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Abstract

The Iheya North hydrothermal field is located in the middle Okinawa Trough, an actively spreading backarc basin that extends for 1200 km between the Ryukyu arc-trench system and the Asian continent, in a transitional region between continental and oceanic crust. Because the Okinawa Trough contains both hemipelagic and volcanic sediment, in some places >1000 m thick, its hydrothermal systems provide abundant H_2, CO_2, CH_4, NH_4, H_2S, and CO derived from sedimentary organic matter and from magmatic gases that could feed a variety of microbial communities, sustained by different chemolithoautotrophic primary producers within a range of subseafloor habitats (Nakagawa et al., 2005). Integrated Ocean Drilling Program (IODP) Expedition 331, the Deep Hot Biosphere project, drilled into the Iheya North hydrothermal system in order to investigate metabolically diverse subseafloor microbial ecosystems and their physical and chemical settings.

We drilled five sites during Expedition 331: the active hydrothermal vent site and sulfide-sulfate mound at North Big Chimney (NBC) (Site C0016); three sites east of NBC at distances of ~100, 450, and 1550 m from the active vents (Sites C0013, C0014, and C0017, respectively); and one site on a hill ~600 m northwest of the active vents that represents a potential migration path for hydrothermal fluid (Site C0015). Our maximum penetration was 151 meters below seafloor (mbsf) at recharge Site C0017. We used heavy, triangular, gimbaled guide bases at three holes, one each at Sites C0013, C0014, and C0016, for reentry, casing, and capping, including installation of a steel mesh platform with valve controls for postcruise sampling of fluids.

At Site C0016, drilling at the summit of the active hydrothermal mound failed to recover core, and drilling at the base of the mound yielded only 2.1 m of core from 45 m of penetration, but the core included the first Kuroko-type, sphalerite-rich black ore ever recovered from the modern seafloor. The other four sites yielded interbedded hemipelagic and volcaniclastic sediment and volcanogenic breccias and pumice that are variably hydrothermally altered and mineralized, in the zeolite to greenschist facies. Temperature gradients decrease greatly with distance from the active vents at Site C0016, from >7°C/m at Site C0013, to 3°C/m at Site C0014, to 0.6°C/m at Site C0017. Detailed temperature profiles at Sites C0014 and C0017 display irregularities suggestive of lateral flow. The profile at Site C0017 is concave-upward, consistent with recharge of cold seawater into the hydrothermal system at this site.

Analyses of interstitial water and headspace gas yielded complex patterns with depth and laterally at most sites over distances of only a few meters. Documented processes include formation of brines and vapor-rich fluids by phase separation and segregation, uptake of Mg and Na by alteration minerals in exchange for Ca, leaching of K at high temperature and uptake at low temperature, anhydrite precipitation, microbial oxidation of organic matter and anaerobic oxidation of methane utilizing sulfate, microbial methanogenesis, abrupt changes in composition with depth that result from sealing by relatively impermeable cap rock, and generation of hydrogen at depth, apparently by hydrothermal rather than microbial processes.

Shipboard analyses have not confirmed presence of an active deep hot biosphere. Cell abundances are much lower than those found in previous Ocean Drilling Program/ IODP sites on continental margins, and attempts at culturing were generally unsuccessful. We did find ample evidence for microbial activity supported by sedimentary organic matter, but only in sediments within the upper 10–30 mbsf where temperatures were relatively low. At the recharge Site C0014 we found a community of Fe-oxidizers that was successfully cultured.

Background

Active subseafloor hydrothermal systems at mid-ocean ridges, volcanic arcs, backarc basins, and hotspots are environments with extraordinarily high fluxes of energy and matter. The "subvent biosphere" is the subseafloor biosphere that is predicted to exist just beneath active hydrothermal vents and fluid discharge zones (Deming and Baross, 1993). Subseafloor environments within active hydrothermal systems are promising locations for functionally active, metabolically diverse subseafloor microbial ecosystems. The existence of a subvent biosphere has been inferred from many microbiological and geochemical investigations of vent chimney structures and diffuse hydrothermal fluids (Nunoura and Takai, 2009; Nunoura et al., 2010; Takai et al., 2008, 2009; and references in Takai et al., 2006, and Huber and Holden, 2008). High-temperature hydrothermal fluids with focused discharge and little or no dilution by seawater also provide evidence for indigenous subvent microbes active along flow paths of hydrothermal upwelling, in which abundant H_2, CO_2, CH_4, H_2S, and CO provided as magmatic volatiles and by hydrothermal water-rock reactions are metabolized (Takai et al., 2004; Huber and Holden, 2008). Indeed, the possible occurrence of a subvent biosphere has been clearly demonstrated by microbiological and geochemical characterization of high-temperature hydrothermal fluids in several hydrothermal fields (Nakagawa et al., 2005; Nunoura and Takai, 2009; Takai et al., 2004, 2008, 2009). In the Iheya North field, it has been suggested that a variety of microbial communities sustained by different chemolithoautotrophic primary producers is present in subseafloor habitats (Nakagawa et al., 2005). Variability in potential subseafloor microbial communities is likely associated with physical and chemical variation of hydrothermal fluids, controlled by phase separation and phase partition of hydrothermal fluid beneath the seafloor. In addition, the hydrothermal environments hosted by organic-rich sediments provide unusual amounts of C_1 compounds (CO_2 and CH_4) in hydrothermal fluids as carbon sources, as well as unique microbial habitats affected by liquid CO_2 and gas hydrates (Nakagawa et al., 2005; Kawagucci et al., 2010). Thus, the abundant supply of energy and carbon and the richness of the habitats supported by physical and chemical variations in the Iheya North field provide an ideal setting for the formation of functionally and metabolically diverse subseafloor microbial communities associated with hydrothermal activity.

Geological setting and hydrothermal activity in the Okinawa Trough

The Okinawa Trough is a backarc basin extending for ~1200 km, located between the Ryukyu arc-trench system and the Asian continent (Lee et al., 1980; Letouzey and Kimura, 1986) (Fig. **F1**). It is presently undergoing rifting, which began at ~2 Ma and was preceded by an earlier rifting episode during the Miocene. Lee et al. (1980) proposed that the Okinawa Trough is presently in a "drifting phase" (i.e., oceanic crust spreading characterized by short spreading centers and concomitant transform faults), as typically occurs along mid-ocean ridges and in other actively spreading backarc basins such as the Lau Basin and Mariana Trough. Seismic reflection data also suggest a typical geologic structure for the Okinawa Trough (Letouzey and Kimura, 1986): a high-velocity mantle at ~6000 meters below seafloor (mbsf) overlain by potentially young basalt with an average velocity of 5.8 km/s between ~3000 and 6000 mbsf, an igneous rock layer (4.9 km/s) between ~1000 and 3000 mbsf, and ~1000 m of sediment at the seafloor. Geochemical features of hydrothermal fluids in the Okinawa Trough hydrothermal systems clearly demonstrate a significant contribution from felsic rocks and magma, as described below (Glasby and Notsu, 2003). Thus, it seems likely that the Okinawa Trough is an actively rifting transitional region between continental and oceanic crust.

Integrated Ocean Drilling Program (IODP) Expedition 331 provided an opportunity to drill into an active hydrothermal system and associated deposits within a backarc basin in a continental margin setting. Characteristics of this tectonic setting are reflected in the chemical composition of hydrothermal sulfide deposits there. Sulfide samples collected from the Iheya North field show a Zn- and Pb-rich signature that is distinctive from the Fe-rich signature of mid-oceanic ridge sulfides (Halbach et al., 1993) (Fig. **F2**). This chemical signature is similar to that of Kuroko-type hydrothermal deposits formed during the Tertiary in northeast Japan and related to the opening of the Japan Sea. Moreover, many volcanic massive sulfide ore deposits formed throughout geologic time are related to felsic and/or intermediate magmatism rather than to basaltic volcanism at typical mid-ocean ridges (Urabe and Marumo, 1991).

Hydrothermal fluid chemistry in the Okinawa Trough hydrothermal systems is characterized by higher concentrations of CO_2, CH_4, NH_4, I, and K and higher alkalinity than those in typical sediment-free mid-ocean-ridge hydrothermal fluids (Sakai et al., 1990a, 1990b; Gamo et al., 1991; Konno et al., 2006; Takai and Nakamura, 2010; Kawagucci et al., 2010). The distinctive hydrothermal fluid chemistry is strongly linked with the geologic setting and the thick terrigenous sediments of the Okinawa Trough. Philippine plate subduction along the Ryukyu arc-trench system supplies dacitic-rhyolitic magma to the Okinawa Trough that is rich in K and volatile components (Sakai et al., 1990b; Gamo et al., 2006). Organic-rich terrigenous sediment filling the Okinawa Trough (Narita et al., 1990) supplies not only the sedimentary chemical inputs (NH_4, I, etc.) (Gamo et al., 1991; You et al., 1994) but also promotes the widespread occurrence of functionally active microbial communities that impact hydrothermal fluid chemistry and circulation (Nakagawa et al., 2005; Inagaki et al., 2006; Nunoura and Takai, 2009; Nunoura et al., 2010; Takai and Nakamura, 2010). In addition to the chemical aspects, the relatively shallow water depth of many Okinawa Trough hydrothermal systems serves to induce boiling (Suzuki et al., 2008) (subcritical phase separation) and subsequent phase segregation, as the boiling temperature of seawater decreases steeply with decreasing pressure at ~100 bar. Phase separation and segregation sometimes produce hydrothermal fluids of quite different chemical composition at different vent sites in the same hydrothermal field, even though they are derived from the same source fluid (Kawagucci et al., 2010).

Since the discovery of submarine hydrothermal activity at Iheya Knoll and Izena Hole (Halbach et al., 1989; Sakai et al., 1990b) in the mid Okinawa Trough, six active representative hydrothermal fields (Minani-Ensei Knoll, Iheya North, Iheya Ridge, Izena Hole, Hatoma Knoll, and Yonaguni Knoll IV) have been discovered. The Iheya North field has been investigated by interdisciplinary methods, specifically geochemistry of hydrothermal fluids and sulfide/sulfate deposits (Glasby and Notsu, 2003; Kawagucci et al., 2010) and microbial ecology at the seafloor (Takai and Horikoshi, 1999; Takai et al., 2003; Nakagawa et al., 2005; Takai et al., 2006; Takai and Nakamura, 2010). It has also been monitored for ~15 y.

Seismic studies and site survey data

Hydrothermal activity in the Iheya North Knoll (27°47.50′N, 126°53.80′E; 150 km north-northwest of the island of Okinawa, Japan) was first discovered by a camera survey in 1995. Since then, deep submergence vehicle (DSV) and remotely operated vehicle (ROV) dives have revealed details regarding the location of hydrothermal activity and seafloor events (Fig. **F3**). Recent seismic and geophysical surveys (Table **T1**) have provided insights into the subseafloor geologic structure and the pattern of heat flow through the seafloor, suggesting a possible hydrothermal fluid flow model for the Iheya North hydrothermal system.

A grid of multichannel seismic (MCS) profiles has been completed for the Iheya Knoll region (Fig. **F1**). MCS profiles across the Iheya North Knoll (Fig. **F4**) demonstrate an igneous intrusion penetrating the trough-filling sediments, which are >1000 m thick (Fig. **F4**). In the middle of Iheya North Knoll, a central valley exhibits relatively disordered seismic reflectors as deep as 400–500 mbsf, suggesting the presence of pumicious pyroclastic flow deposits beneath the surficial hemipelagic sediments rather than massive igneous rocks (Fig. **F5**).

Pumicious pyroclastic flow deposits have been recovered by numerous gravity cores obtained from the central valley of Iheya North Knoll (Oiwane et al., 2008). In all cores, thick pumice layers with coarse to fine grain sizes were found just below the seafloor. These layers often contain abundant gas-filled voids accompanied by elemental sulfur and sulfide minerals, probably deposited by gas-rich hydrothermal fluids (Masaki et al., in press). These site survey data consistently suggest that multiple and thick volcanic pyroclastic deposits fill the central valley of Iheya North Knoll, providing both potential hydrothermal fluid recharge paths and reservoir and discharge paths (Kumagai et al., in press).

A significant discovery of the seismic reflection survey is the existence of large-scale negatively polarized reflection sequences deep beneath the hill west of the Iheya North hydrothermal vent sites. The eastern end of these westward-dipping sequences appears to converge at the seafloor where the vent sites are located (Fig. **F6**). These negative polarity sequences are interpreted to consist of layers of highly porous pumicious volcanic deposits, which could serve as potential migration paths for subseafloor hydrothermal fluids. The sequences appear to be as thick as 100 m just beneath the western hill near the vent sites (Fig. F6). The negative polarity sequences could also represent a potential fluid reservoir that feeds hydrothermal solutions to the discharge sites along the eastern- and upward-dipping structure (Fig. F6). To determine the possible role of these structures in the hydrothermal system, it would be desirable to penetrate and sample these negative polarity sequences.

The supporting site survey data for Expedition 331 are archived at the **IODP Site Survey Data Bank.**

Scientific objectives

The goal of Expedition 331, Deep Hot Biosphere, is to determine directly the existence of a functionally active, metabolically diverse subvent biosphere in its physical, geochemical, and hydrogeologic context within the Iheya North field in the mid-Okinawa Trough. This work addresses one of the primary themes of the IODP Initial Science Plan, the Deep Biosphere and the Subseafloor Ocean.

This project was conducted by an interdisciplinary group of onboard and shore-based scientists including microbiologists, geochemists, sedimentologists, petrologists, geologists, and geophysicists. The major scientific objectives of Expedition 331 drilling are

1. To prove the existence of a functionally active, metabolically diverse subvent biosphere associated with subseafloor hydrothermal activity in the Iheya North field;
2. To clarify the architecture, function, and impact of subseafloor microbial ecosystems and their relationship to physical, geochemical, and hydrogeologic variations within the hydrothermal mixing zones around the discharge area; and
3. To establish artificial hydrothermal vents in cased holes, using casing with perforated screen pipes at those depths that exhibit hydrothermal flow, and to prepare a research platform at each cased hole for later study of fluids tapped from various parts of the hydrothermal system and their associated microbial and macrofaunal communities.

In our shipboard studies, and continuing postcruise, we have addressed these objectives by a combination of compositional and isotopic biogeochemistry and cellular, molecular, and functional microbiology. We have used downhole temperature measurements, alteration mineral assemblages, and the chemical composition of hydrothermal fluids to constrain the physical and chemical conditions of mineralization and of the microbial habitats. Studies of fluid inclusions in alteration minerals will constrain present and past fluid temperatures and provide evidence for subseafloor phase separation. Isotopic studies of sulfur, lead, and possibly iron will provide information on magmatic and crustal contributions to the hydrothermal system. Dating of hydrothermal deposits will constrain the duration and timing of hydrothermal activity. Application of recently developed microanalytical techniques such as laser ablation–inductively coupled plasma mass spectrometry (LA-ICP-MS) will provide new insight into the sources of hydrothermal metals. Finally, drilling will provide constraints on the hydrology of the active hydrothermal system and the size and geometry of the associated sulfide deposits and alteration zones. Because microbial habitat relies on hydrologic as well as physical-chemical structure, these studies will provide important insights into the range of conditions within the subseafloor biosphere.

Drilling strategy

We drilled five sites during Expedition 331: the active hydrothermal vent site and sulfide-sulfate mound at North Big Chimney (NBC) (Site C0016) (Fig. **F3**); three sites east of NBC at distances of ~100, 450, and 1550 m from the active vents (Sites C0013, C0014, and C0017, respectively); and one site on a hill ~600 m northwest of the active vents that represents a potential migration path for hydrothermal fluid (Site C0015) (Fig. **F6**).

The NBC hydrothermal mound at Site C0016 is 20 m high and 6 m in diameter (Fig. **F7**). We attempted to drill one hole into an active high-temperature (310°C) vent at its summit in which the pipe broke and we failed to recover any core. We drilled a second hole 20 m away, immediately at the base of the mound on its western side. Because of the high temperatures and because we expected to recover hard rock, we used conventional hard rock drilling equipment supplied by Baker-Hughes Inteq (BHI) specifically for Expedition 331. The BHI system collects 4 inch diameter core in aluminum liners in lengths of 9, 18, or 27 m, but the cores cannot be retrieved by wireline and so require a time-consuming pipe trip for each core.

At Sites C0013, C0014, and C0017, we drilled the relatively high, moderate, and low heat flow areas to the east of the Iheya North hydrothermal field to investigate subseafloor microbial habitats and communities within broad gradients of physical and chemical variation, both laterally and vertically, which could be affected by mixing between discharging hydrothermal solutions and recharging ambient bottom seawater. These sites were drilled using the hydraulic piston coring system (HPCS) to first refusal and then again in softer intervals encountered deeper in the hole, alternating as necessary with both the extended punch (EPCS) and extended shoe (ESCS) coring systems to penetrate harder layers. Both of these latter systems were able to penetrate the harder layers, with a slight edge to the ESCS for the hardest, but the EPCS generally was much better at core recovery.

We penetrated the margin of the local discharge-recharge zone to depths of 55, 137, and 151 mbsf at Sites C0013, C0014, and C0017, respectively, coring variably hydrothermally altered sediment and pumiceous deposits (Fig. **F5**). We were able to measure in situ temperature at two of these sites using the advanced piston corer temperature tool (APCT-3) shoe (upper calibration limit = 55°C) as part of the HPCS, combined with commercial thermoseal (Nichiyu Giken Co. Ltd) strips taped to the outer surface of the core liner that estimate temperature in 5° or 10°C increments, each over one of several limited ranges between 75° and 250°C, using chemically impregnated wafers that turn black when exposed to the designated temperature. Temperature at the distal flank Site C0017 reached 44 °C at 112 mbsf and 90 °C at 151 mbsf. Temperature at intermediate flank Site C0014 was 22°C at 6.5 mbsf. It exceeded 55°C at 16 mbsf and 210°C at only 50 mbsf. We were not able to measure temperature at proximal flank Site C0013, but the gradient was likely higher than at the other two flank sites. This contention is, based on the alteration mineral assemblages and the fact that we started melting the acrylide plastic core liners, which begin to soften and deform at 82°C, at only 12 mbsf. Melting of the plastic core liners severely limited our ability to core deep at Site C0013. Our one attempt to use the BHI system at this site, with its aluminum core liners, recovered only 2.1 m of core from a 9.2 m penetration and required nearly 22 h to collect, mainly because the sediment was soft and the BHI system is optimized for hard rock coring. During the port call in Okinawa halfway through Expedition 331, we acquired eighteen 9 m sections of aluminum core liner, with which we were able to retrieve good quality core to the depth we reached at Site C0014.

