, and to 1076.5 mbsf, all samples were consistent with Pliocene age. Diatoms were identifiable until the base of Unit I and appear to be Miocene in age; however, marker species were not identified and the boundary between the early Pliocene–late Miocene was not observed. Calcareous nannofossils were rare and poorly preserved. Hence, calcareous nannofossils did not inform the age model presented here.

- Unit I (647–1256.5 m mud depth below seafloor [MSF]) consists primarily of diatom-bearing silty clay. This unit resulted from sedimentation in an offshore marine environment. Diatoms were best and most abundantly preserved in Unit I among monitored microfossils together with predominantly heterotrophic dinocysts. Diatom floras in Unit I are consistent with a Pliocene cool-water continental shelf succession. Heterotrophic dinocyst communities feeding off diatom blooms are suggestive of elevated marine productivity.
- Unit II (1256.5–1826.5 m MSF) consists mostly of silty shale with some interspersed intervals of sandstone and siltstone. Cuttings samples show a lower amount of sand and an increase of silt at the Unit I/II boundary. The abundance of biogenic siliceous material, glauconite, and plant remains also differentiate Unit II from the

overlying unit. Unit II was subdivided into two different subunits: sandstone and siltstone associated with marine fossiliferous material (Subunit IIa; 1256.5–1500 m MSF) and organic-rich shale and sandstone associated with plant remains (Subunit IIb; 1500–1826.5 m MSF). The upper part of Unit II rather represents an offshore environment, possibly with a paleoposition close to the shelf margin; with increasing depth the paleoenvironment gradually changes into a shallow-marine setting. The bottom part of Unit II is situated in the intertidal zone. This shift is consistent with microfossil assemblages that exhibit few identifiable diatoms and poor dinocysts; reworked dinocyst in Unit II, as in deeper units, have Paleogene ages that broadly fall in the range of early middle Eocene–late Oligocene. Pollen and spores are moderately well represented but are abundant near the base of Unit II, which is consistent with increasing terrestrial influence in shallow-marine sediment.

- Unit III (1826.5–2046.5 m MSF) is dominated by several coal horizons, which we subdivided into coaly shale, siltstone, and sandstone. Almost all coal horizons consist of fine-detritic to xylodetritic coal with some layers of xylitic coal. Water content, color, and vitrinite reflectance measurements of the coal suggest that the coal has low maturity (Fig. F6). Bioturbation and sedimentary features like flaser bedding, lenticular bedding, or cross bedding suggest a nearshore depositional environment with tidal flats and tidal channels. The presence of siderite bands at the bottom of this unit suggests a back-barrier marine environment in combination with wetlands (e.g., salt marsh or swamp). Small terrestrial influence might occur within sand bodies that overlie coal horizons. This could be due to channels from deltaic environments. Unit III contains excellent pollen and spore assemblages in the coal and associated terrestrial to coastal shallow-marine sediment. However, dinocysts are scarce and contain few useful biostratigraphic markers. The pollen floras tentatively suggest a likely age of early middle Miocene for Unit III.
- Unit IV (2046.5–2466 m MSF) is dominated by silty shales in the upper part, sandstone intercalated with siltstone and shale associated with sand in the middle part, and sandstone intercalated with silt and a thin coal layer in the lower part. Wireline logs and cuttings samples suggest a thick homogeneous shale layer between the Unit III boundary and Core 337-C0020A-27R (2200 mbsf). The depositional environment of Unit IV resembles that of Unit III, except that the former contains only one thin coalbed. Like Unit III, Unit IV experienced high-frequency fluctuations of the depositional environment. Within a few meters, there are sediments related to tidal flats and tidal channels, which are overlain by organic-rich material of a marsh that resulted in formation of peat. The pollen floras place a maximum age of late Oligocene for the base of Unit IV.

Physical properties

A series of physical properties measurements were performed on core and cuttings samples from Hole C0020A. Gamma ray attenuation density, magnetic susceptibility, natural gamma radiation, *P*-wave velocity, and noncontact electrical resistivity were measured with the multisensor core logger. Measurements of thermal conductivity were made mostly on working-half cores. Discrete samples taken from working-half cores and some whole-round core samples were applied to moisture and density (MAD) analyses to calculate porosity, bulk density, grain density, and water content. *P*-wave velocity analysis and electrical impedance analysis were made on cubic discrete samples. Cuttings samples were also applied to MAD analysis. Cuttings samples were separated into four categories: original bulk and sieved size categories of >4.0, 1.0–4.0, and 0.25–1.0 mm. Large-size fraction of the cuttings samples were cut off cubic samples and applied to the *P*-wave velocity analysis and the electrical impedance analysis. Anelastic strain recovery analysis was made on some whole-round cores. Vitrinite reflectance analysis was performed on some coaly samples, indicating generally low maturity of coal in Hole C0020A. Porosity of siltstone, sandstone, and shale gradually decreased with increasing depth (Fig. **F7**). Porosity corresponds to lithologic variation, with carbonate-cemented sandstone and siltstone having much lower values than noncemented sandstone and siltstone. The porosity of coal does not deviate from the major trend of the other lithologies, although the coal may change the figures because of decompaction after the recovery. The cuttings also show a gradual decrease in porosity but have generally higher values than the core samples. Discrete core samples are likely more representative of in situ porosity than cuttings.

Downhole logging

Because of the very good borehole condition and relatively simple lithology, logging data of excellent quality was obtained from Hole C0020A. The relatively simple lithology consists of sandstone, siltstone, shale, coal, and conglomerates, most of which show typical log response and are intercalated by a number of marker layers (i.e., coal and cemented sandstone). This resulted in straightforward interpretation of logging data with respect to lithology, compensated for our lack of cored materials in the majority of the drilled section, and ultimately led to the establishment of a database that fully reconstructs the sedimentation history at Site C0020 (Fig. **F5**).

Log characteristics suggest that Unit I lithology is similar to that of Unit II, which consists of alternation of relatively thick layers of massive sandstones and siltstones. Unit

III is characterized by frequent coal layers in a few meters thickness of sandstone and siltstone alternation sequence. Based on correlations of log data and visual core descriptions, 7 coal layers, including the thickest 2, were acquired by coring from a total of 13 identified layers with thicknesses >30 cm. Unit IV consists of thick (~200 m) massive shale in the upper half; the lower half exhibits alternations of sandstone and shale of a few meters thickness each. One thin coal layer was observed in Core 337-C0020A-30R, but log data suggest that this coal represents a single depositional event in Unit IV.

Resistivity borehole images suggest that the sandstones in Unit II are massive and include conglomerates, whereas those in Units III and IV consist of thin sandstones of centimeter thickness or lamina of this scale, suggesting a change in the sedimentary environment at the Unit II/III boundary. By combining the logging data and core descriptions, sandstones that may be of high permeability were identified in Units II and III.

By using a formation-testing tool, fluid samples were acquired from six permeable sandstone layers. The 31 "pretest" measurements prior to fluid sampling indicate that formation pore pressure is hydrostatic or elevated by only a few percent of the hydrostatic value to depths of at least 2425 m wireline log matched depth below seafloor (WMSF) (the depth of the deepest reliable measurement).

Borehole temperature was measured with two types of logging tools. The maximum temperature at the bottom of Hole C0020A was estimated by examining the temperature build-up pattern during the logging operation. The estimated temperature gradient was 24.0°C/km or slightly lower (Fig. **F8**).

Preliminary log-seismic integration was carried out based on the time-depth curve derived from VSP operation and synthetic seismogram calculation. The time-migrated seismic profile for seismic survey Line ODSR03-BS (Taira and Curewitz, 2005) was converted with the time-depth relationship and compared with logging data. Strong reflectors are basically well correlated with the abrupt change on the logging curves.

Geochemistry

Expedition 337 investigated the role of the Shimokita coalbed as a potential energy and carbon source for the deep biosphere. In this context, geochemical studies are aimed at elucidating the cycling of carbon and nutrients, conversion and transport of

hydrocarbons, the flux of both thermogenically and biologically produced organic compounds, their utilization by the deep biosphere, and the impact of deep hydrocarbon sources on the carbon budget of the shallower surface. To this end, geochemists investigated solid phase, gas, and fluid samples at Site C0020.

Organic matter quantity and origin

Shipboard analysis of solid phase samples showed a strong lithologic control of the TOC contents in sediment: coal had the highest average TOC contents (40.9 ± 9.9 wt%), followed by clayey (1.4 ± 1.0 wt%), silty (0.43 ± 0.29 wt%), and sandy (0.26 ± 0.18 wt%) materials (Fig. **F9**). The coal layers in Unit III were evident not only in the high TOC contents of visually identified coal layers in sediment cores but also in the elevated TOC contents of randomly sampled cuttings. In general, TOC contents in cuttings were slightly higher than in the corresponding cores, possibly because of mixing effects with the drilling mud. Since land plants contain less nitrogen than protein-rich marine plankton, the atomic ratio of TOC and total nitrogen (TN) is a useful first indicator for the origin of organic matter. Throughout cores taken form Hole C0020A, TOC/TN ratios ranged from 3 to 58, with higher values indicating a stronger contribution of higher land plant-derived organic matter to TOC. Like TOC contents, TOC/TN ratios were controlled by lithology. The highest TOC/TN ratios of ~58 were observed in coal. In general, clayey sediment had TOC/TN ratios of ~22, which are still indicative of terrigenous organic matter, whereas sandy and silty lithologies showed lower TOC/TN ratios of ~13 that point to marine organic matter sources. However, the clastic sediment had a large within-group variation in the TOC/TN ratio (1σ = 7–10), making it difficult to link the origin of organic matter (terrestrial or marine) to lithological compositions. The total sulfur (TS) values were generally low (from below detection to 1.4 wt%) and showed no clear association with lithology or specific trends with depth. The cluster of samples with low TS contents in Unit III contained samples of different lithology, including coal. Rock-Eval pyrolysis (e.g., Tissot and Welte, 1984) provided some initial information on the type and maturity of organic matter. In Units II–IV, most of the core samples yielded maximum temperature (T_{max}) values in the range of 400°–440°C, indicating organic matter in a thermally immature to early mature state. The average values of parameters S_2, S_3, and hydrogen index (HI) decreased in the order of coal > clayey materials > silty or sandy materials, suggesting higher contents of hydrogen-rich organic matter in fine-grain sediment and coal (Fig. **F9**).

Biomarker evidence for origin and thermal maturity of organic matter

Further insights into the origin and thermal maturity of organic matter come from the analysis of lipid biomarkers. Shipboard lipid data suggest that terrestrially derived organic material is the main contributor to the organic carbon contained in sediment at Site C0020. A large proportion of *n*-alkanes with an odd carbon number predominance in the C_{29} range and *n*-alkanoic acids with an even carbon number predominance in the C_{26} range, as well as the general predominance of C_{29} sterenes derived from C_{29} sterols abundant in higher plants (Fig. **F10**), suggest that terrestrially derived organic matter substantially contributes to TOC (Huang and Meinschein, 1979). The extent of this contribution appears to reach a maximum close to the transition from Unit I to Unit II (~1200 mbsf), with another maximum near the coal-bearing horizons.

In addition, the degree of thermal alteration of sediment can be gauged from the conversion of sterenes to steranes and using biomarker data previously calibrated against temperature in the Hokkaido region (Amo et al., 2007). A critical point for this process in the subsurface is the temperature range of 40°–60°C (Amo et al., 2007). The extent of sterene to sterane conversion for Site C0020 is consistent with previous work and a thermal gradient in the 24°C/km range, as well as the bottom-hole temperatures measured during the expedition (Fig. **F11**).

Gases

For the composition of gases above, in, and below the Shimokita coalbed, about 1 million discrete data points could be recorded by mud-gas monitoring (i.e., the continuous extraction and on-line analysis of gases that are brought up from the formation to the *Chikyu* with the recycling of drilling mud during riser drilling). Mud-gas monitoring was supplemented by gas analysis in >100 samples from cuttings and cores. Analysis focused on the content and carbon isotopic composition of methane but also included higher hydrocarbon gases, N_2, O_2, Ar, H_2, and CO. Unfortunately, the conversion of mud-gas contents into absolute gas concentrations in the drilled rocks is not straightforward, as the former depend on drilling parameters such as ROP, mud flow, mud weight, and mud-to-headspace ratio in the mud-gas separator, all of which vary in the course of operations. However, the relative ratio of gas species in the mud gas is very informative and this is particularly true for the C_1/C_2 ratio. Since the occurrence of major amounts of higher hydrocarbon gases is associated with thermogenic hydrocarbon generation, high C_1/C_2 ratios indicate biogenic methane formation. The downhole profile of C_1/C_2 ratios at Site C0020 points unambiguously

to the predominance of biogenic methane sources (Fig. F12). Interestingly, the ratios found in mud gas of coal-bearing sediment horizons at ~2000 mbsf showed a strong positive inflection, suggestive of an active source of biologically produced methane. O_2/Ar, N_2/Ar, and H_2/Ar ratios were used to monitor corrosive processes during drilling. O_2/Ar and N_2/Ar ratios resembled atmospheric values throughout the drilling process, and the lack of oxygen consumption suggests that only little corrosion occurred. Nevertheless, H_2 contents were distinctly elevated above atmospheric levels in mud gas and core samples, and the extent to which H_2 concentrations are impacted by drilling activities is not fully resolved at this point.

