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IODP Expedition 336: initiation of long-term coupled microbiological, geochemical, and hydrological experimentation within the seafloor at North Pond, western flank of the Mid-Atlantic Ridge

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Abstract. Integrated Ocean Drilling Program (IODP) Expedition 336 addressed questions concerning subseafloor microbial life and its relation to seawater circulation and basalt–seawater reactions in the basaltic ocean crust. Sediment and basement samples were recovered at three drill sites located in the North Pond area, an 8 × 15 km large sediment pond on the 8 Ma western flank of the Mid-Atlantic Ridge around 22°45′ N and 46°05′ W in roughly 4450 m water depth. The average core recovery rate in basement was approx. 31 %. The subseafloor depth of the basement holes ranges from 90 to 332 m; sediment thickness is between 36 and 90 m. Two of the holes (U1382A, and U1383C) were equipped with advanced Circulation Obviation Retrofit Kit (CORK) observatories, employing – for the first time – fiberglass casing. Another CORK string was deployed in Deep Sea Drilling Project (DSDP) Hole 395A, but the wellhead broke off upon final installment. Nonetheless, the North Pond observatory is fully operational and post-cruise observatory research is already underway. Combined geochemical and microbiological studies of the drill core samples and experimental CORK materials will help understand (1) the extent and activity of microbial life in basalt and its relation to basalt alteration by circulating seawater, and (2) the mechanism of microbial inoculation of an isolated sediment pond.

1 Introduction

The upper ocean crust constitutes a permeable and hydrologically active aquifer holding as much as 1–2 % of the ocean's water (e.g., Fisher, 2005). Seawater circulation within this volcanic crust is well documented, but the extent to which microbes colonize, alter, and evolve in subsurface rock is essentially not known (e.g., Edwards et al., 2012a). It is well known that the geochemical changes associated with basalt alteration in the uppermost oceanic crust play an important role in setting ocean chemistry. Microbial habitats may be developed where the intensity of seawater–rock interaction is high. The role of microorganisms in this seawater–ocean crust exchange is currently unknown. Earlier studies of basement rocks from ocean drilling provided putative textural and isotopic indications of microbial life within the crust (e.g., Fisk et al., 1998; Staudigel et al., 2008). At the EPR 9N, rich and diverse bacterial life was found on young surface, lava flows (Santelli et al., 2008), but subseafloor samples yielded lesser extents of colonization (Santelli et al., 2010). Seafloor observatories provide a promising technique for in situ studies of microbial activity within ridge flank aquifers because they enable analysis of pristine formation fluids and experimental colonization devices, as has been previously demonstrated at the Juan de Fuca Ridge flank system (Cowen et al., 2003; Orcutt et al., 2010; Edwards et al., 2012b).

Expedition 336 was executed to address fundamental microbiological questions concerning the nature of the subseafloor deep biosphere in oceanic hydrological, geological, and biogeochemical contexts. Determining the nature of

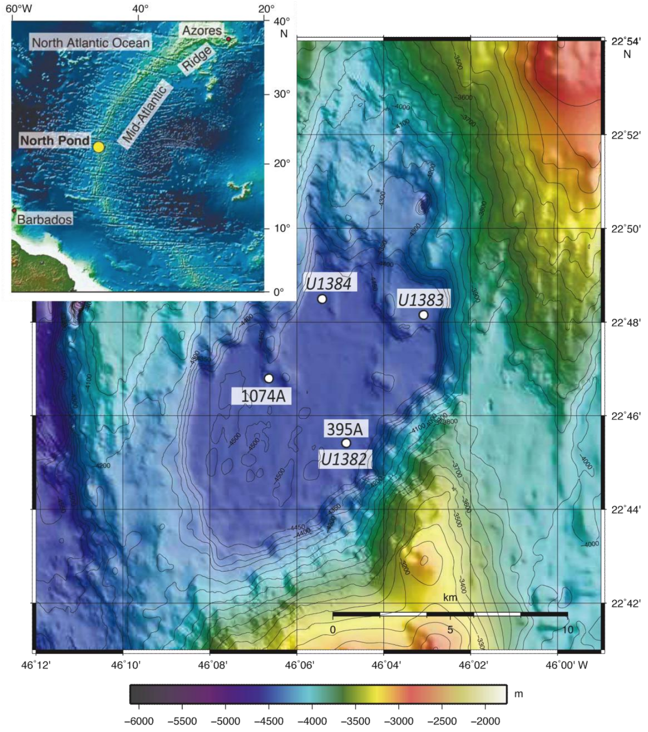

Figure 1. Bathymetric map of the North Pond area (data from Schmidt-Schierhorn et al., 2012) on the western flank of the Mid-Atlantic Ridge (see inset).