Site C0015 is located 600 m northwest of the hydrothermal mounds, where the seismically detected negative polarity sequences become thick. Up to 400 m of penetration would be required to penetrate the deepest of these reflectors, but we had time to take only two HPCS cores, to 9.4 mbsf, before we had to depart for Okinawa for a crew change midway through the expedition.

Operations strategy

All drilling from the D/V *Chikyu* is continuously monitored on the seafloor using a video camera mounted on an ROV operated by Oceaneering Inc. This device was invaluable for observing drilling induced hydrothermal flow. We used heavy triangular, gimbaled guide bases at three holes, one each at Sites C0013, C0014, and C0016, to allow reentry for deep drilling and for postdrilling operations, including casing and capping. The casing in each of these holes was hung from the guide base, a valved wellhead corrosion cap was fit over the hole, and a steel mesh platform with valve controls was installed on its top. Holes C0013E and C0014G were cased to total depth, using perforated screened casing in depth intervals where flow was thought to occur. Hole C0016B was not cased, but it was capped, with a 3 m piece of pipe welded beneath it as a substitute for full casing of the 45 m deep hole. The research group of Co-Chief Scientist Ken Takai has recently developed an in situ cased-hole fluid sampler (DEEP-SAMPLER) and a microbial colonization device (BIO-SAMPLER). These sampling tools will enable them to retrieve indigenous subseafloor fluids and microbes at the seafloor through these cased holes using an ROV. Development of these types of third-party tools greatly extends the usefulness of IODP drill holes, provides research opportunities for investigating subseafloor fluid flow and microbial communities, and permits long-term monitoring of drilling-induced disturbance of seafloor and subseafloor environments.

Site summaries

Site C0013

Operations, lithostratigraphy, minerals, and physical properties

Eight holes (C0013A–C0013H) were drilled at Site C0013, and core was recovered from all but Hole C0013A (Fig. **F8**). Hole C0013E was the deepest (54.5 mbsf) and was cased down to 40.2 mbsf and fixed with a corrosion cap (open outlet pipe) mounted on the guide base. During drilling and coring operations at Site C0013, we encountered many operational and sample-handling problems due to the unexpectedly high temperature gradient at the site and the presence of repeated hard layers that appear to behave as cap rocks, alternating with soft and sticky clay-rich layers. Porosity measurements on the core clearly document the repeated occurrence of low-porosity harder layers (e.g., 0–2, 7–10, and 20–30 mbsf). We observed at several depths that when we drilled through a hard cap rock into the softer intervening layers, subseafloor hydrothermal fluid began to upwell in the hole and exit to the seafloor, where it was imaged by the video camera mounted on the ROV. We surmise that fluids were flowing laterally, trapped beneath the cap rock. To tap this fluid, we used slotted, perforated casing pipe over the depth interval 21–39.8 mbsf in Hole C0013E (Fig. **F9**). Immediately after casing and capping this hole, we observed, in the ROV video image, strong hydrothermal fluid discharge from the casing pipe, which was hung in the guide base. Thermoseal temperature-sensitive strips on the corrosion cap outlet pipe and read in the ROV video imagery indicated that the discharging water was >250°C. When the JAMSTEC ship *Natsushima* visited Hole C0013E with its *Hyper Dolphin* ROV 2 days later, they found that flow from the casing pipe, through the outlet in the corrosion cap, had ceased. Instead, water that appeared blackish was now discharging directly from the hole beneath the guide base. This flow apparently came up through the annulus, between the wall of the hole and the outer surface of the casing pipe. The *Hyper Dolphin* lowered a probe 30 m into cased Hole C0013E and measured a temperature of only 4°C; the hole was plugged below that depth. The *Hyper Dolphin* also measured a temperature of 11°C in diffuse flow from uncased Hole C0013D, higher than the bottom water temperature in the area of 4.51° ± 0.17°C (1σ, $n = 4$, measured at Holes C0014C, C0014D, C0014F, and C0014G). On these same dives the *Hyper Dolphin* revisited the NBC vent about 170 m to the west-northwest, which we drilled as Site C0016, and found it to be venting at 310°C, compared with the highest temperature measured there in previous years of 311°C (Kawagucci et al., in press).

Four lithologic units were identified at Site C0013. The uppermost Unit I (0–4 mbsf) is hydrothermally altered mud containing crystalline pipes of elemental sulfur and sulfide grit. The ROV survey done at the site to choose actual hole locations shows that the seafloor is covered by a thin (<1 m) discontinuous layer of unaltered sediment, as has been documented in previous surveys over the past 10 y. The origin of the sulfide grit is uncertain, and two potential sources, collapsed sulfide mound fragments or in situ deposition by high-temperature hydrothermal fluids, have been proposed. It is apparent in any case that Unit I has been subjected to high temperatures and hosted sulfidic hydrothermal fluids, supporting the in situ formation scenario for at least some of the sulfide.

Unit II (4–14 mbsf) is hydrothermally altered mud with some heavily veined intervals and clastic units containing anhydrite breccia and fragments of metalliferous massive sulfide. Unit II is commonly cut by anhydrite veins, indicating that seawater infiltrated and was heated to >150°C. Unit II represents a possible hydrothermal reservoir or fluid migration path, consistent with our observation of upflow in Hole C0013E.

Unit III (14–26 mbsf) consists of hydrothermally altered mud, some layers of which contain abundant nodular anhydrite. Unit III was distinguished from Unit II by the presence of large whole anhydrite nodules and less abundant vein anhydrite. Unit III likewise experienced high temperatures (>150°C) and hosted hydrothermal flow, of solutions with a different chemical composition than those which infiltrated Unit II, as described below.

Unit IV consists of volcanic breccia with clasts of various volcanic lithotypes. Both monomictic and polymictic breccias were observed, and all were matrix supported and silicified. Other volcanic components within the breccia include clasts of tube pumice and vesicular and flow-banded lavas. The occurrence of various volcaniclastic components such as lavas, vesiculated glasses, breccias, and blocks of polymictic breccia is consistent with breccia formation by mass wasting of a mixture of volcaniclastic units, initially deposited further upslope from Site C0013.

We observed the strongest discharge of hydrothermal fluid after using the BHI system for the first time to drill the interval 26–35.2 mbsf. Beneath that depth, to the total depth of 54.5 mbsf in Hole C0013E, our total recovery was 25 cm of rock. Drilling records indicate that the material we failed to recover became soft again below 35.2 mbsf, suggesting that additional porous and permeable formations exist below Unit IV.

Interstitial water chemistry

Twenty-six whole-round samples were processed for chemical analyses of interstitial water at Site C0013, from five different holes (C0013B and C0013D–C0013G). Chloride, the major anion in seawater, is nearly constant with depth in Holes C0013B and C0013D at the concentration in seawater. In Hole C0013E, however, it varies widely, from 34% lower than seawater to 12% higher in the deepest sample from 17 mbsf. The major cation in seawater, Na, generally follows Cl, as expected from charge balance constraints. Na/Cl and Na/Br are higher than in seawater in Hole C0013B, probably because of expulsion of Na from ion exchange sites in clay minerals by other cations, but these ratios decrease systematically with depth to values lower than seawater in the other two holes, showing that Na is being removed from the seawater-derived pore water into alteration minerals over this interval and at greater depth.

Mg increases with depth in Holes C0013B and C0013D, probably because of ion exchange with clays as for Na, but decreases to only 7 mM in Hole C0013E. Ca increases irregularly with depth in all three holes, to concentrations nearly five times that in seawater. Although some of this increase is typical for hydrothermal solutions, as Ca is leached from the host rock, much of the increase at Site C0013 undoubtedly results from dissolution of the anhydrite that is distributed abundantly throughout much of the core during the 3–25 h that elapsed between the arrival of the core on deck and squeezing of the sediment to separate pore water. Dissolution of anhydrite is implied by the drastic increases in sulfate. By contrast, sulfate decreases with depth in Hole C0013E to less than half the concentration in seawater, as would be expected from anhydrite precipitation within the hydrothermal system with increasing temperature.

The deepest sample obtained from this site, from Hole C0013E at 17 mbsf, is in many ways a typical high-temperature subseafloor hydrothermal fluid. Relative to seawater, it is slightly briny at 623 mM Cl; it has highly elevated Ca, K, Rb, and Cs and greatly depleted Mg and sulfate; and it has lost Na to the altered rocks.

Microbiology

The abundance of microbial cells was evaluated by a fluorescent microscopic analysis using SYBR Green I as a fluorochrome dye. Total cell counts obtained from core samples at Site C0013 are generally lower than the detection limit of our onboard counting method (1×10^5 cells/cm^3 sediment), and the lowest among all coring site in this expedition. Microbial abundance in the sediment of the Iheya North hydrothermal field is known to be relatively low (<2×10^7 cells/mL sediment) (K.T. Ishibashi et al., unpubl. data), but the cell abundances we observed are even lower than those previously found. Cultivation tests were conducted for *Thermococcales* (e.g., *Thermococcus* spp.) and *Aquificales* (e.g., *Persephonella* spp.) and thermophilic *Epsilonproteobacteria* (e.g., *Nitratiruptor* spp.) using core from Site C0013 and different recipes for media at various temperatures. No growth of these organisms was identified in the onboard experiments.

Although onboard microbiological measurements and experiments were quite limited and did not provide any conclusive result, it is evident that no numerically prosperous microbial communities are present in the subseafloor at Site C0013. Except for the upper several meters below seafloor of altered sediment, the subseafloor environments are at higher temperatures (>150°C) than microbes can survive. However, it is still possible that shorebased work will yield evidence for functionally active hyperthermophilic microbial communities at Site C0013.

Site C0014

Operations, lithostratigraphy, minerals, and physical properties

Seven holes were drilled at Site C0014 (Holes C0014A–C0014G) (Fig. **F10**). Hole C0014G was the deepest (136.7 mbsf) and was cased down to 117.8 mbsf and fixed with a corrosion cap (open outlet pipe) mounted on the guide base. As at Site C0013, we encountered repeated hard layers that may behave as cap rock, and we saw discharge from the holes, beyond what may be expelled drilling fluid only, after penetrating these layers (35–44.5 mbsf in Hole C0014B, 25.5–35 mbsf in Hole C0014E, and 37.7–47.2 mbsf and 89.2–93.7 mbsf in Hole C0014G). We again cored multiple low-porosity layers (e.g., in Holes C0014B, C0014E, and C0014G). Based on the low recovery and the lithology of the cores, we infer lateral hydrothermal flow at depths of 35–45 and 90–95 mbsf at Site C0014 (Fig. **F10**). We therefore installed slotted, perforated casing pipe at 29.8–49.2, 78.3–97.8, and 107.5–117.2 mbsf in Hole C0014G (Fig. **F9**). After casing and capping, we saw in the ROV-mounted video diffuse hydrothermal fluid discharge, not from the corrosion cap outlet but from the seafloor, through the annulus, the space between the wall of the hole, and the casing pipe. The temperature of the diffusing fluids was found to be >240°C based on exposure of thermoseal strips mounted on the corrosion cap outlet pipe and observed by ROV.

We measured temperature at Site C0014 using the APCT-3 temperature shoe on the HPCS core barrel for lower temperatures (0°–55°C) and thermoseal temperature-sensitive strips for higher temperatures (75°–250°C). The temperature-depth profile at Site C0014 is shown in Figure **F11.** Temperature increases nearly linearly at 3°C/m to 145° ± 5°C at 47 mbsf and then increases abruptly to >210°C at 50 mbsf, below a hard layer near that depth. Two main lithologic and sediment types were identified at Site C0014: volcanic breccias, often silicified, and hemipelagic hydrothermally altered mud. The dominant depositional process is pelagic sedimentation, although mass wasting and debris flows likely lead to high rates of resedimentation of both hemipelagic and volcaniclastic sediments. Thus the bulk of the sediment volume encountered at the surface is likely to have been resedimented. Furthermore, the bulk of the rock volume cored has been hydrothermally altered. Differing styles of hydrothermal alteration form the basis for the division of Site C0014 sediments and lithologies into lithostratigraphic units.

Unit I (0 to <18 mbsf) is a succession of woody pumice breccias and hemipelagic ooze 12 to 16 m thick that is little altered by hydrothermal activity. It includes a diversity of foraminifers dominated by warm forms of the planktonic *Neogloboquadrina pachy-*

derma, some of them pyritized. Minor coccoliths were present, dominated by *Emiliania huxleyi, Gephyrocapsa oceanica,* and *Reticulofenestra asanoi*. Unit II consists of partially consolidated hydrothermally altered mud with quartz and muscovite as major phases and minor kaolinite and illite/montmorillonite, along with hydrothermally altered pumice breccias. Clasts of pumice have been devitrified to soft clay that has retained the primary woody texture. Unit II is 12 to 15 m thick and extends to a depth of 27 to 30 mbsf. Unit III is defined by the occurrence of consolidated and often cemented volcanic sediments as lithoclasts within breccias or horizons interbedded with hemapalgic and hydrothermally altered mud. Mg chlorite becomes an important alteration phase in Unit III, regardless of lithology. Anhydrite is present below 57 mbsf in Hole C0014G, mostly in millimeter-scale irregular veinlets with halite, but in much lower abundance than was seen at Site C0013. Pyrite occurs in trace amounts as fine-grained disseminations and very rare coarser veins throughout the sequence. Unit III is 128 m thick in Hole C0014G, extending to 136.7 mbsf, and has a gradational contact with Unit II.

Interstitial water chemistry

A total of 75 whole-round samples were processed at Site C0014 from four different holes. Within the upper ~30 m of sediment, these holes exhibit considerable spatial variation in the concentrations of those chemical species that are most influenced by organic matter diagenetic reactions.

The most sulfate-depleted interval, at 38 mbsf, also has the highest NH_4 and notable depletions in Cl and possibly Br. High NH_4 and low Cl typically result from high-temperature phase separation into a brine and a gas-rich, Cl-depleted vapor, as has happened at high-temperature vents in the Iheya North field (Kawagucci et al., 2010; Takai and Nakamura, 2010). Surprisingly, NH_4 in Hole C0014G is also inversely correlated with sulfate, as would result from the anoxic ammonia-oxidizing sulfate-reducing microbial metabolism recently predicted by thermodynamic calculations of microbial energy metabolisms (Schrum et al., 2009).

Below 50 mbsf in Hole C0014G, Cl and Br are enriched relative to seawater as they would be in a brine. Sodium, the major cation in seawater, generally follows the distribution of Cl, but it is often slightly depleted in hydrothermal solutions because of removal into alteration minerals, particularly albite. In contrast to Na, K is enriched relative to seawater at depth at this and all the other sites we drilled. The initial depth (~17.7 mbsf) at which K increases is slightly deeper in Hole C0014G than in Holes C0014B and C0014D, consistent with other observations of hydrothermal fluid influence. This difference in depth implies that the thick pumice deposit in Hole C0014G acted as a barrier for the remixing of the phase-separated and phase-segregated hydrothermal fluids and kept them spatially stratified, with gas-rich fluid at shallower depths and brine-rich fluid at deeper depths, probably because of the difference in their relative density or buoyancy.

Microbiology

Maximum cell number was found just below the seafloor in Hole C0014B (1.8×10^7 cells/cm^3 at 0.33 mbsf), Hole C0014G (3.3×10^7 cells/cm^3 at 0.27 mbsf), and Hole C0014D (5.6×10^8 cells/cm^3 at 0.23 mbsf). Cell numbers generally decrease with increasing depth, although there are some secondary peaks in biomass within shallower intervals at Holes C0014B and C0014D. Numerically abundant microbial communities at these shallow depths are not regarded as deep hot biosphere.

Microbial biomass at Site C00014 is generally similar to that found at Sites C0015 and C0017 and in a gravity core taken previously at Site C0014 (K.T. Ishibashi et al. unpubl. data). By contrast, the maximum microbial cell count in Hole C0014D is the highest we saw during Expedition 331. This densest microbial population was obtained from the CH_4-enriched, sulfate-depleted sediments from just beneath the sulfate-enriched pumice layer. A second peak in microbial population in Hole C0014D was found in the sulfate-depleted sediments at 8.7 mbsf. All the microbial population peaks occur within CH_4-enriched and sulfate-depleted layers, strongly suggesting the occurrence of functionally active AMO communities within the shallow subsurface.

Microbial populations in the deeper and hotter zones were numerically less abundant ($<1 \times 10^5$ cells/cm^3). Overall, however, the microbial population at Site C0014 is more abundant than that at Site C0013.

Cultivation tests for *Thermococcales* (e.g., *Thermococcus* spp.), *Aquificales* (e.g., *Persephonella* spp.), and thermophilic *Epsilonproteobacteria* (e.g., *Nitratiruptor* spp.) produced no positive enrichment. Enrichment experiments for marine iron-oxidizing bacteria (FeOB) such as the Zetaproteobacteria (e.g., *Mariprofundus ferrooxydans*), however, showed limited growth within the top two meters of sediment, to be further investigated during shore-based research.

Fluorescent microspheres were used to check for contamination in cores taken with HPCS, and perfluromethylcyclohexane (PFC) was used as a tracer for contamination in all cores. Fluorescent microspheres were not detected in most of the core samples from Site C0014, even within exterior parts of the core. High numbers of microspheres were detected in pumice layers, however, such as those sampled at 6.67 mbsf in Hole C0014D and 7.84 mbsf in Hole C0014G. Contamination by drilling fluids and/or by core disturbance would be difficult to avoid for such layers because of their high porosity and heavy fragmentation. Because such samples are important for hydrogeological controls on formation of subseafloor microbial communities, such samples need to be studied carefully in future research.

Site C0015

We spent only 10 h at Site C0015. Three holes were drilled there, to a maximum depth of only 9.4 mbsf, but only two recovered core (Fig. **F12**).

Despite our shallow penetration, we recovered several different lithologies: volcanic breccias, siliciclastic sands, and hemipelagic mud. The dominant depositional process is pelagic sedimentation, although resedimented volcanic lithoclasts make up the bulk of the sediment encountered. Siliciclastic sedimentation is evidenced in the deposition of texturally mature sands, and most intervals in the core are very porous. Despite the short interval cored, these holes provide good geological control and useful insight into the prealteration sedimentary architecture at the other Expedition 331 sites.

Interstitial water at Site C0015 is generally indistinguishable from seawater. Methane and hydrogen are very low and provide no evidence for hydrothermal input. Nevertheless, the microbial population is more abundant (4.8×10^6 to 1.2×10^7 cells/cm^3 sediment) than at those sites that exhibit hydrothermal flow in the subseafloor. Over the entire depth interval cored (0–9.4 mbsf), the mud, sand, and pumiceous gravel show weak oxidation, expressed as a yellow to brown discoloration of the mud and orange to brown iron oxide staining on some pumice fragments. Scanning electron microscope (SEM) imaging of orange-brown botryoidal aggregates of Fe-Si oxyhydroxides shows a filamentous structure typical of oxyhydroxides. Subseafloor microbial communities at Site C0015 may thus be sustained by both organotrophic and Fe-oxidizing chemolithotrophic production. Indeed, enrichment experiments conducted at Site C0015, selecting for the growth of marine FeOB, were successful for most samples to ~9 mbsf, exhibiting cellular morphologies common for known iron-oxidizers. FeOB communities are commonly found at low-temperature hydrothermal systems (Rassa et al., 2009), and further exploration of samples collected at Site C0015 will enhance our understanding of FeOB biodiversity.

