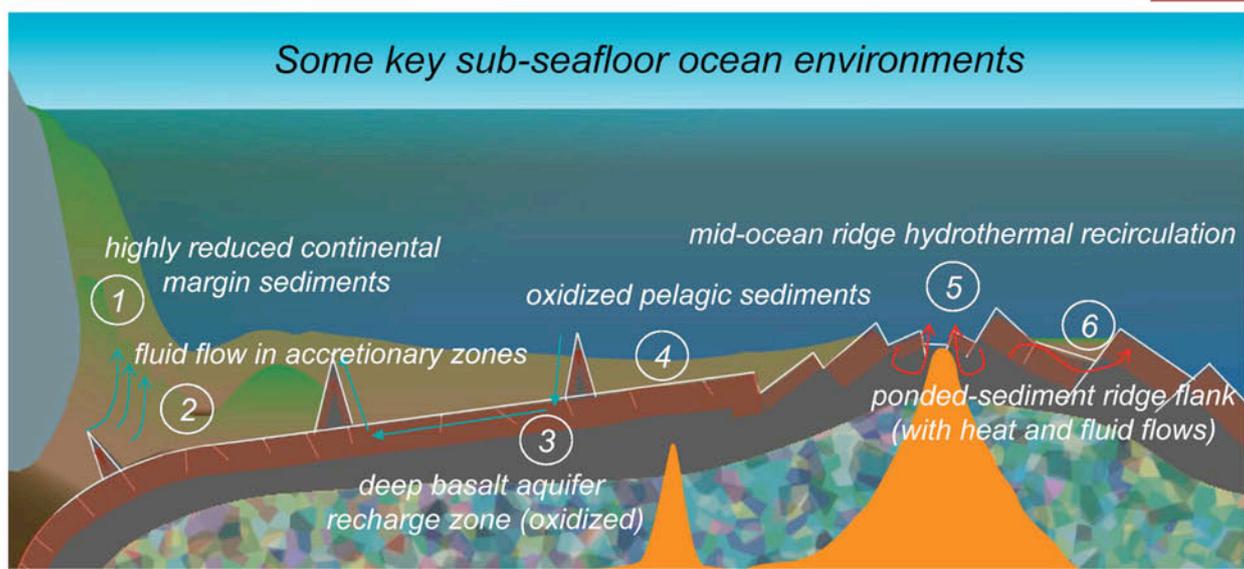


# Thematic Review

Report Series No. 3

## Deep Biosphere and Sub-seafloor Ocean

*IODP and ODP Achievements January 2002 - September 2009*



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## Executive Summary

### Background

"*Deep-biosphere and Subseafloor Ocean*" is one of the major three research themes of the Integrated Ocean Drilling Program (IODP) Initial Science Plan (ISP). Within this theme, "*Deep-biosphere*" and the exploration of "*Gas Hydrates*" were named special research initiatives. An IODP thematic review panel on "*Deep-biosphere and Subseafloor Ocean*" met for two days in September 2009. It reviewed IODP projects comprising the three disciplinary dedicated expeditions (301, 308 and 311). The panel also briefly reviewed Deep-biosphere and Subsurface Ocean components of seven expeditions (302, 307, 310, 323, 315, 316 and 322).

### Specific Achievements

Specific achievements were reviewed with reference to the following sub-themes: (1) Subsurface Ocean; (2) Deep-biosphere; (3) Gas hydrate. In addition to these, the committee reviewed current technological needs.

#### *Subsurface Ocean:*

- Three-dimensional hydrogeological state and processes (physical, chemical and microbiological) in young upper oceanic crust were investigated first time using multi-level CORK observatories on the eastern flank of the Juan de Fuca Ridge in NE Pacific during Expeditions 301 and 327.
- CORK Observatories of the Juan de Fuca Ridge in NE Pacific are successfully connected to NEPTUNE Canada cable network.
- Operational signals created during Expedition 301 were recorded over 2 km away in Hole 1027C. This result demonstrated the feasibility for cross-hole hydrological testing.
- Overpressure profiles at two sites (U1322 and U1324) in the Ursa Basin, Gulf of Mexico were successfully obtained through the combination of *in situ* pore pressure measurements and laboratory studies during Exp. 308.
- Pore pressure data clearly demonstrated the existence of overpressure in the shallow part of the sedimentary formation at the Ursa Basin, Gulf of Mexico (Exp. 308).

#### *Deep Biosphere:*

- Microbiological investigation of "black rust" on the CORK recovered from Hole 1026B on the eastern flank of the Juan de Fuca Ridge in NE Pacific (Exp. 301) indicated presence of a hydrogen-utilizing thermophilic methanogenic archaeon, *Methanothermococcus thermolithotrophicus*, thermophilic sulfatereducers such as the bacterial genera *Desulfomonas*, *Desulfonatovibrio*, *Desulfotomaculum* and archaeal *Archaeoglobus*.
- Contamination test of highly fractured subseafloor basalts on the eastern flank of the Juan de Fuca Ridge in NE Pacific indicated that in most cases the diffusion of contaminated fluids and particles does not cause a significant microbiological

contamination towards the central region of the core, if the samples are stored under ultra cold (<-80°C) conditions soon after core recovery.

- Investigation of microbiological population and metabolic/enzymatic activities using sediment core samples from Site 1301 (Exp. 301) indicated that the diffusive flow of young crustal fluids has an important ecological influence on microbes in sediments above the basalt interface.
- Microbiological investigation of basaltic core samples (Exp. 301) demonstrated for the first time that both carbon and sulfur cycles mediated by microbial activities occur in the deeply buried crustal habitat.
- Microbiological investigation of sediments deposited in different regimes (Exp. 308) indicated that microbial community compositions and structures in the deep seafloor biosphere strongly related to the depositional environment and sediment properties (porosity).

#### *Gas hydrate:*

- Observation of sediment cores indicated that gas hydrate forms preferentially in coarser-grained sediments in Cascadia margin (Exp. 311).
- The primary source of hydrocarbons in Cascadia margin are microbial (Exp. 311).
- Gas hydrate in Cascadia margin appears to be controlled by several factors: (1) local methane solubility linked with pore-water salinity, (2) fluid and gas advection rates, and (3) the availability of suitable host material such as coarse-grained sediments.

### ***Technological developments***

#### *Subsurface Ocean:*

- Weighted mud was found to be useful for non-riser drilling at the overpressured realm and in the place that had been long difficult to access by non-riser drilling.

#### *Deep Biosphere:*

- Semi-aseptic diamond-saw system proved to be effective for sub-sampling frozen cores without melt for microbiological analysis.
- SYBR Green I fluorescent dye produces more bright and specific fluorescent signals of double stranded DNA than other fluorescent dye and therefore serves better for enumeration of microbial life.
- Computer-based automated cell enumeration image system enables to evaluate seafloor biomass quickly and precisely.

### ***Concluding comments***

Between 2003-2009, IODP has addressed the theme "Deep Biosphere and Subsurface Ocean" and both "Deep-biosphere" and "Gas hydrate" initiatives of the Initial Science Plan in three dedicated and seven related expeditions. IODP has probed different geological setting: Ridge flank (Exp. 301), Subduction zone (NanTroSEIZE), Carbonate Mound (Exp. 307) and Carbonate platform (Exp. 310) for this theme.

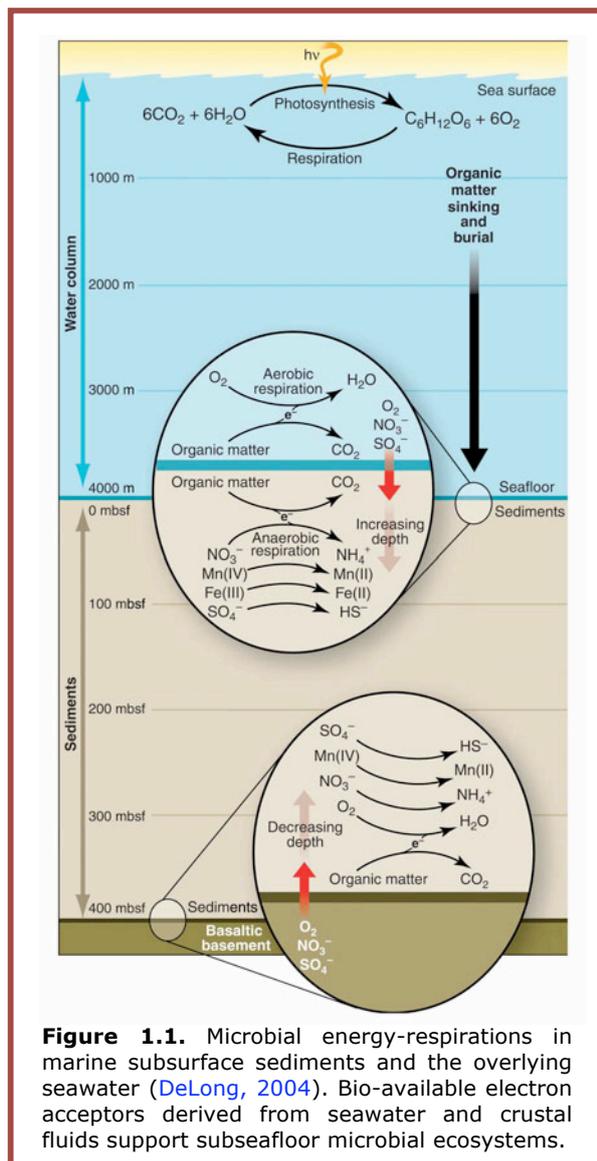
In 2010-2013, IODP plans to address this theme with several dedicated expeditions. IODP plans to investigate Juan de Fuca Ridge (2<sup>nd</sup> part of Exp. 301), Cascadia Margin (2<sup>nd</sup> part of Exp. 311), Mid-Atlantic Ridge, South Pacific Gyre and hydrothermally active mound in Okinawa Trough. Several borehole observatories are planned to be installed in different geological settings during these expeditions (Juan de Fuca, Cascadia margin, Mid-Atlantic Ridge and Okinawa Trough). Successful installation of borehole observatories will allow monitoring of fluid movement and microbiological activities continuously and for long time. Furthermore, connection of borehole observatories with seafloor cable networks (e.g. NEPTUNE Canada) will enable real-time observation of fluid and microbiology activities. Thus, future expeditions will provide truly groundbreaking findings by providing the first compressive characterization of ocean below seafloor, and expand our knowledge of subsurface life.

## 1. Introduction and Background

Deep subsurface environments have long been considered inanimate world harboring only fossil records buried beneath the seabed. However, initial microbiological studies of marine sediments recovered by Ocean Drilling Program (ODP) revealed that over  $10^5$  microbial cells are present in  $1 \text{ cm}^3$  of sediment cores down to 800 meter below the seafloor (mbsf) at various oceanographic locations (Parkes *et al.*, 1994, 2000). The cell numbers are generally logarithmically decreasing with depth, suggesting correlations to geophysical and nutrient conditions in the habitat. The remarkably abundant microbial biomass in subsurface sediments is considered to represent one-tenth to one-third of total living biomass on Earth (Whitman *et al.*, 1998).

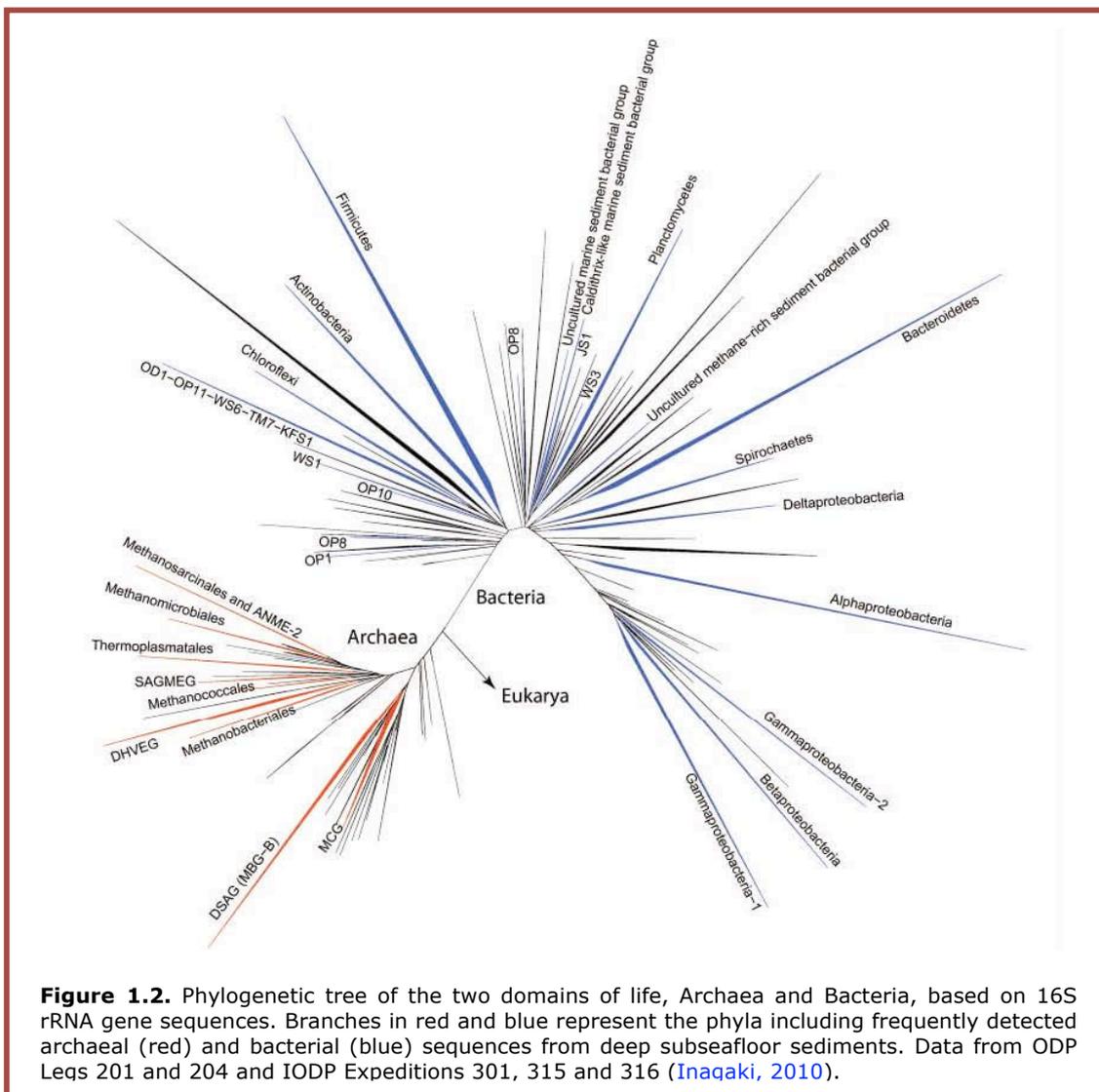
ODP Leg 201 was the first microbiology and biogeochemistry-dedicated scientific drilling expedition at the eastern Equatorial Pacific and the Peru margin, which significantly expanded our knowledge of deep subsurface life and the biosphere. During Leg 201, the core contamination was continuously monitored using perfluorocarbon tracer and fluorescent micro-sphere beads, resulted in successful sampling of least or uncontaminated sediment cores down to 420 mbsf (House *et al.*, 2003). Microbial

populations were evaluated onboard by microscopic direct count of acridine orange (AO)-stained cells (AODC). At Site 1229 in the Peruvian shelf, significant sulfate reduction occurred in sediments at the lower sulfate-methane transition zone (SMTZ) where extremely high number of microbial cells ( $10^{10}$  cells/ $\text{cm}^3$ ) were observed by AODC (Parkes *et al.*, 2005). Digenetic calculations of porewater chemical constituents suggested that microbial activities represented by sulfate reduction and methanogenesis are relatively high in near surface and organic-rich Peru margin sediments, whereas organic-poor sediments in the eastern Equatorial Pacific sites appeared to be characterized by lower activity and oxidative processes (D'Hondt *et al.*, 2004). This has been attributed to very low flux of nutrient and energy substrates from photosynthetic primary organic production in overlying seawater. During the Leg 201, the existence of significant amount of potential electron acceptors, such as sulfate, metal oxides, nitrate, or even oxygen were found in sediments above basaltic basement (D'Hondt *et al.*, 2004; DeLong *et al.*, 2004) (Fig. 1.1). These data consistently suggest that



metabolic activity of deep seafloor life is generally extremely low, and the microbial population and activity are tightly correlated to the availability of electron donors and acceptors for ATP (adenosine triphosphate)-yielding metabolic respirations, which are derived either from the seawater or the crustal fluids underlying sediments.

During the ODP period, the diversity of deep seafloor microbial communities was investigated by culture-independent molecular ecological techniques using polymerase chain reaction (PCR)-amplified 16S rRNA genes. Phylogenetic analysis of 16S rRNA gene-clone libraries revealed that seafloor microbial communities are mainly composed of previously uncultured, hence unidentified archaeal and bacterial components: e.g., Deep-Sea Archaeal Group (DSAG: alternatively designated as Marine Benthic Group-B [MBG-B]), Miscellaneous Crenarchaeotic Group (MCG) and South African Gold Mine Euryarchaeotic Group (SAGMEG) for the domain Archaea, *Chloroflexi*, *Planctomycetes* and Candidate Division JS1, for the domain Bacteria (e.g., Inagaki *et al.*, 2006; Teske, 2006; Webster *et al.*, 2006) (Fig. 1.2). At Site 1227 in the Peru Margin, notably high RNA concentrations were recovered from sediments at the SMTZ, in which DSAG Archaea were predominantly detected by sequencing the reverse transcribed 16S rRNA (Sørensen and Teske, 2006). These molecular results consistently suggested that metabolically active microbial components are living at geochemical interfaces within the



deep seafloor biosphere, although metabolic functioning of these uncultured microbes remains largely unknown.