In summary, shipboard solid phase and gas analysis suggests that organic matter from predominantly terrigenous sources is available for the deep biosphere not only in coal but also in TOC-rich clay. Moreover, organic matter is apparently thermally relatively immature to 2466 mbsf and rich in hydrogen, which might be released as molecular H_2 in the course of diagenesis. Finally, C_1/C_2 ratios suggest that methane is predominantly formed by biogenic processes.

Fluids

Expedition 337 is the second riser drilling expedition, and Hole C0020A is also only the second hole from which interstitial water has been retrieved from riser drilled cores (following IODP Expedition 319 Hole C0009A; Expedition 319 Scientists, 2010). During this expedition we recovered interstitial water from 2405 mbsf, extending the world record for deepest interstitial water recovered by 820 m (Expedition 319 Scientists, 2010). In addition, this is the first cruise to obtain larger volume formation water samples from specific lithologic intervals in the borehole using Schlumberger's Quicksilver device. A total of six formation water samples were sampled downhole, from 1279 to 1978 mbsf, also breaking the previous world record for deepest fluids recovered from marine sediment during scientific ocean drilling.

Recovering fluids from such deep sediment is not without its challenges, however. Sediment cores at this depth have decreased porosity (see Fig. F7), are prone to fracturing along laminations, and are easily contaminated by drilling mud fluids. Pressure changes during core recovery are also likely to provoke phase changes in dissolved gases as well as expansion of the cores along fracture planes. Because of low porosity and permeability, only 24 of the processed 48 whole-round cores yielded interstitial fluid through squeezing, with low volumes between 0.2 and 33.5 mL despite large subsamples (up to 70 cm per whole-round core for selected segments) (see Fig. F13). Of the 24 cores that yielded pore water, 15 were from sediment in Unit II, 7 from

Unit III, and 2 from Unit IV; the relationship between interstitial water yields and lithology is also illustrated in Figure **F13.**

The shipboard inorganic chemistry during this expedition was carried out in conjunction with a variety of microbiological and biogeochemical analyses aimed at characterizing the processes involved in methanogenesis within deeply buried coal layers. The core section intervals devoted to pore water squeezing were selected adjacent to the intervals used by the microbiologists. As such, one important objective of the inorganic chemistry group was to characterize the degree of contamination of the interstitial water caused by the drilling process and also assess contamination of the sampled formation water (see below).

In addition, this was the first scientific expedition where the inorganic chemistry of both drilling mud and drilling cuttings data were analyzed systematically. Because there was no coring through Unit I (i.e., 647–1256.5 mbsf), the only geochemical information over that depth interval was from the drilling cuttings recovered from the circulating drilling mud and mud-gas logging. Whereas the cuttings did not, in the end, provide extensive information about the pore water from the depths at which they were obtained, they did provide some information about the interaction between drilling mud and highly contaminated samples.

Microbiology

Expedition 337 was the first riser ocean drilling expedition to incorporate extensive shipboard microbiological and molecular biological analyses. These were performed with state-of-the-art equipment in the designated microbiology laboratory aboard the *Chikyu*. Because the target sedimentary habitat is strictly anaerobic, all the cored materials recovered on board the ship were immediately processed for shipboard and shore-based microbiological analyses under the N_2-flushed anaerobic condition.

Previous studies in continental margin sediment showed that microbial population and activity generally decrease with increasing sediment depth (e.g., Parkes et al., 2000). However, various geophysical and geochemical factors that constrain the extent of the deep biosphere remain unknown. Therefore, one of the key objectives of the Expedition 337 microbiology program was the quantification of microbial cells and the detection of molecular signatures of "indigenous" deep subseafloor life from sediment cored by riser drilling. In deeply buried horizons below 1000 mbsf, microbial cell numbers approach the detection limits of most established protocols. There-

fore, various cell counting methods, partly in combination with techniques separation and concentrations of cells, were applied to Expedition 337 samples (e.g., manual cell count, image-based cell count, high-spec flow cytometry, and density gradient cell separation). To implement these ultra-sensitive assays for the detection of deep life, QA/QC is extremely important. The rather high microbial cell concentrations in riser drilling mud and fluid of at least 100 million cells/cm^3 required careful monitoring of potential core samples contamination by chemical tracers added to the drilling mud, examination of samples for molecular (DNA) fingerprints of contaminant microbes, and monitoring of pore fluid for ionic species enriched in drilling mud.

During Expedition 337, all microbiological sample processing, cell counts, cultivation, and molecular studies were successfully performed with special aseptic care in the microbiology laboratory on the *Chikyu*. Preliminary results of cell detection and enumeration show that extremely small cells are present at very low cell concentrations in deep samples below 1000 mbsf. These cells potentially represent the deepest subseafloor life that has ever been studied through scientific ocean drilling (Fig. **F14**). Together with geochemical anomalies that suggest the presence of biological activity related to the deep coalbed, some important functional genes that mediate carbon-conversion metabolisms were consistently detected in our samples. However, community-fingerprinting analysis based on PCR-amplified 16S rRNA genes showed that even very carefully collected samples are not free of signals from contaminant microbes; careful examination of all available lines of evidence is required for obtaining a comprehensive view of the potentially deepest subseafloor ecosystem ever studied.

To more fully address some of the primary scientific objectives, various types of microbiological and biogeochemical samples were prepared for shore-based studies, which include more than 1700 samples that will be used for stable isotope probing (SIP) combined with NanoSIMS experiments, quantitative functional gene surveys, whole shot gun metagenomics and single cell genomics, batch-type and bioreactor cultivation experiments, and geobiological application of CO_2 capture and sequestration (see Fig. **F15**). In addition, biomineralogical studies are planned for some minerals that likely precipitated in the course of modern and/or past geomicrobial processes, such as pyrite and siderite formation associated with the deeply buried coalbeds. In conclusion, the goals of the shipboard microbiology program were successfully accomplished, and extensive research using samples and data collected during Expedition 337 will significantly expand our knowledge of the deep, dark, and old

subseafloor biosphere and contribute to the better understanding of the biogeochemical carbon cycle.

Expedition-specific challenges, risks, and future perspectives

Quality assurance/Quality control

The riser drilling technology and the associated use of drilling mud had considerable impact on our scientific program. Whereas contamination control has become an integral measure of quality assurance in ODP/IODP expeditions with focus on subseafloor life (Smith et al., 2000a, 2000b; House et al., 2003; Lever et al., 2006), the riser drilling procedure required a more rigorous QA/QC program during Expedition 337. Most severely affected by the contamination risk were the scientific objectives of the fluid chemistry and microbiology disciplines. Therefore, our sampling programs of both fluid chemistry and microbiology included routine monitoring of the integrity of samples selected for interstitial water and microbiology analysis by X-ray CT scanning (see Fig. **F16**). Heavily disturbed or fractured samples were returned into the normal core flow before processing, and alternative samples with lower risk of contamination were selected. An example of such heavily disturbed, and thus contaminated, sediment interval is shown in Figure **F17.** Since drilling mud was highly divergent in chemical composition from sediment pore water, even minor contamination would complicate the accurate analysis of routinely measured interstitial water parameters such as sulfate, pH, or alkalinity, all of which are directly relevant to the study of subseafloor life. Moreover, microbial cell concentrations in drilling mud were consistently $>10^8$ cells/cm^3. This is at least three orders of magnitude higher than pelagic seawater used during past riserless drilling expeditions (Lever et al. 2006) and 2–5 orders of magnitude higher than expected concentrations of indigenous cells in sediment deeper than 1000 mbsf (Parkes et al., 2000), and thus increases the risk of contamination with nonindigenous cells substantially compared to previous riserless drilling operations.

The use of both chemical and microbial contamination tracers were pioneered for riser scientific ocean drilling as part of an extensive QA/QC program. Contamination monitoring with a perfluorocarbon (PFC) compound as a chemical tracer utilized a modified version of past protocols (Smith et al., 2000a, 2000b; House et al., 2003; Lever et al., 2006). PFC tracer was added daily to drilling mud tanks. Detailed sampling and analyses of drilling mud, sediment cuttings, and core samples provided valuable

quantitative estimates of the volume of drilling mud and number of cells introduced into samples during riser drilling (Fig. **F16**). PFC concentrations monitored within drilling mud in tanks, core liner, and in the mud ditch after recovery show consistently high values and low loss during drilling operations (mean concentration > 100 µg/L). PFC measurements within cores show a wide range of values, with high contamination near the core liner (exterior; typically 1–100 µL drilling fluid/g sediment) and low values in the core center (interior; 0.01–1 µL/g). DNA-based contamination tests targeting organisms associated with surface seawater, drilling mud viscosifiers, and sewage reveal drilling mud viscosifiers as the main source of drilling-induced microbial/DNA contamination. Cell counts on deep sediment cores indicate detectable, but very low cell densities to the bottom of the borehole.

Data obtained during Expedition 337 demonstrate the suitability of PFC tracers to monitor contamination during riser drilling operations and indicate that many core samples obtained have low to nondetectable levels of contamination at the core center. The successful detection of cells and DNA demonstrates that monitoring of microbial populations in cores obtained by riser drilling is possible on board the ship. Shore-based molecular analyses and cultivation experiments in the coming years will reveal the extent to which cells and DNA detected represent indigenous microbial communities, and, if so, what the metabolism of these microbes is. Cultivation assays will be carefully monitored for marker genes of potential contaminants and designed to specifically select for deep subseafloor rather than contaminant microbial populations (cf. Fig. **F15**).

During fluid sampling and analyses of inorganic constituents, we sought to minimize contamination by relying on prior information of X-ray CT scan images and by thoroughly peeling off outer layers that were in contact with the core liner and thus drilling mud and avoiding fractures as much as possible. Nevertheless, residual levels of contamination were not avoidable as illustrated in Figure **F18.** The fraction of drilling mud contamination in the total interstitial water was calculated assuming binary mixing of several major ions that are present in much greater concentrations in the drilling mud than in interstitial water. Likewise, we assumed that the degree of natural variation of these ions in the interstitial water composition was small compared to variation caused by contamination. The most contaminated samples were typically sandy mudstone at the top of Unit II. Results were variable based on the ion chosen but provide some indication of the degree of contamination. For example, the mud-derived water fraction of pore water in the most severely contaminated, shallowest whole-round sample (337-C0020A-1R-2, 0–65 cm, sandy, 1278 mbsf) varies from 65%

to 88% depending on whether salinity, K^+, SO_4^{2-}, or Cl^- are considered as the interstitial water end-members in binary mixing. Consequently, corrections of other interstitial water constituents for their dilution with drilling mud is associated with inherent, residual uncertainties. For the majority of interstitial water samples obtained toward the bottom of Unit II and deeper, contamination is significantly lower. In these horizons, we estimate a range of contamination from 2% to 20%.

Considerable challenges were also posed on organic geochemical and micropaleontological analyses by contamination from drilling mud. TOC concentrations of cuttings were suspiciously elevated relative to core samples in the nearby horizon. Lipid extracts of cuttings and some core samples showed molecular signatures of the asphalt-based drilling additive ASTEX (trade name; also known as sulfonated asphalt sodium salt [SAS]); cuttings in shallower horizons of Hole C0020A showed other unidentified, highly concentrated contaminants that probably result from the drilling technology (Fig. **F19**). During micropaleontological analysis, core samples, core catchers, and especially cutting samples for diatom analysis were affected by a memory effect of younger assemblages admixed to the drilling mud. For example, relatively young diatom assemblages representative of shallower, late Miocene and Pliocene strata were abundant in drilling mud and complicated the use of cuttings for diatom-based chronostratigraphy. Focus on undisturbed core samples eliminated this problem but also substantially lowered the resolution in our analysis of age-relevant marker fossils.

Preliminary evidence for deep life and its stimulation by coalbed

The main goals of Expedition 337 are related to life associated with deeply buried coalbeds. In order to tackle this set of goals within the Shimokita coalbeds, we had to drill deeper than any previous expedition of scientific ocean drilling. Both microbial activity and cellular concentrations are expected to decrease with sediment depth and age (e.g., Parkes et al., 2000; Jørgensen, 2012). Even if our samples were uncontaminated, the detection and examination of such deep life would not be trivial and would require both state-of-the-art methodology and utmost scientific scrutiny. With the added difficulty of contamination, we will need to build a case that integrates various lines of biological and chemical evidence for the presence and activity of microbes and the nature of microbially mediated processes.

This said, we have begun to assemble preliminary lines of evidence that are suggestive of microbial life associated with the coalbed and provide first answers to some of our scientific questions. The most compelling evidence for microbially mediated metha-

nogenesis is found in our gas compositional data. In particular, C_1/C_2 ratios (Fig. **F12**) analyzed during mud-gas monitoring are generally in the range suggesting biological methanogenesis as the major source of methane (e.g., Whiticar, 1999). Most striking, however, is the strong positive inflection of this ratio associated with the major coal-bearing horizons at Site C0020; this trend to higher values is consistent with coalbeds being an active source of methane to the deep formation. This interpretation of an important role of methanogenesis is supported by stable carbon isotopic compositions of methane that have been recorded in real time during mud logging (data not shown). Further validation of isotopic relationships of different carbon pools and determination of stable hydrogen isotopic compositions of methane will provide more detailed information regarding the pathways and substrates utilized by methanogens.

