# IODP Expedition 331: Strong and Expansive Subseafloor Hydrothermal Activities in the Okinawa Trough

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### **Abstract**

Integrated Ocean Drilling Program (IODP) Expedition 331 drilled into the Iheya North hydrothermal system in the middle Okinawa Trough in order to investigate active subseafloor microbial ecosystems and their physical and chemical settings. We drilled five sites during Expedition 331 using special guide bases at three holes 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 base of the North Big Chimney (NBC) mound yielded low recovery, but core included the first Kuroko-type black ore ever recovered from the modern subseafloor. The other four sites yielded interbedded hemipelagic and strongly pumiceous volcaniclastic sediment, along with volcanogenic breccias that are variably hydrothermally altered and mineralized. At most sites, analyses of interstitial water and headspace gas yielded complex patterns with depth and lateral distance of only a few meters. Documented

processes included 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, potential microbial oxidation of organic matter and anaerobic oxidation of methane utilizing sulfate, and methanogenesis. Shipboard analyses have found evidence for microbial activity in sediments within the upper 10–30 m below seafloor (mbsf) where temperatures were relatively low, but little evidence in the deeper hydrothermally altered zones and hydrothermal fluid regime.

#### **Introduction and Goals**

Active seafloor 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 and is sustained from the

hydrothermal energy and matter inputs (Deming and Baross, 1993; Takai et al., 2001). 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). In the Iheya North field, a typical deep-sea hydrothermal system in the Okinawa Trough, it has been suggested that a variety of microbial communities based on different chemolithoautotrophic primary producers is present in subseafloor habitats (Nakagawa et al.,

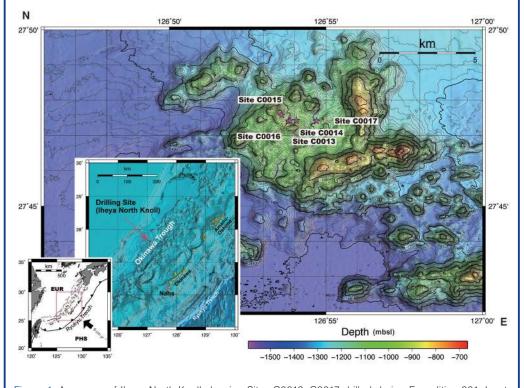


Figure 1. Area map of Iheya North Knoll showing Sites C0013-C0017 drilled during Expedition 331. Insets show the Iheya North Knoll in relation to Okinawa and Okinawa in relation to major tectonic components. EUR= Eurasian Plate, PHS = Philippine Sea Plate. Modified from Takai et al. (2011).

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 overall hydrothermal environments associated with organic-rich sediments provide unusual amounts of C1 compounds (CO2 and CH4) in hydrothermal fluids as carbon sources, as well as unique microbial habitats affected by liquid CO<sub>2</sub> and gas hydrates (Nakagawa et al., 2005; Kawagucci et al., 2011). 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.

There were three major scientific objectives of Expedition 331 drilling were.

- to test for the existence of a functionally active, metabolically diverse subvent biosphere associated with subseafloor hydrothermal activity;
- to clarify the architecture, function, and impact of subseafloor microbial eco-systems and their relationship to physical, geochemical, and hydrogeologic variations within the hydrothermal mixing zones around the discharge area; and
- to establish artificial hydrothermal vents in cased holes from potential subseafloor hydrothermal flows, 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.

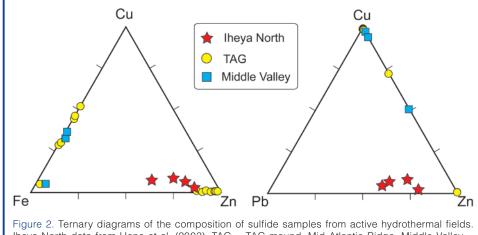
#### **Geological Setting and Earlier Work**

The Okinawa Trough is a backarc basin extending for ~1200 km, between the Ryukyu arc-trench system and the Asian continent (Fig. 1; Lee et al., 1980; Letouzey and Kimura, 1986). Seismic reflection data suggest a typical backarc structure (Letouzey and Kimura, 1986) with a high-velocity mantle below ~6000 mbsf overlain by potentially young basalt with an average velocity of 5.8 km s<sup>-1</sup> between ~3000 mbsf and 6000 mbsf, an igneous rock layer  $(4.9 \text{ km s}^{-1})$  between ~1000 mbsf and 3000 mbsf, and ~1000 m of sediment immediately beneath the seafloor. Since the discovery of submarine hydrothermal activity at Iheya Ridge and Izena Hole in the middle Okinawa Trough in 1988 (Halbach et al., 1989; Sakai et al., 1990), six active hydrothermal fields have been discovered-Minani-Ensei Knoll, Iheya North, Iheya Ridge, Izena Hole, Hatoma Knoll, and Yonaguni Knoll IV.