Recent progresses in high-throughput DNA sequencing and molecular ecology have enabled the retrieval of metagenomic information from natural microbial habitats, even for relatively low biomass or low DNA-recovery environments like the deep-biosphere. Using ODP Leg 201 samples from the Peru Margin, the small amount of DNA was extracted and purified, and then amplified using a multiple displacement amplification technique (MDA, [Dean et al., 2001](#)). This whole genome amplified material was used for pyrosequencing, yielding approximately 62 Mbp of genetic information from 4 sediment horizons down to depths of 50 mbsf at Site 1229 ([Biddle et al., 2008](#)). The results of similarity analysis against known sequences revealed that a large fraction of total 16S rRNA genes (up to 88% at 50 mbsf) were affiliated to Crenarchaeota (see Chapter 2). Interestingly, in the metagenomic libraries, only 3% to 8% of sequences from seafloor sediments showed detectable homologies to functionally known sequences in the databases: *i.e.*, over 90% of metagenomic sequences in the deep seafloor biosphere remain unknown.

If the activity of microbes at SMTZs is supported by anaerobic oxidation of methane, the carbon isotopic compositions ( $\delta^{13}\text{C}$ ) of cellular bio-molecules must correspond to isotopically light carbon (*i.e.*,  $^{12}\text{C}$ -enriched carbon) derived from methane as the carbon source. Using secondary ion mass spectrometry (SIMS) coupled with fluorescence *in situ* hybridization (FISH-SIMS; [Orphan et al., 2001](#)), carbon isotopic compositions of intact archaeal cells were measured and compared with the  $\delta^{13}\text{C}$  values of archaeal intact polar lipids from the same samples ([Biddle et al., 2006](#)). The  $\delta^{13}\text{C}$  values obtained by SIMS and intact polar lipid analysis were in good agreement and suggested that most metabolically active archaeal components at SMTZs were not assimilating methane, but rather might be mixotrophs, using buried organic matter as a carbon source, while gaining energy from sulfate-dependent methane oxidation. Nevertheless, the microbes mediating the anaerobic oxidation of methane have remained largely unknown in the deep seafloor biosphere. In addition, based on the  $\delta^{13}\text{C}$  values of porewater dissolved inorganic carbon (DIC), acetate, and hydrocarbons, it has been suggested that other novel microbial metabolisms may also contribute to the production of ethane and propane using hydrogen and acetate ([Hinrichs et al., 2006](#)).

It has been long unknown if the deeply buried seafloor microbes are alive, just surviving, or dead. Activity measurements using radiotracers such as  $^{35}\text{S}$ -labeled sulfate for sulfate reduction and  $^{14}\text{C}$ -labeled bicarbonate or acetate for methanogenesis indicate that microbial activities in deep seafloor sediments are extremely low, with mean generation times of up to thousands of years ([Jørgensen and Boetius, 2007](#)). More recently, the proportion of living microbial cells in the deep seafloor was independently assessed using RNA-based molecular techniques on ODP Leg 201 samples. Using a fluorescence microscopy method known as catalyzed reporter deposition fluorescence *in situ* hybridization (CARD-FISH), [Schippers et al. \(2005\)](#) reported a large fraction of RNA-containing bacterial cells and no visible (*i.e.*, hybridized) archaeal cells. Cultivation efforts also resulted in the successful isolation of a numbers of (mostly facultative) anaerobic bacteria from deep sediments ([D'Hondt et al., 2004](#)), although the predominant microbial components (identified with cultivation-independent molecular methods) were resistant to growth in the culture media. These data demonstrated that some deep seafloor microbes are alive, although their *in situ*

physiological state as well as the mechanisms of long-term growth and/or survival is still poorly understood.

Within the subseafloor biosphere, the deep aquifer in the permeable upper oceanic crust –so called "*Subseafloor Ocean*" –represents a large space that is potentially habitable for microbial life. Microscopic observations of basaltic core samples recovered by ODP showed that cell-like fabrics are present in the volcanic glass and fractures (Fisk *et al.*, 1998). Based on the volume of glass as the microbial habitat, the total global biomass of basalt-hosted microbes was estimated to be 0.6 Tg of cellular carbon, representing only a small fraction of total living biomass on Earth. However, the real abundance of microbial cells in the rock aquifer remains unknown. During the ODP Leg 186, an experimental borehole seal, so called Circulation Obviation Retrofit Kit (CORK), was deployed at Site 1026B in the eastern flank of the Juan de Fuca ridge, where 247 m of sedimentary column and 48 m of basaltic ocean crust was drilled. Although the CORK at Site 1026B did not completely seal the borehole and may have introduced ambient seawater, molecular analysis of the borehole fluids collected from a BioColumn device on the CORK revealed diverse bacterial and archaeal sequences related to thermophilic microorganisms that are potentially derived from the crustal aquifer (Cowen *et al.*, 2003). Thermodynamic and bioenergetic calculations suggest that water-rock reactions such as iron-oxidation and hydrogen production may support significant population of subseafloor life within the upper basaltic crustal habitat in ridge flank hydrothermal systems (Bach and Edwards, 2003).

Given these pilot and ongoing studies as the background, the understanding of "*Deep-biosphere*" and "*Subseafloor Ocean*" is selected as one of the important scientific objectives that should be addressed during the Integrated Ocean Drilling Program (IODP) (IODP Initial Science Plan [ISP], 2001). Together with deep-biosphere and subseafloor ocean initiatives, the exploration of "*Gas Hydrates*" on the continental margins is also listed as one of the IODP scientific initiatives in terms of formation/accumulation mechanisms, structure and stability, and microbiological associations such as methanogenesis and methane-consumption. Since the ODP Leg 201, unfortunately no microbiology-biogeochemistry-dedicated drilling expeditions have been completed thus far. On the other hand, microbiologists and biogeochemists have participated in multiple IODP expeditions, providing valuable information that may help the identification and planning of more comprehensive microbiology-biogeochemistry focused expeditions in the future.

## 2. Archaea vs. Bacteria: A Hypothesis of an "Archaeal World"

All living organisms on Earth are classified into three major Domains of life: Archaea, Bacteria and Eukarya. Pilot studies on PCR-amplified 16S rRNA genes revealed the presence of diverse uncharacterized phylotypes within the domains of Archaea and Bacteria in deep seafloor sedimentary habitats. However, the quantitative balance between these two domains of life in the deep subsurface has been long debated. This is a question fundamental to understanding the Earth's biosphere. On the other hand, microbiological studies of deep seafloor environments pose some unique challenges. Most primers used for PCR are constructed based on known microbial sequences, thus may have strong biases against the indigenous, unknown microbial communities of the Earth's deep biosphere. A recent review by [Teske and Sørensen \(2008\)](#) highlighted some of the biases and variation in specificity of current archaeal PCR primers, illustrating that our knowledge of archaeal diversity is ultimately linked to the robustness of primer design. For example, high mismatch frequencies for some of the most widely used archaeal 16S rRNA gene primers and probes (ARC915R and ARC958R), have significant mismatches against the members of the seafloor-associated archaeal groups SAGMEG and DSAG, respectively. Studies using quantitative-PCR with known primer sets indicates the deep seafloor sediment communities are dominated by Bacteria with extremely low or no recovery of archaeal 16S rRNA genes ([Schippers \*et al.\*, 2005](#); [Schippers and Neretin, 2006](#); [Inagaki \*et al.\*, 2006](#)).

Similarly, using CARD-FISH with general archaeal probe (AR915) and bacterial probe (Eub338), [Schippers \*et al.\* \(2005\)](#) reported a large fraction of 16S rRNA hybridized bacterial cells to depths of >400 mbsf and a low number of Archaea. However, archaeal cells, even those in culture can be difficult to visualize by FISH or CARD-FISH, possibly due to problems with permeabilizing their unique cell wall structure. This can be particularly problematic with the CARD-FISH technique that amplifies the fluorescent signal within the cell by using an oligonucleotide DNA probe modified with horseradish peroxidase; hence cell wall permeability is a critical step for successful hybridization of some Archaea (and Bacteria enveloped with thick outer cell membrane).

Using IODP Expeditions 301 (Juan de Fuca ridge flank) and 311 (Cascadia Margin) samples together with ODP Legs 201, 204 and 207 samples down to 367 mbsf, [Lipp \*et al.\* \(2008\)](#) studied the structure and amount of the extracted intact polar lipids (IPLs). Bacteria have bi-layer lipid membranes composed of glycerol-ester-bonded fatty acids, whereas Archaea, with a small number of exceptions, have mono- and bi-layer membranes of glycerol-ether isoprenoid hydrocarbons. In most living cells, the glycerol of membrane lipids is bound to polar head groups such as phosphate and sugars, which are enzymatically degraded soon after the cell death. In the IPL fractions from sediments deeper than 1 m from the seafloor, approximately 87% of IPLs are attributable to glycerol dibiphytanyl glycerol tetraether (GDGT) and the derivatives, the typical lipid structure of the domain Archaea. Based on the amount of IPLs, the total microbial biomass in the seafloor biosphere was estimated to be 90 Pg of carbon, which is nearly consistent (difference within an order of magnitude) to the previous estimates for seafloor microbial biomass.

The IPL data, on one hand, suggest that seafloor biosphere is an "Archaeal World", however these results contrast some of the previous molecular results (*e.g.*, [Schippers \*et al.\*, 2005](#)). One possible explanation for the discrepancy in datasets may lie in the experimental methods applied. Differences in DNA extraction techniques, sample

preservation and preparation methods, and differential bias in the primers and probe sets, may all influence the detection of the *in situ* microbial assemblage. In the [Lipp \*et al.\* \(2008\)](#) study, DNA was recovered from microbial cells that were physically lysed directly in the sediment matrix using bead beating in liquid nitrogen, and then amplified using MDA with phi29 polymerase ([Dean \*et al.\*, 2001](#)) to increase the total genomic DNA concentrations prior quantifying archaeal and bacterial 16S rRNA genes with quantitative-PCR and slot-blot-hybridization. The physical destruction of cells in the sediment is believed to increase the cell lysis efficiency, reducing potential bias associated with the DNA extraction procedure. Whole genome amplification using phi29 polymerase generates enough DNA for purification and molecular analysis from low biomass samples, but also suffers from possible exogenous DNA contamination in the reagents and has the potential to artificially skew the microbial assemblage composition. Differences in molecular detection methods may additionally result in variation in the recovered microbial diversity. For example, slot-blot-hybridization uses a single oligonucleotide probe for signal detection, while PCR-based quantification requires a forward and reverse primer. Each of these molecular techniques may contain mismatches to the 16S rRNA genes from unknown subseafloor microorganisms, as documented by [Teske and Sørensen \(2008\)](#). By modifying the primer sequence used for detecting microorganisms in the deep subsurface and by combining independent molecular detection methods on the same samples (qPCR, slot blot hybridization and IPLs), [Lipp \*et al.\* \(2008\)](#) reported a greater proportion of archaeal 16S rRNA genes than had been previously observed (approximately 40% of the total archaeal and bacterial 16S rRNA gene abundance recovered). These recent findings illustrate the importance of applying complimentary methods to the study of microorganisms in this challenging environment, and suggest that we may have been overlooking the importance and distribution of the Archaea within the subseafloor sediments and crust.

While recent data points to an archaeal world, dominated by GDGT-type lipids and 16S rRNA sequences ([Lipp \*et al.\*, 2008](#) and [Biddle \*et al.\*, 2008](#)), in depth molecular studies have only been conducted at a handful of sites and the nature and adaptation of Archaea and other microorganisms residing in the deep subseafloor still remains as one of the fundamental questions to be addressed in future IODP expeditions. For example, there are exceptions in the lipid classification scheme: *i.e.*, some cultured bacteria within *Aquificales* and *Thermodesulfobacteriaceae* as well as uncultured sulfate-reducing bacteria from methane seep habitats ([Hinrichs \*et al.\*, 2000](#)) also have glycerol-ether lipids ([Jahnke \*et al.\*, 2001](#)). It has been pointed out that mono-layer lipid structure has been developed by adapting to extremely energy-limited or -starved conditions because of the low substrate-permeability while bi-layer fatty acids-structure like bacterial cell membrane is fit to high-energy habitats ([Valentine, 2007](#)). Hence, it is conceivable that some uncultured bacteria well adapted to energy-limited subseafloor habitats may also have GDGT-like IPLs. The efficiency of lipid-recovery from subseafloor sedimentary biomass as well as the turnover rates of phosphate or sugar head groups remains unknown.

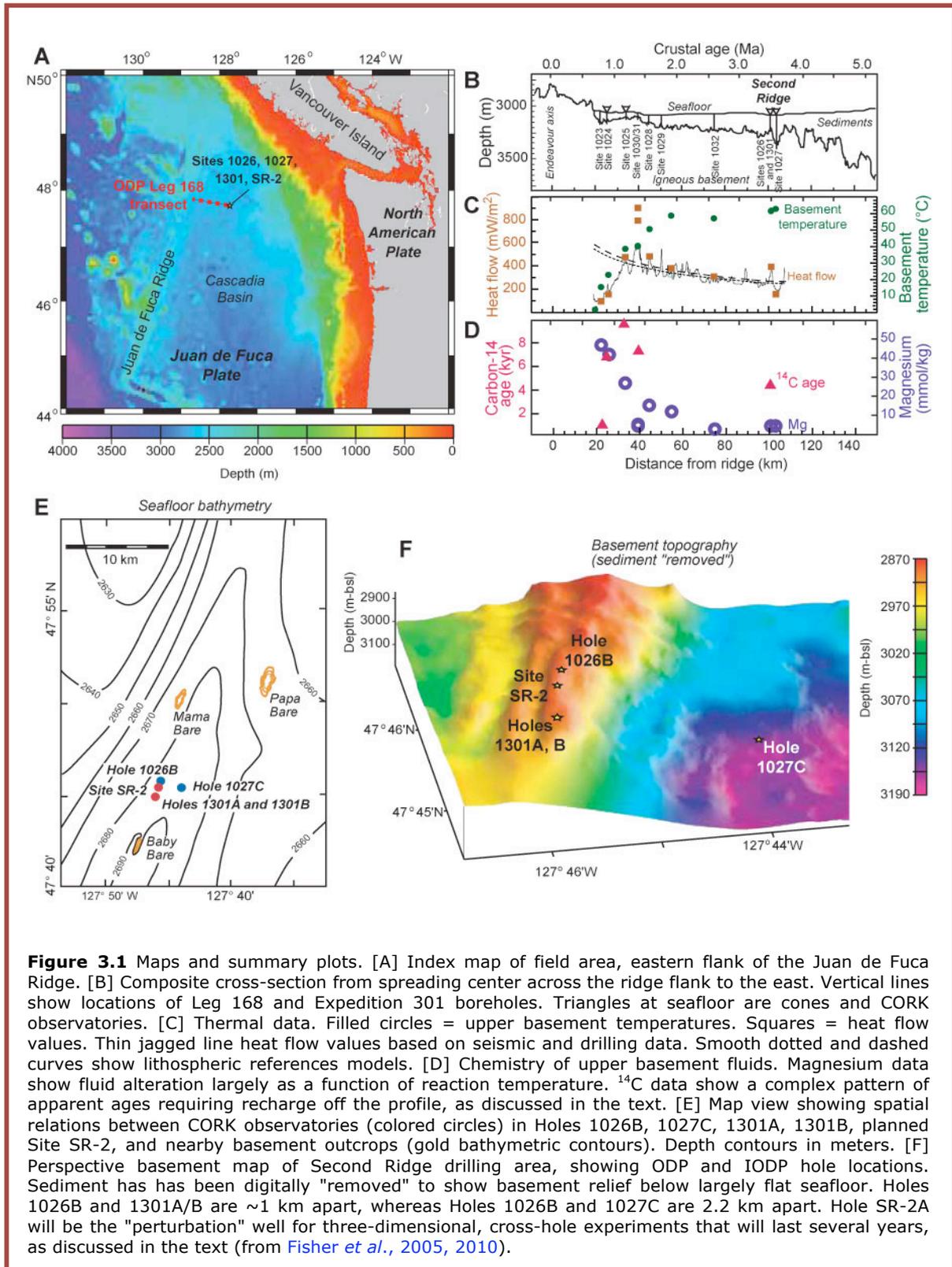
### 3. Expedition 301: Hydrogeology in the Juan de Fuca Ridge Flank

Expedition 301 was the first IODP expedition directed primarily at understanding three-dimensional hydrogeological state and processes in young upper oceanic crust on the eastern flank of the Juan de Fuca Ridge. It was the first of a two-expedition program designed to address these objectives using basement coring, logging, installation of multi-level CORK observatories, and controlled source cross-hole experiments. As such, a significant component of Expedition 301 was largely operational, in terms of installing CORKs to set the stage for the cross-hole, three-dimensional assessments to be conducted on the second expedition, now scheduled for summer of 2010. Nevertheless, Site U1301 was an important success in terms of coring several hundred meters into young oceanic basement, and there have been important scientific results from the cores, logs, and CORK experiments from that site. These include focused microbiological studies in both the basement section and the overlying sediments.