Site C0016

Operational observation, lithostratigraphy, minerals, and physical properties

Two holes were drilled at Site C0016, but core was recovered from only one of them (Fig. **F13**). Hole C0016A was drilled with the BHI system, without a guide base, directly into the top of the 30 m high NBC hydrothermal mound that was thickly covered with Galatheid crabs and was discharging fluid at temperatures as high as 311°C. We spudded the hole at the crest of the mound within 1–2 m of a vent that was steadily and continuously phase separating, which made it look as if it was flaming. The drill bit penetrated easily and quickly. We noticed almost immediately that the drill string was canted over by 10°, which made the pipe move erratically as it rotated and caused it to enlarge the top of the hole. That the drill pipe was listing 10° from vertical was later attributed by the drillers to the effect of the Kuroshio Current. The designated 18 m of pipe was lowered within minutes at the rig floor, although we determined later that only about 15 m actually went into the hole; the rest just added to the bowing of the drill string in the water column. When 18 m of pipe had been lowered (with ~15 m of penetration), the drillers attempted to pull out of the hole. Two small steam explosions were seen around the pipe in the ROV video image, the flow of hot water and "black smoke" increased dramatically, and then the end of the pipe snapped off. The steam explosions were probably triggered by pressure transients induced by movement of the pipe in the hole in a fluid that was already on the verge of boiling. The drillers recorded a series of brief, sharp incidents of overpull that ended abruptly with the breaking of the pipe. A short section fell onto the mound and was recovered by the ROV, but the bit and most of the BHA stayed in the hole. The most likely explanation is that the pipe became bent in the hole, largely because of the stress put on it by rotating 10° from the vertical, and then broke when it was pulled out vertically. For the pipe to bend, though, it must have encountered some hard material within the mound, most of which appeared to be very soft. According to the drillers, it is not likely that either the high temperature or the small steam explosions contributed to the break. The major cause appears to have been the Kuroshio Current, which caused the drill string to buckle and so deviate 10° from vertical. The increase in black smoker flow that we observed with the ROV was almost certainly the result of our enlarging the orifice at depth, possibly by poking a larger hole into a cap rock that was inhibiting flow. This black smoker flow was still vigorous 1 day later but had diminished somewhat and become a clear, dark fluid 5 days after drilling. When first seen >10 y ago, the NBC vent site was emitting a clear, dark, single-phase fluid, which subsequently evolved to a boiling fluid. The fluid emitted since our drilling appears to resemble this earlier fluid, and so may have a different composition from that described by Kawagucci et al. (2010).

Hole C0016B was again drilled with the BHI system, this time with a guide base. It was positioned at the foot of the NBC mound, immediately adjacent to it and 20 m west of the NBC vent and Hole C0016A. It penetrated to 45 mbsf in three runs of 9, 18, and 18 m. Each run recovered several large pieces of core, but the total recovery was only 2.095 m (Fig. **F13**), nearly all of it hard rock. The only evidence for the actual depths of the recovered rock thus comes from the drilling records. Hole C0016B was not cased, but it was fitted with a corrosion cap with 3 m of 5.5 inch pipe hanging beneath and extending 0.4 m into the seafloor. ROV video images showed vigorous black smoker discharge from the corrosion cap outlet immediately after its deployment. This hydrothermal emission began only after the third coring run, which penetrated 27–45 mbsf, and was probably derived from a depth below 38 (Fig. **F13**).

The overall core recovery was only 4.7% from Hole C0016B, consisting mostly of hard rock. The first core comprises two pieces of massive sulfide totaling 61 cm, separated by 3 cm and underlain by another 15 cm of highly silicified and mineralized volcanic material altered to illite/muscovite clay, which probably came from 6–9 mbsf. The massive sulfide ore contains 40%–60% sphalerite (ZnS), 10%–40% pyrite (FeS_2), and a few percent each of galena (PbS) and chalcopyrite ($CuFeS_2$). It closely resembles the classic “black ore” of the Kuroko deposits of Miocene age in Japan and represents the first time such ore has been recovered from an active hydrothermal system on the seafloor. The second core consists of three pieces of three different lithologies, totaling 31 cm, from 9–27 mbsf: two pieces of altered, silicified, mineralized volcanic rock, above and below a 12 cm piece of very coarsely crystalline, snow white anhydrite with a sphalerite-pyrite vein running along one side. The third coring attempt recovered 99.5 cm of quartz-chlorite altered volcanic rock with abundant stockwork veining filled with quartz, chlorite, pyrite, and late anhydrite. The rocks from all three cores show a consistent paragenesis, from early formed sphalerite, to pyrite and galena, to chalcopyrite with increasing temperature, followed by a second generation of sphalerite with decreasing temperature. The last mineral to precipitate was anhydrite, as seawater penetrated into the waning system. Pyrite increases relative to sphalerite, and illite/muscovite alteration gives way to chlorite-quartz, with increasing depth and temperature. The textures and relationships seen in thin section for the massive sulfide require that a significant proportion of the sulfide mineralization occurred via subseafloor precipitation, with at least some sphalerite precipitating into void space in the rock.

Microbiology

Cultivation tests for *Thermococcales* (e.g., *Thermococcus* spp.), *Aquificales* (e.g., *Persephonella* spp.), and thermophilic *Epsilonproteobacteria* (e.g., *Nitratiruptor* spp.) produced no positive enrichment from any of the samples from Hole C0016B. We did detect PFC contamination on the surfaces of several core samples from Hole C0016B, from PFC added to the drilling fluid.

Site C0017

Operational observation, lithostratigraphy, minerals, and physical properties

Hole C0017D was the deepest (150.7 mbsf) of four holes drilled at Site C0017 and is located ~1500 m east of the high-temperature vents. Based on its low heat flow it was inferred to be a location of probable recharge of the hydrothermal system. We observed no evidence for discharge of water from any of these holes, but the concave-upward temperature profile we measured (Fig. **F14**) can be fit with an exponential function ($r^2 = 0.92$) and is consistent with overall downwelling, with kinks that suggest lateral flow that may be influenced by the variable lithologies we cored.

We measured temperature successfully at seven depths in Holes C0017B, C0017C, and C0017D over the interval from 18.3 to 150.7 mbsf; combined with the ocean bottom water temperature at the site of 4.87° ± 0.54°C (1σ, $n = 8$), our profile is defined by eight points. Six of the downhole measurements were made using the APCT-3 temperature shoe on the HPCS. The seventh and deepest measurement, at 150.7 mbsf, was made in triplicate using three identical thermoseal temperature-sensitive strips with chemically impregnated beads for 75°, 80°, 85°, 90°, and 95°C taped to the bottom outer surface of the plastic core liner. These beads were darkened and thus exposed at maximum temperatures of 85°, 90°, and 90°C on the three strips, which we are reporting as 90° ± 5°C. The APCT-3 shoe recorded a temperature in excess of its maximum range of 55°C for this core (but did not burn up; we recovered the entire temperature record). Down to ~50 mbsf, where we encountered a hard layer that is probably pumice (no core was recovered), temperature remains low, <15°C (Fig. **F14**). Beneath this hard layer it jumps up to 25°–39°C at 69–85 mbsf and then appears to level off for a short interval, reaching only 44°C at 112 mbsf before increasing nearly exponentially to 90°C at the bottom of Hole C0017D at 150.7 mbsf.

Coring at Site C0017 recovered three different lithologies: homogeneous hemipelagic mud (Unit I), pumiceous sediment (Unit II), and volcaniclastic-pumiceous breccia and mixed sand with erosional bases (Unit III). None of these showed obvious evidence of hydrothermal alteration, except weak alteration to clay within the deepest part of Hole C0017D. These three units together resemble the lithologies within Unit I defined at Sites C0014 and C0015. At Site C0017, the volcanic lithologies are relatively more abundant from 18 to 70 mbsf, such that the total section drilled could be interpreted as two layers of hemipelagic mud separated from each other by a pumiceous horizon. The thicknesses of mud and pumice beds at Site C0017 are significantly greater than at Site C0014, consistent with its location downslope.

Different lithologies of widely varying porosity and permeability alternate within the upper 37 mbsf at Site C0017. In particular, the pumiceous gravel/clast layers (Unit II) and the volcaniclastic-pumiceous sand layers (Unit III) are likely to be much more permeable than the clay layers (Unit I). As such, they represent potential pathways for recharge of cold bottom seawater into the hydrothermal system. One such example is the prominent oxidized interval from 26 to 35 mbsf in Hole C0017C. These observations and inferences are consistent with the low thermal gradient in the shallow subseafloor and the concave-upward temperature profile that increases exponentially with depth. Similar lithologies occur over the depth interval 60–100 mbsf, with probable high porosity and permeability and a lower thermal gradient, suggesting the possibility of lateral flow within this deeper interval. No core was recovered between 37 and 60 mbsf, but the drilling record indicates the presence of a hard layer at ~50 mbsf that could represent a hydrologic barrier between upper and lower permeable zones. At greater depth, the only hydrothermal alteration evident at Site C0017 was seen in the deepest core recovered, from 140–150 mbsf, where more permeable pumiceous grit horizons are altered to pale gray clay. These grits are intercalated with apparently unaltered indurated dark gray calcareous clay, again suggesting that the coarser sediment layers acted as flow paths. As noted above, the temperature at this depth is 90° ± 5°C.

As was observed within Unit I at Site C0014, abundant foraminifers and coccoliths dominated by *Emiliania huxleyi*, *Gephyrocapsa oceanica,* and *Reticulofenestra asanoi* were identified within the upper 28 mbsf. Biostratigraphy thus supports the equivalence of Units I through III at Site C0017 with Unit I at Sites C0014 and C0015.

Interstitial water chemistry

A total of 21 whole-round samples were processed at Site C0017 from four different holes. No samples were obtained between 30 and 63 mbsf. At 0–15 mbsf, the interstitial water profiles show the clear effect of microbial sulfate reduction utilizing organic matter in the sediment: sulfate decreases slightly and alkalinity, ammonium, and phosphate increase with depth from 0 to 15 mbsf. From 15 to 30 mbsf, however, all of these chemical species reverse direction and return to their concentrations in seawater, indicating a source of unaltered seawater at ~30 mbsf. This source can be identified as the oxidized layer at 26–35 mbsf discussed above, which is almost certainly a permeable layer through which seawater is flowing laterally, recharging the hydrothermal system 1500 m to the west. This layer presumably outcrops a short distance to the east.

Except for sulfate, the major ions are present over the depth interval 0–30 mbsf at their seawater concentrations. Below 64 mbsf, however, K decreases to a low of 6.5 mM at 106 mbsf before rebounding again to the seawater value in the deepest sample from 141 mbsf. Over this same interval, Ca increases more or less steadily to 18 mM and alkalinity to 6 mM. Changes in other ions are small or nonexistent. The causes of these changes are not obvious. The increase in Ca and alkalinity may result in part from dissolution of $CaCO_3$, but this compound should become less soluble with rising temperature, which increases from 25° to 90°C between 69 and 151 mbsf. K is typically taken up from seawater into clay minerals and zeolites at temperatures below ~150°C, but the abrupt rebound in K to the seawater value that occurs between 131 and 141 mbsf is hard to explain except by another permeable layer providing relatively unaltered seawater to that depth. It would have to do so without affecting the relatively more reactive species Ca and alkalinity.

Concentrations of methane and hydrogen are low and show no evidence for significant input from either hydrothermal processes or a prosperous anaerobic microbial community. Methane increases slightly near the bottom of the deepest Hole C0017D, probably from microbial methanogenesis at greater depth.

Microbiology

Maximum cell number was found within the mud of Unit I at 6.36 mbsf (3.2×10^7 cells/cm^3) and a relatively abundant microbial population (2.4×10^7 to 3.2×10^7 cells/cm^3) was identified above 10.8 mbsf. At greater depths, cell number generally decreased, although we found a substantial population at 20–40 mbsf, within the layer of lateral recharge identified by the oxidized sediment and the return of the interstitial water to seawater composition. This microbial community may thus be energized by iron oxidation under oxidative chemical conditions. Enrichment for FeOB in this zone of lateral recharge showed growth under microaerophilic conditions only. These are the deepest enrichment of putative FeOB to date, though caution is advised for the potentially contaminated samples.

Cultivation tests produced no positive enrichment for any samples from Site C0017, besides those enrichments conducted for the FeOB. Contamination tests found no fluorescent microspheres in most samples even within exterior parts of core at Site C0017. Contamination evaluated by PFC introduced into the drilling fluid was low even for unconsolidated pumice samples, and even at the surfaces of cores consisting mainly of pumice. We did find low levels of PFC in the interior of one core from Hole C0017D taken by ESCS, but even there the concentration of PFC was much lower than in the drilling mud.

Principal results

Lithostratigraphy

The oldest and deepest stratigraphic unit drilled during Expedition 331 is a volcaniclastic rock formed during explosive submarine volcanism (Fig. **F15**). These volcanic sedimentary rocks were subsequently altered by hydrothermal activity, with much of their original composition altered to or replaced by silica (quartz) and phyllosilicates, including kaolinite, illite/muscovite, and Mg chlorite.

On top of the silicified volcaniclastic rocks are scree deposits comprising large pumice clasts interbedded with hemipelagic mud (Fig. **F15**). These deposits are mostly unconsolidated, except where hydrothermal activity is prevalent at and near the surface, as at Sites C0013 and C0016. Debris flows process and rework surface sediment: Site C0017 is dominated by volcaniclastic sediment derived from a variety of pumice types, whereas Site C0015 contains clastic material derived from the continental shelf.

Hydrothermal activity silicified the deeper stratigraphic units and also formed new rock types at and near the surface (Fig. **F15**). Thick anhydrite-bearing horizons are present, both as veins and as nodules, and some of these can be correlated between different holes at the same site, especially at Site C0013, where the anhydrite appears to be laterally extensive. Anhydrite horizons occur at the surface at Site C0016 and at successively greater depths downslope at Sites C0013 and C0014.

Massive sulfide- and sulfide-rich sediment was found at Sites C0013 and C0016. At the moment it is unclear whether the sulfide in the shallow subseafloor at Site C0013 was resedimented from farther upslope or whether it represents an early stage of formation of a massive sulfide deposit, with the potential for thicker and higher temperature deposits to be located upslope (Fig. **F15**).

Hydrothermally altered clay is present at the surface at Site C0013 and in the shallow subsurface at Site C0014 (Fig. **F15**). At Site C0014, a progression can be seen from pumice breccias to hydrothermally altered pumice horizons, in which pumice has been changed to mud (that retains the shape and texture of pumice), and finally from mud-retaining pumice textures to a hydrothermal clay where pumice textures have been replaced by mottling in a gray clay background. Where pumice has been hydrothermally altered to mud at Site C0013, the altered clasts remain unconsolidated to 12 mbsf and would be expected to retain hydraulic conductivity. Given that the top surface of the underlying silicified volcaniclastic sediment is at the same depth at both Sites C0013 and C0014, it is reasonable to interpret the 24 m of hydrothermal clay encountered at Site C0013 as a correlative equivalent of the surface pumice breccias at Site C0014.

Biostratigraphy

The active hydrothermal discharging Site C0016 and the two nearby Sites C0013 and C0015 did not have microfossils in any of the core catcher samples, even those from 0 to 10 mbsf. At Site C0014, which has a moderate temperature gradient of 3°C/m within the uppermost 50 mbsf, microfossils were found only within the uppermost 6.5 mbsf. Recharge Site C0017 yielded microfossils from two depths: 9–28 mbsf, at <6°C, and 112 mbsf, at a measured temperature of 44°C. In all cases, the major microfossils are modern assemblages of planktonic foraminifers. Hydrothermal activity is deleterious both to growth of benthic foraminifers and to the preservation of microfossils, especially coccoliths, which were found rarely and were invariably badly corroded. Some foraminifer tests in near-surface samples from Sites C0014 and C0017 were exquisitely replaced by micrometer- and submicrometer-scale pyrite, so accurately that species could be determined.

Iron oxides in near-surface samples from Site C0015 possess micrometer-diameter, filamentous organic coatings and need to be assessed using high-resolution imaging for the presence of bacterial microfossils.

Petrology

Interpretation of hydrothermal alteration and mineralization for Sites C0013 to C0017 provides a broad overview of the nature, evolution, and architecture of the Iheya North hydrothermal system.

Site C0016 at NBC mound, despite very poor recovery overall, intersected massive sphalerite-rich sulfides, thus recovering for the first time, from an active hydrothermal system on the seafloor, material that strongly resembles the black ore of the Kuroko deposits of Miocene age in Japan. The textures and relationships seen in thin section for the massive sulfide require that a significant proportion of the sulfide mineralization occurred via subseafloor precipitation, with at least some sphalerite precipitating into void space in the rock. Additionally, the sulfide and sulfate paragenesis of the samples shows an evolving system, with early sphalerite mineralization overprinted by pyrite and then chalcopyrite, as temperature increased, before a second sphalerite mineralizing event, as temperatures cooled, and a final seawater influx, indicated by late coarsely crystalline anhydrite.

The underlying altered volcanic rocks recovered at Site C0016 show a similar evolution to the massive sulfide. The silicified volcanic rock from Core 331-C0016-1L shows a similar sulfide paragenesis and, in all cases, anhydrite was among the last phases precipitated during alteration.

With increasing depth at Site C0016, the relative abundance of pyrite increases with respect to sphalerite, both on a local scale within the massive sulfide recovered in Core 331-C0016-1L, and overall within the sequence. This variation is one that is observed in many volcanic-hosted massive sulfide (VHMS) deposits and is interpreted to be a function of increasing temperature with depth. The predominance in Hole C0016B of quartz-muscovite/illite alteration grading to quartz-chlorite alteration at depth is also consistent with the proximal quartz-sericite grading to chloritic alteration commonly recorded in the immediate footwall of ancient VHMS systems, including the Miocene Kuroko deposits of Japan.

At Site C0013, a likely location of recent high-temperature discharge, sediments exhibit kaolinite-muscovite alteration and contain variable thicknesses of sulfide-rich material to ~5 mbsf. Native sulfur is also abundant near-surface, which, together with the abundance of kaolinite, suggests acidic fluids, probably caused by oxidation of H_2S dissolved in the hydrothermal fluid or released from the fluid during decompression. Below ~5 mbsf, Mg chlorite and anhydrite are the dominant alteration phases to ~26 mbsf, at which depth a hard sequence of volcanic breccias altered to quartz and Mg chlorite with scattered quartz veins was intersected that we interpret to represent volcanic basement. This lithology extends to the bottom of the deepest Hole C0013E at the site, at 54.5 mbsf.