Characteristics of the tectonic setting in this active hydrothermal system are reflected in the chemical composition of sulfide deposits. Sulfide samples collected from the Iheya North field are distinctly more enriched with Pb than mid-oceanic-ridge sulfides (Fig. 2). The polymetallic Zn-Pb-Cu chemical signature of Iheya North sulfides is similar to that of Kuroko-type hydrothermal deposits formed during the Tertiary in northeast Japan.

The chemistry of hydrothermal fluids collected from active sulfide chimneys in the Okinawa Trough is characterized by higher concentrations of CO<sub>2</sub>, CH<sub>4</sub>, NH<sub>4</sub>, I, and K and higher alkalinity than those in typical sediment-free midocean-ridge hydrothermal fluids (Sakai et al., 1990; Gamo et al., 1991; Konno et al., 2006; Takai and Nakamura, 2010; Kawagucci et al., 2011). 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 rich in K and volatile components to the Okinawa Trough (Sakai et al., 1990; Gamo et al., 2006). Organic-rich terrigenous sediment filling the Okinawa Trough (Narita et al., 1990) supplies not only the sedimentary chemical inputs (NH<sub>4</sub>, 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; Kawagucci et al., 2011). In addition to the chemical aspects, the relatively shallow water depth of many Okinawa Trough hydrothermal systems serves to induce subcritical phase separation (Suzuki et al., 2008) and subsequent phase segregation, as the boiling temperature of seawater decreases steeply with decreasing pressure at ~100 bar. Phase separation and segregation sometimes



lheya North data from Ueno et al. (2003). TAG = TAG mound, Mid-Atlantic Ridge, Middle Valley Middle Valley, Juan de Fuca Ridge. Modified from Takai et al. (2011).

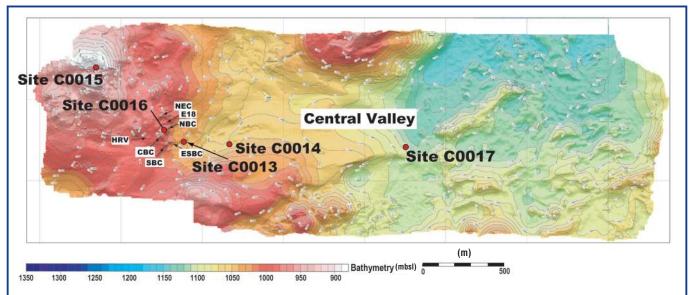


Figure 3. Detailed bathymetry of the Iheya North hydrothermal field and the central valley at Iheya North Knoll, with the location of Sites C0013–C0017 drilled during Expedition 331. HRV=High Radioactive Vent mound, CBC=Central Big Chimney mound, SBC=South Big Chimney mound, NEC=North Edge Chimney mound, E18=event Marker 18 mound, NBC = North Big Chimney mound, ESBC="Ese" South Big Chimney mound. Modified from Takai et al. (2011).

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., 2011).

# **Drilling, Temperature Measurement, Sampling, and Installation of Vents**

We drilled five sites during Expedition 331: the active hydrothermal vent site and sulfide-sulfate mound at North Big Chimney (NBC) (Site C0016, Fig. 3), three sites east of NBC at distances of ~100 m (C0013), 450 m (C0014), and 1550 m (C0017) from the active vents, and one site (C0015) on a hill ~600 m northwest of the active vents that represents a potential migration path for hydrothermal fluid.

The NBC hydrothermal mound at Site C0016 is 20 m high and 6 m in diameter. When we attempted to core this active high-temperature (311°C) vent at its summit, the pipe broke, and core recovery failed (Hole C0016A). We drilled a second hole 20 m away, immediately at the base of the mound on its western side (Hole C0016B). We used conventional hard rock drilling equipment supplied by Baker-Hughes Integ (BHI) specifically for Expedition 331 due to high temperatures and expectation of hard rock. The BHI system collects 4-inch diameter core in aluminum liners in lengths of 9 m, 18 m, or 27 m, but requires a time-consuming pipe trip for each core. It penetrated to 45 mbsf in three runs of 9 m, 18 m, and 18 m. Each run recovered several large pieces of core, but the total recovery was only 2.095 meters, 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 (Fig. 4). 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 mbsf.