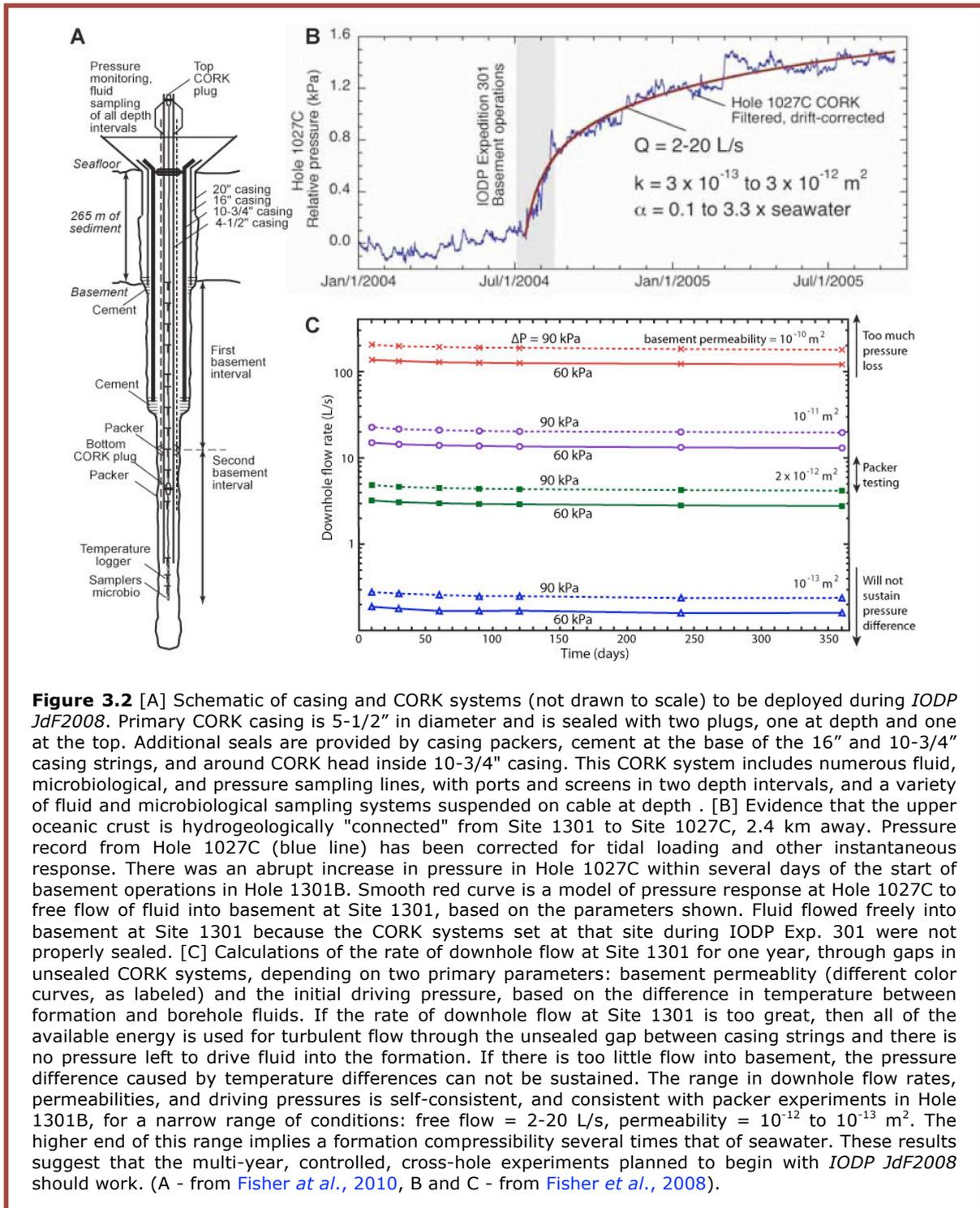
#### 3.1 Hydrogeology: Environmental settings and CORK-installation

Expedition 301 returned to the thickly sediment-covered eastern flank of the Juan de Fuca Ridge, where ODP Leg 168 had cored several sites generally on a crustal age transect from 0.8 to 3.6 Ma. The IODP Expedition 301 focused specifically on the "Second Ridge" area (*i.e.*, second sediment-buried abyssal ridge going off-axis) in ~3.5 Ma crust (Fig. 3.1). This was the location of two primary ODP Leg 168 sites with CORKs installed into the shallowest (<50 m) upper basement: Hole 1026B in the sediment-buried ridge and Hole 1027C in the adjacent sediment-buried basement valley ~2km to the east. Those CORKs had documented slight overpressure of basement fluids in Hole 1026B and slight underpressure in Hole 1027C, nearly isothermal conditions in upper basement (~65°C) despite large differences in the sediment thickness at the two sites and high permeability in both holes. These observations are consistent with very high lateral fluxes of upper crustal fluids that could be interpreted in terms of traditional two-dimensional (depth and distance from axis) models for off-axis hydrothermal circulation. Expedition 301 and a follow-on 2010 expedition were designed to extend the observations to greater depth into basement and to investigate a likely more realistic scenario involving significant components of hydrothermal circulation in the third dimension.

Addressing the three-dimensional aspects (physical, chemical and microbiological) will represent a major advance in our understanding of off-axis hydrothermal circulation, which to date has largely a two-dimensional perspective. The plan for Expedition 301 was to replace the two original CORKs in Holes 1026B and 1027C, and core, log and CORK a new site (U1301) about one km to the south along the buried basement ridge (Fig. 3.1). U1301 was planned to penetrate down to 600 m into the basement where reduced permeability was expected based on regional seismic data and observations of both permeability and velocity variations with depth at other sites (*e.g.*, 504B). For operational reasons (likelihood of unstable hole conditions in the uppermost basement, as had been encountered in Hole 1026B), two separate holes and CORKs were planned at Site 1301: U1301A for the uppermost 100 m of basement, and U1301B for the deeper section. All the Expedition 301 CORKs were newer multi-zone CORK-II models that allow long-term *in situ* geochemical and microbiological monitoring in addition to the traditional CORK pressure and temperature monitoring. The design of these CORK-II



models represents an important technical contribution from Expedition 301 (Fisher *et al.*, 2005). The design (Fig. 3.2) has been used as the starting point in designing CORK installations for the return expedition in 2010 and the planned 2011 expedition to the North Pond near the Mid-Atlantic Ridge.



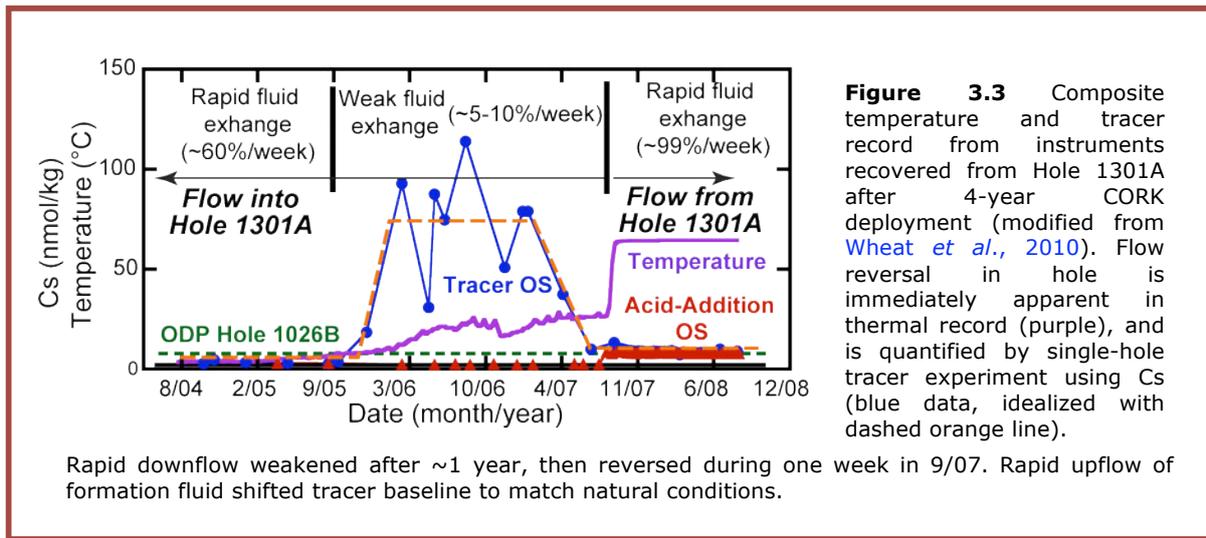
In the original plan, the second expedition (ideally 1-2 years after Expedition 301) would establish a new CORKed site (SR-2) on the buried basement ridge between Sites 1026 and U1301, using this new site as a hydrologic source well for three-dimensional cross-hole hydrologic tracer experiments to the downhole sensors installed on Expedition 301 – experiments designed to elucidate actual flow paths and km-scale connectivity of basement permeability for the first time in an off-axis hydrothermal system.

Not all of the Expedition 301 operational objectives were achieved, as is summarized in bullet form below. The post-expedition operational review task force dealt very carefully with the operational issues. National Science Foundation (NSF) has generously supported ROV *Jason II* or HOV *Alvin* operations to the installations every summer since Expedition 301, and highlights are noted below:

- Basement penetration at Site U1301 was about 350m, somewhat less than the original objective of 600m (but still a significant achievement in coring such young crust).
- Time limits and loss of a CORK-II assembly precluded replacement of the original CORK in Hole 1027C (which has continued to work well as a pressure-monitoring installation).
- Neither of the two CORK-II installations at Site U1301 was properly sealed during Expedition 301, allowing for significant downhole flow of ocean bottom water in both Holes 1301A and 1301B, perturbing the *in situ* hydrogeological state. This required remedial cementing attempts during post-Expedition 301 submersible operations and a June 2009 revisit by *JOIDES Resolution* Expedition 321T.
- The second expedition is now completed as Expedition 327 in summer 2010, six years after Expedition 301, much longer than the original vision for a 1 or 2-year interval. This has required/will require that the original 5-year sensor strings installed during Expedition 301 be replaced during submersible operations in 2008 (1026B) and 2009 (U1301A) and the return IODP expedition (U1301B).

There were additional *Jason II* submersible operations scheduled for June 2010 before the IODP Expedition 327. There will be some modifications required to the operational plans for the IODP Expedition 327, but the overall experiment still seems quite feasible. An important highlight was the summer 2009 connection of the new instruments installed in 2008 in Hole 1026B to the NEPTUNE Canada observatory. There is a high chance of connection of other SR-CORKs to NEPTUNE Canada in future years.

Although Expedition 301 was largely operational, there have been some important scientific results published on the physical and hydrological state of the crust. Expedition 301 operations produced signals in the CORK pressures recorded over 2 km away at Hole 1027C, convincingly demonstrating the feasibility of the overall plan for cross-hole hydrologic testing (Fig. 3.2). Bartetzko and Fisher (2008) analyzed core and log data to compare the physical state of the crust at Site U1301 to other important crustal reference holes. Packer experiments (Becker and Fisher, 2008) showed high permeability at Hole U1301B, with a gradual reduction deep in the hole. The cross-hole signals recorded at Hole 1027C were interpreted by Fisher *et al.* (2008) to constrain a km-scale upper crustal permeability and the potential for permeability anisotropy, and Davis *et al.* (2010) are conducting numerical experiments to further constrain the regional hydrological state. Long-term CORK downhole-pressure and -temperature data recovered in 2008 and 2009 indicate that the downhole flow induced in Hole U1301A during Expedition 301 reversed in September of 2007 as natural formation overpressures reestablished (Fig. 3.3), and that the deeper intervals in the deeper Hole 1301B have also recovered a significant overpressure in 2009.



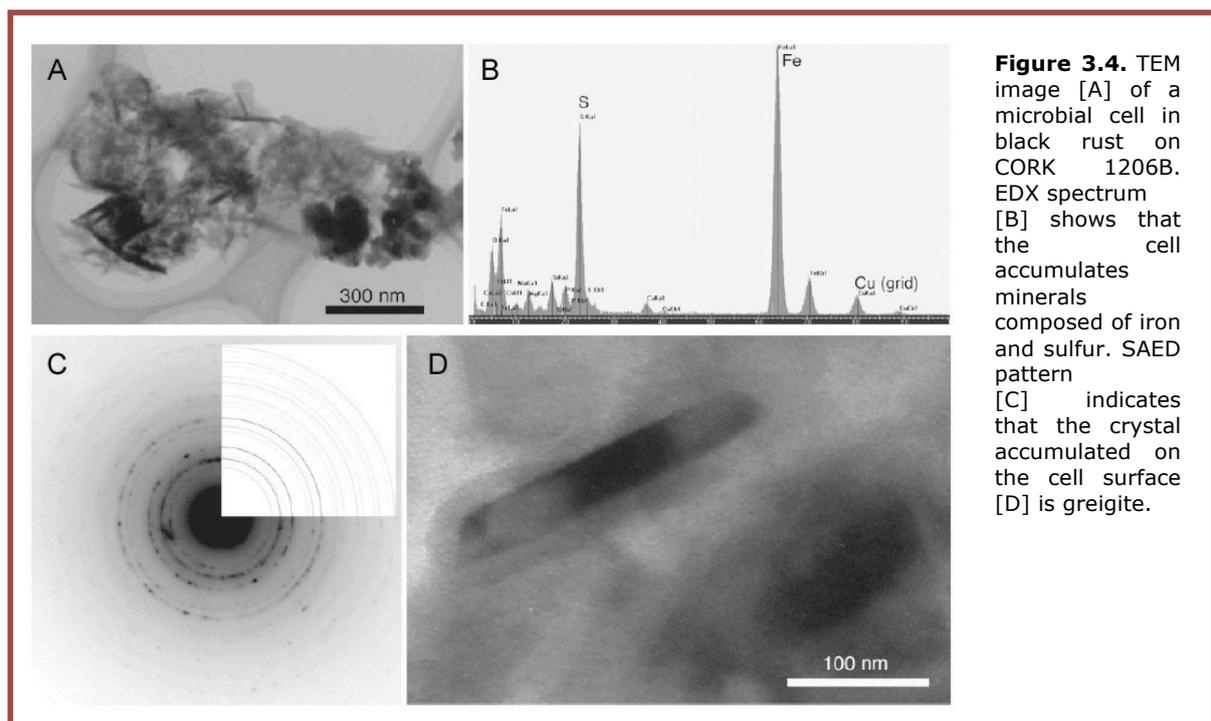
### 3.2 Microbiology: Subseafloor life and ridge flank hydrothermal circulation system

During Expedition 301, shipboard microbiologists collected samples of black rust on the CORK recovered from Hole 1026B, approximately 8 years after installation during ODP Leg 168. Although the CORK at 1026B was not completely sealed, hot crustal fluids measuring temperatures of 64°C were flowing continuously, providing an excellent opportunity to study thermophilic microbial community inhabiting “an artificial hydrothermal vent” in the ridge flank hydrothermal system. Nakagawa *et al.* (2006) studied the microbial community using both cultivation and culture-independent molecular experiments. The slurry sample for cultivation study was successfully prepared with anaerobic artificial seawater and nitrogen on D/V *JOIDES Resolution*. Shore-based cultivation experiments using a most probable number (MPN) method showed that a hydrogen-utilizing thermophilic methanogenic archaeon, *Methanothermococcus thermolithotrophicus*, was abundant in the black rust with the cultivable population at  $1.3 \times 10^3$  at the incubation temperature from 55°C to 70°C. Thermophilic sulfate-reducers such as the bacterial genera *Desulfomonas*, *Desulfonatovibrio*, *Desulfotomaculum* and archaeal *Archaeoglobus* relatives and thermophilic fermenters (*Thermoshipho* and *Halothermothrix* spp.) were also cultured from the black rust. Cultivation-independent molecular ecological surveys targeting 16S rRNA and dissimilatory sulfite reductase (*dsrAB*) genes were also completed for the black rust samples, showing congruence with the cultivation results.

Viable archaeal species were also recovered from the hydrothermal ridge flank. Cultivation studies using ridge flank samples led to the successful isolation of a new *Archaeoglobus* species, *Archaeoglobus sulfaticallidus* sp. nov. (strain PM70-1) (Steinsbu *et al.*, 2010). Like other cultured members of the *Archaeoglobus* genus, strain PM70-1 is a thermophilic, facultatively lithoautotrophic sulfate-reducing archaeon, growing

anaerobically on  $H_2$  and  $CO_2$  coupled to sulfate, sulfite or thiosulfate reduction at an optimum temperature of  $75^\circ C$ .

Quantitative-PCR analysis showed the presence of a remarkably high copy number of *dsrAB* genes ( $9.5 \times 10^7 \pm 2.5 \times 10^7$  copies/cm<sup>3</sup>) in the black rust, suggesting that sulfate reduction is the major metabolic process in the borehole, likely driven by secondary seawater circulation. *Ammonifex*-relatives, related to chemolithotrophic nitrate-reducing gram-positive microorganisms, were also recovered in a Bio-Column experiment from the same CORK at Site 1026B (Cowen *et al.*, 2003), in addition to thermophilic sulfate-reducers and heterotrophic microorganisms using both 16S rRNA gene and *dsrAB* clone libraries. Observations of transmission electron microscopy (TEM) as well as energy-dispersive X-ray spectroscopy (EDX) and selected area electron diffraction (SAED) pattern analysis showed formation of iron sulfide on the cell surface (Fig. 3.4), supporting the predominance of sulfate reduction and sulfide formation in this environment. Further, Nakagawa *et al.* (2006) performed carbon and sulfur isotopic analysis of the total organic carbon (TOC), lipids, and mineral deposits (sulfide) in the black rust habitat. The isotopic data suggest that buried photosynthetic organic matter is the primary carbon source and that seawater sulfate is the source for active sulfate reduction in the black rust on the CORK. While the specific sources of energy for subseafloor microbial life in the crust are unknown, it is conceivable that the growth of *M. thermolithotrophicus* and other potential hydrogen-consuming thermophilic sulfate reducers in the CORK environment may have been stimulated either by the production of molecular hydrogen via steel corrosion or inorganic nutrient and energy sources (*i.e.*, hydrogen and  $CO_2$ ) generated from basalt-water reaction. Comparing the black rust “artificial” hydrothermal vent community to the naturally occurring microbial community in the neighboring Juan de Fuca hydrothermal vents revealed some notable differences, including the absence of commonly recovered cosmopolitan hyperthermophilic and mesophilic vent microorganisms *Thermococcales*, *Aquificales* and *Epsilon-proteobacteria* (Nakagawa and Takai, 2008).



During the ODP Leg 187, [Lysnes et al. \(2004\)](#) have previously reported the presence of phylogenetically diverse microbial components in deeply buried basalts (~28 Ma, 374.2 mbsf) by cultivation and cultivation-independent molecular ecological analyses. Although fluorescent tracer beads experiment showed that most samples recovered by rotary core barrel (RCB) were heavily contaminated with drilling fluids, the growth of strictly anaerobic microbes as well as microbial methane production and iron reduction was observed in anaerobic cultures, which were most likely indigenous anaerobic microbial components within the deep basaltic habitat.