The transition from kaolinite-muscovite-rich to chlorite-rich rocks with increasing depth at Site C0013 is similar to the gradation from paragonitized to chloritized rocks that was documented for the basement underneath the Trans-Atlantic Geotraverse (TAG) hydrothermal mound at the Mid-Atlantic Ridge (26°N) (Humphris et al., 1995). The difference in mineralogy between Iheya North Knoll and the Mid-Atlantic Ridge can be attributed to the lack of iron and the abundance of potassium available within the volcanic rocks in the Okinawa Trough when compared with the mafic volcanic rocks of the mid-ocean-ridge setting.

The latest alteration phase seen at Site C0013 is coarse-grained anhydrite in veins, which overprint both the kaolinite-muscovite and the Mg chlorite alteration between ~4 and ~10 mbsf. This anhydrite likely precipitated when downwelling seawater mixed with upwelling hydrothermal fluid as the system waned, at a temperature of ~150°C.

Geochemistry

Site C0016, drilled directly into and immediately adjacent to a major high-temperature hydrothermal mound of the Iheya North hydrothermal field called Big North Chimney, yielded no samples suitable for interstitial water or headspace gas analyses. Such samples were recovered from Sites C0013 and C0014, drilled ~160 and 450 m east of the high-temperature vents, and they clearly show the chemical signatures of high-temperature fluid-rock interaction.

Depth profiles for CH_4 and H_2 show different patterns at Site C0013: methane is most enriched at 10–12 mbsf, whereas H_2 increases with depth. The peak in methane combined with a consistently high ratio of methane to ethane suggests an input of hydrothermal fluid that already contains unusually high concentrations of microbially produced methane. The CH_4 maximum occurs just beneath a cap rock layer and is consistent with the observed flow of hydrothermal fluid up the hole. The logarithmic increase in hydrogen with depth suggests a deep source for this gas, as well as a relatively shallow sink at ~10 mbsf that is probably microbial consumption. Total sulfur content of the sediment varies from 1% to 66% and is highest within the uppermost

10 mbsf. Total organic carbon and total nitrogen are generally both quite low, <0.2 and <0.02 wt%, respectively, although there are a few high values that cannot be attributed to melting of the plastic core liners. These melted as shallow as 12.5 mbsf, indicating that the temperature at this depth exceeded 82°C. It appears that hydrothermal alteration and possibly also microbial activity remineralize sedimentary organic carbon relatively quickly.

Site C0013 interstitial water is heavily affected by dissolution of the abundant anhydrite in the core prior to separation of the pore water, which greatly increased Ca and sulfate in solution; the increased Ca, in turn, displaced K and Mg from ion exchange sites in clay minerals in the sediment. Chloride varies from 34% lower than in seawater to 12% higher in the deepest pore water sample from the site, from Hole C0013E at 17 mbsf, almost certainly due to phase separation. This deepest sample is a relatively pure hydrothermal fluid, containing only 7 mM Mg and 13 mM sulfate; a Na/Cl ratio 10% lower than that in seawater, indicating uptake of Na into alteration minerals; and elevated Cl (623 mM), Ca (48 mM), K (81 mM, among the highest values ever measured in a seafloor hydrothermal system), alkalinity (22 mM), and ammonium (1.5 mM).

At Site C0014, concentrations of dissolved sulfate and methane are inversely correlated, especially above 30 mbsf. The variable (Hole C0014B) and low (Hole C0014G) methane concentrations in the upper section indicate zone(s) of anaerobic methanotrophy at the sulfate–methane transitions. The observed high methane to ethane ratios further suggest that methane at this site is mainly biogenic. Temperatures within the deeper horizons, which exceed 55°C at only 16 mbsf and 210°C at 50 mbsf, would seem to preclude in situ methanogenesis, however. Biogenic methane could be migrating downward from the biologically active zone or the observed methane at depth (Hole C0013G) could be a component of the subseafloor hydrothermal fluids.

Interstitial water at Site C0014 shows large variations in composition both laterally and vertically as a result of hydrothermal input. At 0–10 mbsf, it closely resembles seawater, except for sulfate, alkalinity, and the nutrient species, which are affected by microbial sulfate reduction and show large lateral variation from hole to hole. Below 10 mbsf, it deviates sharply from seawater, with generally deceasing sulfate, Mg, and Na/Cl and increasing Ca, K, and Si. Chloride varies from that of seawater in the uppermost 27 mbsf, to a vapor at 27–40 mbsf and to a brine at 40–114 mbsf. These large lateral and vertical variations display the role of permeable and less permeable layers in the sediment in permitting and isolating lateral flow of hydrothermally influenced waters.

Interstitial water at Site C0015 is generally indistinguishable from seawater. Methane and hydrogen are very low and provide no evidence for hydrothermal input.

At hydrothermal recharge Site C0017, 1500 m east of the hydrothermal vents, concentrations of methane and hydrogen are low and show no evidence for significant input from either hydrothermal processes or a prosperous anaerobic microbial community. Methane increases slightly near the bottom of the deepest Hole C0017D, probably from microbial methanogenesis at greater depth. As at Sites C0013 and C0014, interstitial water at Site C0017 shows the effects of lateral flow of water through permeable layers, but at this site it is unaltered seawater, through a layer of oxidized pumice at 26–35 mbsf and possibly through deeper layers as well. As at Site C0014, pore water within the upper sediments, at 0–15 mbsf, is affected by microbial reduction of sulfate utilizing organic matter. At greater depth, concentrations generally return to those in seawater because of lateral recharge, probably from an outcrop of pumice to the east. Chloride increases slightly with greater depth along with Ca and alkalinity, and K decreases and then increases again to nearly the concentration in seawater in the deepest sample collected, from 141 mbsf.

Microbiology

We did not obtain immediate evidence of an active deep hot biosphere during Expedition 331. We did, however, firmly establish the hydrogeologic basis for a variety of subseafloor microbial habitats associated with a high-temperature hydrothermal system. We also conducted the first tests for contamination by drilling fluid using PFC as a tracer with a variety of drilling and coring systems. We found that, in general, these systems on the *Chikyu* can collect core that is free of contamination and therefore useful for microbial studies.

Two of the five sites we drilled, Sites C0015 and C0017, hosted relatively abundant subseafloor microbial populations, but these depended on buried sedimentary organic matter. Even at these two sites, the microbial cell abundance was much lower than those found previously at ODP/IODP sites along continental margins (Parkes et al., 1994, 2000; D'Hondt et al., 2004), even though the Iheya North hydrothermal field is located in a backarc basin along a continental margin. These low cell abundances presumably result from the relatively low total organic carbon in the sediments, generally <0.1 wt% at Site C0015 and <0.5 wt% at Site C0017. The abundant occurrence of very permeable layers of pumice and volcaniclastic sediments and the consequent recharge of seawater into the hydrothermal system at Site C0017 generates a locally highly oxic subseafloor environment. This environment promotes the formation of oxidative oligotrophic and/or chemolithoautotrophic microbial communities, rather than obligately anaerobic communities including fermentors, methanogens and sulfate reducers. Methane concentrations at Sites C0015 and C0017 are generally low, and microbial methanogenesis is quite unlikely. Instead, we found layers enriched in iron oxide minerals at these two sites, which were probably produced by aerobic to anaerobic subseafloor microbial communities sustained by oligotrophic and chemolithoautotrophic iron oxidizers such as *Zetaproteobacteria* (Emerson et al., 2007). Successful enrichments of putative FeOB from these two sites will allow us to investigate the relationships of these subseafloor microbial communities.

The other three sites we drilled, Site C0016 at an active hydrothermal mound and Sites C0013 and C0014 about 160 and 450 m to the east, respectively, all had very high temperatures at very shallow depths, limiting microbes to the uppermost part of the sediment column, if they were present at all.

Physical properties and downhole temperature measurements

Physical property measurements including density and porosity, thermal conductivity, formation factor, *P*-wave velocity, and in situ temperature were made at the five sites drilled during Expedition 331. Sediment was cored at Sites C0013, C0014, C0015, and C0017. The major sediment types are pelagic and hemipelagic mud and volcaniclastic deposits, variably altered by hydrothermal processes including silicification and precipitation of anhydrite. At Site C0016 only rocks were recovered, including massive sulfide and sulfate and altered and mineralized volcanic breccias and flows.

At Site C0013, there is evidence from the density, thermal conductivity, and *P*-wave velocities within the uppermost 12 mbsf for hard layers rich in anhydrite, interbedded with softer layers. A hard volcanic layer was penetrated at ~22 mbsf. At Site C0014, physical property results are similar in the surface portions of all seven holes, and density and porosity are consistent with pumicious sediments. At greater depths at Site C0014, sediments become more consolidated, as evidenced by higher thermal conductivity, lower porosity, and higher formation factor, but no clear evidence for a hard basement layer is seen until possibly in our deepest sample, from 128 mbsf. Temperature was measured four times in four different holes at Site C0014 using the APCT-3 shoe, yielding values between 16° and 22°C at 4–9 mbsf and >55°C at 16 mbsf. Thermoseal strips were used three times in Hole C0014G and measured 145°C at 47 mbsf and >210°C at 50 mbsf. At Site C0015, physical properties are relatively constant to the maximum depth of 9 mbsf. There is a slight increase in bulk density and a corresponding decrease in thermal conductivity toward the bottom of the hole that coincide with the presence of sandy layers. At Site C0017, the uppermost 20 mbsf exhibit fairly uniform physical properties consistent with the presence of clay; between 20 and 40 mbsf the density and porosity reflect a higher percentage of pumice in the sediments. The in situ temperature at 37 mbsf is 6°C. Between 70 and 90 mbsf at Site C0017, thermal conductivity and bulk density increase along with pumice content, whereas temperature increases from 25° to 40°C. In the lower portion of Hole C0017D, at 95–148 mbsf, thermal conductivity, bulk density, and formation factor all increase with depth, consistent with the presence of increasingly indurated clays. At the maximum depth drilled of 151 mbsf, the temperature is 90° ± 5°C, as measured in triplicate with thermoseal strips.

Preliminary scientific assessment

Artificial hydrothermal vents created

A total of 24 holes were drilled during IODP Expedition 331, of which 21 recovered core. We recovered 300 m of core from 605 m of penetration, yielding an overall recovery of 49.6%. Holes C0013E and C0014G were cased with stainless steel pipe and capped with stainless steel corrosion caps with open outlets, and Hole C0016B was capped but not cased, above a 3 m insertion pipe. Four artificial hydrothermal vents were created by our drilling operations, in Holes C0013E, C0014G, C0016A, and C0016B, in which hydrothermal fluid formerly trapped in the subseafloor ascended up the hole and exited into the oceans (Fig. **F9**). All the hydrothermal vent emissions were confirmed to be >240°C using thermoseal strips taped to the outlet pipe of the corrosion cap. These newly created hydrothermal vents will serve as windows into the subseafloor and any associated microbial communities entrained into them in post-drilling, long-term monitoring studies of fluid composition and flow and of in situ microbial colonization.

Subseafloor hydrothermal alteration, fluid flow, and reservoirs within the defined hydrogeologic structure

Drilling and coring operations during Expedition 331 provide insight into the hydrothermal flow regime at Iheya North Knoll. Although the thermal gradient was known to be high at Site C0016 at NBC mound, Sites C0013 and C0014 have steeper thermal gradients than we expected.

Site C0013 is located 160 m east of the vigorous high-temperature vents and mounds. On our third coring attempt in Hole C0013C, we melted the core liner, indicating a temperature >82°C at 12.5 mbsf. Immediately on penetrating a cap rock just a few meters below the seafloor, hot hydrothermal fluid began to flow from Hole C0013E. Drilling to our maximum depth of 54.5 mbsf, we penetrated several hard, low-porosity layers that could function as a cap rock and found thick, porous sediment hydrothermally altered at high temperature in between the harder, less permeable layers. The interstitial water showed large changes in composition both laterally and vertically over short distances, suggesting lateral flow in permeable horizons that were separated by impermeable barriers to flow. The lithostratigraphy, the physical properties of the sediment and rock, and the interstitial water chemistry thus all provide insight into the hydrothermal flow regime at Site C0013. This is, in turn, broadly consistent with the seismic reflection structure, which shows strong reflectors connecting several of the sites (Fig. F5).

Site C0014 is located ~450 east of the high-temperature vents and mounds. Even here we found that the temperature exceeds 210°C at only 50 mbsf. The temperature gradient is roughly linear from 0 to 47 mbsf, increasing from the bottom water temperature of 4.5° to 145°C over that depth range, but it deviates greatly from this line at 0–9 and 47–50 mbsf, where it is clearly affected by lateral flow. The interstitial water chemistry demonstrates a vertical stratification, from water of seawater chloride at 0–25 mbsf, to a vapor at 29–38 mbsf, to a brine from 48 mbsf to the deepest sample at 114 mbsf. Within the upper sediments, which consist of pelagic sediments and pumice deposits, there is considerable lateral variability in the intensity of microbial sulfate reduction utilizing organic matter among the four holes that are only meters apart,; clearly there is a functionally prosperous and metabolically diverse subseafloor biosphere here. At 20 mbsf, where the temperature is ~70°C, and to the bottom of the deepest Hole C0014G at 137 mbsf, where a linear fit would have the temperature exceed 400°C, the sediment and rock we recovered is intensely hydrothermally altered. As at Site C0013, lateral flow must be occurring through permeable formations, and is likely separated vertically by impermeable beds that behave as cap rocks. Again as at Site C0013, the seismic reflection structure at Site C0014 is generally compatible with the lithostratigraphic and hydrogeologic architecture we infer from drilling.

Site C0016 is located on and immediately adjacent to the NBC high-temperature hydrothermal mound and vents. We did not recover sufficiently continuous core at this site to determine a detailed lithostratigraphy. We did recover both massive and stockwork sulfide ores, as discussed below.

Site C0017 is located 1540 m east of the high-temperature vents of the Iheya North hydrothermal field, in an area of low heat flow. The overall temperature profile is exponential and concave upward, consistent with downwelling of cold water, implying that this is an area of recharge to the hydrothermal system. We reached a maximum temperature of 90° ± 5°C at the bottom of the deepest Hole C0017D at 151 mbsf. Deviations from a smooth temperature profile again indicate the presence of lateral flow within specific horizons, presumably separated by less permeable layers, consistent with the interstitial water chemistry and with the presence of a highly oxidized layer at 26–35 mbsf that supports a microbial community. We found only a small amount of hydrothermally altered sediment deep in Hole C0017D.

These results taken together provide a more detailed look at the large-scale hydrogeology of the Iheya North Knoll hydrothermal system than has typically been available for subseafloor hydrothermal systems elsewhere, sketched in Figure **F16.** The hydrological regime at Iheya North Knoll is characterized by large-scale hydrothermal alteration, deposition, and fluid migration within permeable rocks and sediments hosted by the Iheya North Knoll volcanic complex. Prior to Expedition 331, we knew little about the spatial and temporal scales and patterns of hydrothermal circulation in subseafloor hydrothermal systems, particularly those in subduction zone settings such as volcanic arcs and backarc spreading centers. Postcruise studies of data and samples recovered during Expedition 331, along with future field investigations that will benefit from the cased and capped holes we left, will further our understanding of these important hydrothermal systems.

Stratification of hydrothermal fluid by subseafloor phase separation and segregation

Interstitial water collected during Expedition 331 demonstrates that the Iheya North Knoll hydrothermal field is chemically stratified with respect to chloride concentration. Pore water near the seafloor has the chloride concentration of seawater. It is underlain by a thin and possibly discontinuous vapor-rich layer that has lower chloride than seawater, at 5 mbsf and deeper at Site C0013 and at 29–38 mbsf at Site C0014. Beneath the vapor-rich layer lies a chloride-enriched brine, at least to the maximum depth of the holes drilled at these two sites. The recharge Site C0017 has no statistically convincing indication of a low-chloride layer. Previous surveys of the Iheya North hydrothermal vents have always found that the discharging fluids are low in chloride and vapor rich (Kawagucci et al., 2010; Takai and Nakamura, 2010). It has been a puzzle where the complementary brines may reside. Several theoretical calculations have predicted that the greater densities of brines cause them to sink to greater depths within subseafloor hydrothermal reservoirs (Fontaine and Wilcock, 2006). Until now there has been no observational evidence to support this hypothesis. Expedition 331 provides tentative evidence of subseafloor stratification of hydrothermal fluids that have phase separated, as two data points deviated significantly from seawater. More evidence is needed, and we will monitor this process in the future using the hydrothermal fluids discharging from the artificial vents.

Is a subvent biosphere present?

So far, the shipboard analyses and experiments have provided little evidence for the existence of a hot subvent biosphere beneath the Iheya North hydrothermal field, though cultivation from a colder, diffusely venting site and a site of lateral recharge provided evidence for a subvent iron-oxidizing microbial community. Prior to this expedition, it was hypothesized that subseafloor mixing between hydrothermal fluids and recharging seawater was sustained by a fine-scale network of narrow hydrothermal fluid flow paths within the subseafloor along the eastern flank of the hydrothermal system. The hydrothermal features we intersected by drilling, including the permeable reservoirs and flow paths, appear to be larger in scale that we had envisioned. Most of the sediment and rock we cored at Sites C0013, C0014, and C0016 has been exposed to much higher temperatures than the microbiologically habitable temperature range (<150°C). Smaller and more limited zones that are habitable by microbes were sampled, however, as well as one large one, the hydrothermal recharge zone at Site C0017. Recharge zones are, of course, an essential part of all hydrothermal systems. Future shore-based, more detailed investigations by a team of international and interdisciplinary scientists will provide multiple lines of evidence for the functionally active, metabolically diverse subseafloor microbial communities that live within the environs of the Iheya North hydrothermal system.

Actively forming Kuroko deposit in the subseafloor environment of the Iheya North field

Much of the sediment and rock we cored at Sites C0013, C0014, and C0016 was intensely hydrothermally altered, silicified, and mineralized. Exceptional among the disseminated and vein, stockwork-type sulfide we recovered is the massive sphalerite-rich ore we cored in Hole C0016B. This marks the first time this type of massive sulfide, which closely resembles the Kuroko black ore, has been recovered from an active deep-sea hydrothermal system. We recovered only 2.1 m of core from 45 m of penetration in this hole, which will make reconstruction of the lithostratigraphy impossible, but the recovered core is diverse and spectacular. Besides the sphalerite-rich black ore, it includes boulder-sized, coarsely crystalline anhydrite veined by pyrite, as well as veined and mineralized volcanic breccia. Detailed studies of these lithologies, in the context of all that is known about the active hydrothermal system at Iheya North Knoll, will provide direct evidence of how VHMS mineral deposits in general, and the Kuroko ores in particular, are formed.