At Sites C0013, C0014, and C0017, we drilled the relatively high, moderate, and low heat flow areas, respectively, 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, that 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 any softer intervals encountered deeper in the hole, alternating as necessary with the extended punch (EPCS) and extended shoe (ESCS) coring systems, and, for one run at Site C0013, the BHI system, to penetrate the harder layers. Both the EPCS and ESCS systems were able to penetrate the harder layers, with a slight advantage to the ESCS for the hardest layers, though the EPCS was generally much better at core recovery. We penetrated the margin of the local discharge-recharge zone to depths of 55 mbsf (C0013), 137 mbsf (C0014), and 151 mbsf (C0017), coring variably hydrothermally altered sediment and pumiceous deposits (Figs. 5, 6). We were able to measure in situ temperature at two of these sites using the advanced piston corer temperature tool (APCT3) shoe (upper calibration limit = 55°C) as part of the HPCS, combined with commercial thermoseal strips (Nichiyu Giken Co., Ltd.) taped to the outer surface of the core liner. 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.

Eight holes (C0013A-C0013H) were drilled at Site C0013, and core was recovered from all but Hole C0013A. 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 (Fig. 4). During drilling and coring operations at Site C0013, we encountered many operational and sample handling problems. These problems were 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 mbsf, 7-10 mbsf, and 20-30 mbsf). We observed at several depths that when a hard cap rock was drilled through into softer underlying layers, subseafloor hydrothermal fluid began to outflow from the hole, where it was imaged by the ROV video camera. To tap this fluid, we used slotted, perforated casing pipe over the depth interval 21-39.8 mbsf in Hole C0013E (Fig. 4). Immediately after casing and capping this hole, we observed in the ROV video image strong hydrothermal fluid discharge from the casing pipe that was hung in the guide base. Thermoseal temperature-sensitive strips on the corrosion cap outlet pipe showed in the ROV video imagery that the discharging water temperature was >250°C.

Seven holes were drilled at Site C0014 (Holes C0014A-C0014G). 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 behaved as cap rock, and discharge from the holes, beyond what may be only expelled drilling fluid, 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 pore water chemistry, density, and porosity (and indicated by low recovery during drilling), we inferred lateral hydrothermal flow at 31–42.5 mbsf and 90–95 mbsf at Site C0014. We therefore installed slotted, perforated casing pipe at 29.8-49.2 mbsf, 78.3-97.8 mbsf, and 107.5-117.2 mbsf in Hole C0014G (Fig. 4). 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. We measured temperature at Site C0014 using the APCT3 temperature shoe on the HPCS core barrel for lower temperatures (0°C-55°C) and thermoseal temperature-sensitive strips for higher temperatures (75°C–250°C). The temperature-depth profile at Site C0014 is shown in Fig. 7a. Temperature increases nearly linearly at  $3^{\circ}$ C m<sup>-1</sup> to  $145^{\circ}$ C  $\pm 5^{\circ}$ C at 47 mbsf and then increases abruptly to >210°C at 50 mbsf, below a hard layer near that depth. The drilled sequence at Site C0014 comprises interbedded, variably altered, and consolidated volcaniclastic gravels and breccias, as well as hemipelagic mud (Fig. 5). Given the location of the site on the upper flank of an active volcanic complex, mass wasting and debris flows are likely to be important sedimentary processes, potentially leading to high rates of redeposition of hemipelagic and volcaniclastic material. The deeper portion of the rock volume cored has been hydrothermally altered. Differing degrees and styles of hydrothermal alteration form the basis for the division of Site C0014 sediments and lithologies into lithostratigraphic units.

Hole C0017D is the deepest (150.7 mbsf) of four holes drilled at Site C0017 and is located 1550 m east of the hightemperature 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 tempera-

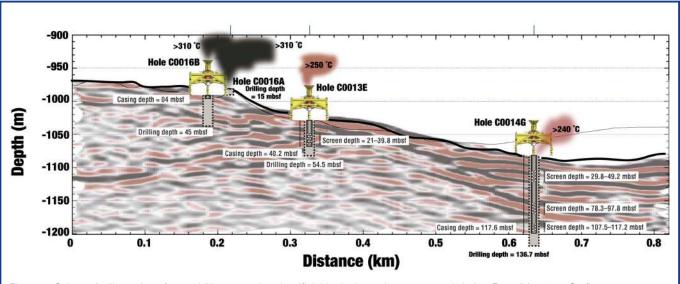


Figure 4. Schematic illustration of post-drilling natural and artificial hydrothermal vents created during Expedition 331. Outflow temperatures measured by ROV (Hole C0013E) and thermal stickers (Holes C0014G and C0016B). Modified from Takai et al. (2011).