Although further validation in shore-based laboratories is required, other lines of evidence such as extracted DNA or visual observation of intact cells are consistent with the presence of indigenous microbial populations at great burial depth at Site C0020. The sole detection of functional genes indicative of methane cycling in sediment samples (and not in drilling mud) is promising. Moreover, intact cells were detected in deep horizons (Fig. **F14**) and show generally very low cellular abundances. These very low concentrations in carefully cleaned samples are on the one hand encouraging as they suggest that contamination has not resulted in vastly elevated cellular counts; on the other hand we are faced with the relatively highest potential impact of contamination when in situ cell densities are already low. Additional molecular work on the single cell level to the system level is thus required to confidently assign these cells to indigenous populations.

We also have obtained the first indication related to the activity of the subseafloor biosphere in deep horizons associated with the coalbed. Whereas the compositions and concentrations of various gases suggest a stimulation of microbial activity through the coalbed, the relatively low levels of alkalinity suggest substantially lower rates of microbially mediated remineralization of carbon than in the upper few hundred meters of previously studied deep biosphere sites (e.g., Shipboard Scientific Party, 2003; Expedition 311 Scientists, 2006), including shallower sediment at this location (Aoike, 2007; Tomaru et al., 2009). Nevertheless, there is roughly a twofold elevation in alkalinity within the coal-bearing Unit III, relative to the overlying Unit II. This supports other lines of evidence suggestive of stimulation of microbial activity through the coalbed. In conclusion, the coalbed is probably not responsible for the presence of methane hydrates found in shallower layers at this site (Aoike, 2007); it rather resembles a slow-paced bioreactor with sustained activity on geologic time-

scales as previously proposed for other organic-rich deeply buried layers such as Cretaceous black shales at Demerara Rise (Arndt et al., 2006).

Preliminary assessment of the sedimentation history at Site C0020

Our sedimentary analysis of the depositional environment, revealed through Hole C0020A, is that >700 m of intertidal and wetland sequences were deposited from late Oligocene/early Miocene through early/middle Miocene. This result is inconsistent with our expectations that such young sequences would not significantly thicken and that Eocene lignite layers would be present under an Oligocene unconformity layer. Our findings suggest that this sedimentary basin had been continuously subsiding in order to generate the accommodation space during this period without an abrupt faulting event and that the rate of basin subsidence had been in balance with the sedimentary input. For a better understanding of such basin dynamics and formation mechanisms, further investigation of the regional geology and tectonics is required.

Accomplishments and future perspectives

During Expedition 337, our major operational objectives (cf. Inagaki et al., 2010) were successfully accomplished through use of the riser drilling system of the *Chikyu*. The bottom depth of Hole C0020A is 2466 mbsf, extending the previous maximum penetration depth in scientific ocean drilling by 355 m and providing the chance that our postcruise research will extend the widely accepted evidence of deepest subseafloor life by up to 800 m. The cored materials provide unprecedented opportunity to address fundamental scientific questions related to the deep coalbed hydrocarbon system and subseafloor life. New shipboard facilities such as the mud-gas monitoring laboratory and the radioisotope laboratory were successfully implemented and strongly contributed to the success of Expedition 337. The core recovery through riser drilling was remarkably high, often close to 100%, even at great burial depths of 2000 mbsf and deeper. The cored materials include diverse lithologies (e.g., lignite coal, sandstone, silty mudstone, beach sand, carbonate minerals, and conglomerate). The condition of the riser borehole was excellent, allowing close-to-perfect acquisition of downhole wireline logging data. The successful accomplishment of the aforementioned tasks required the technological capabilities of the riser drilling vessel *Chikyu*. Therefore, Expedition 337 has been an important step into a new era of scientific ocean drilling in which Earth and life scientists will jointly explore the deep realms of our planet that have never been studied before.

This first deep riser drilling expedition exploring deep life had also important strategic value in that this was the first time that the impact of commercially used drilling technology was rigorously tested by a large team of biologists, chemists, and geologists to test its compatibility with the scientific goals. As a result, a number of recommendations related to the future use of this technology in scientific ocean drilling can be made. These relate to (1) coring technology, (2) drilling mud composition and sterilization, and (3) the use of deep riser holes for experiments.

1. During Expedition 337, we performed spot coring, instead of conventional sequential coring strategy, using standard 8½ inch RCB coring and 10⅝ inch large diameter industry-type coring system (LDC). Both coring systems resulted in excellent quality of cores, including very hard carbonate-cemented nodules and conglomerates, from scientifically significant horizons. This spot coring strategy is essential for reducing the cost and time for riser drilling operation. LDC cores maximize the probability of obtaining noncontaminated massive core samples that are adequate for high recovery of pore water, allowing highly sensitive and specific biogeochemical and microbiological analyses. However, the use of an aluminum core liner required modification of the normal workflow and resulted in much longer time requirements for delivery of core material from the rig floor to the laboratory. Nevertheless, we processed LDC cores under anaerobic conditions and retrieved useful data and samples with relatively low levels of contamination for shipboard and shore-based analyses. Considering the high risk of drilling mud and microbial contamination of the standard RCB core, it would be desirable to explore the potential use of improved LDC-type coring systems with nonmetal core liner (e.g., carbon glass fiber or reinforced plastic liner) as the standard spot-coring tool for future deep scientific drilling on the *Chikyu*.
2. The use of riser drilling mud is essential for future deep scientific explorations. On the other hand, we need to improve the issues identified as contamination paired with very low indigenous signatures of life and geochemical characteristics. The mud used during Expedition 337 contained close to 10^8 contaminants, even though the fluid is alkaline and contains sterilizing chemicals. This high concentration of nonindigenous cells complicated precise detection of deep microbial life and its metabolic activities and influenced the chemical composition of pore water. To minimize the risk of drilling mud–related sample contamination during future scientific riser drilling expeditions, alternative drilling mud compositions should be considered. For example, are there feasible technologies for mud sterilization that could be implemented without conflicting with operational demands for deep drilling; can the organic additives that appear to nour-

ish microbial communities be substituted with inorganic components? Can we develop in situ sampling devices for recovering noncontaminated and biologically pristine core and fluid samples?

3. A positive aspect of the deep-riser drilling is the superior borehole stability supported by the use of high-viscosity mud that prevents possible collapse and flow down of rubbly horizons such as coal and fault layers. This is not only useful for coring materials with high recovery rate, but also essential for successful completion of multiple deployments of logging tools, including downhole in situ fluid sampling and analysis. With the combined use of borehole observatory sensors and subseafloor laboratory equipment, the maintenance of stable deep-riser boreholes will be highly useful for advanced subseafloor research in short- to long-term projects.

Last but not least, this expedition also provided a test ground for the use of riser drilling technology to address geobiological and biogeochemical objectives and was therefore a crucial step toward the next phase of deep scientific ocean drilling. Since the riser system was originally developed by the petroleum industry, the *Chikyu* is equipped with a mature technology. However, the adaptation of this technology to the needs of basic science will be an important challenge that needs to be addressed as integral component in plans for the next riser missions. Implementation of science-oriented deep-riser drilling in IODP would provide grand opportunities for Earth system sciences.

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Table T1. Expedition 337 coring summary.

Hole	Latitude	Longitude	Water depth (mbsl)	Cores (*N*)	Cored (m)	Recovered (m)	Recovery (%)	Drilled (m)	Penetration (m)	Time on site (days)
337-C0020A	41°10.5983′N	142°12.0328′E	1180	32	263.5	200.9	76.2	1555.5	1819	60

Figure F1. Index map of Site C0020 off the Shimokita Peninsula of Japan with bathymetry, seismic survey track lines, and locations of existing drill holes. Inset map exhibits plate configuration around Japanese Islands and the location of the index map (red square).

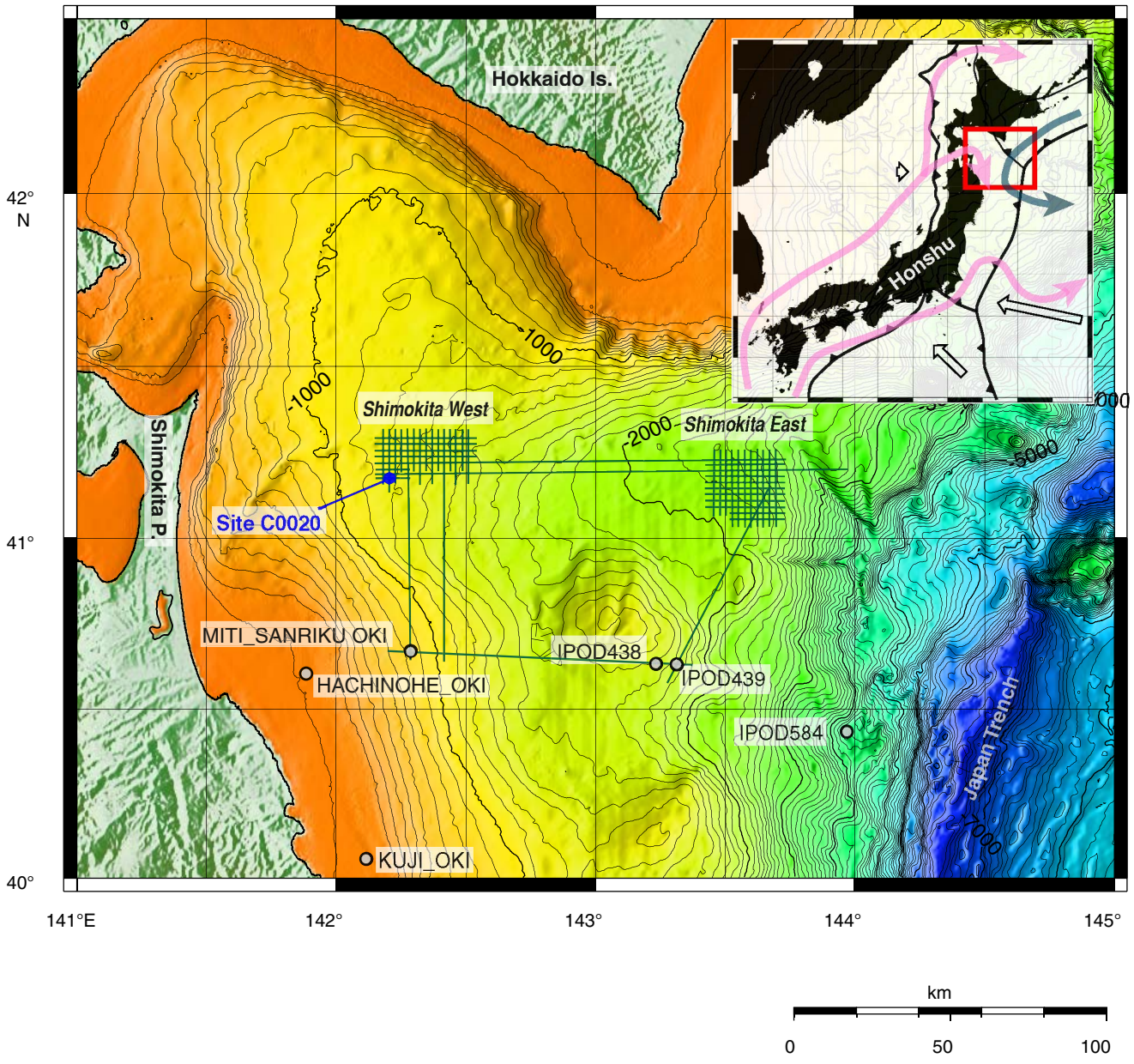

Figure F2. Seismic profile in the vicinity of Site C0020 off Shimokita Peninsula. Gray zones = potential coal-bearing layers. WD = water depth, BSR = bottom-simulating reflector.