Identifying and controlling for contamination by exogenous microorganisms and/or DNA in drill fluids remains a significant concern for the study of low biomass deep biosphere samples, especially when dealing with highly fractured subseafloor basalts. During Expedition 301, [Lever et al. \(2006\)](#) evaluated potential contamination of sediment and basalt cores at Site U1301 with a perfluorocarbon (PFT) tracer method, improving upon earlier methods focusing on subseafloor sediments ([Smith et al., 2000](#); [House et al., 2002](#)). By analyzing contaminated PFT concentrations, it was demonstrated that in most cases the diffusion of contaminated fluids and particles does not cause significant microbiological contamination towards the central region of the core, if the samples are stored under ultra cold (<-80°C) -conditions soon after core recovery. Model calculations of fluid advection varying time and temperature suggest that temperature is more significant in controlling contamination than sampling time. Contamination of basaltic rock core samples was also tested by PFT method. Near-complete removal of the exterior rind with washing using a sterilized buffer or water and/or flame sterilization further minimized microbial contamination.

Using sediment core samples from Site U1301, [Engelen et al. \(2008\)](#) reported total microbial populations, cultivable populations, and metabolic/enzymatic activities in sediments containing SO<sub>4</sub> levels of 16mM from upward diffusion of hydrothermal fluids from the basement. Total cell abundance was evaluated using both AODC and SYBR Green I methods. Both methods showed the presence of high numbers of microbial cells reaching >10<sup>7</sup> cells/cm<sup>3</sup>. The cell abundance generally decreased with depth, with the exception of some local maxima at and below the SMTZs and close to the basement. Using <sup>14</sup>CH<sub>4</sub> and <sup>35</sup>SO<sub>4</sub> radiotracers, weak (a few pmol/cm<sup>3</sup>/day level) but obviously enhanced sulfate reduction and anaerobic methane oxidation potentials were documented below the lower SMTZ at around 150 mbsf. Consistently, relatively high number of cultivable anaerobic microbes (1.1 × 10<sup>7</sup> MPN counts/cm<sup>3</sup>: cultivation efficiency of 3.57%) was observed near the lower SMTZ. Interestingly, enzymatic activities of extra-cellular phosphatase show an increase with depth from the lower SMTZ to basement, where no or little phosphate was measured. These data demonstrate that, in the ridge flank hydrothermal circulation systems, the diffusive flow of young crustal fluids has an important ecological influence on microbes in sediments above the basalt interface.

Although young oceanic crustal environments are postulated as potential deep subseafloor habitats that harbor chemolithoautotrophic microbial communities ([Bach and Edwards, 2003](#); [Edwards et al., 2005](#)), direct evidence for the indigenous microbial activity within the crust still remain poorly demonstrated. Using U1301 basaltic core samples from Expedition 301, [Lever et al. \(submitted\)](#) studied endolithic microbial activities with molecular and biogeochemical techniques. Basaltic whole round core samples containing altered vein structures were aseptically collected for microbiological analyses by removing potential contamination from core exterior ([Lever et al., 2006](#)),

and then stored at  $-80^{\circ}\text{C}$  prior to use. The endolithic DNA was subsequently extracted from crushed basaltic samples. Using PCR, the alpha subunit of methyl coenzyme M reductase (*mcrA*), a key gene for methanogenesis and anaerobic oxidation of methane (AOM), were successfully amplified from basalt core DNA extracts. Phylogenetic analysis of *mcrA* genes indicated the potential occurrence of AOM-associated Archaea within the basalt. The sulfur isotopic analysis of reduced sulfur compounds consistently showed notably depleted sulfur isotopic compositions in the same horizons that *mcrA* genes were detected, most likely suggesting the *in situ* occurrence of thermophilic AOM coupled with sulfate reduction. These data from basaltic cores demonstrate for the first time that both carbon and sulfur cycles mediated by microbial activities occur in the deeply buried crustal habitat.

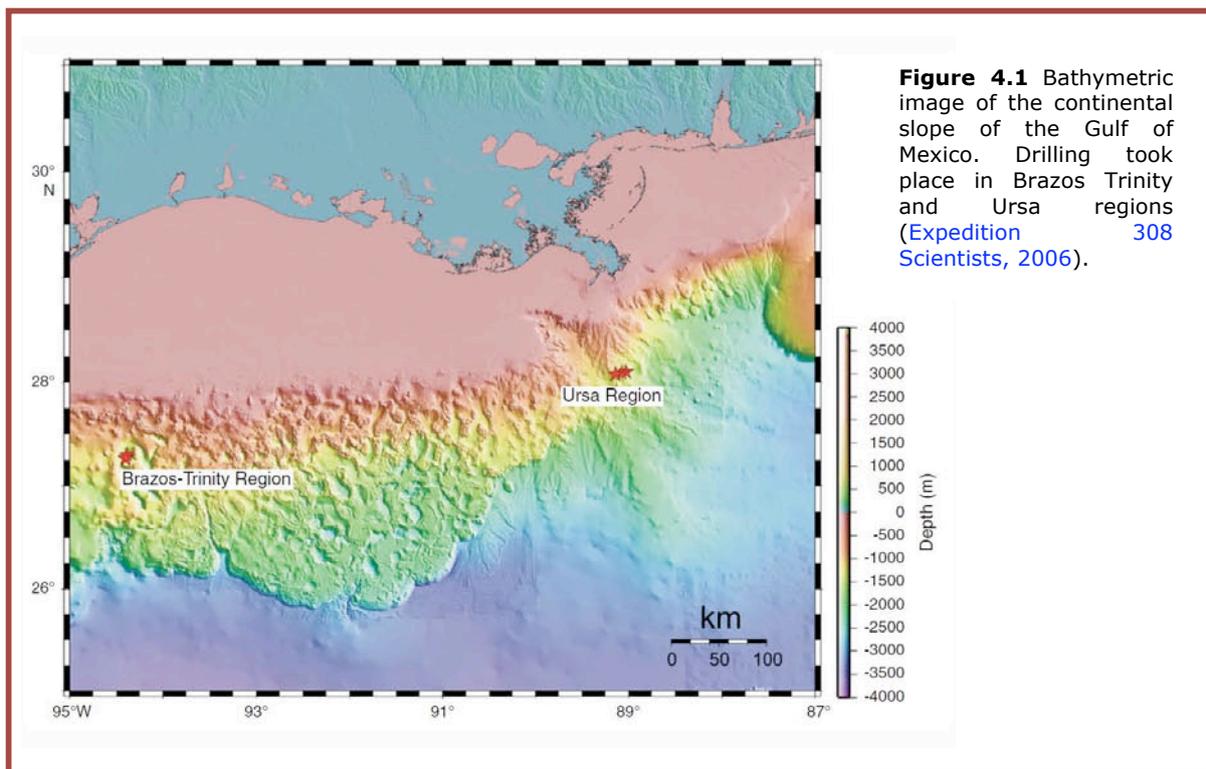
Lever *et al.* (2010) performed thermodynamic calculations of microbial acetate-producing (acetogenesis) pathways based on geochemical porewater profiles and carbon isotopic compositions. The results suggested that fermentation products or lignin monomers are sustainable for various types of acetogenesis pathways: *e.g.*, auto-, mixo- and methylo-trophic acetogenesis. However, *in situ* concentration of hydrogen is a key factor controlling the thermodynamic thresholds of competing hydrogen-consuming processes such as sulfate-reduction and methanogenesis (see also Hoehler *et al.*, 1998; Heuer *et al.*, 2009). Phylogenetically diverse and novel formyl tetrahydrofolate synthetase (*fts*) genes, key functional genes for acetogenesis via acetyl-coA pathway, were detected from the upper half of cored sediments, in which depth-range, relatively high concentrations of acetate were obtained. Carbon isotopic compositions of the acetate were also depleted in  $^{13}\text{C}$  from 3‰ to 9‰ relative to TOC and from 9‰ to 24‰ relative to DIC. These thermodynamic, geochemical, and molecular data consistently indicate that a substantial fraction of acetate in deep sediments may be sourced from microbial trophic interactions driven by fermentation, sulfate reduction, and diverse acetogenesis pathways.

### 3.3 *In situ* technology for studying indigenous microbial communities and activity

To understand the indigenous microbial community within the pristine crustal habitat, the CORKed borehole observations based on microbiological *in situ* sensors and colonization devices has been vital to characterizing indigenous communities and microbial activity. In 2008, the CORK sensor strings were recovered from Hole U1301A, which contained prototype *in situ* microbial colonization device (Orcutt *et al.*, in prep.). The colonization device captured two distinct microbial communities, directly reflecting the change in hydrological state at three years from downflow of seawater to production of formation fluids.

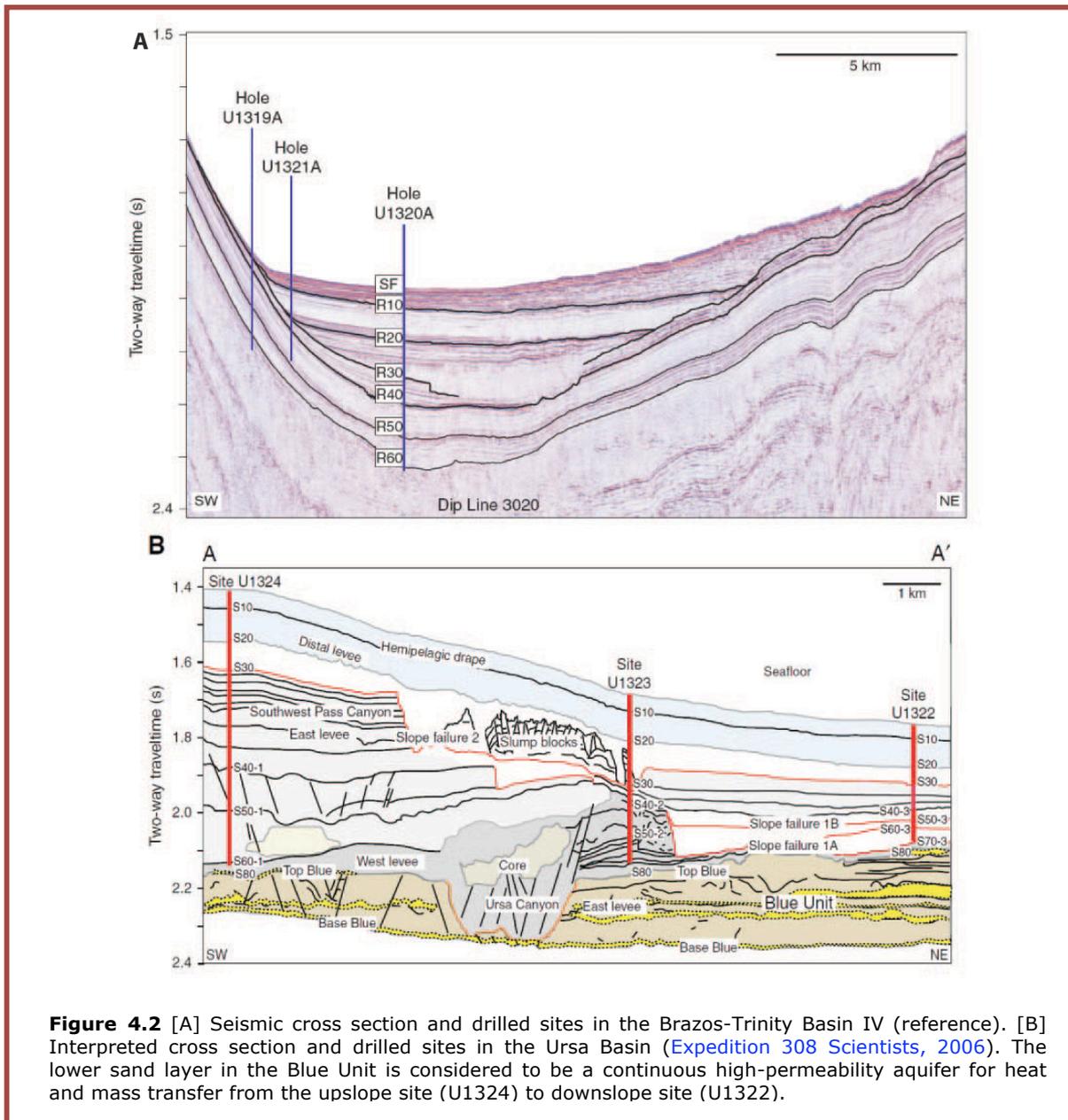
#### 4. Expedition 308: Gulf of Mexico Hydrogeology

IODP Expedition 308 (Gulf of Mexico Hydrogeology; [Flemings \*et al.\*, 2006](#)) was dedicated to study overpressure and fluid flow on the continental slope of the Gulf of Mexico. This expedition examined how sedimentation, overpressure, fluid flow, and deformation are coupled in a passive margin setting, and investigated how extremely rapid deposition of fine-grained mud might lead to a rapid build-up of pore pressure and sedimentary mass wasting. The expedition also tried to understand the state and evolution of geotechnical and petrophysical properties of shallow marine section, and to characterize ponded and channeled turbidites. For these purposes, two different locations were drilled ([Fig. 4.1](#)), one in the Brazos-Trinity Basin IV ([Fig. 4.2A](#)) where overpressure is hypothesized to be zero and the other in the Ursa Basin ([Fig. 4.2B](#)) where overpressure is known to exist and flow focusing phenomenon is expected to be present.



Measurement-while-drilling (MWD) approach to monitor downhole pressure during drilling operation was used together with the weighted mud to drill through the overpressured formation by non-riser drilling. In addition, many whole-round core samples were taken for the laboratory geotechnical experiments to infer in situ stress states. Also, this expedition was the first one that the spatial variation of the pore pressure field has been documented in detail ([Fig. 4.3](#)). Thus, future expeditions which focus on subsea hydrogeology might benefit from both technical and scientific achievements from this expedition.

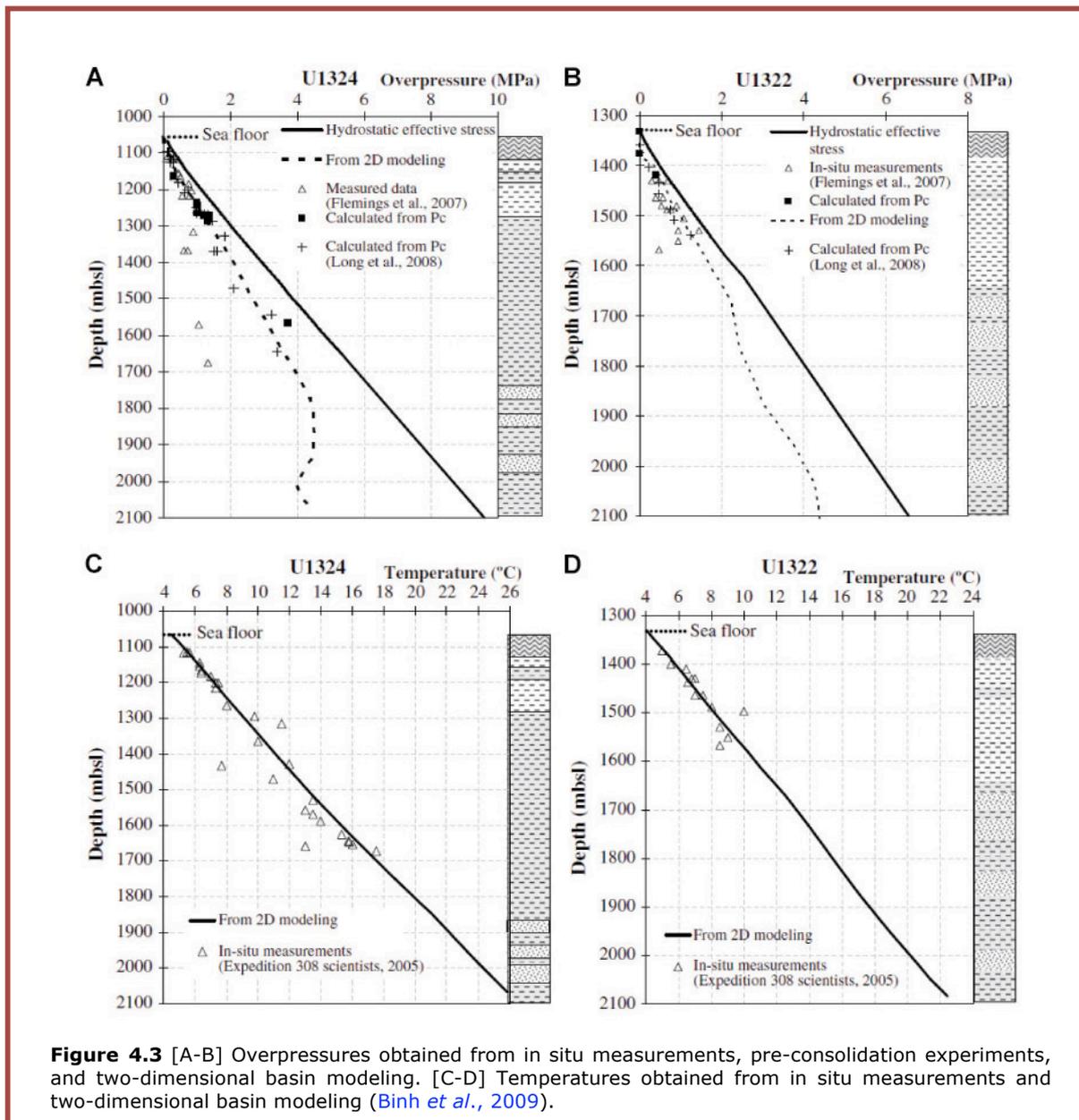
A fundamental achievement of this expedition is that the overpressure profiles at two sites (U1322 and U1324) in the Ursa basin were obtained through the combination of in situ pore pressure measurements and laboratory studies ([Figs. 4.2B and 4.3](#)).



Because of the difficulty of deploying devices for pore pressure measurements (Expedition 308 Scientists, 2006; Flemings *et al.*, 2008) and the ambiguity of pore pressure estimation based on the laboratory consolidation experiments due to sample deformation (Long *et al.*, 2008), pore pressure data were rather scattered at the measured sites. However, pore pressure data clearly indicate the existence of overpressure in the shallow part of the sedimentary formation at the Ursa basin. Temperature measurements showed the different geothermal gradients at two sites, *i.e.*, 18.4 and 26.2 K/km at the site U1324 and U1322, respectively (Fig. 4.3). Based on the fact that the thermal conductivities and specific heats are similar between two sites, the temperature data were considered to be suggestive of the existence of lateral fluid flow from the upslope site (U1324) to the downslope site (U1322) in the Ursa basin. Recent numerical experiments based on this expedition have also supported the existence of lateral fluid flow (Binh *et al.*, 2009).