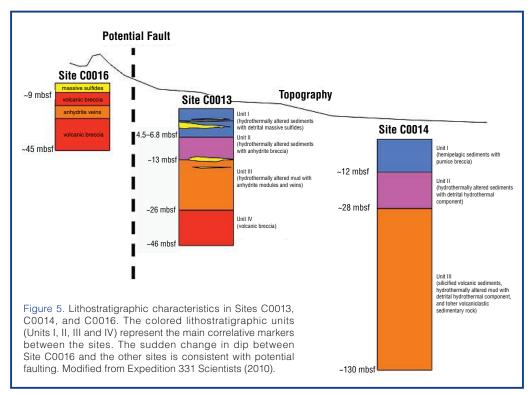
ture profile we measured (Fig. 7b) can be fit reasonably well with an exponential function and is consistent with overall downwelling, with distinct perturbations in the temperature gradient that suggest localized lateral flow that may be influenced by the variable lithologies we cored (Fig. 6). We measured temperature successfully at seven depths in Holes C0017B-C0017D over the interval 18.3–150.7 mbsf: combined with the ocean bottom water temperature at the site of  $4.9^{\circ}$ C  $\pm 0.5^{\circ}$ C, our profile is defined by eight points. Six of the downhole measurements were made using the APCT3 tempera-

ture 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°C, 80°C, 85°C, 90°C, 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°C, 90°C, and 90°C on the three strips, which we reported as 90°C ± 5°C. The APCT3 shoe recorded a temperature in excess of its maximum range of 55°C for this core. Down to ~50 mbsf, where we encountered a hard layer that was probably pumice (no core was recovered, Fig. 6), temperature remained low, <15°C. Beneath this hard layer, temperature jumped to 25°C-39°C at 69-85 mbsf and then appeared 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.

Three holes were drilled at Site C0015 (Holes C0015A-C0015C). Although a relatively short interval was cored (0–9.5 mbsf), a broad diversity of sediment types was drilled, with coarse pumiceous gravel and grit, siliciclastic sand, hemipelagic mud, bioclastic gravel, and foraminiferal sediment all recovered. In contrast to the other sites, the samples of Site C0015 do not have any significant hydrothermal input, nor do they appear at present to support a robust microbial community associated with hydrothermal activity. Thus, Site C0015 is excluded from further description and discussion.

#### **Preliminary Scientific Results**

Drilling and coring operations during Expedition 331 provided insight into the hydrothermal flow regime at Iheya North Knoll. While 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 was located 100 m east of the vigorous high-temperature vents and mounds (Fig. 3). 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 between the harder, less permeable layers. The interstitial water showed large changes in composition both laterally and vertically over short distances, suggesting chaotic lateral flow in permeable horizons separated by impermeable barriers. The lithostratigraphy, physical properties of the sediment and rock, and interstitial water chemistry thus all provided insight into the hydrothermal flow regime at Site C0013.

Site C0014 was located ~450 m east of the high-temperature vents and mounds (Fig. 3). The temperature gradient was roughly linear from 0 mbsf to 47 mbsf, increasing from the bottom water temperature of 4.5°C to 145°C over that depth range, but it deviated greatly from this line at 0-9 mbsf and 47–50 mbsf, where it was clearly affected by high-temperature fluid pooling or lateral flow. Interstitial water chemistry demonstrated vertical stratification, from water with seawater chloride values at 0-25 mbsf, to a vapor at 29-38 mbsf, and to a brine from 48 mbsf to the deepest sample at 114 mbsf. Within the upper sediments, which consisted of pelagic sediments and pumiceous gravel, there was considerable lateral variability in the intensity of microbial sulfate reduction between the four holes that were only a few meters apart; clearly there was a functionally robust and metabolically diverse subseafloor biosphere here. At 20 mbsf, where the temperature was ~70°C, and to the bottom of the deepest Hole C0014G at 137 mbsf, where a linear fit would have the