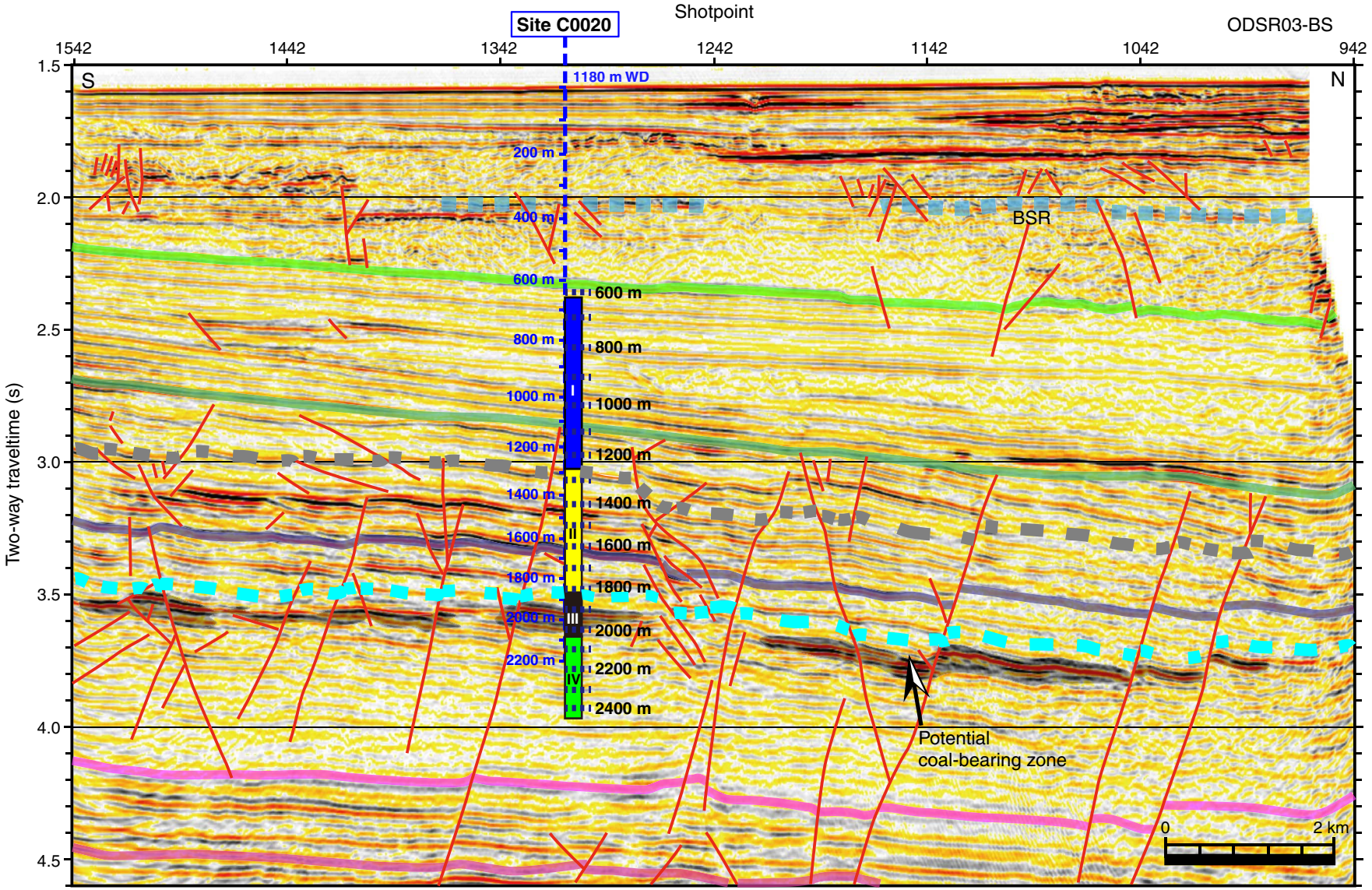

Figure F3. Lithostratigraphic profiles derived from macroscopic observation of cuttings samples and cores in Hole C0020A with ages; wood/lignite, glauconite, diatom, and sponge spicule relative abundance; depositional environment; and subdivision into different units.

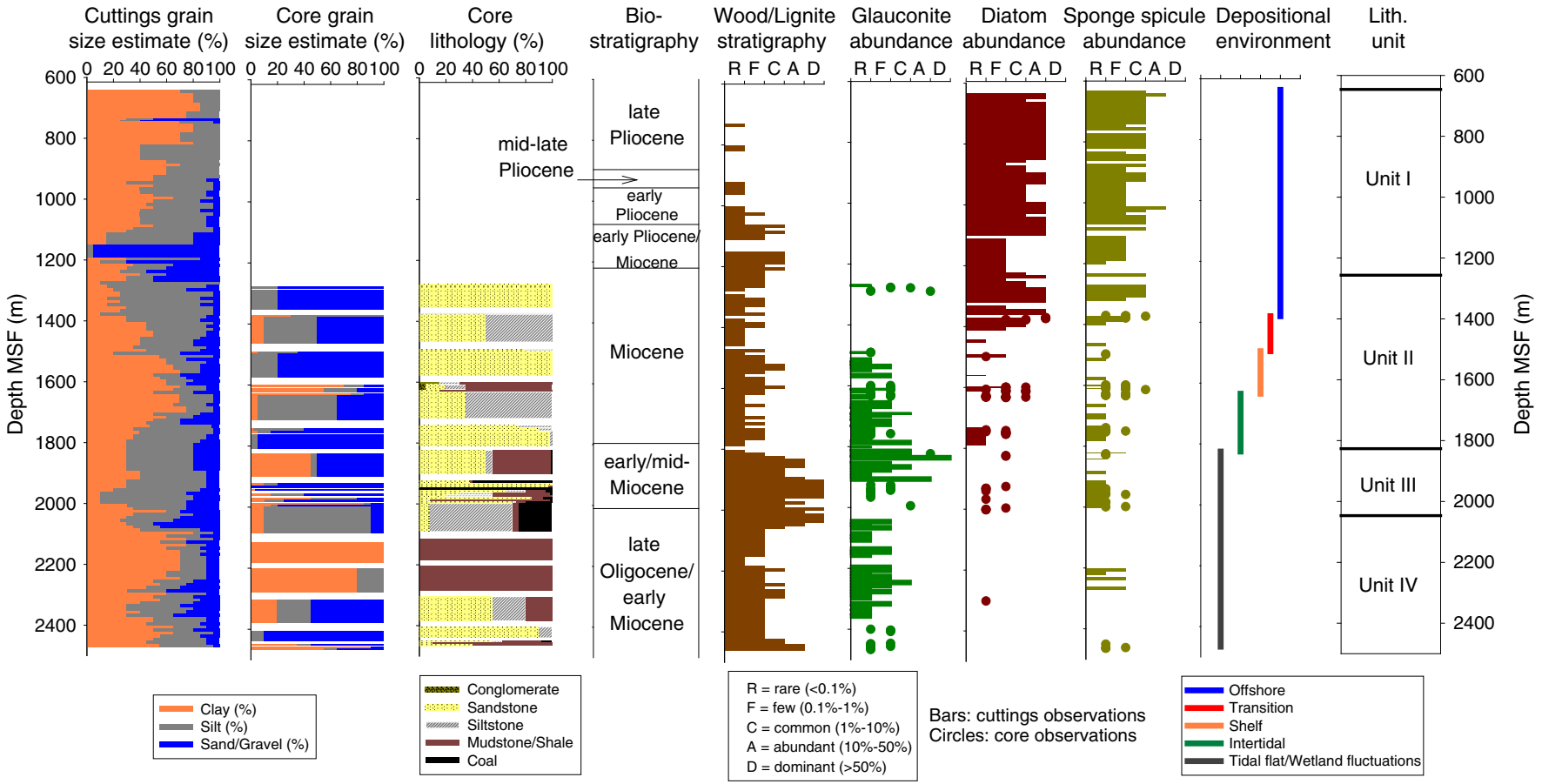

Figure F4. Section photos and X-ray CT scan images of selected intervals of different units. **A.** Sandstone with pebbles of igneous rocks and few cherts, Subunit IIa (Sample 337-C0020A-4R-2, 58–71 cm). **B.** Vertical burrows filled with medium sandstone in a silty shale, Subunit IIb (Sample 12R-2, 25–43.5 cm). Shell fragments appear in the lower part. **C.** Photo shows coaly shale with pyrite (upper part) in contact with coal interval (lower part), Unit III (Sample 19R-7, 98–115.5 cm). **D.** Light brown cemented (sideritic) mudstone and concretions, Unit III (Sample 24R-2, 24–39 cm). In the middle of this brown horizon, a thin coaly layer appears. **E.** Siltstone with siderite concretions and siderite band in silty shale, Unit IV (Sample 30R-1, 47.5–64 cm). Lenticular bedding is visible in this section.

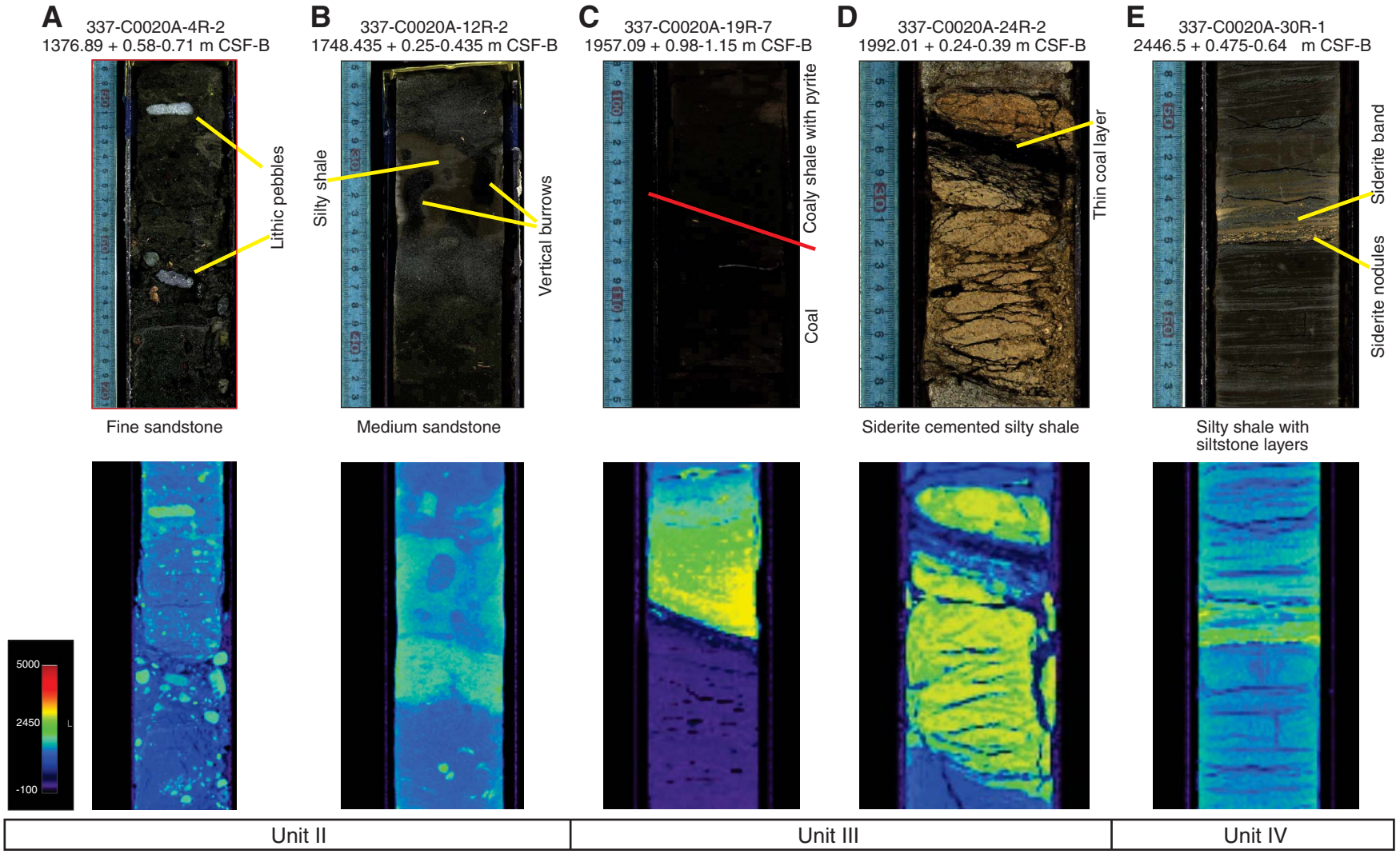

Figure F5. Site summary diagram of downhole logging operations for Site C0020 from 1200 to 2466 m wireline depth below seafloor (WSF), showing caliper (hole diameter), natural gamma ray, spontaneous potential, laterolog resistivity (RLA) of five different depths of investigation (RLA5 = deepest), neutron porosity, photoelectric factor, density, *P*- and *S*-wave velocities and Poisson's ratio calculated from these velocities, natural gamma ray from three different radioactive materials, a series of permeability and porosity measurements by nuclear magnetic resonance (NMR) method together with their original data (combinable magnetic resonance tool, CMR), and borehole resistivity image.