At the Brazos-Trinity Basin IV where pore pressure was hypothesized to be hydrostatic (Fig. 4.2A), pore pressures in the central part of the basin (site U1320) were overpressured. Recent study (Schneider *et al.*, 2009) showed that even at the margin of the ponded basin (site U1319), pore pressure may be overpressured, showing that the rapid sedimentation of low permeability fine-grained sediments can produce shallow overpressures in the passive margin setting.

Data on the geotechnical and petrophysical properties of shallow sediments have been reported in the proceedings volume as data reports (Ask, 2009; Dugan and Germaine, 2009; Long *et al.*, 2008a, 2008b), which have filled the gap of our knowledge between deeper intervals based on petroleum geology/reservoir engineering fields and very shallow intervals based on geotechnical engineering analyses. Studies on the relationship among sedimentation, overpressure development, and slope stability, and the estimation of the timing of the formation of mass transport deposits are under progress (e.g., Urgeles *et al.*, 2009; Sawyer *et al.*, 2009).



**Figure 4.3** [A-B] Overpressures obtained from in situ measurements, pre-consolidation experiments, and two-dimensional basin modeling. [C-D] Temperatures obtained from in situ measurements and two-dimensional basin modeling (Binh *et al.*, 2009).

Overall, the result of this expedition has shown that the IODP program has potential to advance understanding of geohazards, *i.e.*, slope stability in this case, and has demonstrated the relevance of IODP science to industry partners.

The subseafloor microbial communities in sediment of the Brazos-Trinity Basin IV and the Mars-Ursa Basin were evaluated by DNA-based molecular analyses. Nunoura *et al.* (2009) reported that microbial cell counts or 16S rRNA gene copy numbers in both basin sediments were less than  $1 \times 10^7$  cells or copies/wet g sediments, respectively. The subseafloor biomass in this area was found to be overall low; however, the firm reason of low biomass remains currently unknown. The phylogenetic analysis of PCR-amplified 16S rRNA gene clone libraries revealed that the microbial communities were composed of diverse bacterial and archaeal subseafloor microbial components, such as sequences affiliated to the phylum *Chloroflexi*, JS1, *Spirochetes*, NTB6, *Bacterioides*, *Planctomycetes* for Bacteria, and DSAG, MCG, and SAGMEG for Archaea. Principal coordinate analysis showed that microbial community structures in turbidite deposits of the Brazos-Trinity Basin IV were statistically distinguishable from those in clay and basement horizons, suggesting that sedimentation regimes such as the rapid turbidite deposition have affected microbial community compositions and structures in the deep subseafloor biosphere. Using extracted DNA solutions, the copy number of two functional genes for dissimilatory sulfite reductase alpha subunit (*dsrA*) and methyl coenzyme M reductase alpha subunit (*mcrA*), key enzymes of sulfate reduction and methane-production/oxidation pathways, was measured by quantitative PCR analysis. Although relatively high concentrations of biogenic methane were observed in the research field, neither *mcrA* genes nor 16S rRNA genes of putative methanogens or ANME (which stands for "anaerobic methanotroph") Archaea could be detected from the extracted DNA solutions. In addition, the measurable *dsrA* genes were only obtained from upper sediments of the sulfate-reduction zone, although the copy numbers were significantly low.

These phylogenetic and quantitative molecular data suggest that we cannot rule out the potential bias caused by DNA extraction, purification and PCR-amplification: *i.e.*, the present data is true but can only indicate "detectable/amplifiable/quantifiable-DNA sequences" in "DNA-extractable" populations. To solve these problem and grab the true nature of the deep subseafloor life and the biosphere, new technological improvements or developments for bias-less molecular analyses will be required.

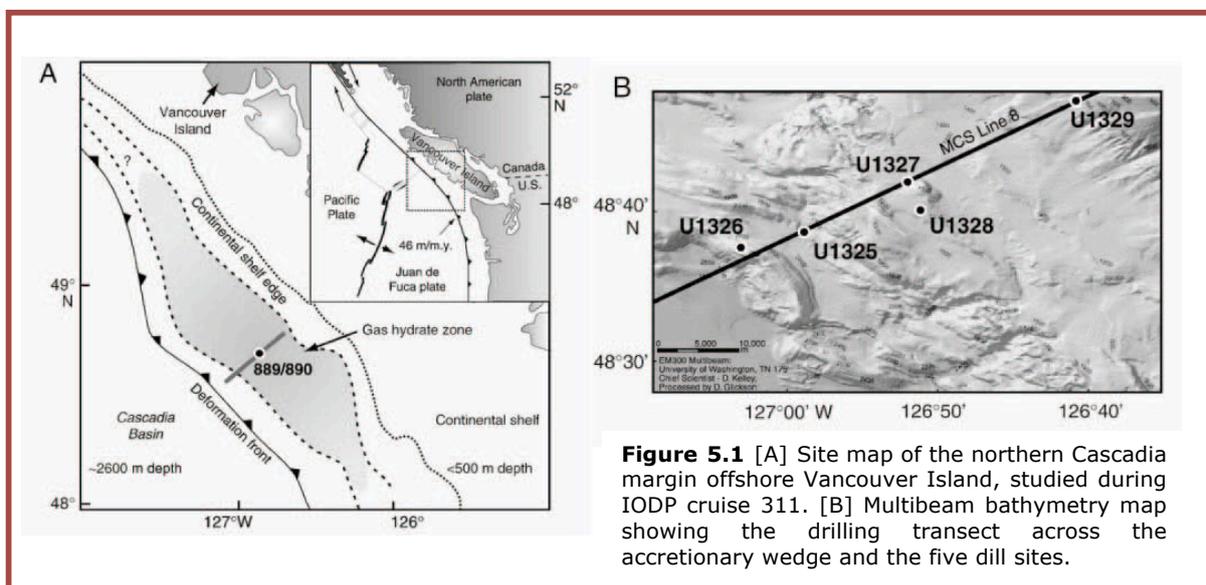
Using Expedition 308 core samples, Nunoura *et al.* (2009) reported the profiles of hydrogenase activity. Interestingly, hydrogenase activities in the Ursa Basin sediments showed a good correlation to the sediment porosities, suggesting that the pore-space (and likely dissolved nutrients in porewater) is an important factor for metabolic activity and habitability in the subseafloor biosphere.

## 5. Expedition 311: Gas hydrate drilling transect across northern Cascadia margin

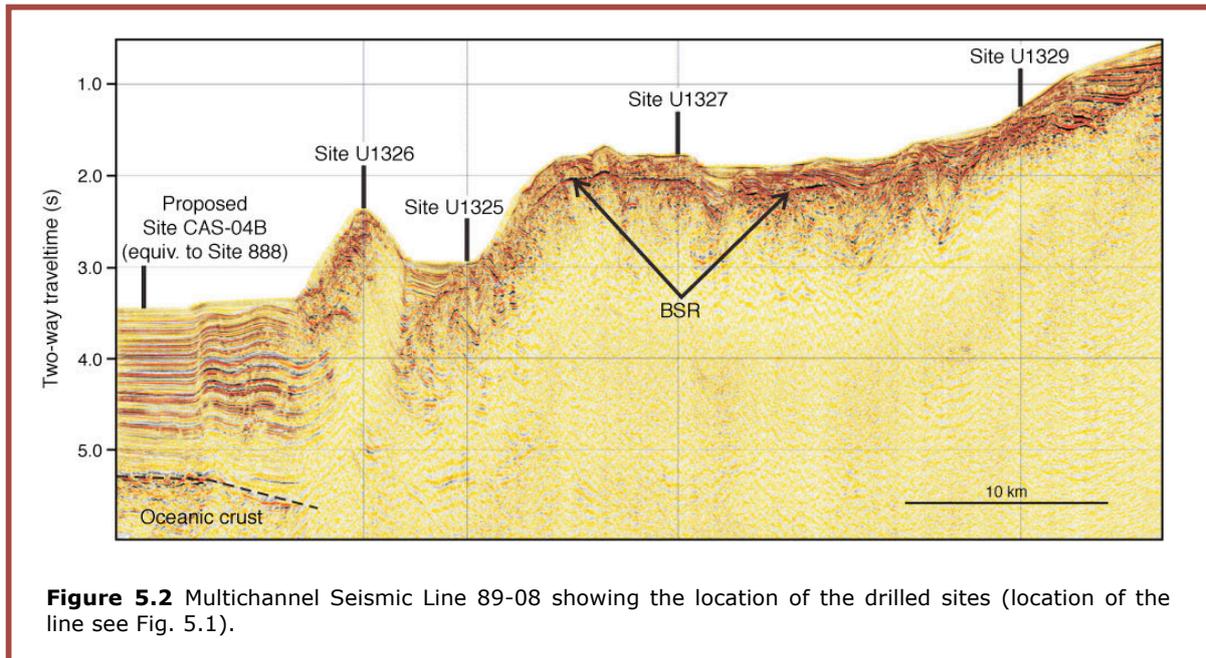
IODP Expedition 311 drilled a margin-wide transect of drill sites to study gas hydrates through an accretionary prism. Five sites represent typical accretionary wedge environments that show various stages of gas hydrate evolution on the Northern Cascadian Margin (Figs. 5.1 and 5.2). The gas hydrate occurrences as well as gas hydrate concentrations have been studied within their local environment. The westernmost Site 1326 is located on the first uplifted accretionary ridge which showed heavily faulted sediments intersecting the entire sediment column down to the well developed BSR. Site 1325 is located within an extensive slope basin eastward to the first ridge. Site 13127 was drilled at the mid-slope of the accretionary prism where 90 m slope-sediments are underlain by older accreted sediments containing a clearly defined BSR. Apart from the transect Site 1328 was drilled where seafloor seepage is documented by chemosynthetic organisms, methane-derived carbonate precipitates and shallow massive gas hydrates. Site 1229 represents the eastern limit of the gas hydrate occurrence along the margin where still a faint BSR was identified in seismic data.

As developed during previous ODP campaigns (ODP Legs 164 and 204; [Tréhu et al., 2004](#)) various gas hydrate proxies have been used like, electrical resistivity measurements and *P*-wave velocities on downhole logs, negative chloride interstitial water anomalies, infrared low-temperature anomalies, as well as hydrate-related sedimentological textures. In previous models for gas hydrate formation and distribution in accretionary prisms, the highest concentrations of gas hydrate were predicted to occur at the base of the gas hydrate stability zone, right above the BSR. The concentrations were expected to decrease gradually upwards in the sediment column as a result of widespread fluid advection caused by the tectonically driven fluid circulation. However, the results of the cruise show that there are different variations in the gas hydrate distribution leading to the conclusion that this model is too simple ([Riedel et al., 2010](#)).

Analysis of the hydrocarbon and isotopic composition of gas extracted from the sediments along the transect of drill sites suggests that microbial methane generated by CO<sub>2</sub> reduction is the primary hydrocarbon with acetate fermentation to much less extent at all sites. The predominant fraction of the methane is interpreted to be formed within



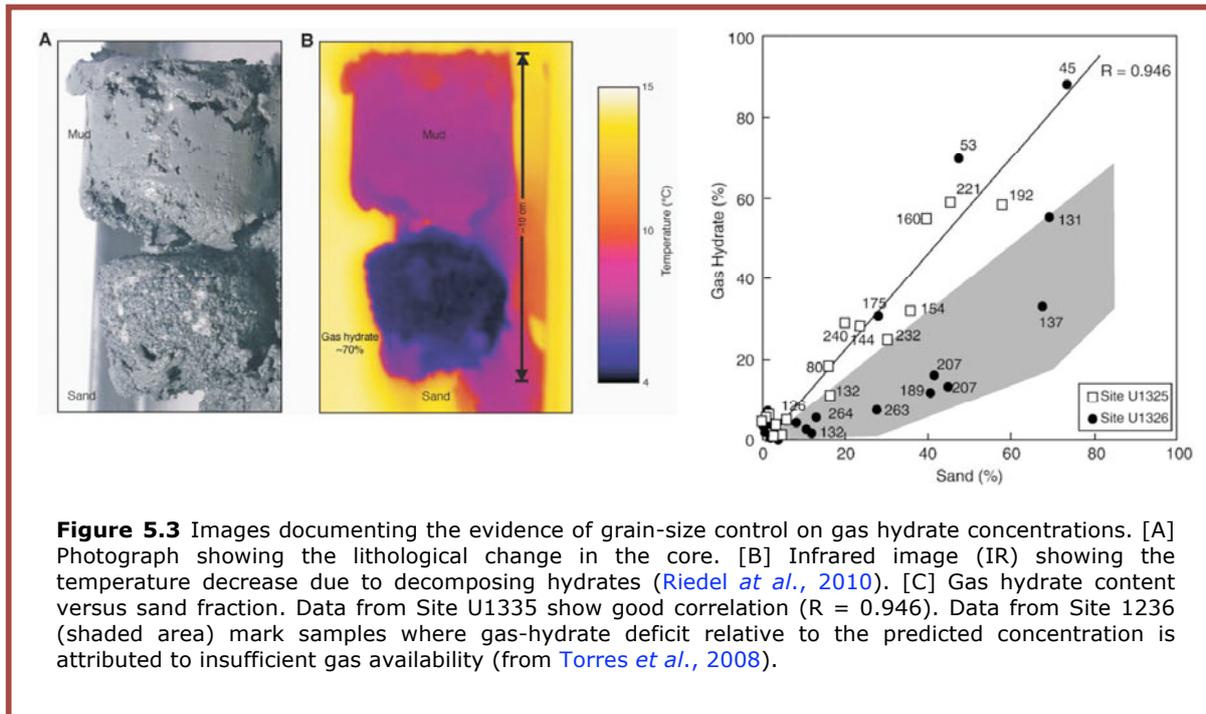
**Figure 5.1** [A] Site map of the northern Cascadia margin offshore Vancouver Island, studied during IODP cruise 311. [B] Multibeam bathymetry map showing the drilling transect across the accretionary wedge and the five drill sites.



the Gas Hydrate Stability Zone (GHSZ). The hydrocarbon sources are microbial and significant thermogenic gases were not detected (Pohlmann *et al.*, 2008). Evidence for a deep-migrated source of methane was observed in shallow gas hydrate accumulations at the cold vent Site U1328, where near-vertical fracture systems delivered methane from a deep source to the surface.

The fundamental outcome of the expedition is the overall observation that gas hydrate grows preferentially in coarser-grained sediments. Although the preference of gas hydrate occurrence in coarser grained sediments has been observed in some cases, specifically in terrestrial locations, direct observation of gas hydrate in sands on the Cascadia margin was systematically documented for the first time during Leg 311 (Torres *et al.*, 2008). The analyses of contiguous sediment samples quantitatively document the preferential formation of gas hydrate in medium to coarser-grained intervals and show about 90% of the variance in hydrate saturation is explained by the sand content of the sediments (Fig. 5.3; Torres *et al.*, 2008). The coarser-grained sediments permit most probably the nucleation and migration of free gas, which facilitates gas-hydrate formation. Other forms of gas hydrate were recovered at the cold vent site U1328 and document dipping veins or fracture fillings. Such hydrates which clearly represent precipitates filling tectonic fracture have been shown during ODP Leg 204 on Hydrate Ridge and seem to represent the major type of hydrate in very fine-grained sediments (Abegg *et al.*, 2008; Weinberg and Brown, 2006).

A primary objective of Expedition 311 was to define the gas hydrate concentration within the GHSZ using a multi-proxy approach very much similar as during ODP Leg 204 on Hydrate Ridge (Tréhu *et al.*, 2004). The combined data from IODP Cruise 311 confirm that the gas hydrate concentrations at the drill sites are generally less than 5% of the pore space. Gas hydrate concentrations locally can exceed 50% of the sediment pore space, e.g. Site 1326, 50-120 mbsf.



## 6. Microbiology: Linking between seafloor life and paleoceanographic events

### 6.1 Expedition 302: Arctic Coring Expedition

The Arctic Ocean plays a fundamental role in the global ocean and climate system. The dense, cold bottom waters strongly influence global thermohaline circulation, driving the world climate. During the Arctic Coring Expedition (ACEX) 302, approximately 428m-thick of sedimentary sequence was successfully recovered by mission-specific platforms from the Lomonosov Ridge in the central Arctic, located at approximately 250km distant from the North Pole. The lithological changes from organic-rich euxinic black layers to grayish oxic layers represented the ventilating history of redox-conditions tectonically driven by opening and closing events of the gateway (*i.e.*, the Fram Strait) (Brinkhuis *et al.*, 2006). The early Miocene Arctic events were appeared to be concomitant to the climate changes (Jakobsson *et al.*, 2007). The sediment cores contained the Cenozoic paleoenvironmental evolutionary records such as ice-rafted debris and diatomaceous fossils, representing the climatic transition from a warm, ice-free Arctic environment from the ice-covered cooling phase (Stickley *et al.*, 2009).