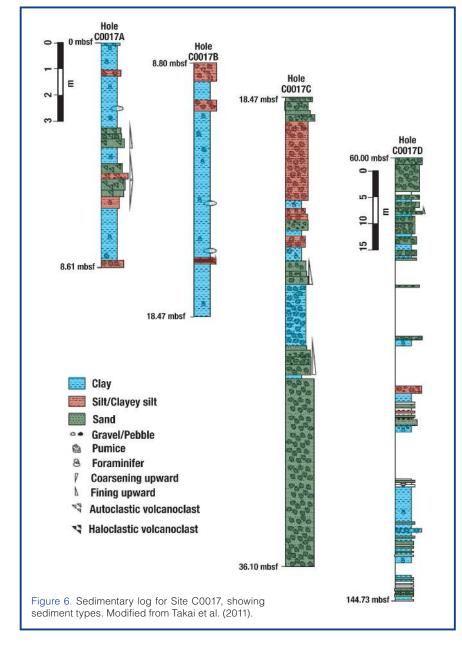
temperature exceed 400°C, the sediment and rock we recovered was intensely hydrothermally altered. As at Site C0013, chaotic flow should be occurring through permeable formations and along fault structures, and was likely separated vertically by impermeable beds that behave as cap rocks.

Site C0017 was located 1550 m east of the high-temperature vents of the Iheya North hydrothermal field, in an area of low heat flow (Fig. 3). The overall temperature profile was exponential and concave upward, consistent with downwelling of cold water, implying that this was an area of recharge to the hydrothermal system. We reached a maximum temperature of 90°C ± 5°C at the bottom of the deepest Hole C0017D at 151 mbsf. Deviations from a smooth temperature profile indicated the presence of a discrete zone of cold water recharge, consistent with interstitial water chemistry and the presence of a highly oxidized layer at 26-35 mbsf that supported a microbial community. We found only a small amount of hydrothermally-altered sediment deep in Hole C0017D. These results, taken together, provided a more detailed look at the large-scale hydrogeology of the Iheya North Knoll hydrothermal system than previously observed at subseafloor hydrothermal systems elsewhere (Takai et al., 2011). The hydrological regime at Iheya North Knoll was 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.

Much of the sediment and rock we cored at Sites C0013, C0014, and C0016 was intensely hydrothermally altered, and mineralized (Fig. 5). Exceptional among the disseminated and vein, stockwork-type sulfide we recovered was the massive sphalerite-rich ore we cored in Hole C0016B. This

marked the first time this type of massive sulfide, which closely resembles the Kuroko black ore, has been recovered from the subseafloor environment of an active deep-sea hydrothermal system. We recovered only 2.1 meters of core from 45 m of penetration in this hole, which made reconstruction of the lithostratigraphy impossible, but the recovered core included a wide diversity of lithologies that were typically associated with volcanic-hosted massive sulfide (VHMS) mineralization. In addition to the sphalerite-rich black ore, recovered core included a boulder-sized, coarsely crystalline piece of anhydrite veined by sphalerite-pyrite, as well as pyrite-veined and pyrite-altered volcanic

The shipboard microbiological analyses and experiments provided little evidence for the existence of a hot subvent biosphere beneath the Iheya North hydrothermal field, though direct microscopic cell count and cultivation from a colder, diffusely venting site and a site of lateral recharge provided evidence for subseafloor psychrophilic to mesophilic microbial communities. 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 regimes we intersected by drilling, including the permeable reservoirs and flow paths, appeared to be larger



in scale than we had envisioned. Most of the sediment and rock we cored at Sites C0013, C0014, and C0016 were exposed to much higher temperatures than the microbiologically habitable temperature range (<122°C). However, smaller and more limited zones that were habitable by microbes were sampled, as well as a larger one at the hydrothermal recharge zone at Site C0017. Recharge zones were, of course, an essential part of all hydrothermal systems.

# **Plans for Future Investigations**

A total of twenty-four holes were drilled during Expedition 331, of which twenty-one resulted in recovered cores. We drilled 708 m and recovered 312 meters of core from 560 m attempted, yielding an overall recovery of 56%. This compares highly favorably with the only other attempt to drill a submarine felsic-hosted hydrothermal system during ODP Leg 193, which recorded an overall core recovery of 10.7% at the PACMANUS site in the Eastern Manus Basin, Papua New Guinea

(Shipboard Scientific Party, 2002). The improved core recovery during Expedition 331 will provide excellent opportunities to assess the existence of a functionally active, metabolically diverse subvent biosphere and to clarify the architecture and function of subseafloor microbial ecosystems and the physical, geochemical, and hydrogeologic variations.