Operations

To achieve the scientific objectives of Expedition 331, drilling was planned at three main sites (proposed Sites INH-1D, INH-4D, and INH5-D, which were drilled as Sites C0016, C0013, and C0014, respectively). No less than nine additional sites were approved and considered as potential contingency sites. Of these, Proposed Sites INH-1C, INH-6B, and INH-11A ended up being drilled as Sites C0016, C0017, and C0015, respectively.

The drilling plan was designed to penetrate the hydrothermal flow path, including the main discharge at NBC (Site C0016), a high heat flow area (Site C0013), and a low heat flow area (Site C0014). Drilling was prioritized according to potential scientific return, as well as operational feasibility. Main holes at Sites C0013 and C0014 were to be drilled through guide bases, cased with perforated casing, and supplied with corrosion caps. We carried a third guide base for a possible contingency, which ended up being used in Hole C0016B. A complete list of sites and holes can be found in the general coring summary in Table T2.

Unusual aspects of Expedition 331 include the high heat flow encountered especially at Sites C0013, C0014, C0015, and C0016. It also includes very short distances between sites, allowing the ship to reach even the most remote Site C0017 within hours of operating at the other sites. High levels of H_2S gas from the cores required most of the core recovery operation to be undertaken wearing breathing apparatus at the rig floor and core cutting areas. Finally, coring systems with two different diameters were used: IODP standard systems as carried by the *Chikyu* (HPCS, EPCS, and ESCS) with core diameters of 2.44 inches and a commercial system taking 4 inch diameter cores.

Initial drilling at Site C0013 quickly lead to the realization that the heat flow indeed was high there, causing the plastic liners to melt, which added issues to core extraction as well as concerns about contamination. Site C0013 was approved to a total depth of 100 m, but the main Hole, C0013E, achieved a total depth of only 54.5 mbsf. The *Chikyu* eventually returned there after completing Site C0017 to core with aluminum core liner and thereby retrieve cores not disturbed by thermal alteration of the liner material. Drilling at Site C0014 encountered the same issue, only slightly deeper.

Site C0014 is another discharge-recharge zone similar to Site C0013. The target depth was 200 mbsf, but drilling was terminated at 136.7 mbsf. The main objectives were reached by then, and the borehole assembly seals were starting to show damage from the heat, which was estimated to be at least 300°C based on the composition of precipitates in the recovered rocks. Hole C0014G was used for the casing operation there.

The heat issue led to drilling at the first contingency Site C0015 to use up about half a day until it was time for crew change and the opportunity to load aluminum core liners for the IODP standard coring systems. This site is a potential methane and fluid source west of the vent axis. (Most other sites are located east of the axis.)

Site C0016 targeted the active hydrothermal vent system and was drilled using the commercial 4 inch coring system. Hole C0016A targeted the crest of the NBC mound but recovered no core because the core barrel severed, leaving the drill bit and full-closure core catcher of the commercial drilling assembly in the hole. The ship left the third guide base at the location for Hole C0016B before relocating to Site C0017. Hole C0016B was drilled as the last hole of the expedition, using the third guide base at the base of NBC mound. A depth of 45 mbsf was reached, but only 2 m of core was recovered. The guide base was capped with a 3 m section of casing added to function as an entry guide for downhole tools.

Site C0017 is a site of suspected cool seawater recharge to the hydrothermal system. Holes C0017A and C0017B were abandoned because the bit pulled out while retrieving the core barrel. Hole C0017C was abandoned when the core barrel came back without shoe or core catcher. The total depth of 150.7 mbsf was reached at Hole C0017D after a successful unguided reentry. This site provided a good temperature record using a combination of APCT-3 and thermoseal strips, in addition to good core recovery. A happy surprise was very good recovery with the ESCS assembly of firm, sticky mud. When operations at Site C0017 were completed, the *Chikyu* returned to the guide base NBC mound to drill Hole C0016B.

References

Deming, J.W., and Baross, J.A., 1993. Deep-sea smokers: windows to a subsurface biosphere? *Geochim. Cosmochim. Acta,* 57(14):3219–3230. **doi:10.1016/0016-7037(93)90535-5**

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

Emerson, D., Rentz, J.A., Lilburn, T.G., Davis, R.E., Aldrich, H., Chan, C., and Moyer, C.L., 2007. A novel lineage of *Proteobacteria* involved in formation of marine Fe-oxidizing microbial mat communities. *PLoS ONE,* 2(8):e667. **doi:10.1371/journal.pone.0000667**

Fontaine, F.J., and Wilcock, W.S.D, 2006. Dynamics and storage of brine in mid-ocean ridge hydrothermal systems. *J. Geophys. Res., [Solid Earth],* 111(B6):B060102. **doi:10.1029/2005JB003866**

Gamo, T., Sakai, H., Kim, E.-S., Shitashima, K., and Ishibashi, J., 1991. High alkalinity due to sulfate reduction in the CLAM hydrothermal field, Okinawa Trough. *Earth Planet. Sci. Lett.,* 107(2):328–338. **doi:10.1016/0012-821X(91)90080-2**

Gamo, T., Ishibashi, J., Tsunogai, U., Okamura, K., and Chiba, H., 2006. Unique geochemistry of submarine hydrothermal fluids from arc-back-arc settings of the Western Pacific. *In* Christie, D.M., Fisher, C. R., Lee, S.-M., and Givens, S. (Eds.), *Back-arc Spreading Systems: Geological, Biological, Chemical, and Physical interactions*. Geophys. Monogr., 166:147–161.

Glasby, G.P., and Notsu, K., 2003. Submarine hydrothermal mineralization in the Okinawa Trough, SW of Japan: an overview. *Ore Geol. Rev.,* 23(3–4):299–339. **doi:10.1016/j.oregeorev.2003.07.001**

Halbach, P., Nakamura, K., Wahsner, M., Lange, J., Sakai, H., Käselitz, L., Hansen, R.-D., Yamano, M., Post, J., Prause, B., Seifert, R., Michaelis, W., Teichmann, F., Kinoshita, M., Märten, A., Ishibashi, J., Czerwinski, S., and Blum, N., 1989. Probable modern analogue of Kuroko-type massive sulfide deposits in the Okinawa Trough back-arc basin. *Nature (London, U. K.),* 338(6215):496–499. **doi:10.1038/338496a0**

Halbach, P., Pracejus, B., and Maerten, A., 1993. Geology and mineralogy of massive sulfide ores from the central Okinawa Trough, Japan. *Econ. Geol.,* 88(8):2210–2225. **doi:10.2113/gsecongeo.88.8.2210**

Huber, J.A., and Holden, J.F., 2008. Modeling the impact of diffuse vent microorganisms along mid-ocean ridges and flanks. *In* Lowell, R.P., Seewald, J.S., Metaxas, A., and Perfit, M.R. (Eds.), *Magma to Microbe: Modeling Hydrothermal Processes at Oceanic Spreading Centers*. Geophys. Monogr., 178:215–231.

Humphris, S.E., Herzig, P.M., Miller, D.J., Alt, J.C., Becker, K., Brown, D., Brügmann, G., Chiba, H., Fouquet, Y., Gemmell, J.B., Guerin, G., Hannington, M.D., Holm, N.G., Honnorez, J.J., Itturino, G.J., Knott, R., Ludwig, R., Nakamura, K., Petersen, S., Reysenbach, A.-L., Rona, P.A., Smith, S., Sturz, A.A., Tivey, M.K., and Zhao, X., 1995. The internal structure of an active sea-floor massive sulphide deposit. *Nature (London, U. K.),* 377(6551):713–716. **doi:10.1038/377713a0**

Inagaki, F., Kuypers, M.M.M., Tsunogai, U., Ishibashi, J.-I., Nakamura, K.-I., Treude, T., Ohkubo, S., Nakaseama, M., Gena, K., Chiba, H., Hirayama, H., Nunoura, T., Takai, K., Jørgensen, B.B., Horikoshi, K., and Boetius, A., 2006. Microbial community in a sediment-hosted CO2 lake of the southern Okinawa Trough hydrothermal system. *Proc. Natl. Acad. Sci. U.S.A.,* 103(38):14164–14169. **doi:10.1073/pnas.0606083103**

Kawagucci, S., Chiba, H., Ishibashi, J., Yamanaka, T., Toki, T., Muramatsu, Y., Ueno, Y., Makabe, A., Inoue, K., Yoshida, N., Nakagawa, S., Nunoura, T., Takai, K., Takahata, N., Sano, Y., Narita, T., Teranishi, G., Obata, H., and Gamo, T., in press. Hydrothermal fluid geochemistry at the Iheya north field in the mid-Okinawa Trough: implication for origin of methane in subseafloor fluid circulation systems. *Geochem. J.*

Konno, U., Tsunogai, U., Nakagawa, F., Nakaseama, M., Ishibashi, J., Nunoura, T., and Nakamura, K., 2006. Liquid CO_2 venting on the seafloor: Yonaguni Knoll IV hydrothermal system, Okinawa Trough. *Geophys. Res. Lett.,* 33(16):L16607. **doi:10.1029/2006GL026115**

Kumagai, H., Tsukioka, S., Yamamoto, H., Tsuji, T., Shitashima, K., Asada, M., Yamamoto, F., and Kinoshita, M., in press. Hydrothermal plumes imaged by high-resolution side-scan sonar on a cruising AUV, Urashima. *Geochem., Geophys. Geosys.*

Lee, C.-S., Shor, G.G., Jr., Bibee, L.D., Lu, R.S., and Hilde, T.W.C., 1980. Okinawa Trough: origin of a back-arc basin. *Mar. Geol.,* 35(1–3):219–241. **doi:10.1016/0025-3227(80)90032-8**

Letouzey, J., and Kimura, M., 1986. The Okinawa Trough: genesis of a backarc basin developing along a continental margin. *Tectonophysics,* 125(1–3):209–230. **doi:10.1016/0040-1951(86)90015-6**

Masaki, Y., Kinoshita, M., Inagaki, F., Nakagawa, S., and Takai, K., in press. Possible kilometer-scale hydrothermal circulation within the Ihyea-North field, mid-Okinawa Trough as inferred from heat flow data. *JAMSTEC Res. Devel.*, 12.

Nakagawa, S., Takai, K., Inagaki, F., Chiba, H., Ishibashi, J., Kataoka, S., Hirayama, H., Nunoura, T., Horikoshi, K., and Sako, Y., 2005. Variability in microbial community and venting chemistry in a sediment-hosted backarc hydrothermal system: impacts of subseafloor phase-separation. *FEMS Microbiol. Ecol.,* 54(1):141–155. **doi:10.1016/j.femsec.2005.03.007**

Narita, H., Harada, K., and Tsunogai, S., 1990. Lateral transport of sediment particles in the Okinawa Trough determined by natural radionuclides. *Geochem. J.,* 24:207–216.

Nunoura, T., Oida, H., Nakaseama, M., Kosaka, A., Ohkubo, S.B., Kikuchi, T., Kazama, H., Hosoi-Tanabe, S., Nakamura, K., Kinoshita, M., Hirayama, H., Inagaki, F., Tsunogai, U., Ishibashi, J., and Takai, K., 2010. Archaeal diversity and distribution along thermal and geochemical gradients in hydrothermal sediments at the Yonaguni Knoll IV hydrothermal field in the southern Okinawa Trough. *Appl. Environ. Microbiol.,* 76(4):1198–1211. **doi:10.1128/AEM.00924-09**

Nunoura, T., and Takai, K. 2009. Comparison of microbial communities associated with phase-separation-induced hydrothermal£uids at the Yonaguni Knoll IV hydrothermal field, the Southern Okinawa Trough. FEMS Microbiol. Ecol., 67:351–370.

Oiwane, H., Kumagai, Y., Nakamura, Y., and Tokuyama, H., 2008. Characteristics of sediment in Iheya North Knoll and “the acoustic blanking layer.” *JPGU Meeting,* Abstract J164-P013. **http://wwwsoc.nii.ac.jp/jepsjmo/cd-rom/2008cd-rom/program/pdf/J164/J164-P013_e.pdf**

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

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

Rassa, A.C., McAllister, S.M., Safran, S.A., and Moyer, C.L., 2009. *Zeta-Proteobacteria* dominate the colonization and formation of microbial mats in low-temperature hydrothermal vents at Loihi Seamount, Hawaii. *Geomicrobiol. J.,* 26(8):623–638. **doi:10.1080/01490450903263350**

Sakai, H., Gamo, T., Kim, E.-S., Shitashima, K., Yanagisawa, F., Tsutsumi, M., Ishibashi, J., Sano, Y., Wakita, H., Tanaka, T., Matsumoto, T., Naganuma, T., and Mitsuzawa, K., 1990a. Unique chemistry of the hydrothermal solution in the mid-Okinawa Trough backarc basin. *Geophys. Res. Lett.,* 17(12):2133–2136. **doi:10.1029/GL017i012p02133**

Sakai, H., Gamo, T., Kim, E.-S., Tsutsumi, M., Tanaka, T., Ishibashi, J., Wakita, H., Yamano, M., and Oomori, T., 1990b. Venting of carbon dioxide–rich fluid and hydrate formation in mid-Okinawa Trough backarc basin. *Science,* 248(4959):1093–1096. **doi:10.1126/science.248.4959.1093**

Schrum, H.N., Spivack, A.J., Kastner, M., and D'Hondt, S., 2009. Sulfate-reducing ammonium oxidation: a thermodynamically feasible metabolic pathway in subseafloor sediment. *Geology,* 37(10):939–942. **doi:10.1130/G30238A.1**

Suzuki, R., Ishibashi, J., Nakaseama, M., Konno, U., Tsunogai, U., Gena, K., and Chiba, H., 2008. Diverse range of mineralization induced by phase separation of hydrothermal fluid: case study of the Yonaguni Knoll IV hydrothermal field in the Okinawa Trough back-arc basin. *Res. Geol.,* 58(3):267–288. **doi:10.1111/j.1751-3928.2008.00061.x**

Takai, K., Gamo, T., Tsunogai, U., Nakayama, N., Hirayama, H., Nealson, K.H., and Horikoshi, K., 2004. Geochemical and microbiological evidence for a hydrogen-based, hyperthermophilic subsurface lithoautotrophic microbial ecosystem (HyperSLiME) beneath an active deep-sea hydrothermal field. *Extremophiles,* 8(4):269–282. **doi:10.1007/s00792-004-0386-3**

Takai, K., and Horikoshi, K., 1999. Genetic diversity of archaea in deep-sea hydrothermal vent environments. *Genetics,* 152:1285–1297.

Takai, K., Inagaki, F., Nakagawa, S., Hirayama, H., Nunoura, T., Sako, Y., Nealson, K.H., and Horikoshi, K., 2003. Isolation and phylogenetic diversity of members of previously uncultivated ε-Proteobacteria in deep-sea hydrothermal fields. *FEMS Microbiol. Lett.,* 218(1):167–174. **doi:10.1111/j.1574-6968.2003.tb11514.x**

Takai, K., Nakagawa, S., Reysenbach, A.L., and Hoek, J., 2006. Microbial ecology of mid-ocean ridges and backarc basins. *In* Christie, D.M., Fisher, C.R., Lee, S.M., and Givens, S. (Eds.), *Back-Arc Spreading Systems: Geological, Biological, Chemical and Physical Interactions.* Geophys. Monogr., 166:185–213.

Takai, K., and Nakamura, K., 2010. Compositional, physiological and metabolic variability in microbial communities associated with geochemically diverse, deep-sea hydrothermal vent fluids. *In* Barton, L., Mendl, M., and Loy, A. (Eds.), *Geomicrobiology: Molecular and Environmental Perspective*: New York (Springer), 251–283. **doi:10.1007/978-90-481-9204-5_12**

Takai, K., Nunoura, T., Ishibashi, J., Lupton, J., Suzuki, R., Hamasaki, H., Ueno, Y., Kawagucci, S., Gamo, T., Suzuki, Y., Hirayama, H., and Horikoshi, K., 2008. Variability in the microbial communities and hydrothermal fluid chemistry at the newly discovered Mariner hydrothermal field, southern Lau Basin. *J. Geophys. Res., [Biogeosci.],* 113(G2):G02031. **doi:10.1029/2007JG000636**

Takai, K., Nunoura, T., Horikoshi, K., Shibuya, T., Nakamura, K., Suzuki, Y., Stott, M., Massoth, G.J., Christenson, B.W., deRonde, C.E.J., Butterfield, D.A., Ishibashi, J., Lupton, J.E., and Evans, L.J., 2009. Variability in microbial communities in black smoker chimneys at the NW caldera vent field, Brothers Volcano, Kermadec arc. *Geomicrobiol. J.,* 26(8):552–569. **doi:10.1080/01490450903304949**

Ueno, H., Hamasaki, H., Murakawa, Y., Kitazono, S., and Takeda, T., 2003. Ore and gangue minerals of sulfide chimneys from the North Knoll, Iheya Ridge, Okinawa Trough, Japan. *JAMSTEC J. Deep Sea Res.,* 22:49–62. **http://docsrv.godac.jp/MSV2_DATA/23/22_06.pdf**

Urabe, T., and Marumo, K., 1991. A new model for Kuroko-type deposits of Japan. *Epsiodes,* 14(3):246–251.

You, C.-F., Butterfield, D.A., Spivack, A.J., Gieskes, J.M., Gamo, T., and Campbell, A.J., 1994. Boron and halide systematics in submarine hydrothermal systems: effects of phase separation and sedimentary contributions. *Earth. Planet. Sci. Lett.,* 123(1–3):227–238. **doi:10.1016/0012-821X(94)90270-4**

Table T1. Site surveys in the Iheya North hydrothermal field and the surrounding area. (**See table note.**)

Cruise name	Research vessel	Period	Site surveys
KR01-09	*Kairei*	Jul–Aug 2001	Heat flow, bathymetry, gravity, magnetism, subbottom profiling, coring
NT02-06	*Natsushima*	Apr–May 2002	Seafloor survey and sampling by submersible, heat flow
KY02-11	*Kaiyo*	Nov–Dec 2002	Multichannel seismic reflection survey
NT03-09	*Natsushima*	Aug–Sep 2003	Seafloor survey and sampling by ROV, heat flow
KY05-14	*Kaiyo*	Jan 2005	Heat flow, coring
YK06-09	*Yokosuka*	Jul 2006	Seafloor survey and sampling by submersible, single channel seismic reflection survey, heat flow
KY07-03	*Kaiyo*	May 2007	Multichannel seismic reflection survey
YK07-07	*Yokosuka*	May 2007	High-resolution of bathymetry, side scan sonar imaging by autonomous underwater vehicle
NT07-11	*Natsushima*	June 2007	Seafloor survey and sampling by ROV, heat flow
NT07-13	*Natsushima*	July 2007	Seafloor survey and sampling by ROV, heat flow
KY08-01 Leg2	*Kaiyo*	Jan 2008	Coring
KY08-01 Leg3	*Kaiyo*	Jan–Feb 2008	Coring
KR10-02	*Kairei*	Jan 2010	Multichannel seismic reflection survey

Note: ROV = remotely operated vehicle.

