doi : 10.17596/0002667

KAIREI Cruise Report

KR18-04

Recovery of temperature data and shallow sediment at IODP Site C0023

Nankai Trough off Cape Muroto

March 14 - 21, 2018

Kochi - Shimizu

Japan Agency for Marine-Earth Science and Technology

(JAMSTEC)

JAMSTEC KR18-04 cruise report: Recovery of 1.5 years-temperature observatory data and shallow piston-core sediments from IODP Site C0023

Fumio Inagaki[1,2*], Masa Kinoshita[1,3], Akira Ijiri[2], Keita Akiyama[4], Verena B. Heuer[5], Kira Homola[6], Carmen Li[7], Takashi Morisawa[8], Yuki Morono[2], Donald Pan[9], Tianhaozhe Sun[10], Man-Yin Tsang[11], Tomohiro Toki[12], and the International Ocean Discovery Program (IODP) Expedition 370 Scientists

*Correspondence author (Chief scientist and Representative of Science Party)

[1] Research and Development Center for Ocean Drilling Science, Japan Agency for Marine-Earth Science and Technology (JAMSTEC), Japan.
[2] Kochi Institute for Core Sample Research, JAMSTEC, Japan.
[3] Earthquake Research Institute, The University of Tokyo, Japan.
[4] Center for Deep-Earth Exploration, JAMSTEC, Japan.
[5] MARUM-Center for Marine Environmental Sciences, University of Bremen, Germany.
[6] Graduate School of Oceanography, University of Rhode Island, U.S.A.
[7] Department of Biological Sciences, University of Calgary, Canada.
[8] Graduate School of Integrated Arts and Sciences, Kochi University, Japan.
[9] Department of Subsurface Geobiological Analysis and Research, JAMSTEC, Japan.
[10] Department of Geosciences, Pennsylvania State University, U.S.A.
[11] Department of Earth Sciences, University of Toronto, Canada.
[12] Department of Chemistry, Biology and Marine Science, University of the Ryukyus, Japan.

Abstract

During the International Ocean Discovery Program (IODP) Expedition 370 in 2016, the drilling vessel *Chikyu* has established Site C0023 Hole A (Hole C0023A) off Cape Muroto, Japan. Because 20’’-casing was installed down to 181 meters below seafloor (mbsf) without coring, sedimentological, biogeochemical and microbiological processes occurring in the shallow sedimentary sequence remained unexplored. During Expedition 370, 13 temperature sensors were deployed in the cased borehole down to 863 mbsf, including the horizons associated with the décollement, which data have been continuously recorded in the data logger equipped with the CORK wellhead at Hole C0023A. To recover the missing part of shallow sediment and temperature observatory data, we revisited Site C0023 using the JAMSTEC RV *Kairei* and ROV *Kaiko* during the KR18-04 cruise in March 2018. During the KR18-04 cruise, we obtained two ~560 cm-sediment cores using 6 m-long heat flow-piston coring system nearby Site C0023, and we successfully recovered a 1.5-year record of borehole temperature data during the *Kaiko*’s Dive #377. The geophysical, geochemical, microbiological data and samples obtained during the KR18-04 cruise provide crucial information to understand how microbial communities in shallow sediments of the protothrust subduction zone off Cape Muroto are sensitive and/or persistent to environmental factors, such as elevating temperature with increasing burial depth and time at Site C0023.

Introduction

Scientific ocean drilling has revealed the existence of live microbial cells in deeply buried sediments and basement rocks all over the ocean-floor. The metabolic activities of these organisms are very low (D'Hondt et al., 2002, 2004), but most deeply buried microbial cells are physiologically active (Morono et al., 2009; Trembath-Reichert et al., 2017), quiescent as dormant phase or spore (Lomstein et al., 2012). In total, the sub-seafloor biosphere is thought to contain as many microbial cells as all the aquatic habitat in the global oceans, but its exact size is still a matter of debate (Parkes et al., 1994, 2000; Whitman et al., 1998; Lipp et al., 2008; Kallmeyer et al., 2012; Hinrichs and Inagaki, 2012). This is partly due to the fact that energetic and environmental factors constraining the limit of the biosphere extent remain poorly understood (Hinrichs and Inagaki, 2012). For example, temperature is commonly used as the variable defining the deepest boundary of the deep biosphere in estimates of its size (Whitman et al., 1998; Parkes et al., 2000; Lipp et al., 2008; Heberling et al., 2010; Kallmeyer et al., 2012; Parkes et al., 2014); however, only little is known about the effects that temperature and fluid supply exert on deep subseafloor life (Inagaki et al., 2015; Ijiri et al., 2018). The currently known upper temperature limit of life for microorganisms inhabiting comparatively energy-rich hydrothermal vent environments ranges around 113° to 122°C (Blöchl et al., 1997; Kashefi and Lovley, 2003; Takai et al., 2008). However, studies of petroleum biodegradation in deeply buried basins suggest that sterilization takes place at formation temperatures between 80° and 90°C (Head et al., 2003; Wilhelms et al., 2001), and this finding might be more relevant for the energy-limited subseafloor sedimentary biosphere (Hoehler and Jørgensen, 2013).