subseafloor microbiological communities in young igneous ocean crust and their role in ocean crust alteration was a primary objective. Specifically, we wanted to test the hypothesis that microbes play an active role in ocean crust alteration, while also exploring broad-based ecological questions such as how hydrological structure and geochemistry influence microbial community structures. We also intended to study the biogeography and dispersal of microbial life in subseafloor sediments.

2 Geological setting

All sites are located in the North Pond area at 22°45′ N, 46°05′ W in 4414 to 4483 m water depth (Fig. 1). From previous ocean drilling and site survey investigation, this 8×15 km basin was known as a site of particularly vigorous circulation of seawater in permeable 8 Ma basaltic basement underlying a < 300 m thick sedimentary pile. Heat flow surveys indicate that recharge occurs dominantly in the southeastern part of basin, which is consistent with basement fluid flow generally directed to the northwest (Langseth et al., 1992).

Deep Sea Drilling Project (DSDP) Leg 45 rotary core barrel (RCB) cored two holes at Site 395 (Fig. 1), revealing a 93 m-thick sediment sequence, consisting of 89 m of foraminifer-nannofossil ooze underlain by 4 m of calcareous brown clays with manganese micronodules (Melson et al., 1978). Basement penetration was 91.7 m (Hole 395) and 576.5 m (Hole 395A); a re-entry cone and casing to basement were installed in Hole 395A. The basement lithology is dominated by several units of massive and pillow lava flows, typically several 10s of meters in thickness, which are separated by sedimentary breccia (Bartetzko et al., 2001; Melson et al., 1978). A several meters thick peridotite–gabbro complex with brecciated contacts was cored in Hole 395 (Melson et al., 1978).

During several return missions to Hole 395A, logging operations, packer testing, and borehole fluid sampling were conducted (e.g., Becker et al., 1998). Temperature and flow logs indicated rapid fluid flow (~ 1000 $\mathrm{L\,h^{-1}}$) down into Hole 395A and low formation pressures. Comparison of lithologic and downhole electrical resistivity logs for Hole 395A suggested a series of discreet basalt flows (i.e., different volcanic flow units with distinct geochemical and petrographic characteristics; Bartetzko et al., 2001). Most of the seawater recharge in Hole 395A is accommodated by aquifers tied to flow contacts within the uppermost 300 m of basement. Below that depth, temperature increases (Becker et al., 1998), and borehole fluid chemistry indicates significant chemical exchange with the rocks in the borehole walls (Gieskes and Magenheim, 1992).

During IODP Leg 174B, Hole 1074A was cored near the northwestern margin of North Pond (Fig. 1). Temperature and geochemical profiles are diffusive, indicating there is no upward advection of basement fluids through the sediments, even in an area of local high heat flow (Becker et al., 1998). This observation is consistent with the hydrologic model of Langseth et al. (1992) that indicates fluid flow is predominantly lateral beneath all of North Pond, and recharge/discharge is taking place through basement outcrops that surround the basin.

3 Science and operational objectives

The primary operational goal for Expedition 336 was installations of subseafloor borehole observatories for long-term coupled microbiological, geochemical, and hydrological experiments. The observatories were designed to allow monitoring conditions and study processes in situ after the drilling-induced disturbance and contamination of the borehole environment have dissipated. Sampling of basement and sediment for microbiological and geochemical studies was conducted on cores retrieved from the CORK holes and their immediate vicinity.

A specific goal was to drill a basement hole to > 500 meters below seafloor (m b.s.f.) at a site in the northern area of presumed fluid discharge. We had also planned on drilling a basement hole to ~ 175 m b.s.f. and ~ 70 m into the basaltic crust in another area in the northern part of the sediment pond. For both sites, downhole hydrologic (packer) tests and wire-line logging were planned, but installation of

Table 1. Overview of operational achievements.