Table T2. Coring summary, Expedition 331.

Hole	Latitude	Longitude	Water depth (mbsl)	Cores (*N*)	Cored (m)	Recovered (m)	Recovery (%)	Drilled (m)	Penetration (m)	Time on site (days)
C0013A	27°47.4150′N	126°53.8605′E	1035	1	7.0	0.00	0.0	0.0	7.0	<1
C0013B	27°47.4140′N	126°53.8602′E	1035	1	9.5	1.44	15.2	0.0	9.5	<1
C0013C	27°47.4119′N	126°53.8606′E	1035	1	9.5	9.70	102.1	3.0	12.5	<1
C0013D	27°47.4130′N	126°53.8609′E	1036.5	4	32.5	17.91	50.0	3.0	35.5	<1
C0013E	27°47.4157′N	126°53.8546′E	1034	8	45.0	11.59	35.4	9.8	54.5	3
C0013F	27°47.4122′N	126°53.8601′E	1035.1	1	7.5	9.50	126.7	0.0	7.5	<1
C0013G	27°47.4100′N	126°53.8554′E	1035.1	1	1.8	1.80	100.0	7.5	9.3	<1
C0013H	27°47.4098′N	126°53.8565′E	1035.1	1	0.6	0.60	100.0	9.3	9.9	<1
			Site C0013 totals:	18	113.4	52.54	66.2	32.6	145.7	7
C0014A	27°47.4140′N	126°54.0487′E	1059.5	1	6.5	5.32	81.8	0.0	6.5	<1
C0014B	27°47.4131′N	126°54.0448′E	1059	5	44.5	45.01	101.5	0.0	44.5	1
C0014C	27°47.4194′N	126°54.0391′E	1060	1	6.5	4.90	75.4	0.0	6.5	<1
C0014D	27°47.4158′N	126°54.0406′E	1060	2	16.0	12.50	79.1	0.0	16.0	<1
C0014E	27°47.4158′N	126°54.0406′E	1060	2	19.0	15.30	80.5	16.0	35.0	<1
C0014F	27°47.4185′N	126°54.0443′E	1060.8	1	4.2	4.20	100.0	0.0	4.2	<1
C0014G	27°47.4165′N	126°54.0463′E	1059.8	28	136.7	74.40	56.1	0.0	136.7	4
			Site C0014 totals:	40	233.4	161.63	82.1	16.0	249.4	9
C0015A	27°47.6678′N	126°53.4981′E	885	0	6.3	0.00	0.0	6.3	6.3	<1
C0015B	27°47.6673′N	126°53.4981′E	886	1	6.5	6.20	95.4	0.0	6.5	<1
C0015C	27°47.6689′N	126°53.4993′E	885.5	1	9.4	2.90	100.0	6.5	9.4	<1
			Site C0015 totals:	2	22.2	9.10	97.7	6.5	22.2	<1
C0016A	27°47.4548′N	126°53.8034′E	982	1	18.0	0.00	0.0	0.0	18.0	1
C0016B	27°47.4538′N	126°53.7860′E	995	3	44.9	1.74	3.9	0.0	44.9	3
			Site C0016 totals:	4	62.9	1.74	2.0	0.0	62.9	4
C0017A	27°47.5030′N	126°54.7176′E	1129	1	8.8	8.80	100.0	0.0	8.8	<1
C0017B	27°47.5027′N	126°54.7176′E	1129	1	9.5	9.50	100.0	8.8	18.3	<1
C0017C	27°47.5039′N	126°54.7202′E	1129.6	2	19.0	18.00	94.8	31.0	50.0	<1
C0017D	27°47.5049′N	126°54.7217′E	1129.5	12	90.7	50.59	49.2	60.0	150.7	3
			Site C0017 totals:	16	128.0	86.89	86.0	99.8	227.8	3
			Expedition 331 totals:	78	559.9	311.90	66.8	154.9	708.0	23

Figure F1. Area map of Iheya North Knoll showing Sites C0013–C0017 drilled during Expedition 331. Inserts show the Iheya North Knoll in relation to Okinawa and Okinawa in relation to major tectonic components. Yellow lines on the map show a grid of MCS and SCS survey lines. EUR = Europe, PHS = Philippine Sea Plate.

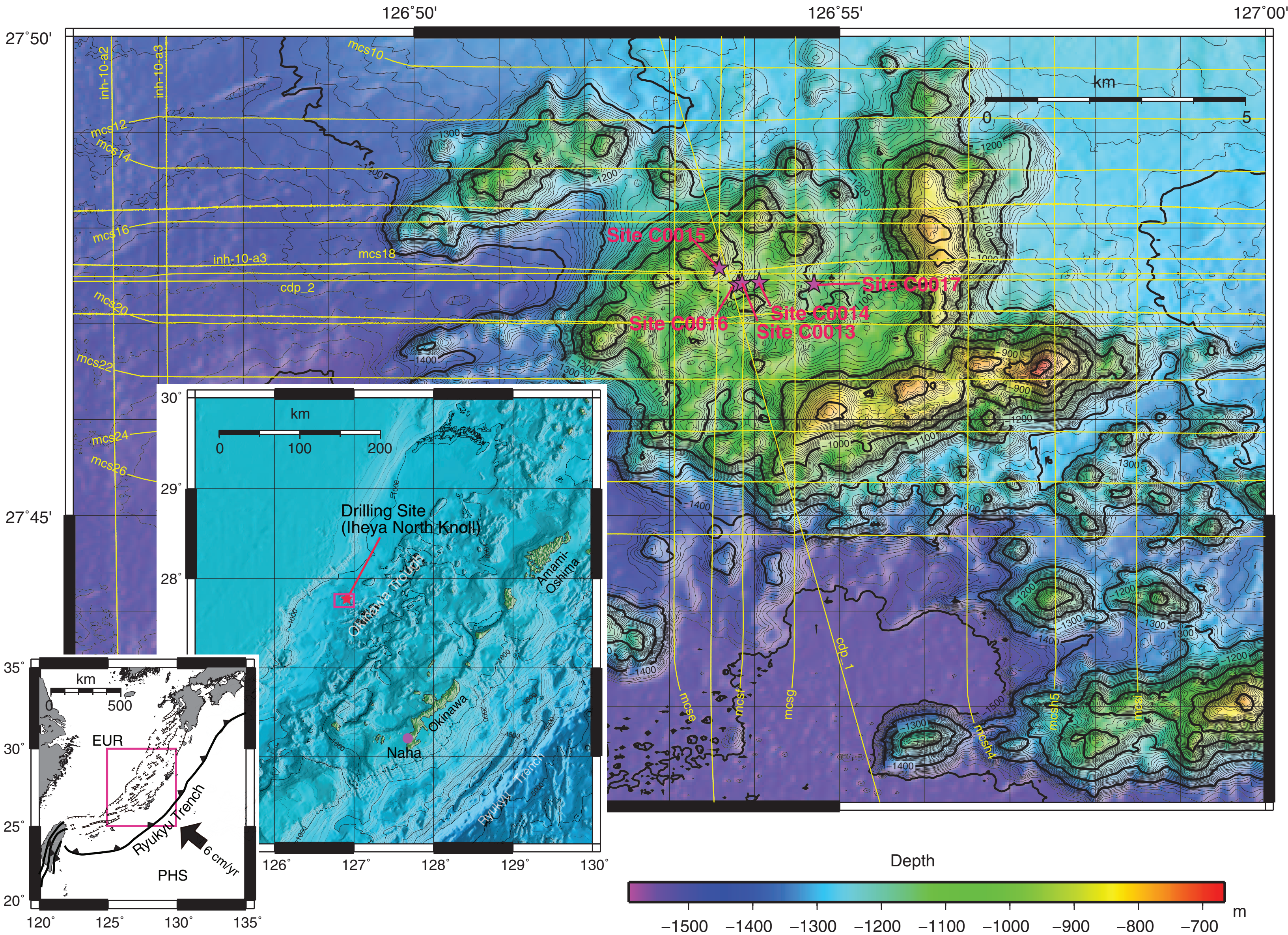

Figure F2. Ternary diagrams of the composition of sulfide samples from active hydrothermal fields. Iheya North data from Ueno et al. (2003). TAG = TAG mound, Mid-Atlantic Ridge, Middle Valley = Middle Valley, Juan de Fuca Ridge.

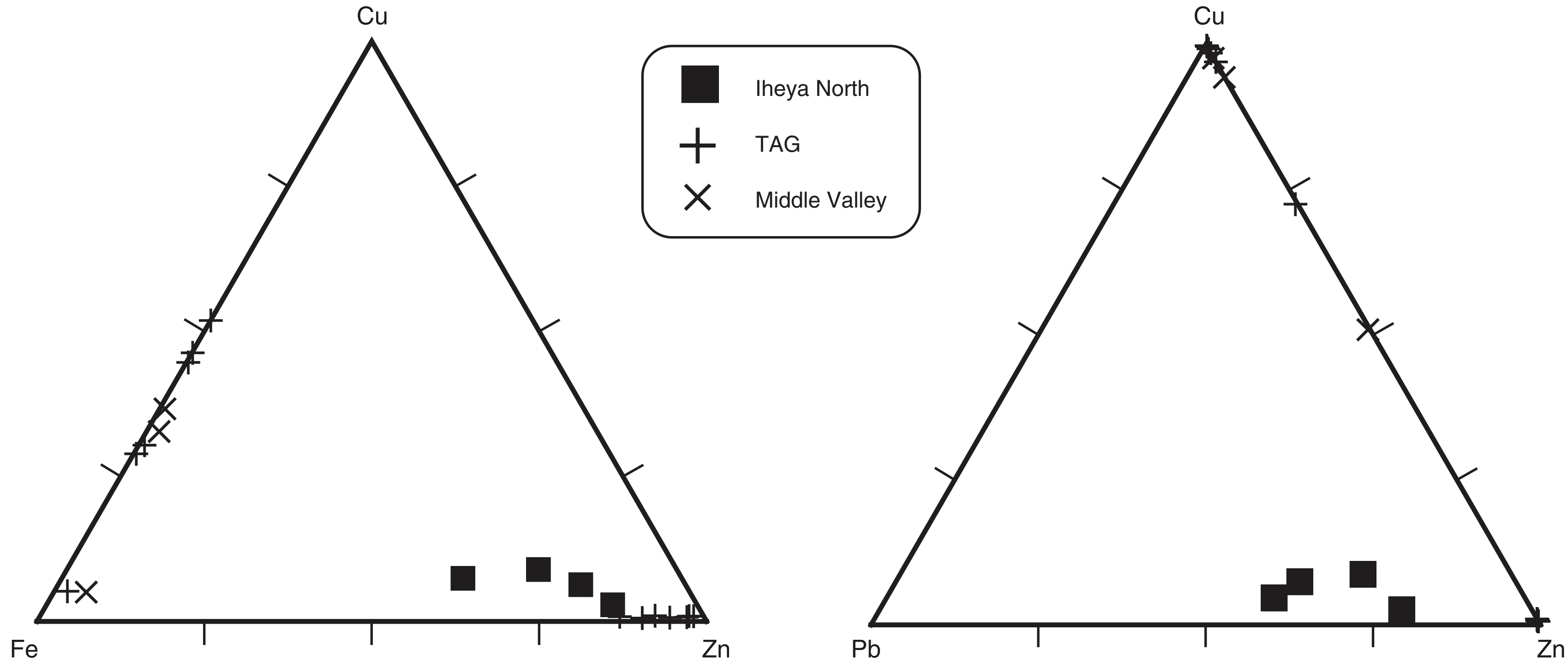

Figure F3. Detailed map of the Iheya North hydrothermal field and the central valley at Iheya North Knoll, with the location of Sites C0013–C0017 drilled during Expedition 331. HRV = High Radioactive Vent mound, CBC = Central Big Chimney, SBC = South Big Chimney mound, NEC = North Edge Chimney mound, E18 = event Marker 18 mound, NBC = North Big Chimney mound, ESBC = "Ese" South Big Chimney mound.

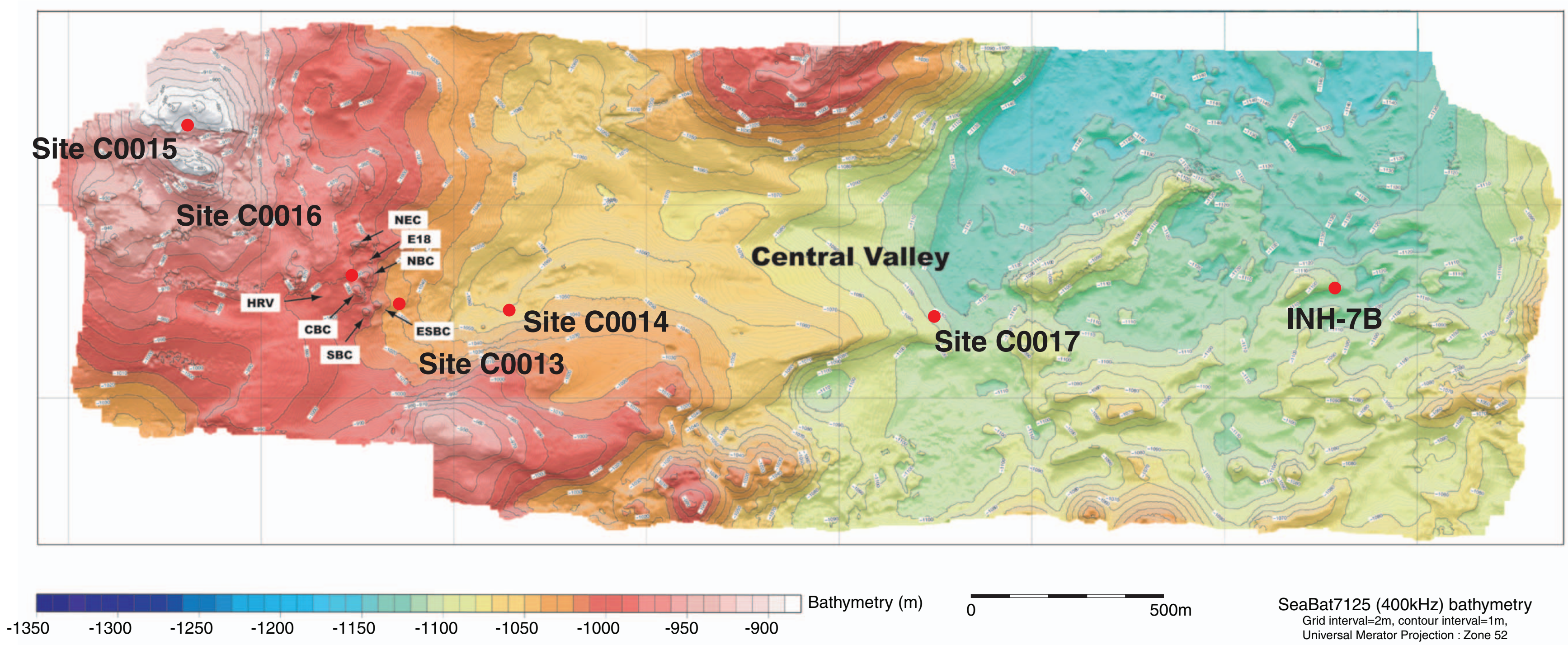

Figure F4. A cross section constructed from MCS profiles around Iheya North Knoll. Black bars indicate sites drilled during Expedition 331 and their proposed (not actual) depths assuming that 1 s of two-way traveltime corresponds to 750 m. Green bars indicate sites proposed but not drilled and their prospective depths.

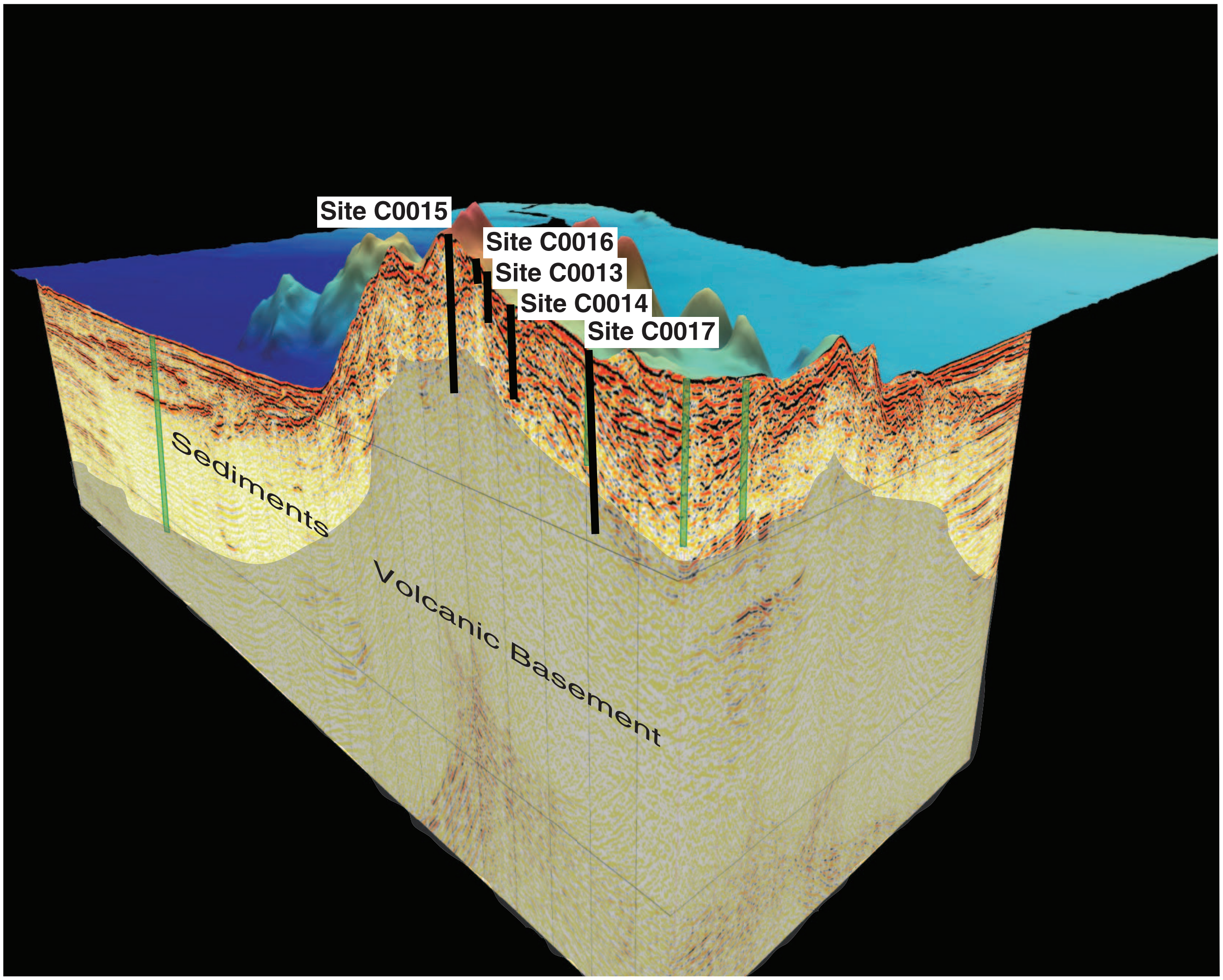

Figure F5. Subseafloor structure of the hydrothermal field and the central valley of Iheya North Knoll based on the profile of multichannel seismic Line MCS18. Expedition 331 drilling sites and their proposed (not actual) depths are shown as blue bars along with possible lithological interpretation assuming that 1 s of two-way traveltime corresponds to 750 m. Units (A, B, and C) are those identified from the seismic reflection profile prior to drilling, set against our lithological interpretation based on the cores recovered. Curved colored lines represent an attempt to correlate significant reflectors. CDP = common depth point.