Aiming to rigorously study the influence of temperature on the size, activity, and taxonomic composition of subseafloor sedimentary microbial communities, IODP Expedition 370 (Temperature Limit of the Deep Biosphere off Muroto, 10 September – 23 November 2016) revisited an already well-characterized geological setting: the Muroto Transect in the Nankai Trough off Japan (Heuer et al., 2017). IODP Expedition 370 established Site C0023 in the vicinity of Ocean Drilling Program (ODP) Sites 1173, 1174 and 808 (Moore et al., 1991; Moore, Taira, Klaus, et al., 2001), about 125 km offshore Kochi City, Japan (**Figure F1**). Site C0023 locates at the seaward end of the Nankai Trough accretionary prism, the so called proto-thrust zone, where the Shikoku Basin is subducting underneath Japan. Previous studies demonstrated that heat flow is exceptionally high in this area, resulting in temperatures of ~110° to 130°C at the sediment-basement interface at ~1.2 kmbsf (Moore et al., 1991; Moore and Saffer, 2001). This particular geological setting enables in-depth examination of the putative temperature-dependent biotic–abiotic transition zone(s) that possibly occurs within the temperature range of the sediment column. At the same time, the increase of temperature with depth is still gradual enough for the establishment of distinct depth horizons with suitable conditions for psychrophilic (optimal growth temperature range < 20°C), mesophilic (20° to 43°C), thermophilic (43° to 80°C) and hyperthermophilic (>80°C) microorganisms. In this particular setting, Expedition 370 aimed (1) to study the factors that control biomass, activity, and diversity of microbial communities in a subseafloor environment where temperatures increase from ~2° at the sediment-water interface on the seafloor down to ~110° to 130°C at the sediment-basement interface and thus likely encompasses the biotic–abiotic transition zone, and (2) to determine geochemical, geophysical, and hydrogeological characteristics in sediments and the underlying basaltic basement and elucidate if the supply of fluids containing thermogenic and/or geogenic nutrient and energy substrates may support subseafloor microbial communities in the Nankai accretionary complex (Heuer et al., 2017).

Site C0023 was established on September 17, 2016, at 32°22.00'N, 134° 57.98'E in a water depth of 4,776 m. After stabilizing Hole C0023A with a 20'' drill-in casing down to 181 mbsf, 112 cores were taken from 189 mbsf to 1180 mbsf. In situ temperature measurements were conducted down to 410.5 mbsf using a modified Hydraulic Piston Coring System (HPCS) equipped with the APCT-3 tool. In order to stabilize the borehole condition, coring operations were interrupted for the installation of a casing down to 858 mbsf. After completion of all coring operations, a temporary temperature observatory was installed; to this end, the Circulation Obviation Retrofit Kit (CORK)-wellhead was equipped with a landing platform for remotely operated vehicles (ROV), and thermistor arrays were installed in the borehole down to 863 mbsf. The thermistors monitor temperature mainly inside the cased borehole at 13 depth horizons and data are recorded continuously by data loggers mounted in the Site C0023 CORK (**Figure F2**).

In March 2018, JAMSTEC RV *Kairei* KR18-04 cruise returned to Site C0023 with the mission (1) to retrieve the first 1.5 year-long record of temperature data from the temporary temperature observatory using the ROV *Kaiko,* (2) to perform temperature measurements in surface sediments, and (3) to collect samples for microbiological, geochemical, and geological investigations from shallow sediments using both push corers during ROV *Kaiko* dives and a 6-m-long piston core system. These data and samples are valuable additions for the comprehensive understanding of the limits and habitability of the deep subseafloor biosphere in the Nankai subduction zone off Cape Muroto.

Cruise Operations

Using the RV *Kairei* and the ROV *Kaiko,* Site C0023 was investigated during KR18-04 cruise from March 14 (Kochi port) to March 21 (Shimizu port) in 2018.