Site	395	U1382		U1383					U1384
Hole	395A	U1382A	U1382B	U1383A	U1383B	U1383C	U1383D	U1383E	U1384A
Latitude (N)	22°45.3519′	22°45.3531′	22°45.3528′	22°48.1229′	22°48.1328′	22°48.1241′	22°48.1316′	22°48.1283′	22°48.7086′
Longitude (W)	46°04.8609′	46°04.8911′	46°04.8748′	46°03.1661′	46°03.1556′	46°03.1662′	46°03.1628′	46°03.1582′	46°05.3464′
Seafloor depth (m b.r.f.)	4484.0	4494.0	4494.0	4425.2	4425.2	4425.2	4425.2	4425.2	4475.9
Interval cored	0.0	100.0	98.8	0.0	0.0	262.0	44.3	44.2	96.2
Length recovered	0.00	31.79	84.28	0.00	0.00	50.31	48.65	50.28	94.09
Percent recovered	–	32 %	85 %	–	–	19 %	110 %	114 %	98 %
Section drilled w/o coring	0.0	110.0	0.0	36.0	89.8	69.5	0.0	0.0	0.0
Total penetration	0.0	210.0	98.8	36.0	89.8	331.5	44.3	44.2	96.2
Total depth of hole	5084.0	4704.0	4592.8	4461.2	4515.0	4756.7	4469.5	4469.4	4572.1
Operations conducted	Retrieved Leg 174 CORK, logged, installed CORK (failed wellhead)	Cored basement, logged, installed CORK	Cored sediment and basement contact	Jet-in test	Installed cone and casing; bit lost in hole, 35 m open hole, ROV platform deployed	Cored basement, logged, installed CORK	Cored sediment and basement contact	Cored sediment and basement contact	Cored sediment and basement contact

CORK observatories was the primary objective in order to conduct experiments in the uppermost basement hydrological environment. A third goal was to recover the existing first-generation CORK at Hole 395A in the SE corner of the pond and install a multi-level subseafloor observatory there. Advanced piston coring (APC) of the < 90 m sediment covers in the different areas was another objective. Enhanced education and outreach programs were intended to communicate the excitement and importance of scientific drilling and exploration to a broad audience, build educational curricula, and create media products (photographic, sound, video, and web based) that help achieve critical outreach goals.

Our science objectives for Expedition 336 were to recover sediment and basement and establish subseafloor basement observatories to address two major scientific questions:

1. What is the nature of microbial communities harbored in young ridge flanks and what is their role in ocean crust weathering? What role do microorganisms play on a global basis in promoting seafloor weathering? What energy sources do the microorganisms utilize? How different are communities in different zones in the subseafloor that are characterized by different fluid fluxes and temperature? Geochemical data indicate that oxidative seafloor alteration occurs mostly during the first 10–20 Ma of crustal age, and thereafter slows or even ceases. These records suggest that the most reasonable place to search for active subcrustal microbial communities should be in young, cold ridge flank environments. Chemical reaction kinetics are inhibited at low temperatures, providing a window of opportunity for biological catalysts to take advantage. Yet, the extent and activity of microbial life in these settings remains undetermined. Also, the extent of direct participation in alteration by extant communities in the subseafloor is not clear.

2. Where do deep-seated microbial communities come from? Were they derived from overlying bottom seawater, which acts as a steady source of inoculum that seeds microorganisms (particle-attached and free-living) to sediments? Or is the microbial inoculum provided by advective transport from the basement (passive transport) or by lateral active transport (swimming) from adjacent, older sediments following redox gradients? North Pond was considered an ideal location to test these opposing hypotheses, which have important mechanistic implications concerning dispersal in the deep biosphere, and evolutionary consequences for microbial life on earth.

The low heat flow ridge flank at North Pond represents an ideal model system for studying biologically mediated oxidative basement alteration via the use of CORKed microbial observatories. The work will also provide an excellent point of comparison for the studies taking place at the Juan de Fuca Ridge, which represent the warm, sedimented endmember in the global spectrum of ridge flanks.

4 Expedition summary

Expedition 336 successfully initiated subseafloor observatory science at a young mid-ocean ridge flank setting. A summary of the operational achievements is presented in Table 1. Basement was cored and wire-line-logged in Holes U1382A and U1383C. Upper oceanic crust in Hole 1382A, which is only 50 m west of DSDP Hole 395A, was cored between 110 and 210 m b.s.f.; 31 % of the penetrated basement was recovered, producing different volcanic flow units with distinct geochemical and petrographic characteristics (Fig. 2).