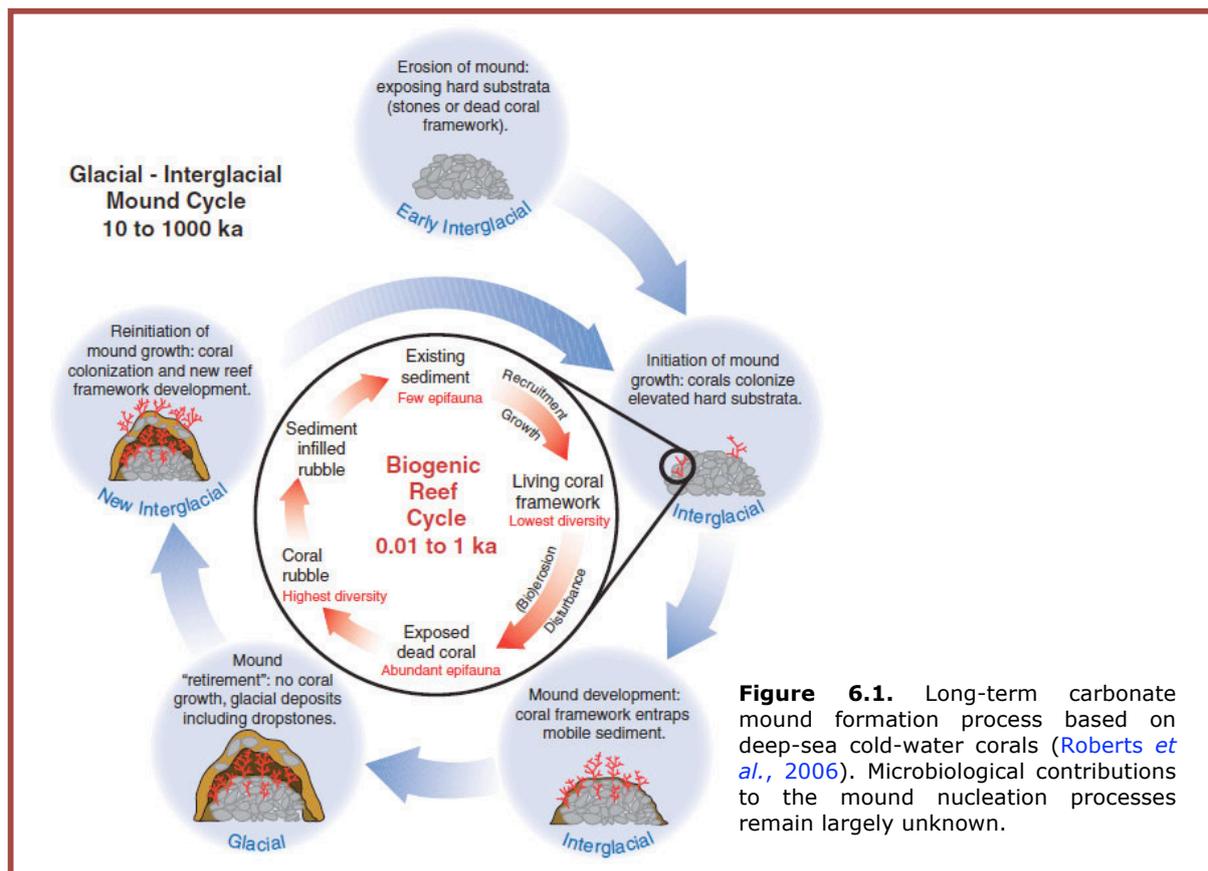
Previous studies of the seafloor biosphere were based largely on samples collected from continental margins in tropical to middle latitudes. Expedition 302 expanded the geographical range of the seafloor biosphere to the Arctic Ocean. Forscher *et al.* (2009) reported microbial communities inhabiting two silty clay layers at 55 and 103 mbsf and a diatomaceous organic-rich layer at 242 mbsf, representing the oxic and euxinic paleoceanographic redox-conditions, respectively. The cell numbers observed in those samples were 1.2 to  $2.7 \times 10^7$  cell/cm<sup>3</sup> (Kallmeyer *et al.*, 2008), indicating the presence of relatively high numbers of cells in the cold and deep Arctic seafloor habitats. For molecular analysis of the seafloor microbial communities, the PCR-inhibitors (likely humic acid-substances) were removed by a size-exclusion and gel-filtration technique, resulting in a small amount of high quality DNA. To study the diversity of Arctic seafloor microbial communities, bacterial and archaeal 16S rRNA genes were amplified by PCR using several primer sets with either phi29 polymerase (MDA)-amplified DNA or unamplified genomic DNA as the template. Interestingly, bacterial 16S rRNA genes were successfully obtained from all three samples while archaeal 16S rRNA genes were detected only from the euxinic black layer at 242 mbsf. Analysis of the sequences showed that approximately 50% of bacterial 16S rRNA gene sequences were highly similar to environmental sequences from glacial or frozen sedimentary environments (*e.g.*, permafrost, Antarctic), suggesting the potential adaptation/association to cold habitats. *Stenotrophomonas*- and *Sphingobacterium*-relatives as well as unknown *Betaproteobacteria* were dominant in the bacterial 16S rRNA clone libraries from grayish layers, whereas the members of phyla *Chloroflexi* and *Bacterioides*, which have been commonly observed in various seafloor sedimentary environments, were predominant in blackish anoxic event layers. Soffientino *et al.* (2009) measured potential hydrogenase activities in sediments of both oxic silty clay and euxinic organic-rich diatom ooze layers with a new radiotracer (<sup>3</sup>H<sub>2</sub>)-based method, and showed that significant hydrogenase activity occurred in the deep organic-rich black layers, while the hydrogenase activity was below the detection limit in the upper sedimentary unit. These data indicate that microbial communities are stratified and highly associated with lithological and geochemical characteristics. This importantly

implies, paleoceanographic events related to the climate and/or redox changes might consequently impact on the modern seafloor microbial habitat, activity and community composition. For example, Brinkhuis *et al.* (2006) reported the evidence records from Expedition 302 that oxic fresh waters were episodically supplied into the Eocene Arctic Ocean, potentially resulting in unique seafloor microbial habitats harboring distinct bacterial components such as *Stenotrophomonas* spp.

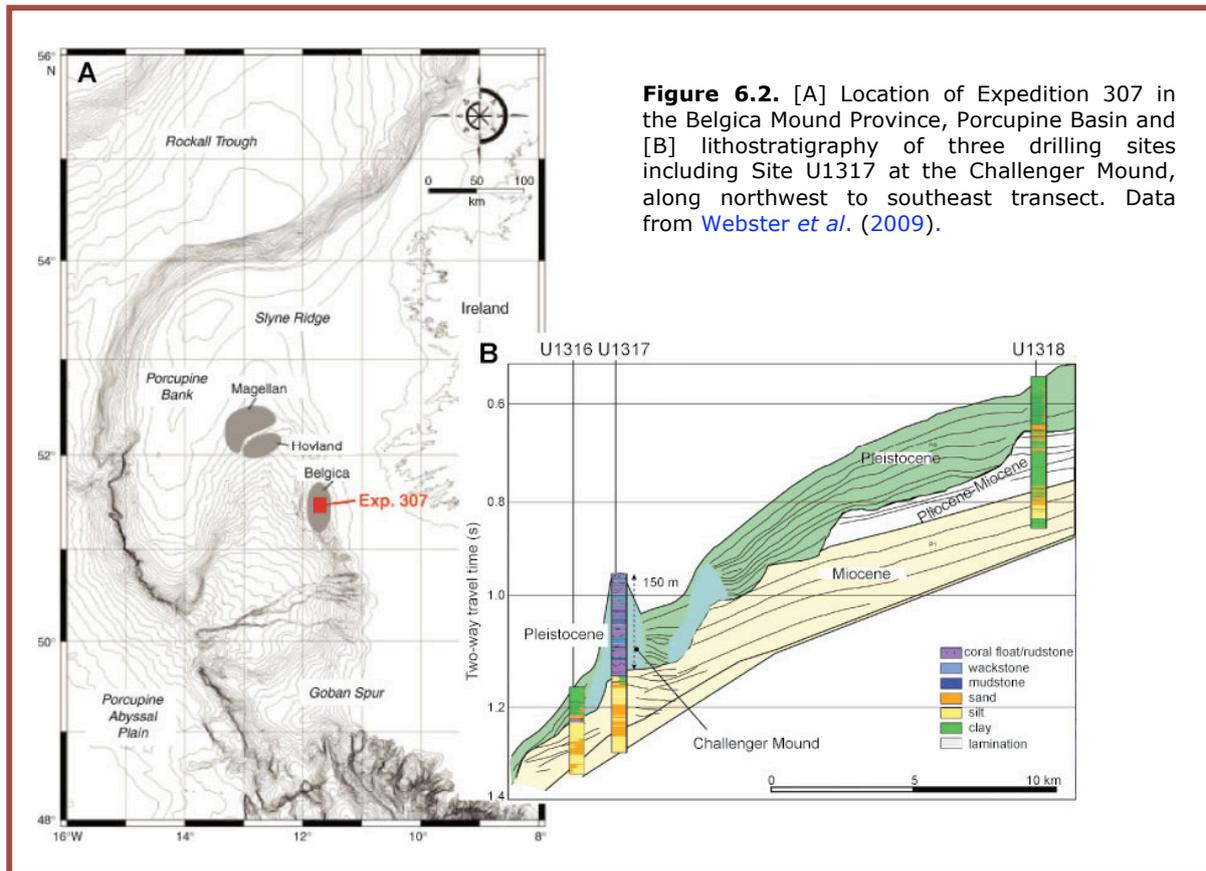
## 6.2 Expedition 307: Carbonate Mound Drilling at the Porcupine Basin

Recent deep-sea explorations using advanced acoustics and submersibles have revealed the widespread occurrence of cold-water ecosystems in continental margins and ridge systems (Roberts *et al.*, 2006). Cold-water corals form large reefs and giant carbonate mound structures over geologic timescales (Fig. 6.1). In marked contrast to coral reefs inhabiting shallow tropical seas, cold-water coral reefs are restricted to temperatures between 4°C and 12°C in the deep-sea. *Lophelia pertusa* and *Madrepora oculata* are the most commonly observed sunlight-independent cold-water corals. Since cold-water corals are highly sensitive to temperature, the deeply buried coral records may provide important paleoceanographic information for the past sea-bottom current and sedimentation processes by analyzing the skeletal dating and chemical characteristics.

During Expedition 307, the Challenger Mound in the Porcupine Basin off the southwestern continental shelf of Ireland was drilled for the first time down to the basement (Fig. 6.2). The expedition aimed toward unveiling the mound formation and

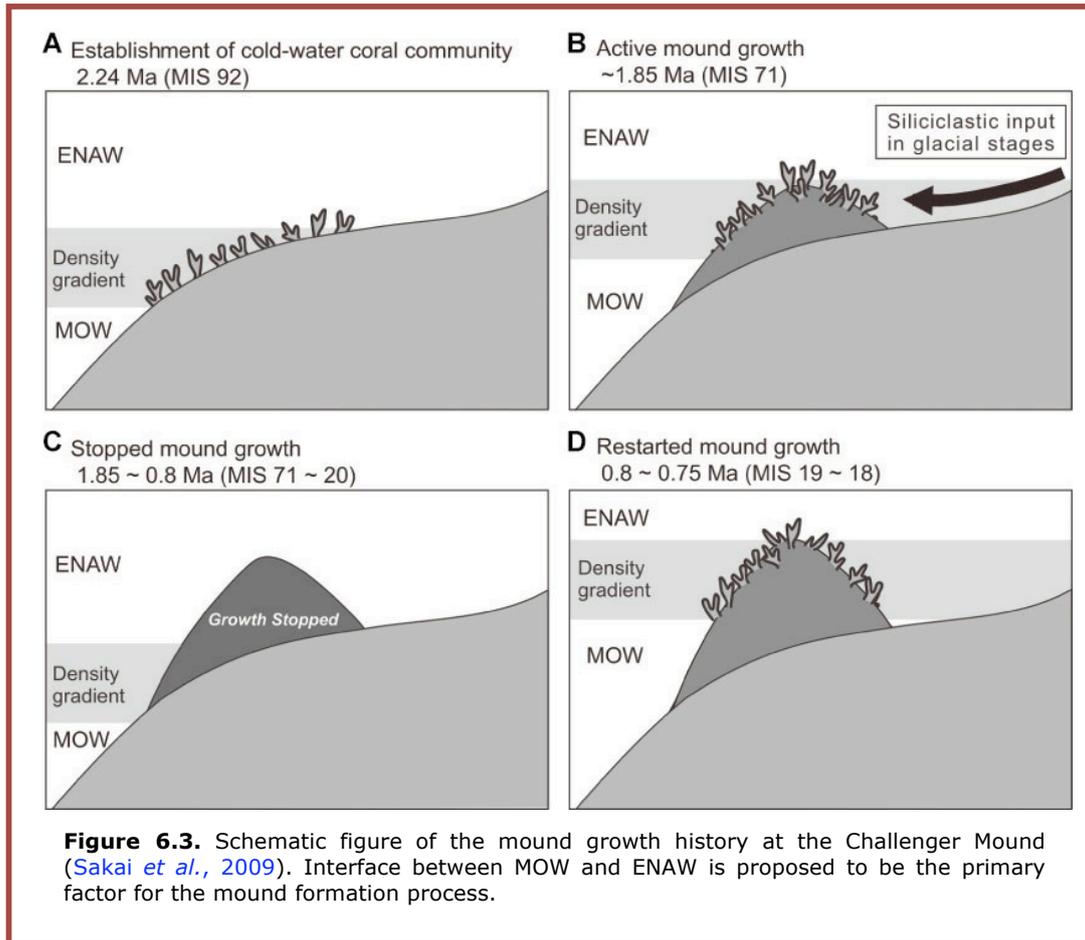


**Figure 6.1.** Long-term carbonate mound formation process based on deep-sea cold-water corals (Roberts *et al.*, 2006). Microbiological contributions to the mound nucleation processes remain largely unknown.



sedimentation processes. By analyzing strontium isotope stratigraphy, Kano *et al.* (2007) reported that the initial formation of the Challenger Mound began at 2.6 Ma and rapidly developed at rates up to 24 cm/ka until 1.7 Ma, which were associated with the density gradient reinforced by the Mediterranean Outflow Water (Sakai *et al.*, 2009). In some areas, the origin of mound formation is linked to isotopically light hydrocarbon (*i.e.*, CH<sub>4</sub>) oxidation and nutrient-rich pore waters, stimulating alkalinity and microbiological carbonate formation (Hovland *et al.*, 1998). Does sulfate-dependent AOM occur and promote carbonate mound formation? How does seafloor microbial metabolic activity contribute to carbonate dissolution? Do chemoautotrophic microbial populations exist in this habitat and are they a sink for DIC? The biogeochemical and ecological roles of seafloor microbial activities in this environment remain largely unknown.

In contrast to the expectation of hydrocarbon hypothesis, onboard geochemical and lithostratigraphic analyses showed no or very little hydrocarbon seepage in the Challenger carbonate mound (Ferdeman *et al.*, 2006). At Site U1317, sulfate penetrated deeply into the carbonate mound and the SMTZ was located in the underlying Miocene mound-base at approximately 120 to 150 mbsf. No microbially associated authigenic carbonates such as massive dolomite or hard carbonate pavements were observed, however beautiful coral skeletons were preserved at this site. These onboard data suggest that hydrocarbon seepage as well as seep-associated microbial activity may not always be a strong driving force for deep-water coral mound development. The trigger for the Challenger mound development likely relies on other factors. For example, oceanographic and paleo-environmental conditions such as salinity, temperature and seafloor currents control carbonate mound formation and the growth of cold-water



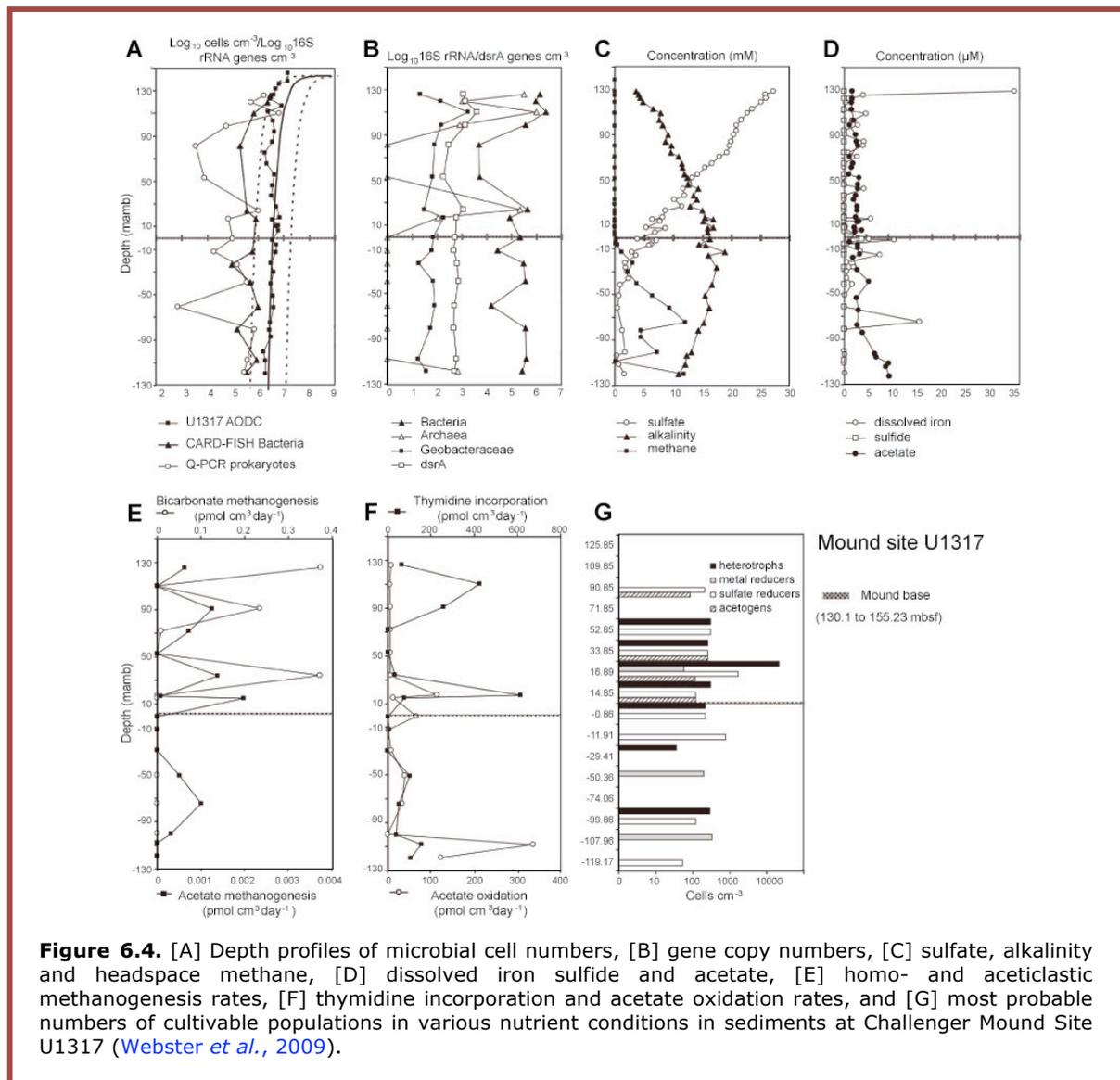
corals. Sakai *et al.* (2009) suggest that the interface between two density/temperature-different water masses, Mediterranean Outflow Water (MOW) and Northeastern Atlantic Water (NEAW), contributes to the mound initiation and volume oscillation processes (Fig. 6.3).