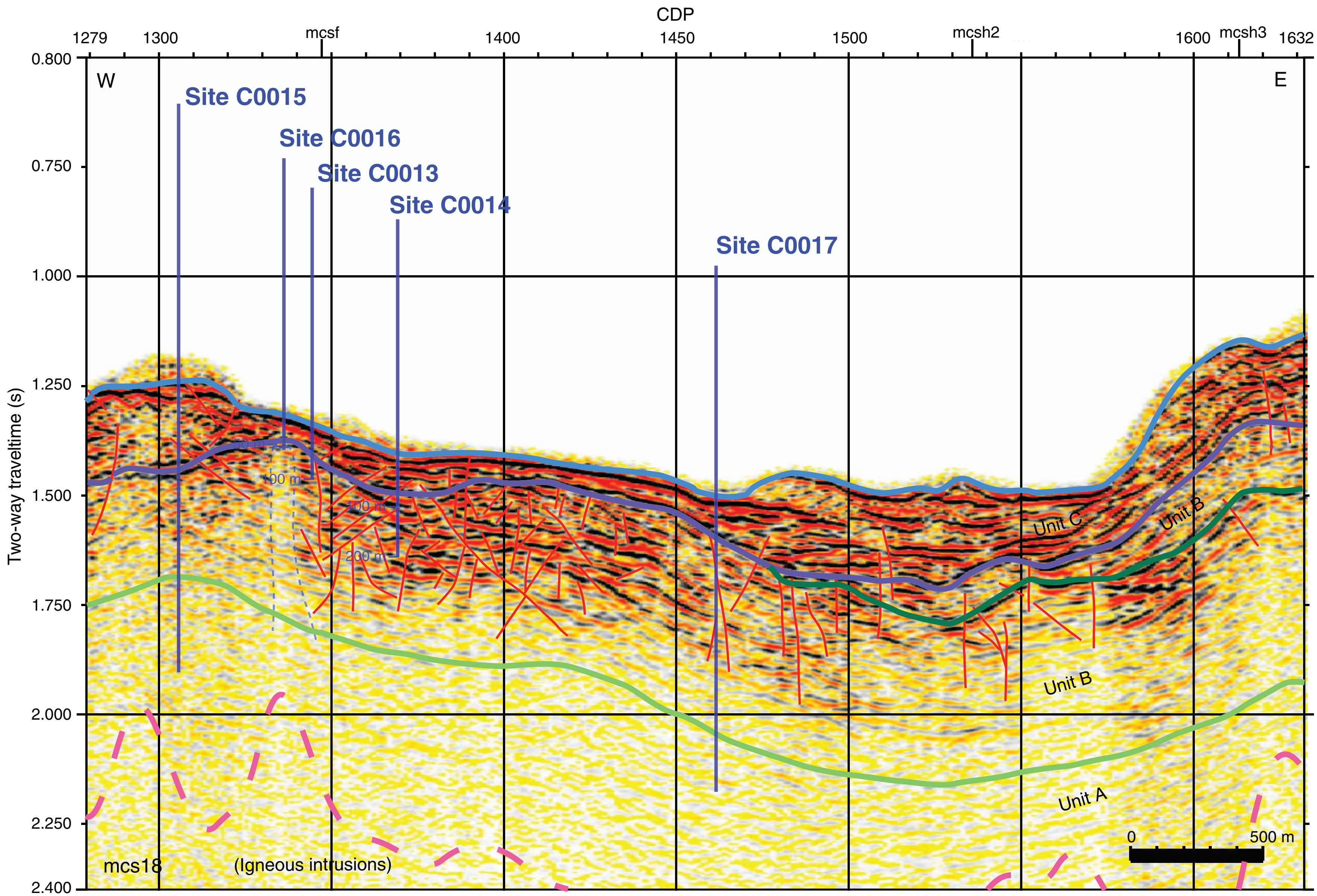

Figure F6. Subseafloor structure of the hydrothermal field and the central valley of Iheya North based on the profile of multichannel seismic Line MCS18. Expedition 331 drilling sites and total drilled depths tentatively converted to two-way traveltime are shown as blue bars, along with a possible hydrogeologic interpretation assuming that 1 s of two-way traveltime corresponds to 750 m. Black and gray blank diamonds show the upper and lower reflections of potential pyroclastic flow deposits beneath the central valley of Iheya North Knoll. These volcanic deposits are possible subseafloor hydrothermal migration paths. In contrast, black and gray blank circles show the upper and lower bounds of negatively polarized reflection sequences beneath the western hill structure near the vent sites. These negative polarity sequences are presently interpreted as very porous, fractured deposits that may host a hydrothermal reservoir and fluid migration paths.

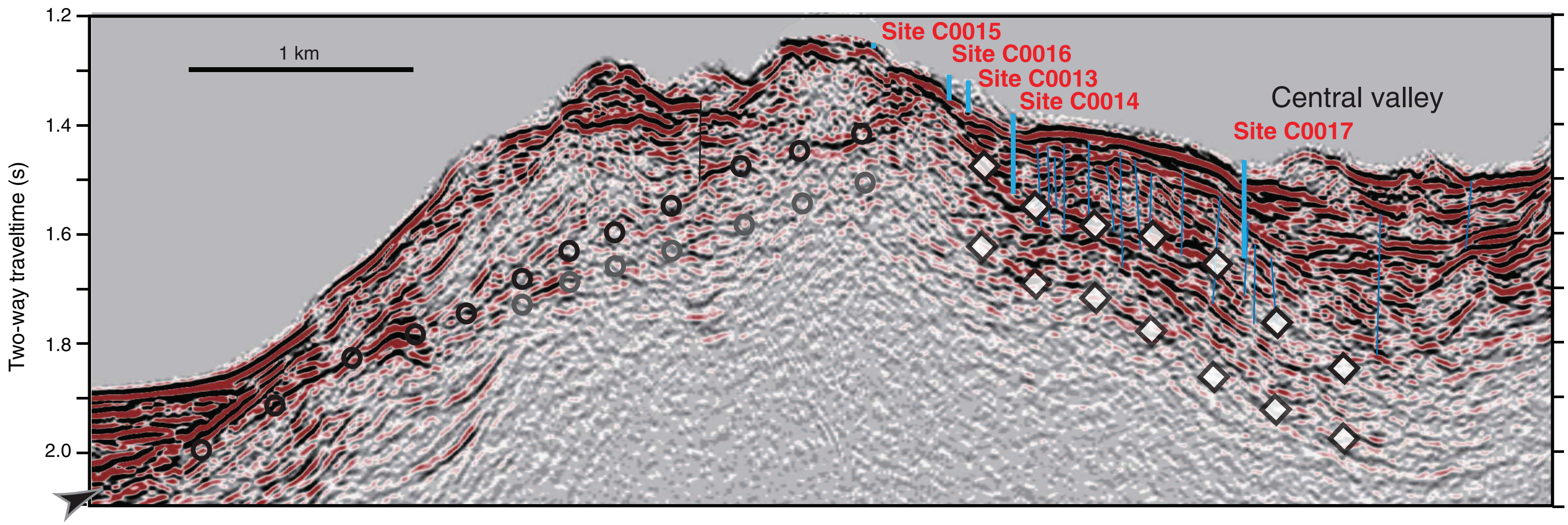

Figure F7. A photomosaic of the North Big Chimney (NBC) hydrothermal mound and photos of associated chemosynthetic animal communities at the Iheya North field.

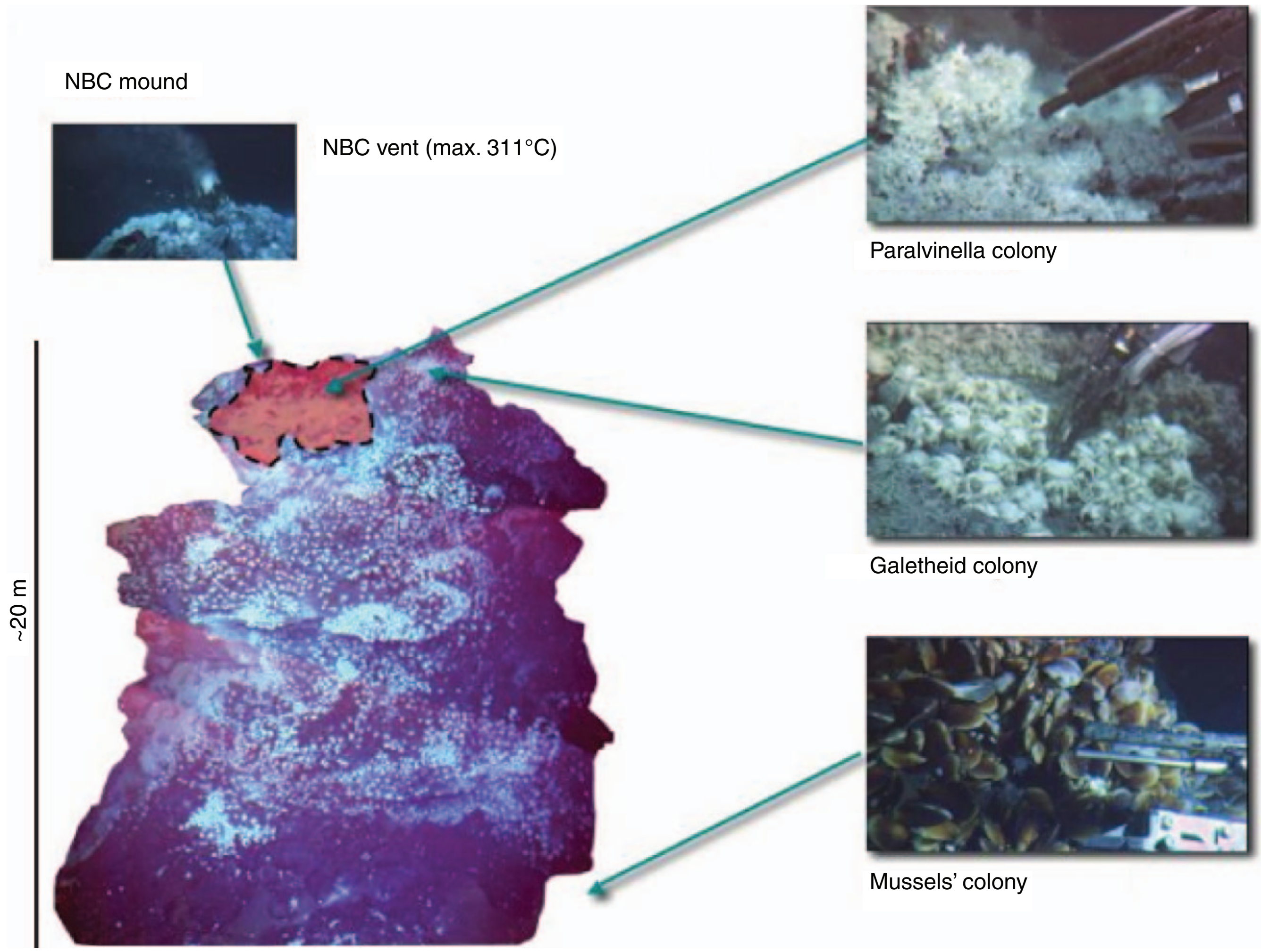

Figure F8. Schematic illustration of drilling and coring systems used, drilling and core recovery from Site C0013. EPCS = extended punch coring system, HPCS = hydraulic piston coring system, ESCS = extended shoe coring system, BHI = Baker-Hughes Inteq.

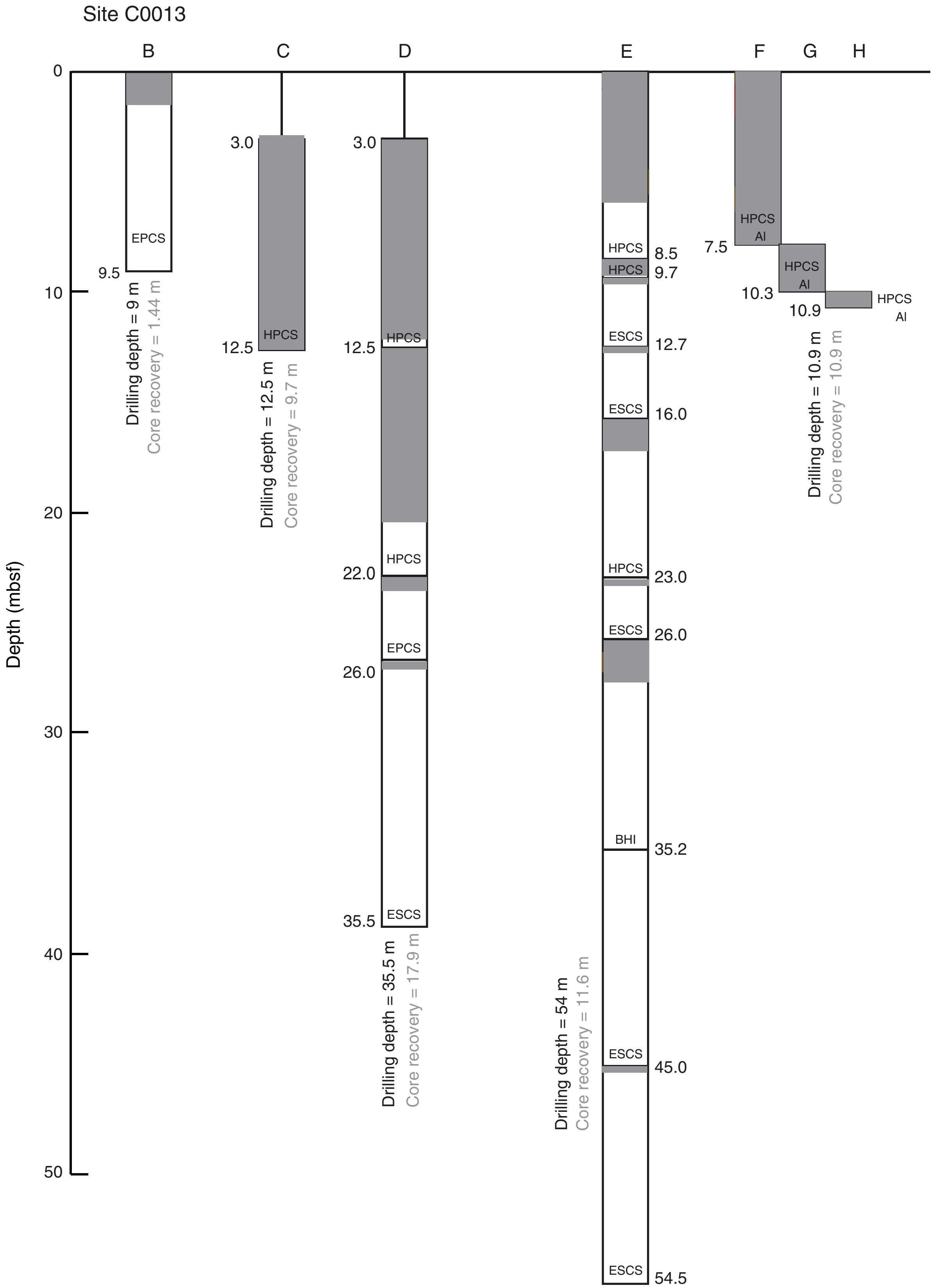

Figure F9. Schematic illustration of artificial hydrothermal vents created during Expedition 331. Outflow temperatures measured by the ROV *Hyper Dolphin* (Hole C0013) and thermal stickers (Holes C0014 and C0016). HPCS = hydraulic piston coring system, ESCS = extended shoe coring system, EPCS = extended punch coring system.

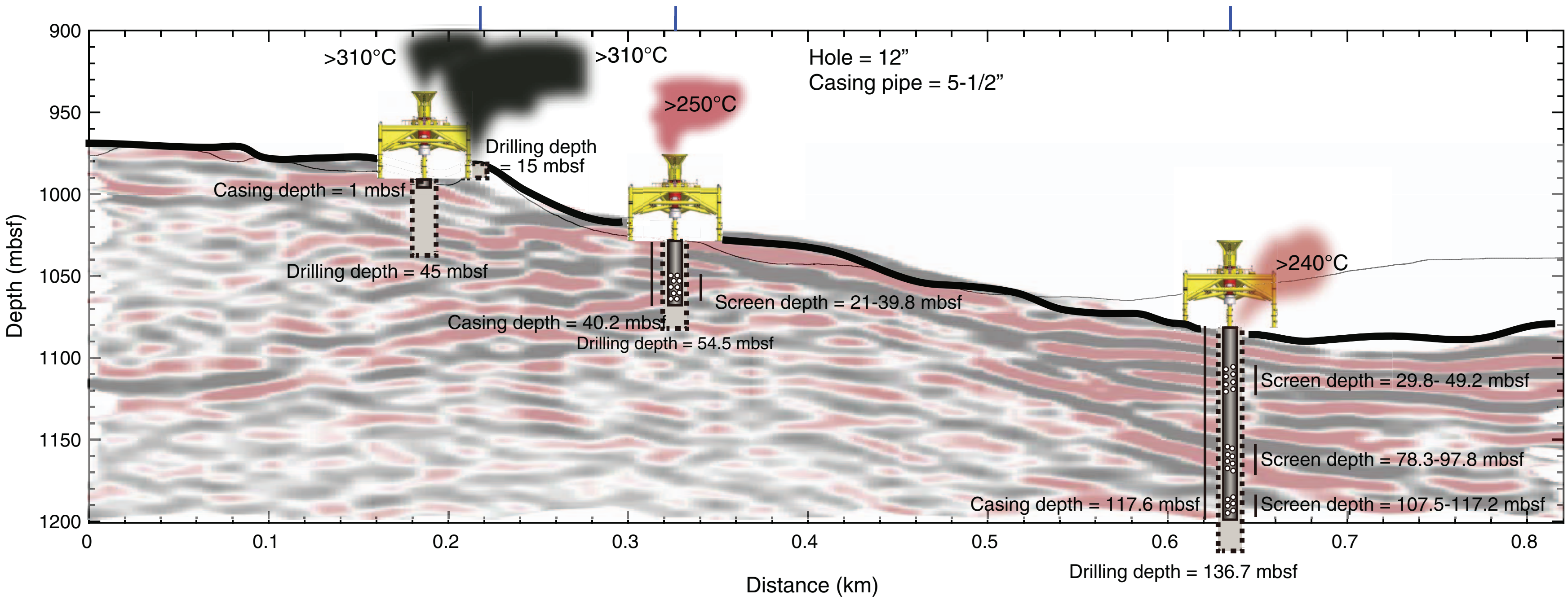

Figure F10. Schematic illustration of drilling and coring systems used, drilling and core recovery depths, and brief remarks on drilling operations and specific features of core recovered from Site C0014. High T = high-temperature.