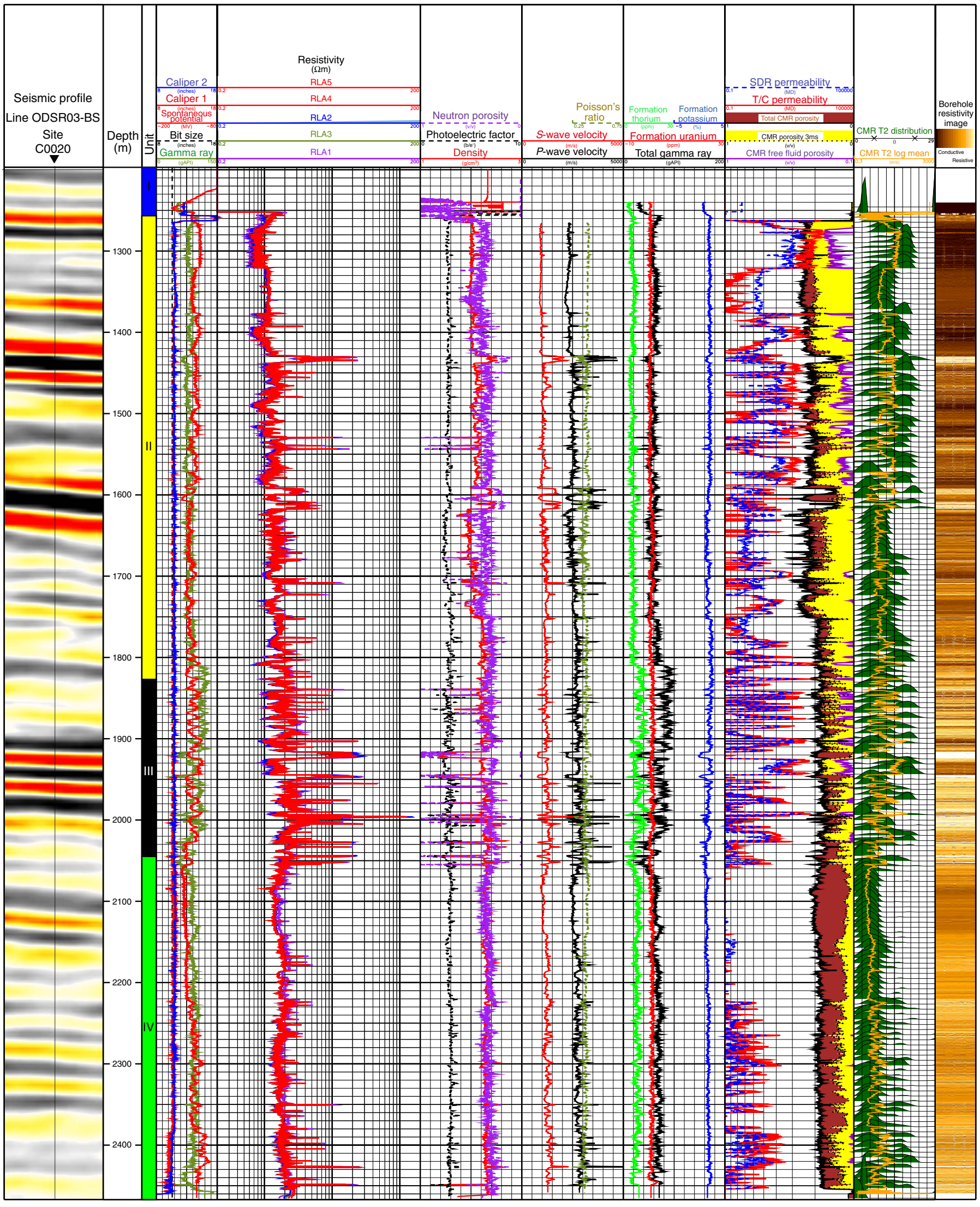

Figure F6. A. X-ray CT scan images from coal of Sections 337-C0020A-18R-1. **B.** Top: SEM picture from a banded coal (xylitic and detritic bands) of Core 18R-1 with some pyrite veins (upper part). Middle: photomicrograph of coal from Core 19R; densinite with some resinite and phlobaphinite. Bottom: photomicrograph of coal from Core 30R; densinite with a large funginite in the lower part and ulminite in the upper third of the photomicrograph.

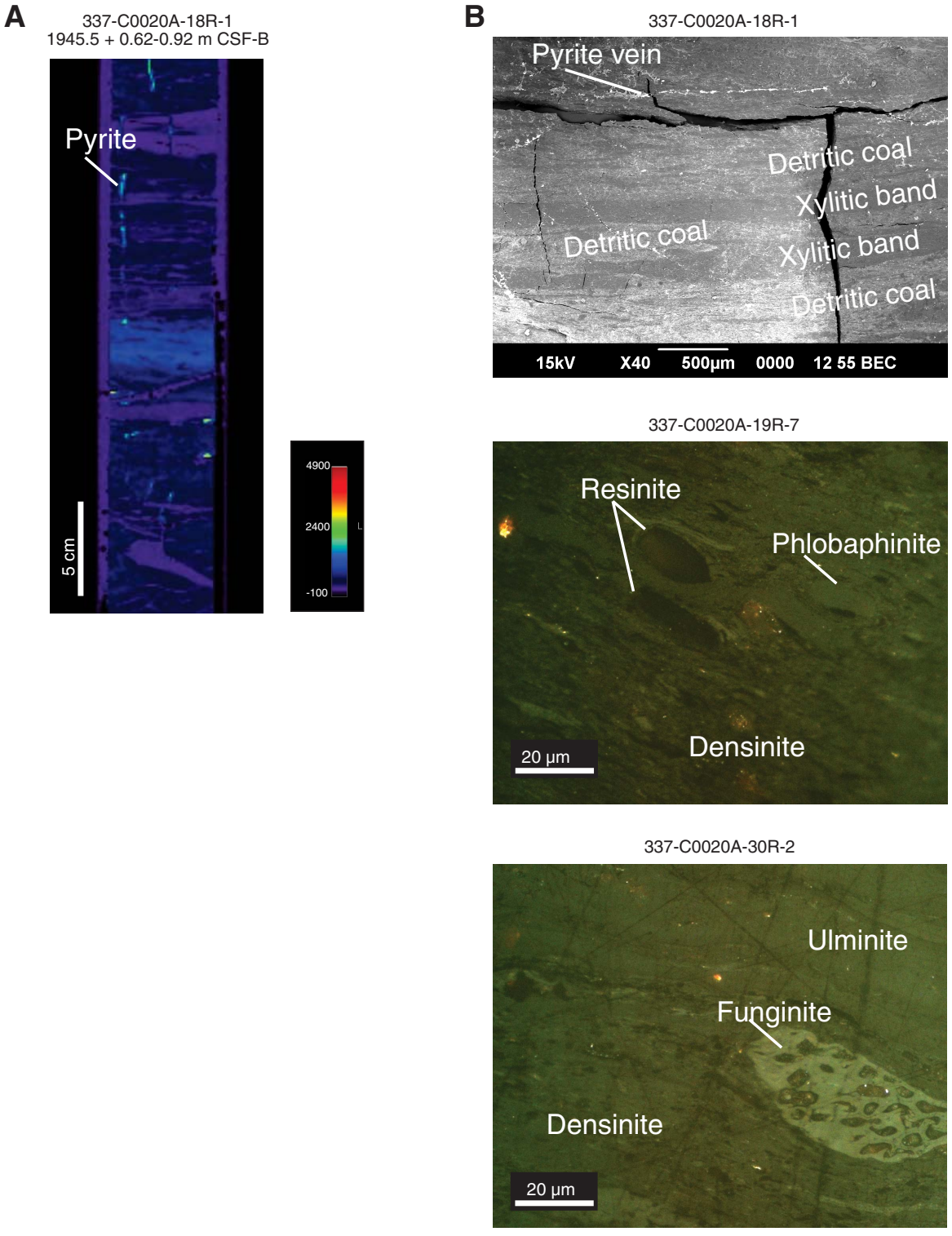

Figure F7. Distribution and lithological variation of porosity in discrete core samples with comparison of cuttings at Site C0020. Porosity of sandstone and siltstone gradually decreased with depth, though carbonate cemented rocks deviate from the consolidation curve with remarkably low porosity. Higher porosity and slower reduction in porosity with depth is observed in cuttings samples. Discrete core samples can be more representative for in situ porosity than porosity of cuttings.

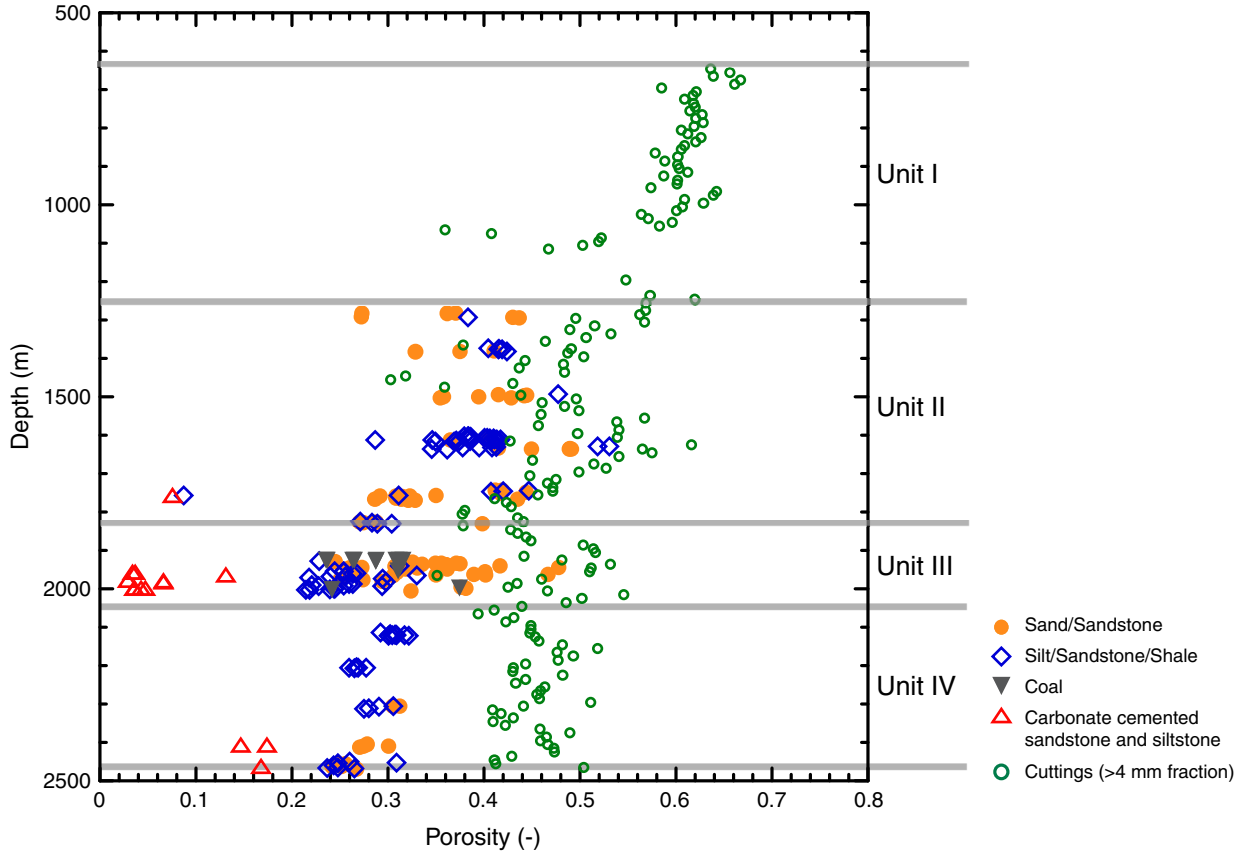

Figure F8. Summary of temperature measurements, Site C0020. Two types of logging tools, i.e., the environmental measurement sonde (EMS) and modular formation dynamics tester (MDT), measured borehole temperature in situ. The MDT tool recorded temperature twice: during the pretests (MDT; yellow) and fluid sampling (Quicksilver [QS]; red). The maximum temperature at the bottom of the hole was estimated by temperature build-up pattern during the logging operation (the Horner Plot method; green; Dowdle and Cobb, 1975) that shows the maximum temperature gradient of 24.0°C/km.

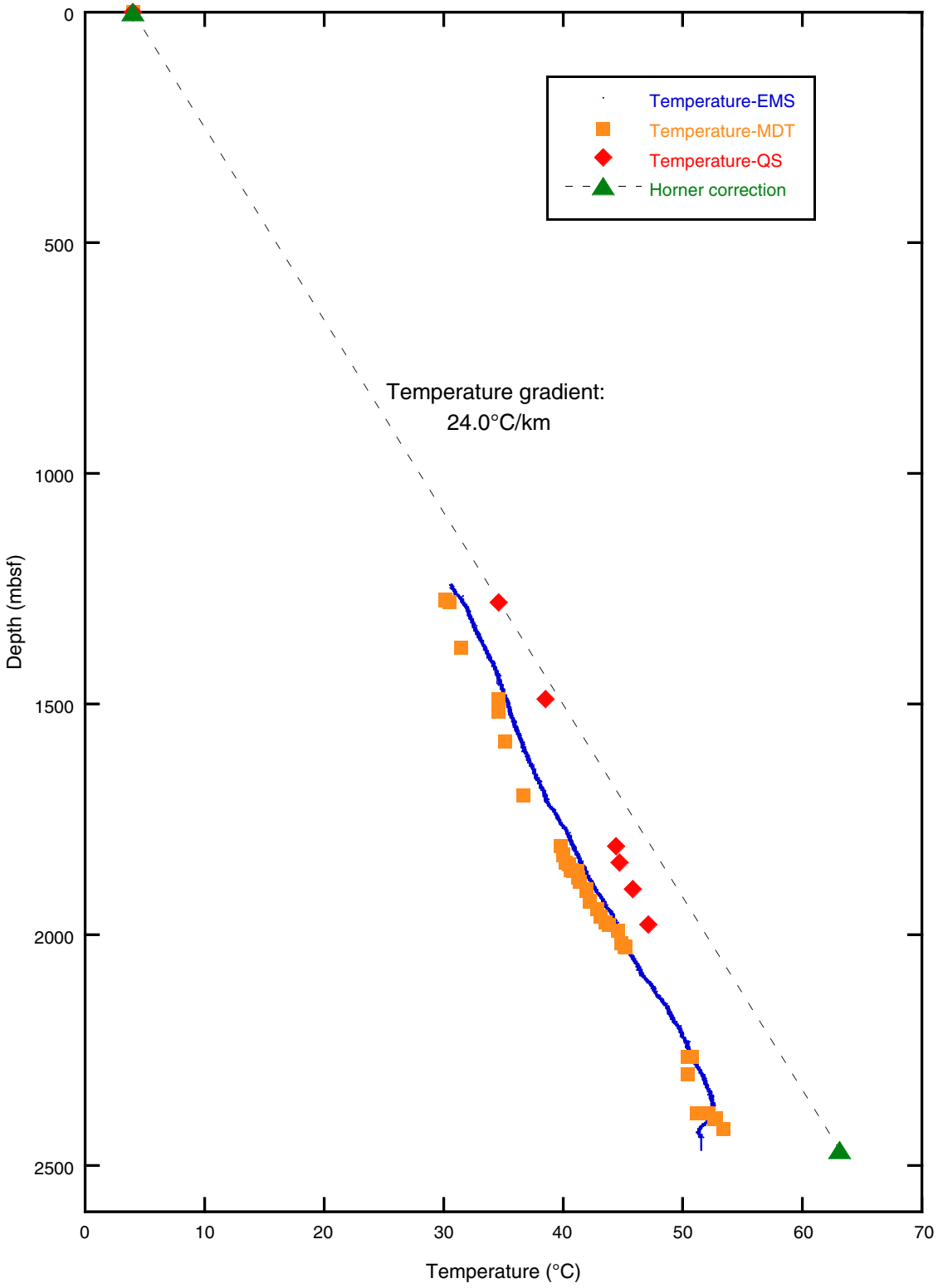

Figure F9. Depth profiles of total organic carbon (TOC), TOC to total nitrogen (TN) ratio, and hydrogen index (HI), Hole C0020A. Open dots = data from cuttings samples, solid dots = data from core samples. All three parameters showed a lithologic control, with values decreasing in the order of coal > clayey materials > silty and sandy materials.