While the Challenger mound itself appears to be formed in large part by non-microbial processes, geochemical pore water profiles from site U1317 showed that, within the Miocene basement underlying the carbonate mounds, AOM coupled with sulfate reduction is active. Increasing dissolved  $\text{Ca}^{2+}$  and decreasing  $\text{Mg}^{2+}$  concentrations in porewater suggest that microbial dolomite is forming in Miocene sediments below the mound base. In shallow sediments down to 50 mbsf, sulfate concentration was decreasing with depth while alkalinity was increasing parallel to sulfate, indicating heterotrophic sulfate reduction is mediating the mineralization of buried organic matter.

Using microbiological samples from Expedition 307, Webster *et al.* (2009) studied microbial population, activities and diversity in seafloor sediments at the mound (U1317), flank (U1316) and reference (U1318) sites of the Porcupine Basin (Fig. 6.4). Microbial cell numbers were evaluated by AODC. Interestingly, microbial cell abundance in the carbonate mound were found to be relatively low ( $\sim 10^6$  cells/cm<sup>3</sup>) but increased slightly with depth down to the mound-basement interface, suggesting that either upward flux of nutrients or depth-dependent organic-mineral alternation may support a seafloor microbial assemblage in the mound subsurface. The sulfate-methane transition occurred in the Miocene mound base: however, no clear indications of cell

proliferation were detected in the mound base as previously observed at ODP Site 1229 in the Peru Margin (Parkes *et al.*, 2005). CARD-FISH detected relatively high numbers of metabolically active bacteria ( $>10^5$  cell/cm<sup>3</sup>) throughout the carbonate mound and mound-base sediments. Methanogenesis based on both hydrogen and acetate as the energy source was detected in the mound subsurface: however, these activities were significantly low overall ( $<1$  pmol/cm<sup>3</sup>/day) as compared to other continental margin drilling sites. MPN cultivations recovered a few hundred cultivable bacteria in 1 cm<sup>3</sup> of sediments, with successful growth detected using media targeting heterotrophs, metal reducers, sulfate reducers, and acetogens. The cultivable microbial populations were mainly observed in the carbonate mound rather than in the mound basement at Site U1317. This trend was in clear contrast to other two drilling locations at Site U1316 and U1318, where sizeable cultivable populations were observed in deep zones near the mound base.

In the study of Webster *et al.* (2009), DNA was extracted by the modified protocol using a commercial FastDNA Spin Kit for Soil (MP Biomedicals) (Webster *et al.*, 2003). 16S rRNA genes were amplified with several combinations of bacterial and archaeal



primers, and re-amplified with GC-anchored primers for denaturing gradient gel electrophoresis (DGGE) analysis. Sequencing analysis of DGGE-bands revealed a range of diverse Bacteria and Archaea, some of which were common seafloor sedimentary microbes such as *Chloroflexi*, JS1 candidate division, *Spirochetes* for Bacteria, and SAGMEG, DSAG, and MCG for Archaea. Interestingly, DGGE analysis of bacterial 16S rRNA genes amplified by using 27F and 907R primers showed a clear difference of amplified bands between the carbonate mound and the Miocene mound base sediments: *i.e.*, *Gamma-* and *Deltaproteobacteria* and OP8 candidate division were predominantly detected in carbonate mound sediments while *Betaproteobacteria* dominated in mound-base sediments. For archaeal 16S rRNA genes, several types of sequences belonged to the SAGMEG were detected in the carbonate mound sediments. The sequences of MCG-Archaea were not detected by the PCR-DGGE analysis.

Using the extracted DNA, the copy numbers of bacterial and archaeal 16S rRNA genes and functional genes for *dsrA* and *mcrA* genes were estimated by quantitative-PCR analysis (Fig. 6.4). At all locations and most depths, 16S rRNA gene copy numbers of Bacteria ( $10^3$  to  $10^6$  copies/cm<sup>3</sup>) were substantially higher than those of Archaea ( $10^6$  copies/cm<sup>3</sup>). The cell abundances estimated by the total 16S rRNA gene copy numbers were approximately an order of magnitude lower than AODC counts. The *dsrA* genes were detected throughout, at a range of relatively low copy numbers from  $10^2$  to  $10^3$  per 1cm<sup>3</sup> of sediments. Although low but significant methanogenesis was observed in sediments, no *mcrA* genes were recovered by PCR from the extracted DNA. Webster *et al.* (2009) suggested this might be due to primer mismatches in *mcr* gene for the *in situ* methanogenic assemblage associated with the Challenger mound. Biases in DNA extraction and purification, especially for archaeal cells, may be another contributing factor.

During Expedition 307, both microbiological and sedimentological studies consistently suggested that hydrocarbon seepage as well as bio-mineralization and – dissolution effect in hydrocarbon seep microbial habitat are not primary driving forces for the Challenger mound nucleation process. However, the cold-water coral mounds and the surrounding subsurface environments represent active seafloor microbial habitats. The biogeochemical and ecological roles of seafloor microbes (*e.g.*, decay of buried organic matter, carbonate dissolution/nucleation and hydrocarbon-oxidation) will need further detailed cross-disciplinary investigations. In addition, the linkage between modern and past ecosystems including not only deep-sea cold-water coral communities but also shallow and deep seafloor microbial communities remains largely elusive.

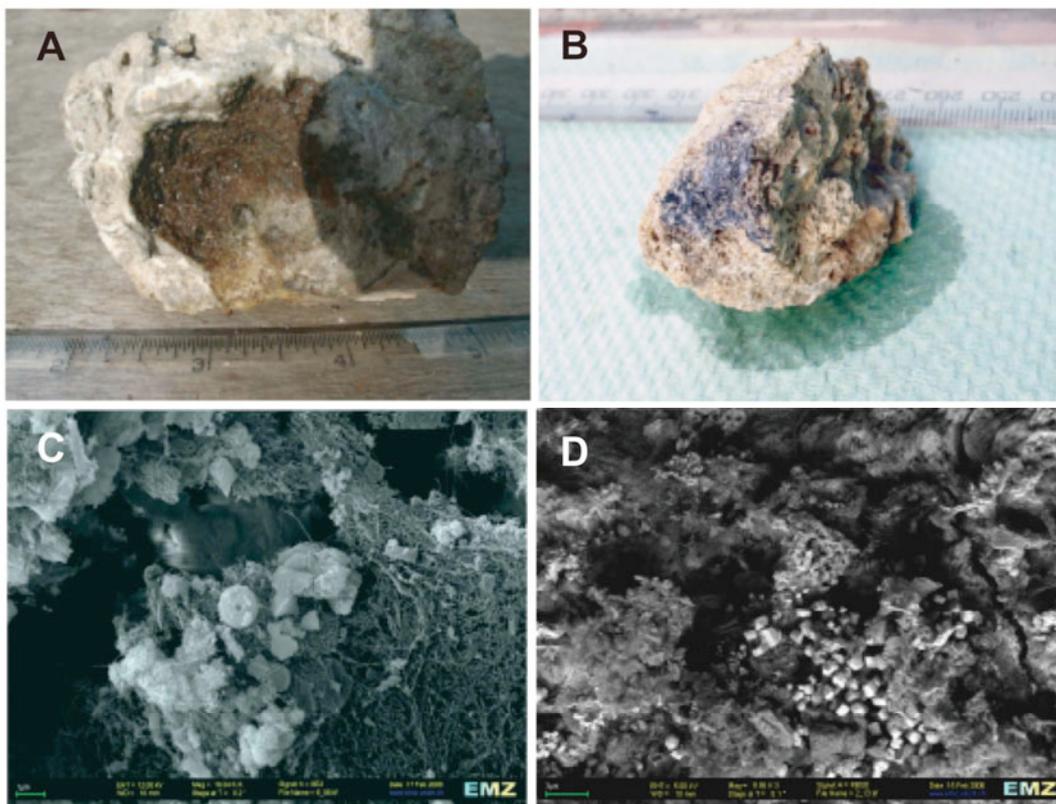
### 6.3 Expedition 310: Tahiti Sea Level

The timing and course of the last deglaciation is an important component for understanding the dynamics of glacial ice sheets and the effect on the global sea level and climate change. Coral reefs are excellent indicators for the dating of glacial-interglacial cycles and the sea surface temperature. During Expedition 310, the coral reef terraces at Tahiti were drilled down to 79 mbsf by the mission-specific platform DP *Hunter* at various water depths ranging from 41.6 to 161 meters.

In the shallow subsurface beneath tropical coral seabed, microbial cell numbers and activity are usually high because of enhanced nutrient and energy supply from the overlying photosynthetic production zone. Interestingly, during Expedition 310,

microbiologists observed dense biofilms associated with ancient reef cavities ([Expedition 310 Scientists, 2007](#)). The biofilms were diverse in their structure and color, some producing visible blue or yellow pigments. The biofilms were associated with brownish iron and manganese deposits and scanning electron microscopic (SEM) observations showed the occurrence of biomineralization such as framboidal pyrites and carbonate minerals ([Fig. 6.5](#)). While characterization of the microbial diversity in the biofilms is still in progress, preliminary studies quantifying ATP, which is a universal substrate for metabolic energy for all living biota, showed the highest levels of ATP-activity (20,600 RFU) in the seafloor biofilms suggesting microbial activity was stimulated within these unique habitats.

Porewater chemical constituents were successfully measured at Site M0008 Hole A. Increasing concentrations of alkalinity, ammonia, iron and manganese concentrations in porewater at around 18 mbsf consistently showed the presence of anaerobic heterotrophic microbial communities that consume buried organic matter derived from seawater photosynthesis and buried corals in this distinct seafloor environment. These preliminary microbiological results onboard suggest that microbes inhabiting coral terrace subsurface may play major biogeochemical roles in organic-decay and metal-reduction, and subsequently contribute to the sedimentation processes.



**Figure 6.5** Microbial biofilms observed in seafloor coral cavities in cores recovered from a coral terrace at Tahiti during Expedition 310. Biofilms were varied in color such as brown [A] and blue [B] derived from microbially produced pigments and/or minerals. [C and D] Scanning electron microscopic image of biofilms. Filamentous microbial network structures [C] and framboidal pyrites [D] were observed. Images from [Expedition 310 Scientists \(2007\)](#).

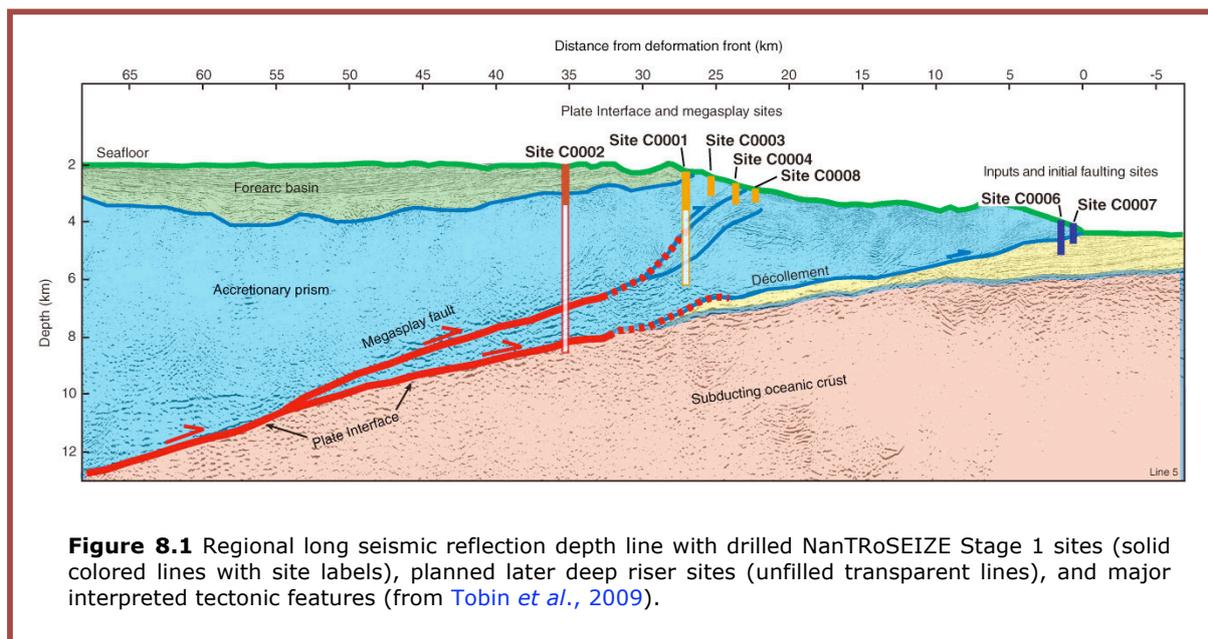
## 7. Expedition 323: Microbial activity and abundance in Bering Sea Microbiology

Relationships between the seafloor biosphere and oceanographic properties such as primary productivity, sedimentation rate, and distance from the continents are beginning to emerge through broad microbiological surveys across a range of oceanic provinces (D'Hondt *et al.*, 2009). Cruises from the Peru Margin (Leg 201); South Pacific Gyre (IODP survey cruise), Cascadia Margin (Leg 311), and Juan de Fuca (Leg 301) provide a spectrum of oligotrophic and organic rich continental margin habitats in which to assess the influence of these parameters on microbial activity and cell abundance in seafloor. The Bering Sea is a region of elevated primary productivity and high sedimentation rates, representing an important endmember for constraining the relationship between chlorophyll-a concentrations and the underlying seafloor microbial community.

Expedition 323 was a paleoceanography dedicated cruise whose primary objectives were to document trends in climate during glacial-interglacial and millennial-scale changes during the Pliocene and Pleistocene. During this expedition, five additional cores were collected for microbiological research using the advanced piston coring system, reaching a depth of approximately 40 m. Four cores were obtained from sites along the shelf, an area characterized by high productivity and sedimentation rates, in addition to a single core collected on the ridge (Bowers Ridge). Microbiological coring sites were selected based upon distance from land and levels of primary productivity in the overlying water column. Samples for microbial community analysis and cell counts were collected every 25 cm for three of the cores and at 75 cm spacing for the remaining samples. Interstitial water chemistry was analyzed on sediment rounds adjacent to sediment horizons collected for microbial community analysis. Contamination tests using a perfluorocarbon tracer (Smith *et al.*, 2000) revealed insignificant levels of contamination of the sediment samples during drilling. Preliminary results (Expedition 323 Scientists, 2010) suggest that microbial activity along the shelf is elevated and more diverse relative to the Bowers Ridge site, with geochemical profiles suggestive of active Fe and Mn-reduction, sulfate-reduction, methanogenesis, and anaerobic oxidation of methane (sulfate-methane transition zone observed between 6-11 mbsf). The data from this shared paleoceanography/ microbiology cruise will lend important data for further development of global models of microbial activity and biomass in the deep seafloor.

## 8. Expedition 315, 316 and 322: NanTroSEIZE Stages 1 & 2

The Nankai Trough Seismogenic Zone Experiment – so called “NanTroSEIZE” is a complex drilling project (CDP) to explore a geologically active subduction zone that has repeatedly generated devastating big earthquakes and tsunamis in the Nankai Trough off Japan. During Expeditions 315 and 316 at the NanTroSEIZE Stage 1, multiple holes in shallow sediments (<1200 mbsf) were drilled and sub-sampled for microbiological and biogeochemical analyses. The samples included not only the forearc basin sediments (Sites C0001 and C0002) but also the old accretionary prism involved in shallow branches of the mega-splay faults (Sites C0004 and C0008) and frontal thrusts at the prism toe (Sites C0006 and C0007) (Fig. 8.1).



Using cored sediment samples from the IODP Expeditions 301, 315 and 316, Futagami *et al.* (2009) studied microbial dehalogenation activities by analyzing anaerobic incubation cultures and the extracted DNA. Previous 16S rRNA gene analyses demonstrated that *Chloroflexi* is one of the most frequently detected phyla from subseafloor sediments. These sequences are closely related to obligatory dehalorespiring bacteria within the genus *Dehalococcoides* that utilize halogenated organic compounds as the terminal electron acceptors. Futagami *et al.* (2009) detected phylogenetically diverse reductive dehalogenase homologous genes (*rdhA*) from various marine subsurface sediments, suggesting the widespread occurrence of dehalogenation activities in the deep subseafloor biosphere. In addition, significant dehalogenation activities were observed in incubation cultures of forearc sediments at Site C0002: *i.e.*, 2,4,6-tribromophenol and trichloroethene were rapidly dehalogenated to phenol and *cis*-dichloroethene, respectively (Fig. 8.2). These molecular and cultivation experiments provided direct evidence that dehalorespiration is a previously unrecognized energy-yielding pathway in the subseafloor microbial ecosystem.