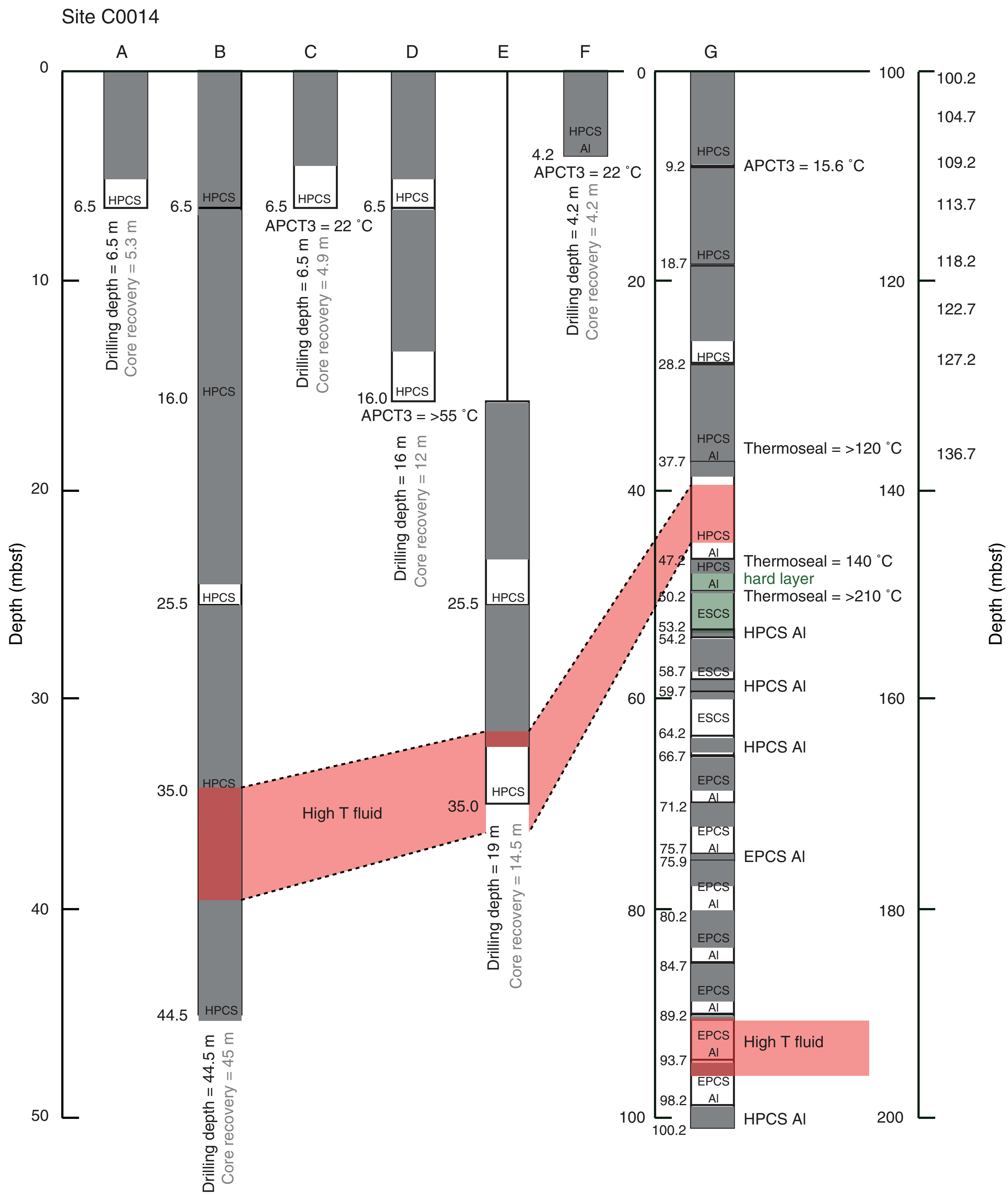

Figure F11. Depth profile of temperature in the subseafloor at Expedition 331 sites, as measured by the by APCT-3 shoe and thermoseal chemically impregnated temperature-sensitive strips. Yellow = APCT-3, black = thermoseal strip. Stars = minimum values.

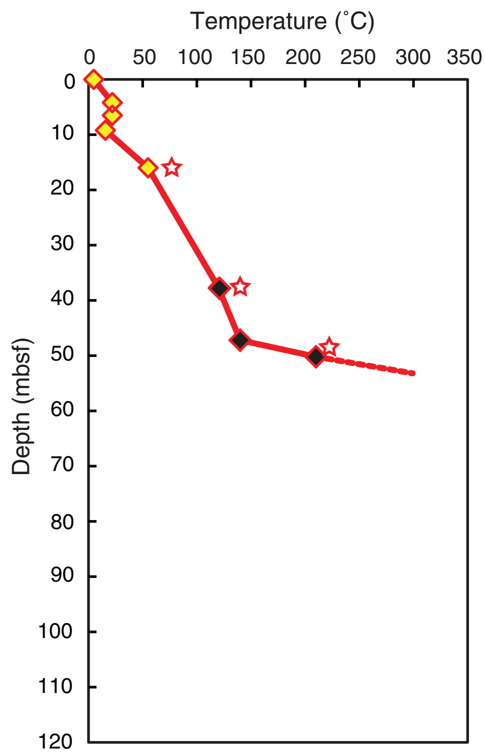

Figure F12. Schematic illustration of drilling and coring systems used, drilling and core recovery depths, and brief remarks on drilling operations and specific features of core recovered from Site C0015.

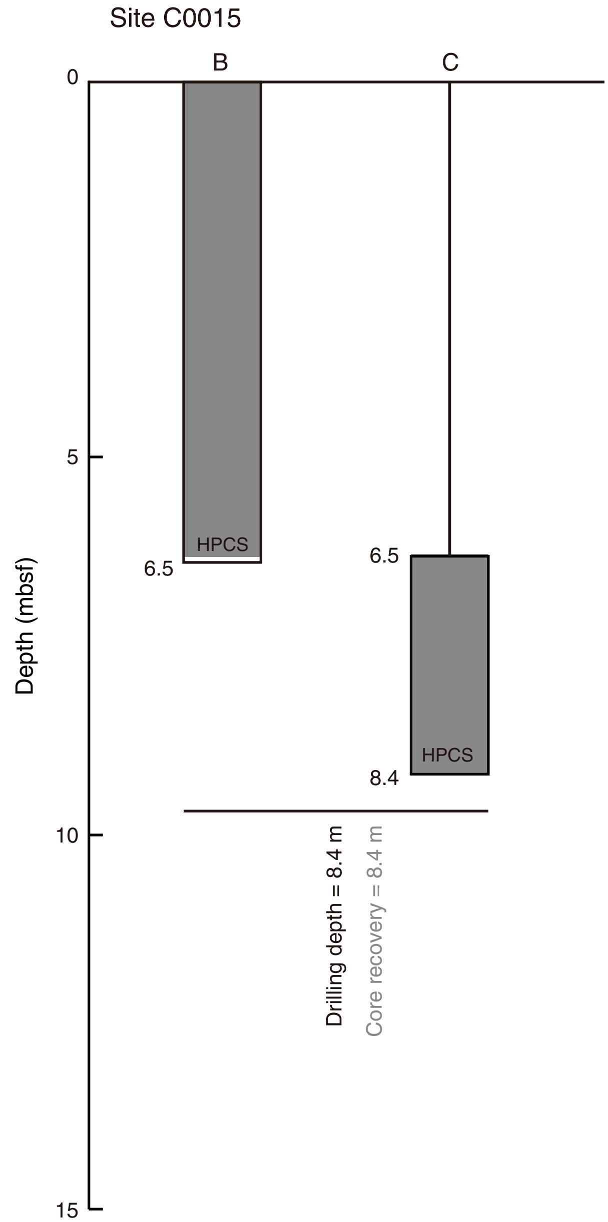

Figure F13. Schematic illustration of drilling and coring systems used, drilling and core recovery depths, and brief remarks on drilling operations and specific features of cores recovered from Site C0016. NBC = North Big Chimney. BHI = BHI = Baker-Hughes Inteq.

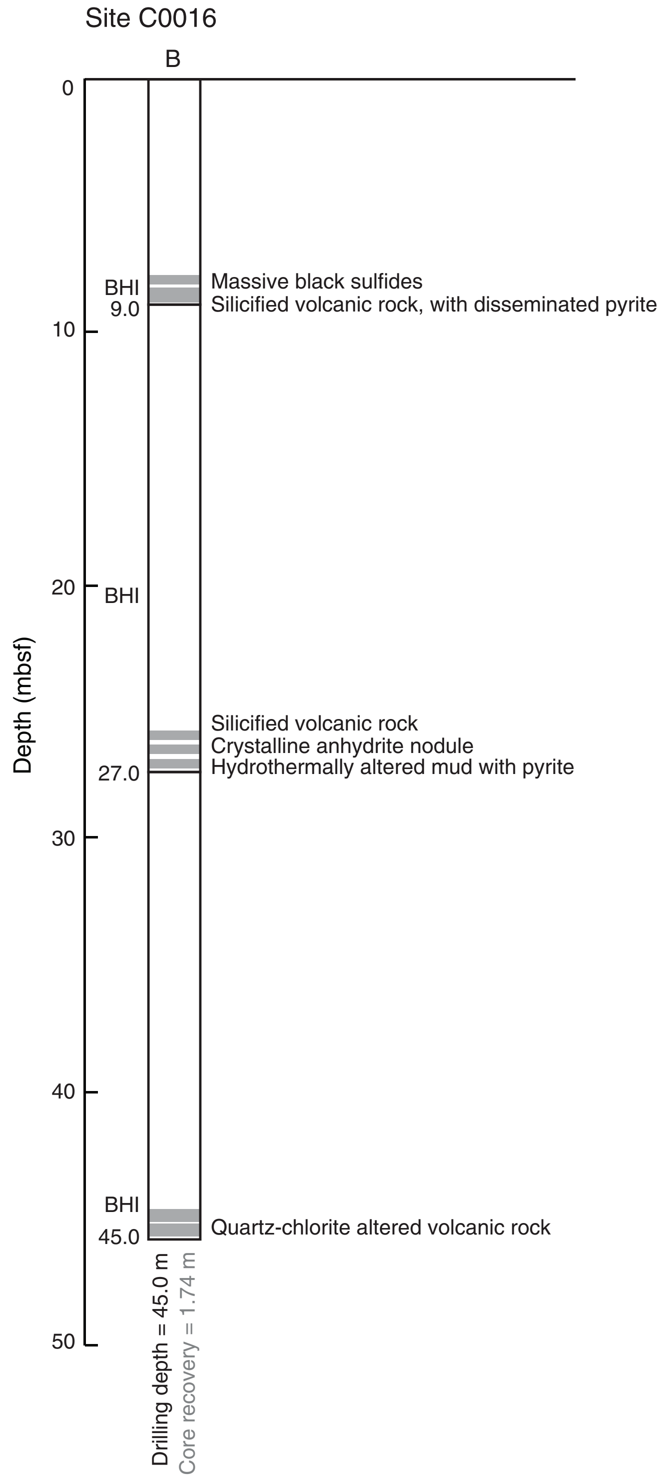

Figure F14. Depth profile of temperature in the subseafloor, as measured by the by APCT-3 shoe and thermoseal chemically impregnated temperature-sensitive strips during Expedition 331. Yellow = APCT-3, black = thermoseal strip. Star = minimum value.

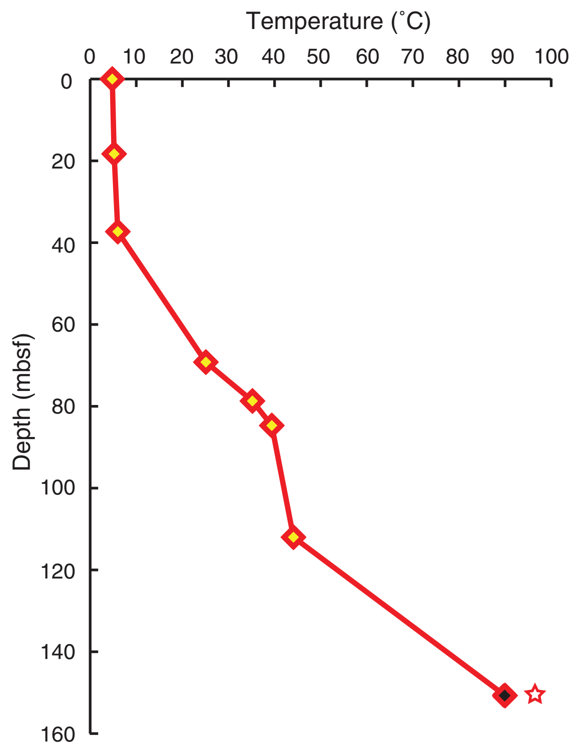

Figure F15. Correlation of lithostratigraphic units between Sites C0013, C0014, and C0016. The top of the silicified volcanic sediment represents the main correlative marker between the sites. The sudden change in dip between Site C0016 and the other sites is consistent with faulting, most likely extensional collapse of a volcanic flank by mass wasting, as seen at Site C0017. The entire section drilled at Site C0013, 0–54.5 mbsf, was hydrothermally altered, with a surface layer of 24 m of pumice altered to gray hydrothermal mud.

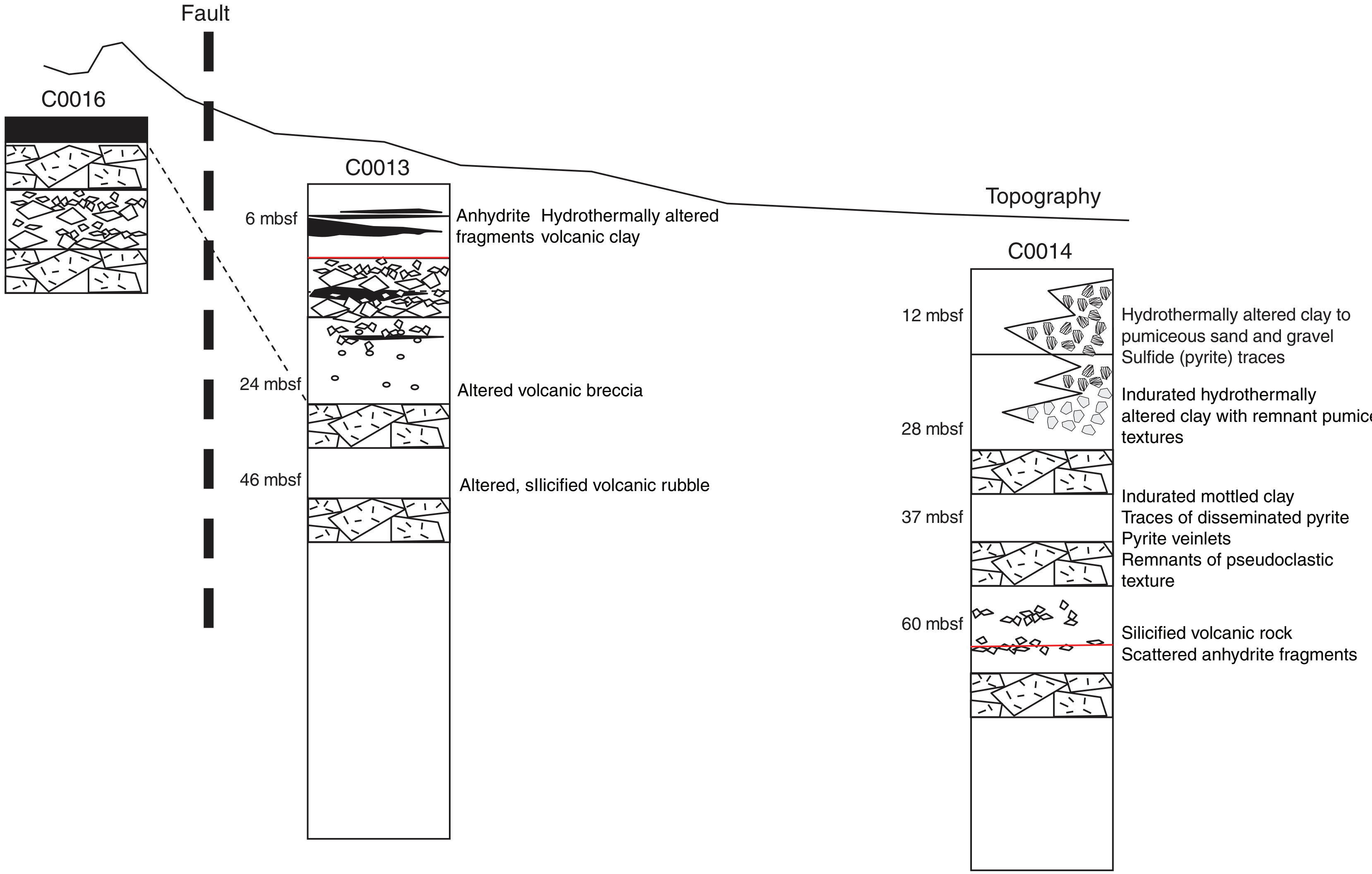

Figure F16. Schematic illustration of the possible spatial extent of subseafloor hydrothermal fluid flow and reservoirs (red) and recharged seawater advection (blue) based on drilling results from Expedition 331. Bars indicate the deeper holes drilled during Expedition 331 and their total penetration depths. The yellow dotted line denotes the shallowest depth of high temperatures of hydrothermal fluids we encountered during the expedition. Hole depths are fit to the seismic reflection profile assuming that 1 s of two-way traveltime corresponds to 750 m.