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 post-drilling natural and artificial hydrothermal vents were created in Holes C0013E, C0014G, C0016A, and C0016B, in which hydrothermal fluid formerly trapped in the subseafloor ascended up the hole and exited into the ocean (Fig. 4). Temperature at all hydrothermal vent emissions was confirmed to be >240°C. These newly created hydrothermal vents will serve as windows into the subseafloor and will facilitate post-drilling, long-term studies of any associated microbial communities, fluid composition and flow, and in situ microbial and macrofaunal colonizations.

Interstitial water collected during Expedition 331 suggested that the subseafloor hydrothermal fluid regime of Iheya North field is chemically stratified with respect to chloride concentration. Pore water near the seafloor had the chloride concentration of seawater. It was underlain by a thin and possibly discontinuous vapor-rich layer at ≥5 mbsf at Site C0013 and at 29-38 mbsf at Site C0014. Beneath the vapor-rich layer lays a chloride-enriched brine, at least to the

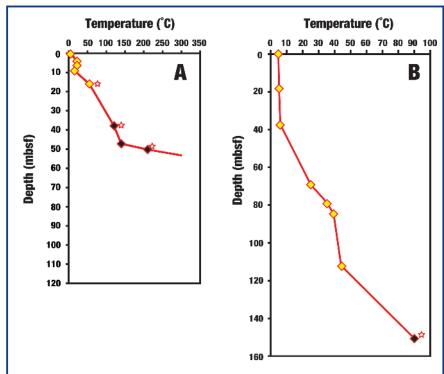


Figure 7. Depth profile of temperature in the subseafloor at Sites [A] C0014 and [B] C0017 as measured by the APCT3 shoe and thermoseal chemically impregnated temperature-sensitive strips. Yellow=APCT3, black=thermoseal strip. Stars=minimum values. Modified from Takai et al. (2011).

maximum depth of the holes drilled at these two sites. Previous surveys of the Iheya North hydrothermal vents always found that the discharging fluids were low in chloride but rich in vapor (Kawagucci et al., 2011). It was a puzzle where the complementary brines resided. Several theoretical calculations predicted that the greater densities of brines caused them to sink to greater depths within subseafloor hydrothermal reservoirs (Fontaine and Wilcock, 2006). Expedition 331 provided tentative evidence of subseafloor stratification of hydrothermal fluids that had separated phases. The post-drilling investigation of the hydrothermal fluids discharging from the natural and artificial hydrothermal vents may provide further evidence of the chemically stratified subseafloor hydrothermal fluid regime of Iheya North field.

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 meters of core from 45 m of penetration, but the core included the first Kuroko-type, sphalerite-rich black ore ever recovered from the modern subseafloor. The other four sites yielded interbedded hemipelagic and strongly pumiceous volcaniclastic sediment, along with volcanogenic breccias that are variably hydrothermally altered and mineralized, in the zeolite to greenschist facies. 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.

Shipboard analyses did not confirm the presence of an active deep hot biosphere. Cell abundances were much lower than those found in previous Ocean Drilling Program/IODP sites on continental margins (8.7 x 10<sup>5</sup>–2.4 x 10<sup>7</sup> cells mL<sup>-1</sup> sediment at Site C0013, 1.3 x 10<sup>6</sup>-4.1 x 10<sup>8</sup> cells mL<sup>-1</sup> sediment at Site C0014 and 5.8 x  $10^5$ –2.4 x  $10^7$  cells mL<sup>-1</sup> sediment at Site C0017; Takai et al., 2011), and attempts at culturing (hyper)thermophiles were generally unsuccessful. In fact, we found evidence for microbial activity in sediments within the upper 10-30 mbsf where temperatures were relatively low but little evidence in the deeper hydrothermally altered zones and hydrothermal fluid regime. Future shore-based, more detailed investigations may provide multiple lines of evidence for functionally active, metabolically diverse subseafloor microbial communities within the environments of the Iheya North hydrothermal system. In addition, the future investigations will seek to clarify the depth limits for the existence of microbial cells, metabolic activities, and living and fossil organic biomarkers in the subseafloor environment. These limits may represent a realistic boundary between the habitable and the uninhabitable zones in the subseafloor environments, namely a thermal barrier of the subseafloor biosphere.

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