One of the intriguing microbiological subjects in scientific ocean drilling is to understand “Geosphere-Biosphere interactions” (D’Hondt *et al.*, 2007). The NanTroSEIZE IODP Expeditions provide unprecedented opportunities to address this issue. How do

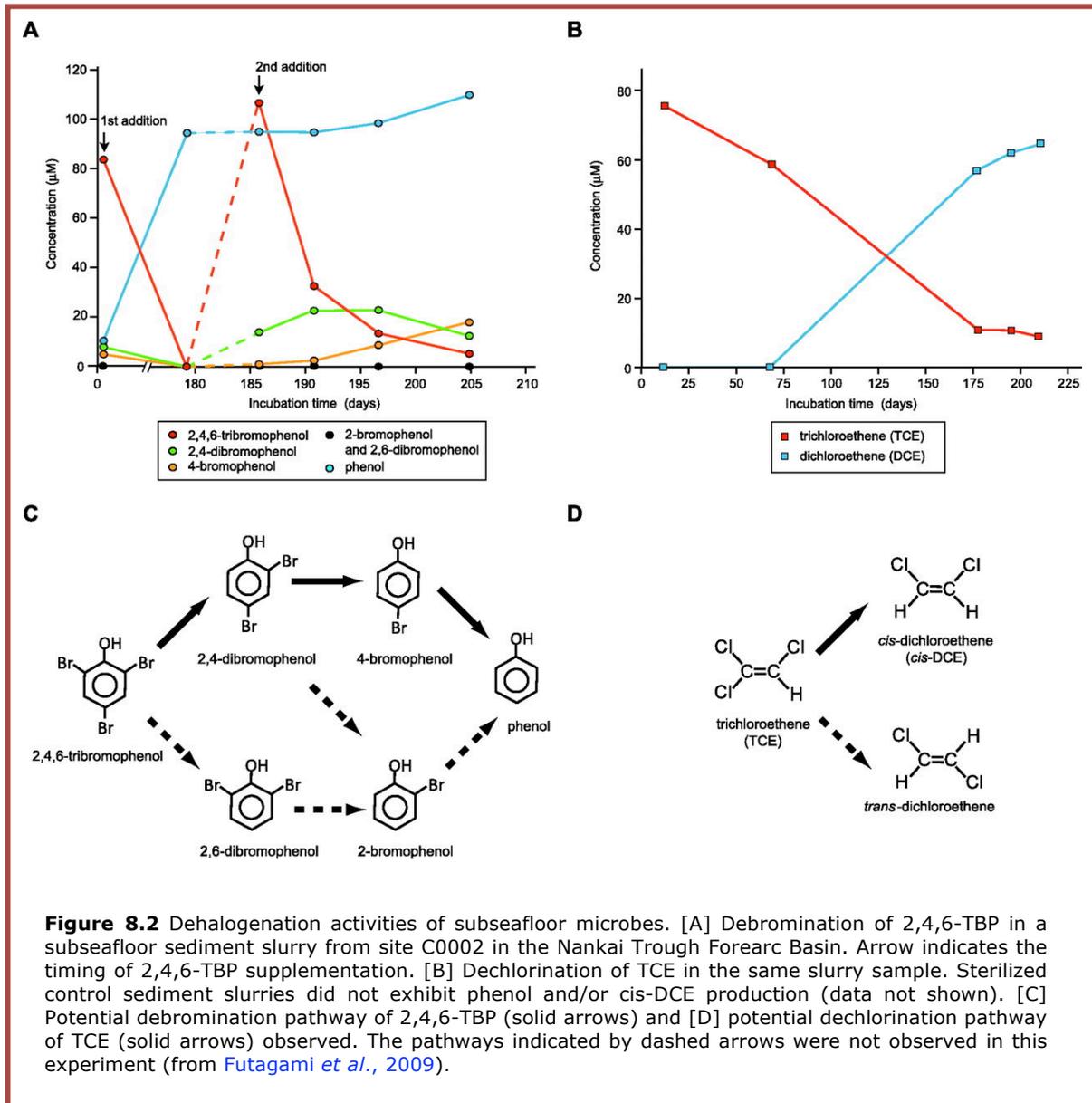


plate tectonics and seismogenic fault activities impact on the distribution of subseafloor life in terms of the ecology, diversity and activity? During Expedition 315 and 316, the cell abundances were investigated using a computer-based microscopic image system ([Morono et al., 2009](#)), indicating that the populations are overall relatively low ( $<10^5$  cells/cm<sup>3</sup>) in the Nankai Trough seismogenic zone as compared to other organic-rich marine sediments. Microbial diversities and the community structures were studied by analyzing 16S rRNA gene-tagged pyrosequence data and lipid compositions. [Kaksonen et al. \(in prep.\)](#) studied spatial distribution and diversity of sulfate-reducing microbes by culture-dependent and -independent molecular approaches. These on-going microbiological studies will illuminate new aspects of Geosphere-Biosphere interactions in geologically active subseafloor environments.

During Expedition 322, pre-subduction open ocean sediments and the underlying basaltic crusts were successfully recovered by *Chikyu* and sub-sampled for

microbiological and biogeochemical analyses. The sediment samples contain a deep sulfate-methane transition zone where the occurrence of sulfate-dependent AOM is expected. Expedition 322 successfully recovered the basalt-sediment interface, some of which samples will be used for microbiological studies to decipher the question whether the old oceanic crust and the aquifer components can support crustal microbial life or not. This is highly relevant to the issues of "Deep-biosphere" and "Subseafloor Ocean" described in the [ISP \(2001\)](#).

## 9. Technological Needs and Developments

Since the first IODP Expedition 301 in 2003, the IODP has made significant progress on the technological developments related to drilling, logging and analytical measurements in multiple drilling platforms. The technological advancements in analytical approaches and measurements offer new opportunities to explore this frontier field and have resulted in the development of new scientific paradigms as well as aided our ability to answer some of the fundamental questions pertaining to the deep seafloor biosphere. In this chapter, we describe some important progresses of technological improvements and developments that have been accomplished during the initial phase of the IODP Expeditions.

### 9.1 Technological developments and issues on hydrogeology: drilling and logging

Technologically, Expedition 308 in the Gulf of Mexico was the first demonstration to monitor the downhole pressure in real time by MWD. During the MWD operation, weighted mud was found to be useful for non-riser drilling at the overpressured realm, resulting in the successful drilling and logging in the place that had been long difficult to access by non-riser drilling. This is indeed a significant technological development that established the methodology to drill overpressured interval by non-riser drilling. In addition, Expedition 308 was first address the issue of spatial variation of pore pressure field in detail (see Fig. 4.3).

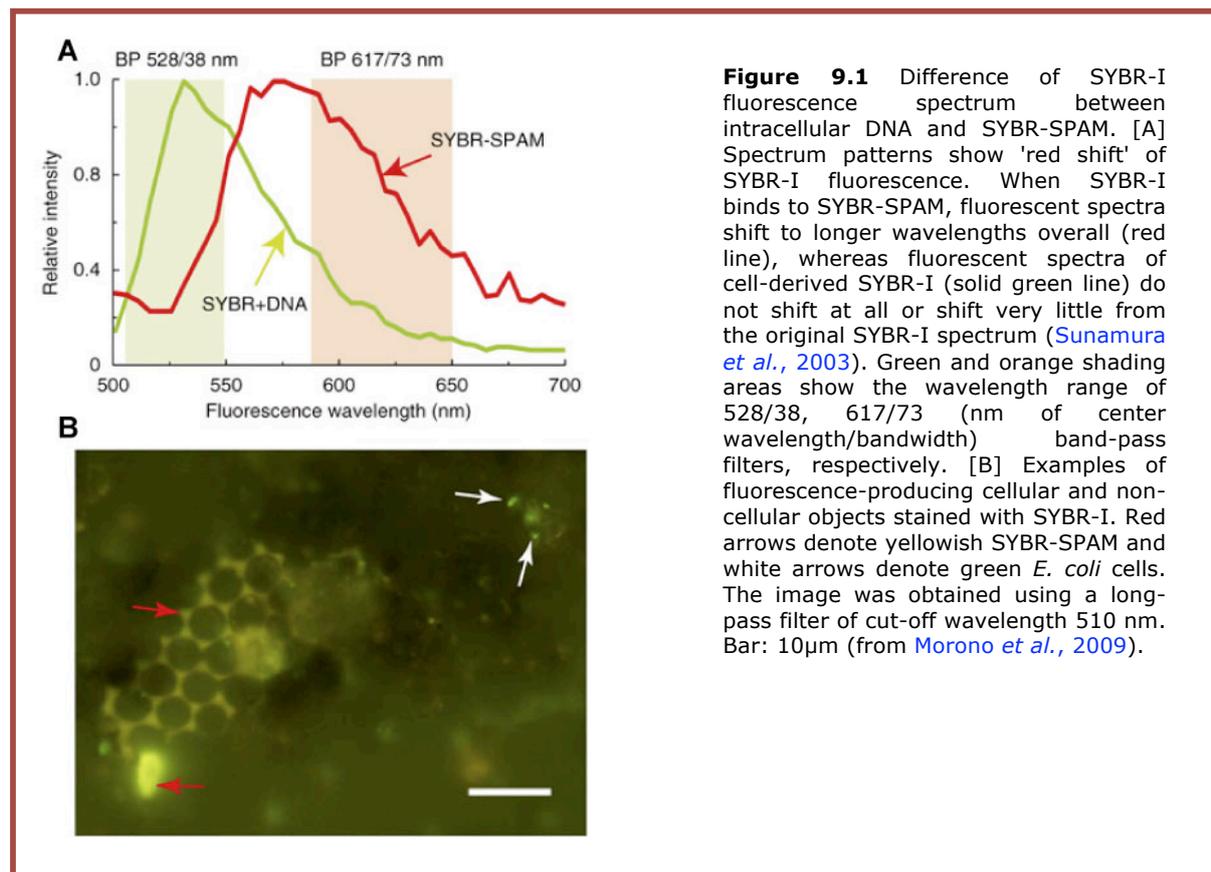
Despite the successful drilling and logging operations in overpressure sediments, the measurement/estimation of *in situ* pore pressure has remained to be improved. During the expedition, a new tool to measure in situ pore pressure and temperature (T2P) was introduced, and both the T2P and the conventional DVTPP (Davis Villinger Temperature-Pressure Probe) sensors were deployed and applied to obtain temperature and pressure profiles at the drilled sites. During the logging operations, it was difficult to obtain better coupling between the pore pressure transducers attached to the T2P and DVTPP sensors and the sedimentary formation. The colleted delivery system did not mechanically decouple to the sensor and the drill strings was not functioning promptly during numbers of sensor deployment. Consequently, the quality of measured pore pressure significantly suffered from this problem at some points. Temperature measurements were considered to be not so sensitive to the decoupling problem, so the temperature data were rather high in quality. Therefore, the improvement of the delivery system should be accomplished prior to the forthcoming hydrogeology-dedicated IODP expeditions. Also, sample disturbance affects the quality assurance of pre-consolidation pressures measured in the laboratory consolidation experiments. This problem should be overcome by the introduction of a thin-wall sampler that has been extensively used in the field of geotechnical engineering.

### 9.2 New technology for detection and enumeration of seafloor life

Detection of microbial life in geologic materials is one of the major scientific challenges in microbial ecology and astrobiology. Enumeration of life forms provides primary information toward the exploration of naturally occurring ecosystems, allowing

subsequent molecular and biogeochemical analyses of the habitat. During the previous scientific ocean drilling explorations, microscopic direct count of AODC successfully resulted in the discovery of deep seafloor biosphere that has a potential to harbor over one-tenth of total living biomass on Earth (Parkes *et al.*, 1994). In the deep-biosphere, the detection and discrimination of buried microbial life is significantly more difficult than in other natural habitats on the surface world, such as aquatic and soil environments, because of the high fluorescent backgrounds of minerals and non-specific binding of fluorescent dye. Moreover, AODC profiles show that cell abundances are generally decreasing with increasing depth, and the buried cells are small and have extremely low metabolic activities, causing the cell-derived AO signals to fade out in a short exposure time. Hence, the recognition and counting of AO-stained cells have required professionally trained microscopic observers, and consequently verification and/or crosscheck of deep seafloor microbial populations, for example on photo-images, has been difficult.

In the deep seafloor biosphere explorations through the IODP, the extent of seafloor life as well as the limit of the biosphere is one of the most significant and fundamental scientific objectives. To address the issue, the accurate enumeration of low microbial biomass in deeply buried core sediments is an essential technological challenge. Recently, Morono *et al.* (2009) developed an innovative technique that solved these fundamental problems. Using SYBR Green I fluorescent dye that produces more bright and specific fluorescent signals of double stranded DNA than other fluorescent dyes (Weinbauer *et al.*, 1998; Lunau *et al.*, 2005), cellular DNA-derived fluorescent signals are successfully distinguished from non-biological backgrounds by computer-based fluorescent image calculation. Analysis of fluorescent spectra from sample slurry-



mounted filters showed that spectrum patterns from all non-specific backgrounds shifted to longer wavelength, whereas those from intracellular double strand DNA shifted no or very little from the original SYBR Green I spectrum. Using the spectrum difference between cellular fluorescent pattern and other backgrounds, seafloor life can be precisely distinguished by dividing green fluorescent image by red per pixel image (Fig. 9.1).

Based on the principle of image analysis, Morono and Inagaki (2010) developed a computer-driven automated cell enumeration system equipped with a slide loader robot that can automatically operate 50 filter slides at once. A new cell detachment protocol by treating sediments with HF and HCl solutions at the SYBR-stainable condition increased cell detachment efficiencies from the consolidated sediment matrix (Morono *et al.*, 2009). The detection limit was also significantly lowered down to  $10^3$  cell/cm<sup>3</sup> by expanding scanning area (*i.e.*, number of microscopic image), approximately two orders of magnitude lower than AODC. Combined the newly developed cell enumeration system with a new cell detachment and concentration method (Kallmeyer *et al.*, 2008), it may be possible to lower the detection limit further more. The computer-based automated cell enumeration image system as well as the slide loader system can be deployed on the drilling platforms, which will enable us to evaluate seafloor biomass onboard more rapidly, precisely and hence high-depth resolution in the future IODP expeditions.

### 9.3 Sampling and storage: bio-archiving for the future

Over the past decade or more, there has been a history of assembling task forces to make recommendations to the ODP/IODP concerning sampling and procedures for archiving microbiological samples (*e.g.*, D'Hondt *et al.*, 2007). An outcome of the earlier task force meeting was the establishment of archived IODP samples "bio-archives" for future shore-based microbiological and biogeochemical studies. This archived material represent tremendous value to the scientific community, in particular for global biomass and molecular surveys at various oceanographic conditions and depths. During the initial phase of the IODP, a report of microbiology task force regarding the bio-archive sampling scheme was recommended by the science technology panel (STP), and then approved by the IODP-MI. The routine microbiology sampling (RMS) was tested during Expedition 322 where archived, ultra-cold ( $\geq -80^\circ\text{C}$ ): a whole round core sample was collected adjacent to or nearby IW (porewater chemistry) samples during all sedimentary drilling expeditions. With regard to the bio-archiving at the core repositories, aseptic sub-sampling procedure is also an important technological challenge in terms of the quality assurance and control (QA/QC). Masui *et al.* (2009) developed a semi-aseptic diamond-saw system for sub-sampling of frozen cores without melt. Although the initial RMS strategy has not yet been fully satisfied to the community requirements for some recommendations (*i.e.*, the communities requested multiple sample processing even in the small volume: *e.g.*, not only deep-frozen cores but also paraformaldehyde-fixed slurries and anaerobic fresh core samples), the RMS framework was an important implementation step for the advanced future researches of the deep seafloor biosphere.

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## Acronyms

ACEX – Arctic Coring Expedition	mbsf – meters below the seafloor
ANME – Anaerobic Methanotroph	MCG – Miscellaneous Crenarchaeotic Group
AO – Acridine Orange	mcrA – methyl co-enzyme M reductase
AODC – Acridine Orange Direct Count	MDA – Multiple Displacement Amplification
AOM – Anaerobic Oxidation of Methane	MOW – Mediterranean Outflow Water
ATP – Adenosine Tri-Phosphate	MPN – Most Probable Number
BSR – Bottom Simulating Reflector	MWD – Measurement-while-drilling
CARD-FISH – Catalyzed Reporter Deposition Fluorescence In Situ Hybridization	NanTroSEIZE – Nankai Trough Seismogenic Zone Experiment
CORK – Circulation Obviation Retrofit Kit	NEAW – Northeastern Atlantic Water
DGGE – Denaturing Gradient Gel Electrophoresis	NSF – National Science Foundation
DIC – Dissolved Inorganic Carbon	ODP – Ocean Drilling Program
DNA – Deoxyribonucleic acid	PCR – Polymerase Chain Reaction
DSAG – Deep-Sea Archaeal Group	PFT – Per-Fuorocarbon Tracer
dsrAB – dissimilatory sulfite reductase	Pg – petagram ( $10^{15}$ grams)
dsrA – dissimilatory sulfite reductase	QA/QC – Quality Assurance and Quality Control
DVTPP – Davis Villinger Temperature Probe	qPCR – quantitative PCR
EDX – Energy-Dispersive X-ray spectroscopy	RCB – Rotary Core Barrel
FISH-SIMS – Fluorescence In Situ Hybridization – Secondary Ion Mass Spectrometry	rdhA – Reductive Dehalogenase Homologous Genes
GC anchored primers – guanine-cytosine anchored primers	RFU – Relative Fluorescence Units
GDGT – Glycerol Dibiphytanyl Glycerol Tetraether	RMS – Routine Microbiology Sampling
GHSZ – Gas Hydrate Stability Zone	ROV – Remotely Operated Vehicle
HOV – Human Occupied Vehicle	rRNA – Ribosomal Ribonucleic Acid
ICDP – International Continental Scientific Drilling	SAED – Selected Area Electron Diffraction
IODP – Integrated Ocean Drilling Program	SAGMEG – South African Gold Mine Euryarchaeotic Group
IODP-MI – Integrated Ocean Drilling Program Management International	SEM – Scanning Electron Microscopy
IPLs – Intact Polar Lipids	SIMS – Secondary Ion Mass Spectrometry
ISP – Initial Science Plan	SMTZ – Sulfate-Methane Transition Zone
IW – Interstitial Water	STP – Science Technology Panel
MBG-B – Marine Benthic Group-B	SYBR Green I – asymmetrical cyanine dye used as nucleic acid stain
Mbp – million base pairs	T2P – Temperature 2 Pressure Probe
	TEM – Transmission Electron Microscopy
	Tg – tetragram ( $10^{12}$ gram)
	TOC – Total Organic Carbon