On March 15 after a day transit with the RV *Kairei* from the Kochi port to Site C0023 off Cape Muroto, we conducted the ROV *Kaiko* Dive #733. We confirmed the seabed at ~90 m from Site C0023, heading north to the CORK-wellhead where the acoustic sonar indicated. We approached to the CORK at Site C0023, and then the *Kaiko* landed on the ROV platform. First, we tried to connect the communication and battery cable of the *Kaiko* system to the temperature-data logger kit ("plan A"). However, no data signals could be detected from the logger. Then, we took "plan B" to recover all the temperature-data logger module including the battery, detached it from the CORK, and then retrieved by locking with the *Kaiko* (**Figure F3**). During the operation, we took three 30 cm-long push core samples (one of three cores was empty) at 50 m southwest from Site C0023 (**Table F1**), and then the *Kaiko* took off the seabed after the 3 hours dive. The temperature data accumulated over 1.5 years were successfully recovered by direct communication to the retrieved data logger onboard.

Unexpectedly, another attempt for the direct communication to the data logger was not successful, indicating some fatal malfunction of the data logger right after the first data retrieval. Consequently, we decided not to re-install the same data logger to Site C0023 and thus the continuous temperature monitoring is suspended from March 15 in 2018. During the night, we performed side-scan sonar at around Site C0023.

On March 16 and 17, no research operations were conducted because of bad weather.

On March 18, we obtained two heat flow-piston cores at ~460 m south (~210°) from Site C0023. Using 6 m-length piston core barrel, two sediment cores were retrieved, which are almost full recovery of this equipment (**Table F1**). The heat flow-temperature data in the shallow sediment were also simultaneously obtained. The first piston core (HFPC-01) was processed onboard for microbiological and biogeochemical analyses, whereas the second piston core (HFPC-02) was used for the onboard measurement of thermal conductivity, followed by the X-ray computer tomography (CT) image scan of whole round core-sections and sedimentological core description at the Kochi Core Center. During the night, we performed again side scan sonar around Site C0023.

On March 19, we tried to recover shallow sediment cores using the heat flow-piston coring system at ODP Site 1173. However, during the system deployment under the sea, it was found to be difficult to control the stable positioning of the coring system by the operation of *Kairei* because of the bad weather and very strong Kuroshio sea current, which made the winch cable notably bending. Because of the safety reason, we pulled up again the coring system onboard without sediment coring.

Then, it was decided by the captain and chief scientist that no operations could be done in the remaining dates during this cruise, and therefore we started moving to the Shimizu port toward the end of the KR08-04 cruise.

Data, Sample, and Preliminary Results

Physical Property

Temperature data recovery from thermister strings

Figure F3 shows the configuration of the connector-battery module system. After data recovery, it was found that some sensors did not work after the end of May in 2017; i.e., temperature data are too noisy and thus not trustable. Nevertheless, 6 (or 7) thermistor sensors were found to remain safely recording in-situ temperatures for 1.5 years. **Figure F4** shows the time-series data plot of in-situ temperature recorded on the logger. The observed temperatures were more than 70% of the original formation temperatures at the time of deployment over the period of 1.5 years, whereas some sensors reached their maximum temperatures in 200 days, followed by a gradual decrease with time. In those profiles, we also observed some simultaneous steps and/or spikes in temperatures (e.g., end of May 2017), which might be caused by either artificial noise in the data logger or some transient natural phenomena, such as fluid flow events. The reason for temperature anomalies will need further onshore investigations. Overall, we successfully confirmed the temperature prediction based on APCT-3 and thermal conductivity data during Expedition 370 by the direct measurement of in-situ temperatures down to the décollement layer (**Figure F5**).

Thermal conductivity and heat flow measurement

Two heat flow measurements were carried out during piston coring operations (HFPC-01 and -02). We deployed a stand-alone heat flow probe (SAHF) and five temperature loggers (MTLs: Multiple-Temperature Loggers) to the piston core barrel (**Figure F6**). Just before the penetration, the piston corer was kept at ~100 meters above the seafloor for 5 minutes to measure the reference bottom-water temperature. After that the piston corer was lowered slowly until the pilot corer triggered the balance for a free-fall launching of the piston corer, core-penetration was identified on board of the RV *Kairei* as a sudden decrease in tension to the wire. After that, the corer stayed in the sediment for 15 minutes to record the changing temperatures, which were used to infer the in-situ formation temperatures by extrapolation.