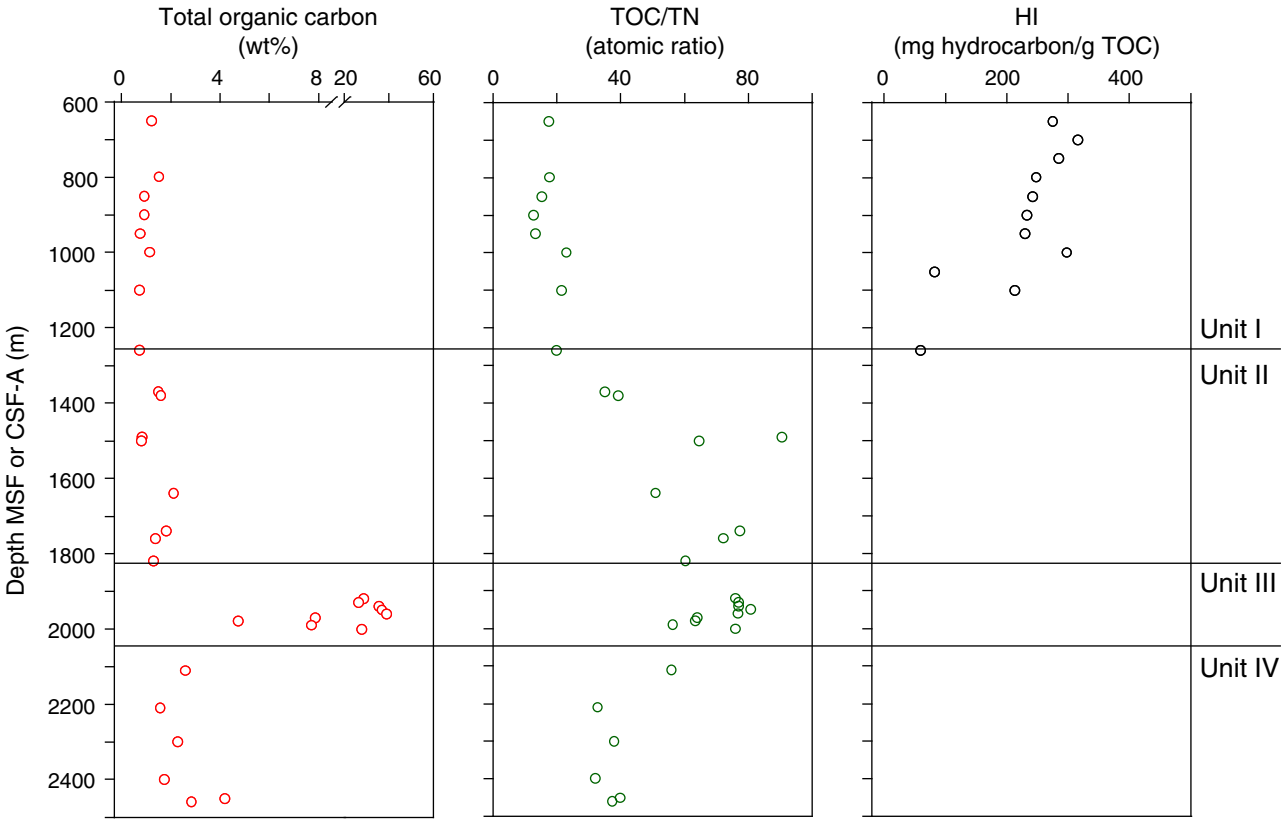

Figure F10. A. Occurrence of wood and lignite fragments in drill cuttings, Site C0020. **B.** Proportion of C_{29} Δ4 and Δ5 sterenes as a proportion of C_{27} to C_{29} Δ4 and Δ5 sterene-homologues (solid circles = coal, open circles = mudstone, x = drill cuttings, + = siltstone). **C.** 215 *m/z* ion chromatogram displaying doublets of Δ4 and Δ5 sterene. More detailed labeling provided in report.

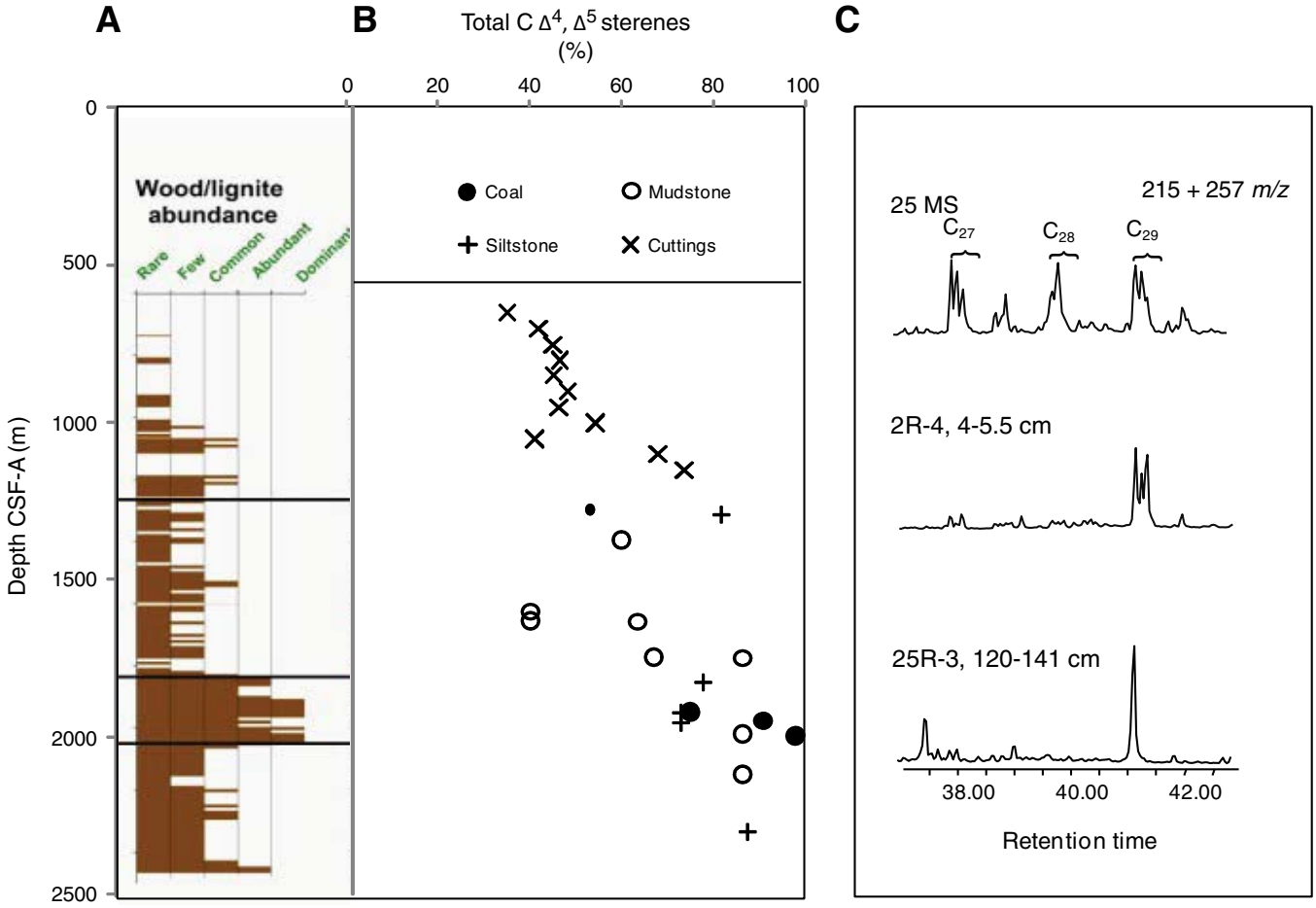

Figure F11. Ratio of C_{29} 5α,14α,17α(H)20R sterane to C_{29} Δ4 and Δ5 sterenes, Site C0020. Data plotted by sample type (solid circles = coal, open circles = mudstone, x = drill cuttings, + = siltstone). Inset displays data from Hokkaido (Amo et al., 2007) used to calculate lines for 22° and 25°C/km geothermal gradient.

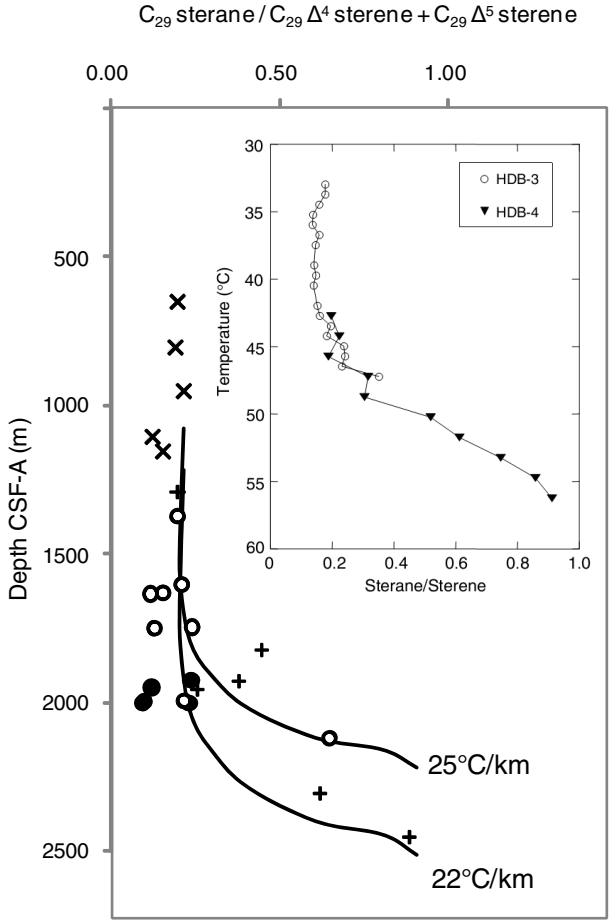

Figure F12. Downhole profile of C_1/C_2 ratios in mud gas analyzed by online mud-gas monitoring during the drilling of Hole C0020A. High C_1/C_2 ratios are characteristic for biogenic methane formation and low ratios indicate thermogenic sources because higher hydrocarbon gases are mainly formed by thermal alteration of organic matter. However, minor amounts of C_2 can also be generated during early diagenesis of organic matter. At Site C0020, C_1/C_2 ratios point altogether to biogenic methane sources. C_1/C_2 ratios generally decrease with increasing depth as expected, but they show a distinct excursion toward higher values between 1840 and 2054 m MSF (i.e., the depth interval in which 12 coal layers were observed [black lines]). The excursion of the C_1/C_2 ratio signals the presence of biogenic methanogenesis in coal-bearing horizons.