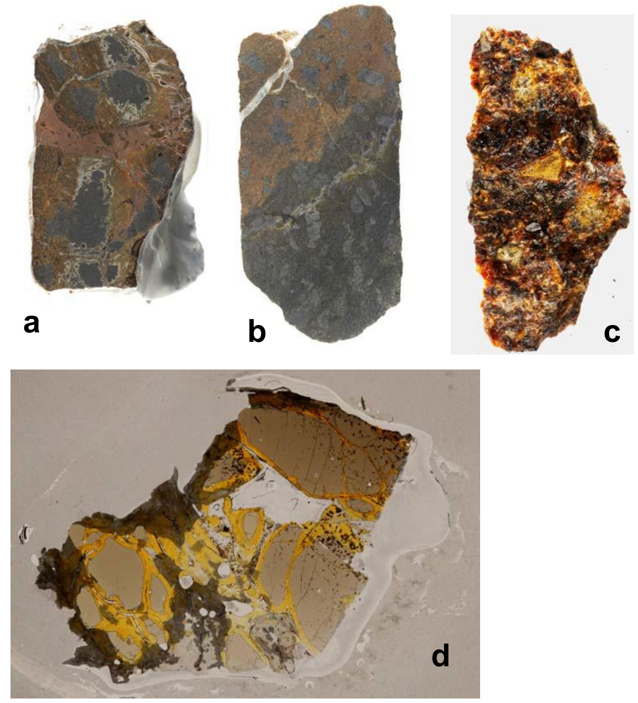

Figure 2. **(a)** Photograph of sample U1382A-5R-1, 116–119 cm, a strongly altered hyaloclastite (length of piece: 3 cm). **(b)** Photograph of sample U1382A-8R-4, 50–63 cm, a strongly serpentinized mantle peridotite with an oxidation halo along a prominent aragonite vein (length of piece: 13 cm). **(c)** Photograph of sample U1383C-17R-1, 120–123 cm, a strongly altered hyaloclastite dedicated for microbiological studies (length of piece: 3 cm) **(d)** Thin section photomicrograph (Sample U1383C-20R-1, 111–113 cm) showing glassy to spherulitic fragments of a hyaloclastite with pronounced palagonite rims (brown) and zeolite (colorless) filling void space. Width of image is 1.2 cm.

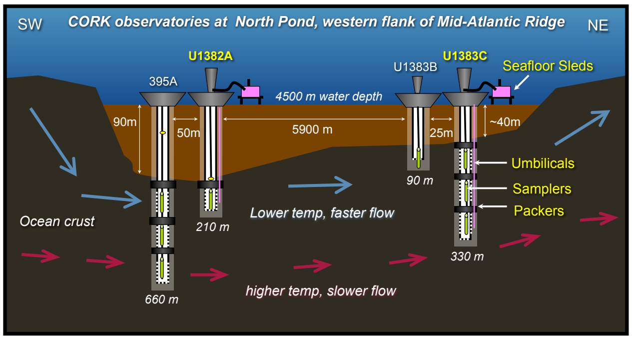

Figure 3. Simplified sketch of the geological and hydrological framework and the observatory layout at North Pond. Two observatories (U1382A ad U1383C; large wellhead) are fully operational and sampling/experimentation using seafloor sleds (Cowen et al., 2012) and incubation chambers (FLOCs; Orcutt et al., 2011). In Hole U1383B, a CORK-lite (small wellhead) was installed using a remotely operated vehicle (Wheat et al., 2012). Flow lines of formation water are highly schematic and adapted from Langseth et al. (1992).