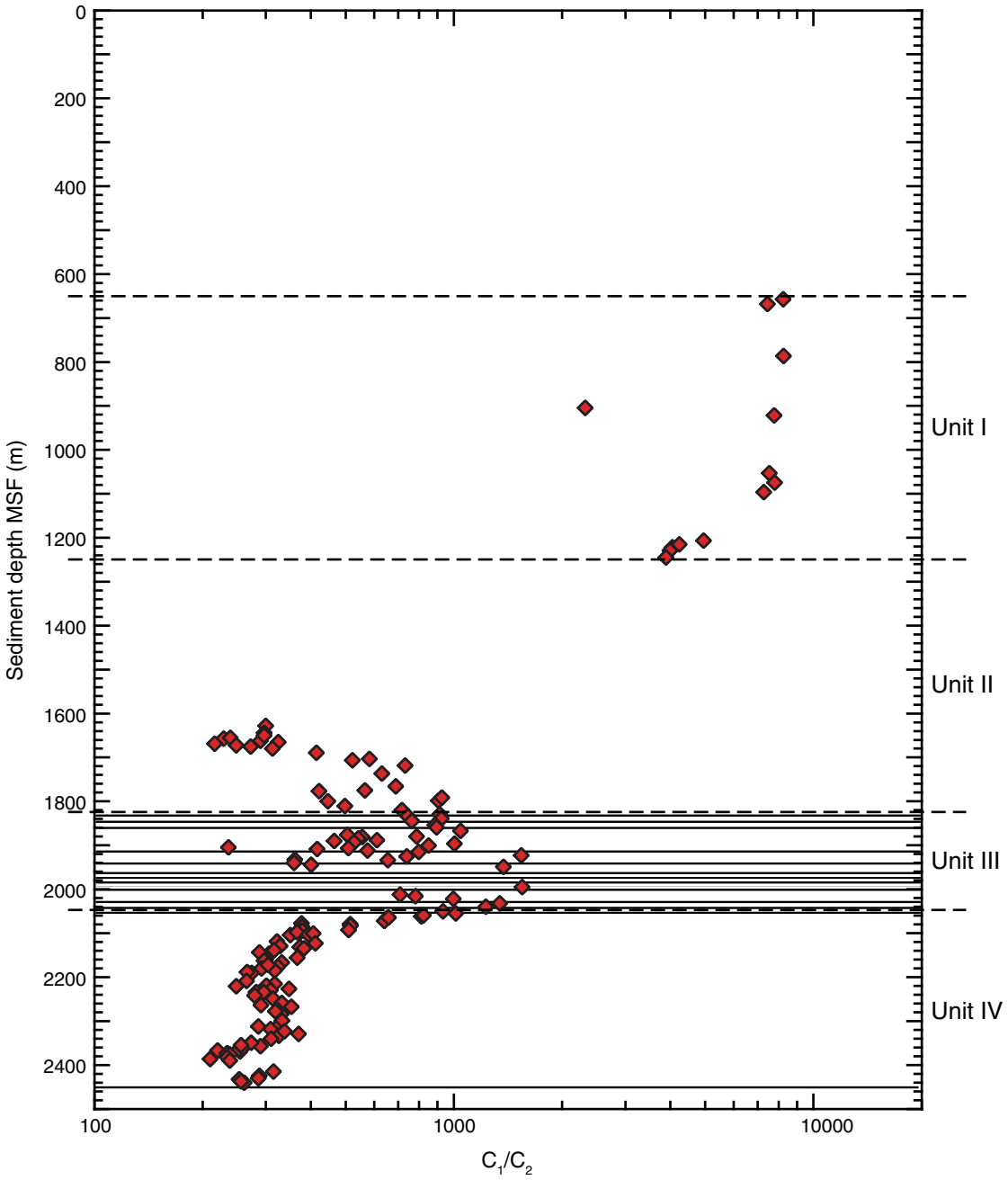

Figure F13. The yield of interstitial water squeezed from whole-round cores in relation to lithology.

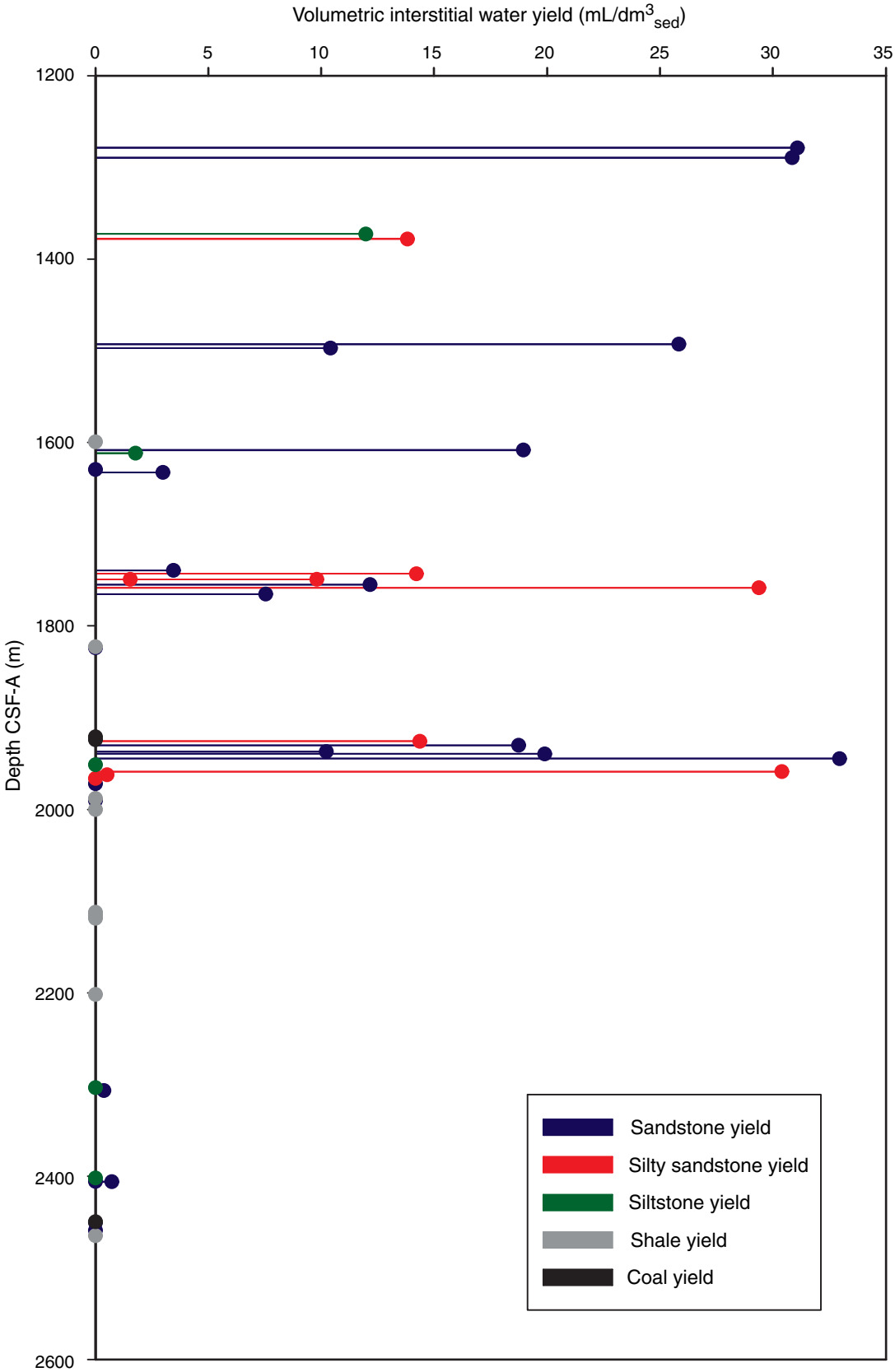

Figure F14. Fluorescent microscopic images of microbial cells from core samples. Each picture shows SYBR Green I-stained microbial cells observed in (**A**) Sections 337-C0020A-1R-2 (1278 mbsf), (**B**) 15R-2 (1920 mbsf), (**C**) 25R-2 (1997 mbsf), (**D**) 26R-7 (2117 mbsf), and (**E**, **F**) 32R-1 (2457 mbsf).

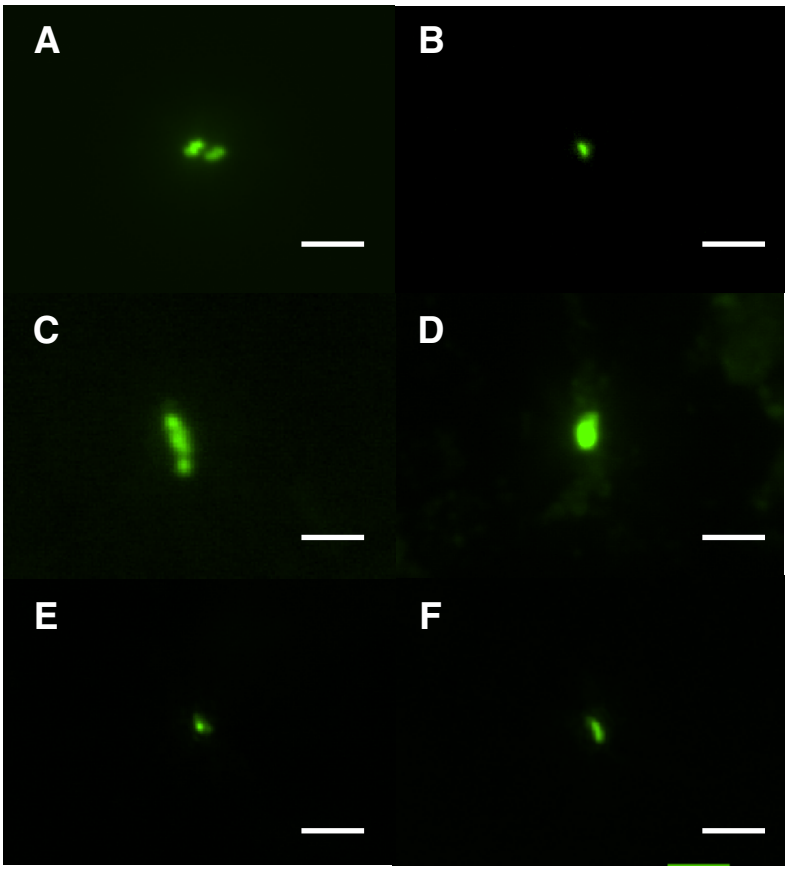

Figure F15. Sampling scheme for microbiology sampling and analyses flow, including whole-round core (WRC) distribution to specific analyses procedures, Expedition 337. Green = shipboard experimental processes, blue = shore-based experiments and analyses. MBIO = microbiology, IW = interstitial water, Sed. = sedimentology, PFC = perfluorocarbon, pSRR = potential sulfate reduction rate, H_2ase = hydrogenase, NanoSIMS = nanoscale secondary ion mass spectrometry, GC-ECD = gas chromatography-electric conductivity detection, FCM = flow cytometry, FISH = fluorescence in situ hybridization, PCR = polymerase chain reaction, RT-PCR = real time-polymerase chain reaction, T-RFLP = terminal-restriction fragment length polymorphism, subst. = substrate, cont. = contaminated.

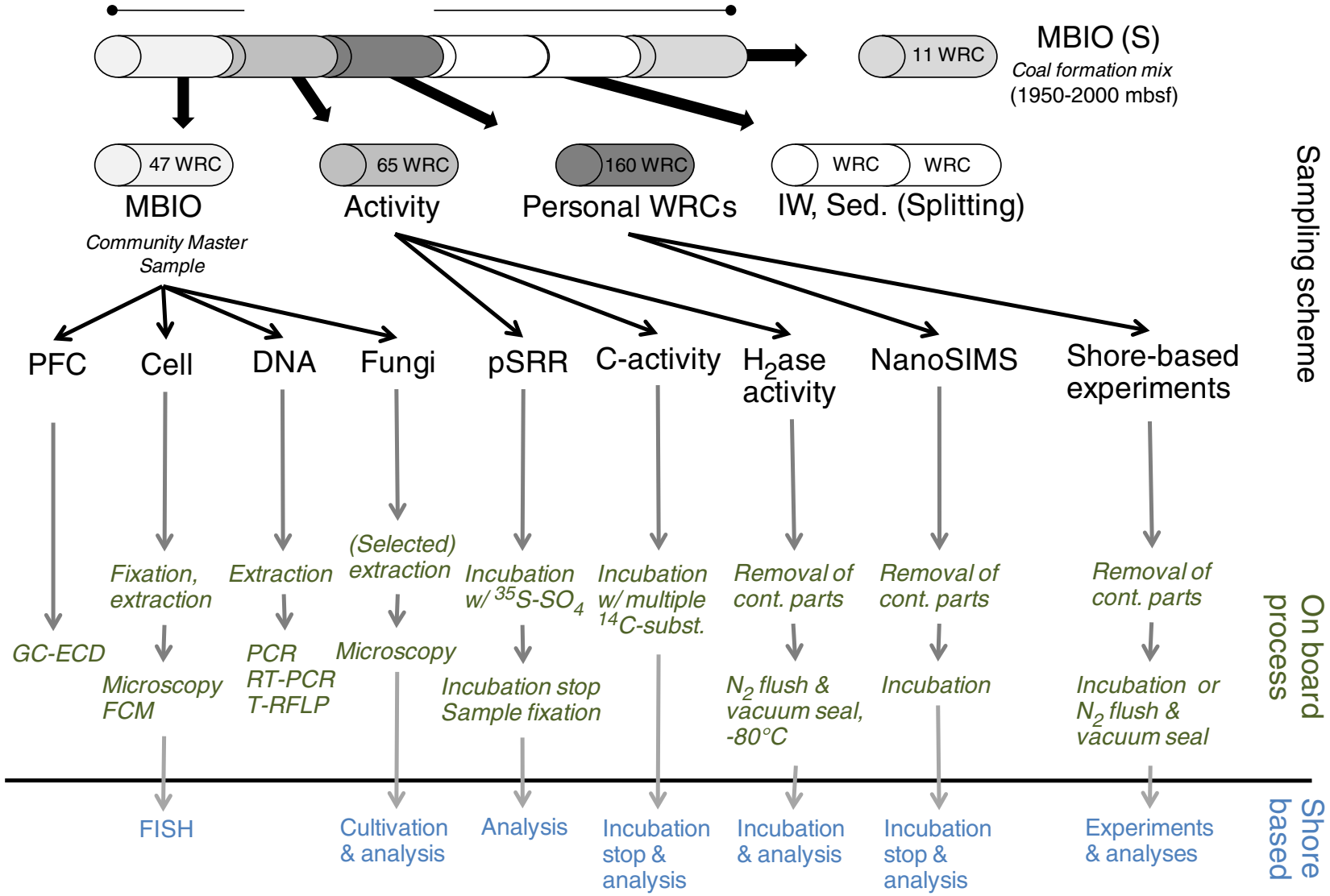

Figure F16. Change in drilling fluid contamination and predicted number of drilling-induced contaminant cells, from the outer centimeter of cores (X), to halfway between the core liner and core center (H), to the innermost part (IN), using Sample 337-C0020A-26R-7, 84–101 cm (Code: MBIO-COM) as an example. PFC concentrations were below detection (BD) in the innermost part. The empty column shown illustrates the expected contamination at the detection limit and should thus be treated as a maximum value. Only the innermost parts of cores were used for cell counts and DNA extractions on board the ship. The boxed photos show X-ray CT scan images of Core 26R-7, 84–101 cm.