Intercalated between two flow units is a sedimentary breccia, containing clasts of basalt, gabbroic rocks, and mantle peridotite; this unit was interpreted as a rockslide deposit. Hole 1383C recovered 50.3 m of core from an interval between 69.5 and 331.5 m b.s.f. (Fig. 2). The basalts are aphyric to highly plagioclase–olivine-phyric tholeiites that fall on a liquid line of descent controlled by olivine fractionation. They are fresh to moderately altered, with clay minerals (saponite, nontronite, celadonite), Fe oxyhydroxide, carbonate, and zeolite as secondary phases replacing glass and olivine to variable extents. Sediment thickness was about 90 m at Sites 1382 and 1384 and varied between 38 and 52 m at Site 1383. The sediments are predominantly nannofossil ooze with layers of coarse foraminiferal sand and occasional sand- to pebble-sized clasts of basalt, serpentinite, gabbroic rocks, and bivalve debris. The lowermost meters of the APC-cored sections feature brown clay at Sites 1382 and 1384. XCB-coring at the sediment–basement interface recovered < 1 m of brecciated basalt with micritic limestone at all three sites. XCB cores from Hole 1382B also contained ultramafic clasts, suggesting that the uppermost meters of basement at Site 1382 may be talus rather than lava flow. Sediments were intensely sampled for geochemical pore water analyses and microbiological work. Shipboard determination of dissolved oxygen concentrations in the pore waters indicate pronounced C-shaped profiles, indicating diffusion of oxygen into the sedimentary pile from both the water–sediment and sediment–basement interfaces and oxygen consumption by aerobic microbial activity within the sediments (Orcutt et al., 2013).

Expedition 336 installed fully functional observatories in two newly drilled holes (U1382A and U1383C). The principle layout of the observatory as of May 2012 is depicted in Fig. 3. The CORK wellhead at Hole 395A broke off, and another Hole (U1383B) was abandoned after a bit failure. Hole 1383B was penetrated 89 m into the seafloor and is equipped with a re-entry cone and remotely operated vehicle (ROV) landing platform for future observatory science objectives. This hole has, in the meantime, been equipped with a Miniature-CORK that was installed during a joint US–German cruise in April/May of 2012 (Wheat et al., 2012). Details of the CORK observatory layout and installation are reported in Edwards et al. (2012c). In principle, the observatories consist of sensors for temperature, pressure, oxygen concentration and osmotically driven fluid sampling and microbial incubation devices. Fluid sampling lines run down to packer-sealed compartments in the subseafloor and allow post-drilling sampling using ROVs. During a first ROV cruise in April/May of 2012, first fluid samples were collected and osmo-samplers as well as other instrument packages (GeoMicrobe sleds; Cowen et al., 2012) were deployed. A repeat visit was recently conducted (April, 2014), and the overall duration of the CORK experiment is 6–8 yr. The

CORK observatory in Hole U1382A has a packer seal in the bottom of the casing and monitors/samples a single zone in uppermost oceanic crust extending from 90 to 210 m b.s.f. Hole U1383C was equipped with a three-level CORK observatory that separates a zone of thin basalt flows with intercalated limestone (56–142 m b.s.f.) from one within glassy, thin basaltic flows and hyaloclastites (142–196 m b.s.f.) and a lowermost zone (196–331.5 m b.s.f.) of more massive pillow flows with occasional hyaloclastite in the upper part. The instrument strings are inside fiberglass casing, which, unlike the traditional steel casings, are noncorrosive and hence will not produce hydrogen upon reaction with borehole water – a critical achievement for subseafloor microbiologic and geochemical experiments. The use of fiberglass casing is a novel development in the history of scientific ocean drilling and one of the operational highlights of the expedition.

Major strides in ridge flank studies have been made by employing CORK subseafloor observatories, as they facilitate combined hydrological, geochemical, and microbiological studies and controlled experimentation within the subseafloor. The North Pond observatory is representative of young and cold ridge flanks and complements similar observatories on the eastern flank of the Juan de Fuca Ridge. These observatories will help constrain the importance of ridge flanks as microbial habitats and the role of seawater circulation in crust–ocean transfers of heat and matter.

The IODP Expedition 336 Scientific Party

Members of the the IODP Expedition 336 Scientific Party included Louise Anderson, Nicolas Backert, Keir Becker, Dale W. Griffin, Amanda G. Haddad, Yumiko Harigane, Hisako Hirayama, Samuel M. Hulme, Steffen Leth Jørgensen, Tania Lado Insua, Paul Le Campion, Heath J. Mills, Kentaro Nakamura, Beth Orcutt, Young-Soo Park, Victoria Rennie, Olivier Rouxel, Joseph A. Russel, Kasumi Sakata, Everett C. Salas, Fengping Wang, and C. Geoffrey Wheat

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