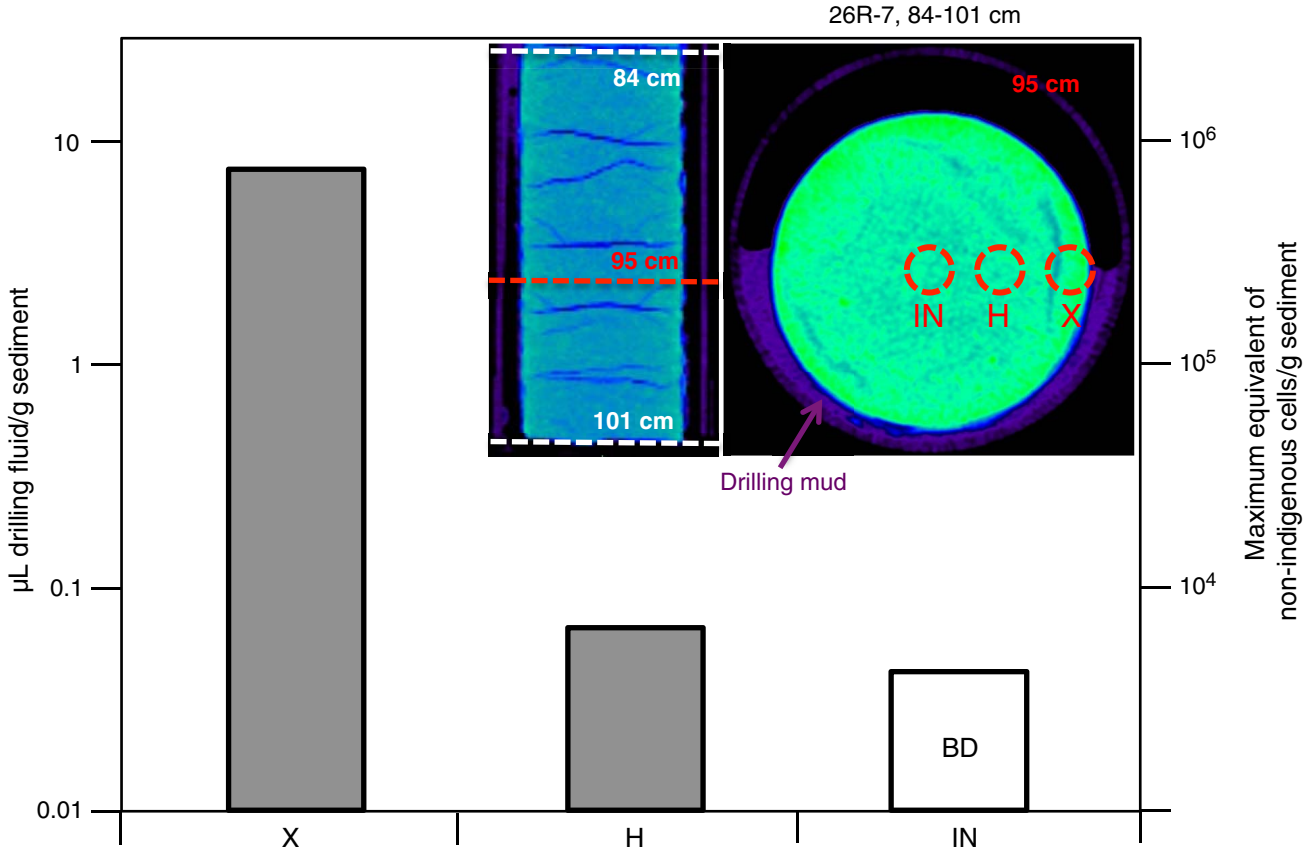

Figure F17. A. Section photo and X-ray CT scan image of Sample 337-C0020A-1R-CC, 0–14.5 cm. Drilling mud injection is causing some lamination in semi-consolidated sandstone. **B.** Section photo and X-ray CT scan image of partial Section 19R-3. An example of "nugget structures" caused by drilling mud injections is shown.

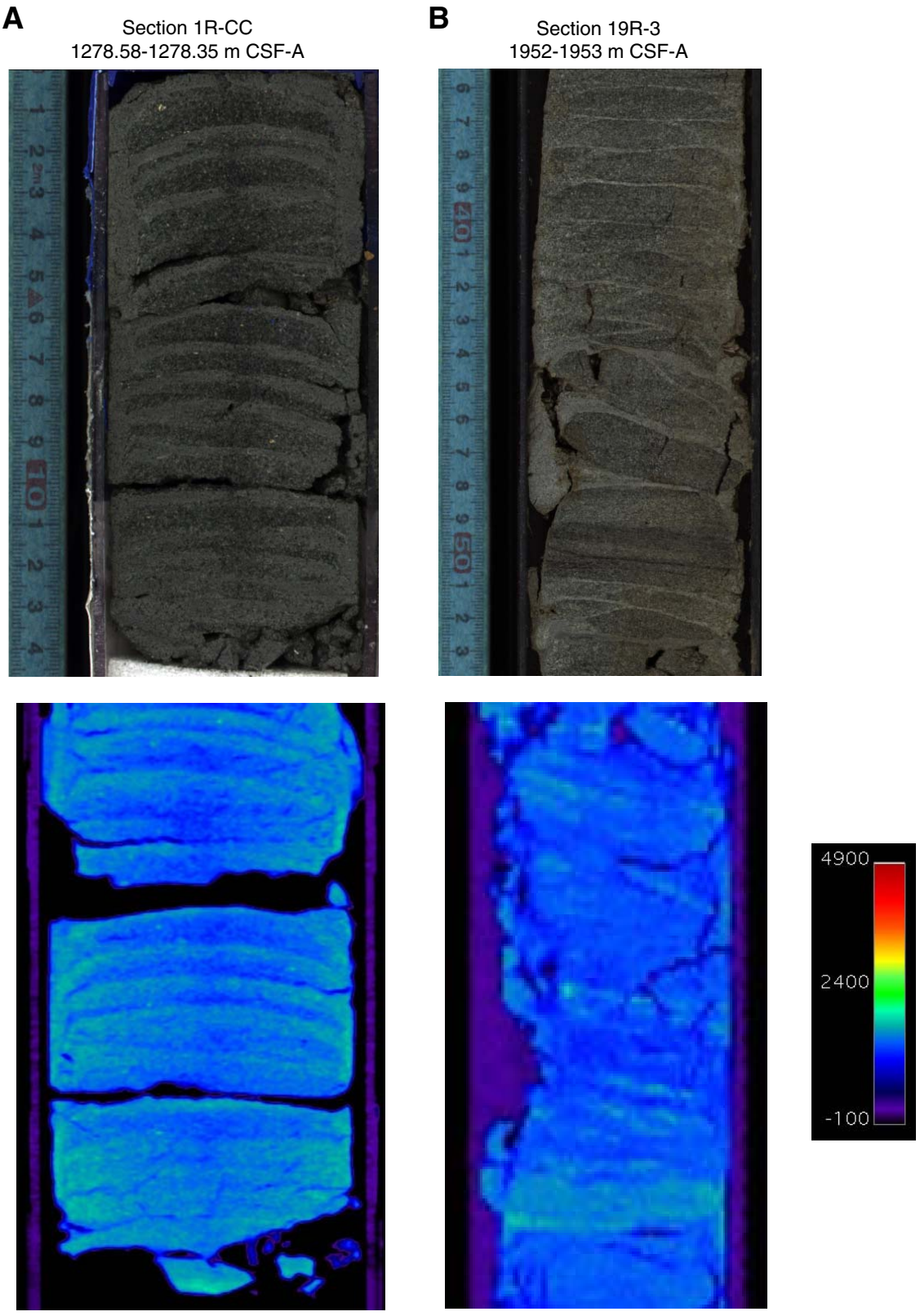

Figure F18. Estimates of fraction drilling mud liquid (X_{DML}) incorporated into interstitial water samples from Site C0020. X_{DML} was estimated based on concentrations of sulfate, potassium, and salinity. Shaded area represents the –1σ and +1σ region of the average X_{DML} values (not shown) of these three estimates.

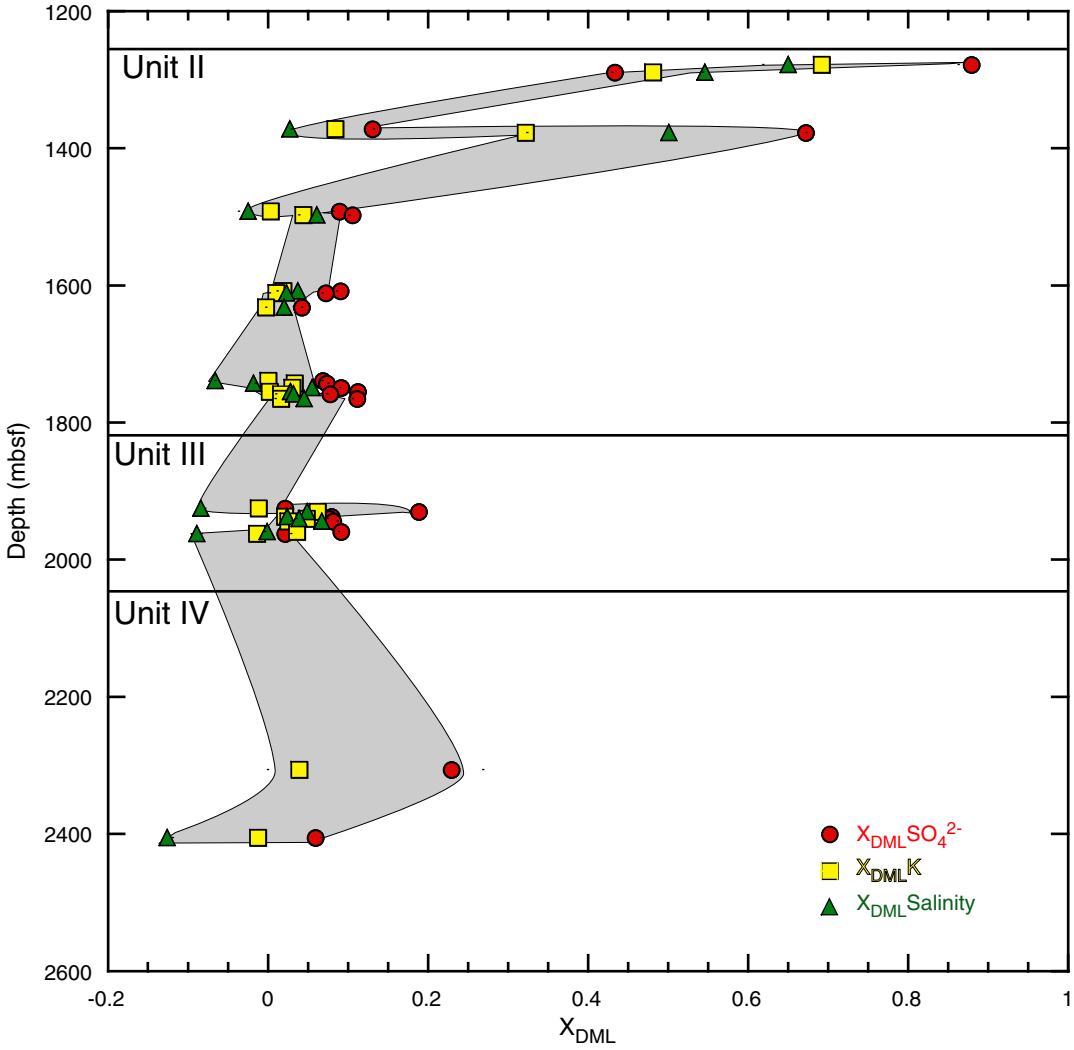

Figure F19. A. Total ion chromatograms for sequential extraction of drill cuttings. The extract from the exterior is dominated by drilling mud components (marked by *). After cleaning by sonication in solvent, a repeat extraction (second extract) yields far less organic matter, suggesting that the surface has been cleaned. The solvent extract obtained from the now clean sample after it has been crushed contains far less drilling components (ASE-extract for sample MS 25). **B.** Biomarker contamination 2: 191 *m/z* ion chromatograms for ASTEX (a drilling additive), the extract from a sandstone (Sample 337-C0020A-16R-2, 12–45 cm) and from a siltstone (Sample 15R-5, 0–21 cm). ASTEX contains thermally mature hopanes; C_{29} and C_{30} αβ hopanes (hopanes with a 17α, 21β [H] configuration) and a distinctively high proportion of C_{35} 22S and 22R hopanes relative to the C_{34} 22S and 22R hopanes. A sample from a more contamination resistant lithology (a siltstone from Section 15R-2) contains C_{30} and C_{31} hopanes with the thermally immature 17β, 21β (H) configuration. The sandstone from Section 16R-2 contains a mixture of hopanes and has a hopane-fingerprint that is a mix of the indigenous formation and the infiltrated ASTEX—both thermally mature and immature hopanes that would not be expected to be found together. (Figure shown on next page.)

Figure F19 (continued). (Caption shown on previous page.)