The first piston core (HFPC-01, **Table F1**) was obtained from ~467 meters to 210° from Site C0023. During this piston coring operation, we could recover temperature data from SAHF only. The core recovery was almost 100%, indicating the penetration of all SAHF sensors into sediments. The second piston core (HFPC02, **Table F1**) was ~458 meters to 209° from Site C0023. We recorded temperatures from both SAHF and other temperature sensors. The core recovery was almost 100%, again indicating the penetration of all SAHF and other temperature sensors deployed on the core barrel.

Thermal conductivity (K) was measured at HFPC02 core section ends by a needle probe method (von Herzen and Maxwell, 1959) using a commercial instrument (KD Pro-2, Decagon Devices, Inc., Pullman WA, U.S.A.). Measurements were made at every 50 cm interval.

Profiles for temperature vs. time and vs. depth are both linear, indicating that the thermal conduction regime near the seafloor is constant (**Figure F7**). By applying a least-squares fitting to the estimated formation temperatures, we obtained geothermal gradients (G). Heat flow at each site was calculated as a product of G and K, which was measured on HFPC-02 cores (see next section). Consequently, heat flow obtained from HFPC-01 and HFPC-02 data ranges from 154 to 172 mW/m^2 (**Table F2**). It is higher than the value estimated from APCT-3 temperature data during Expedition 370 (i.e., 140 to 145 mW/m^2; Heuer et al., 2017).

Geochemistry

Porewater extraction by squeezer and Rhizon^{TM} sampler

Porewater was extracted from all core types using Rhizon^{TM} samplers, 0.12-0.18 µm cylindrical filters (0.2-µm Millipore PTFE filters). Push cores were sampled by inserting Rhizons^{TM} horizontally through pre-drilled holes in the core liner, enabling room temperature vertical sampling at 4 cm-depth intervals. The pilot and piston cores were sub-sampled in the form of 10 cm whole rounds at 40 cm-depth intervals. Whole round core sample (10 cm in length) were collected from the HFPC01 and pilot cores at 40-cm intervals, and pore water samples were extracted onboard using a stainless-steel squeezer (Manheim and Sayles, 1974). A Rhizon^{TM} sampler was also inserted vertically into the center of each whole round core and porewater was extracted into 24-mL syringes at 4°C. The retrieved pore water was subsequently split into sub-samples as shown in **Table F3**. In addition, for shipboard analyses 2.5 mL aliquots of the acquired pore water samples were secured in 5-mL Eppendorf tubes, and pH, alkalinity, and ammonia concentration were measured within a day.

pH/alkalinity measurement

A 2.0 mL aliquot of the acquired pore water was transferred to a 3-mL screw bottle, and an electrode was inserted into the water to measure its pH value. Subsequently, a 0.2 mL aliquot of pore water was transferred to a 5-mL screw bottle, 1.8 mL of ultrapure water was added to achieve a 1:10 dilution of the water sample before its alkalinity was measured. Alkalinity measurements were carried out by Gran's plot titration using 0.01N HCl. The standard seawater, IAPSO, was measured once a day, and the measured alkalinity of samples was corrected by IAPSO to be 2.45 mM.

Ammonia concentration

Ammonia concentration was measured by phenol blue colorimetry on 0.1 mL aliquots of the distributed pore water samples. The concentration was calibrated by prepared NH_4Cl solutions (0, 10, 20, 50, 100, 200, 500, 750, and 1000 µM) that were colored simultaneously. Samples below Section 2 in the HFPC-1 core showed much higher absorbance than the calibration curve (absorbance > 1) and were re-measured after 10 times dilution.

Shipboard examination of sulfate reduction

0.5 mL of saturated zinc acetate were added into 2 to 3 mL of pore water to precipitate dissolved total sulfide. The zinc sulfide appeared as pale-yellow precipitates in the solution. Zinc sulfide precipitated from total dissolved sulfide was visible with naked eyes on board from 0.46 to 5.6 mbsf (C0023-HFPC-01-sec1-IWRh-46-56cm to sec6-IWSQ-91-101cm), suggesting that the piston core penetrates a part of the sulfate reduction zone.

Microbiology

Sectioned cores (1 m in length for HFPC-01 and 30cm for push cores) were further sub-sectioned into 10 cm sub-sections using sterile tools and capped prior to further sub-sampling. Sub-sections for microbiological analyses were sub-sampled by sterile tip-cut syringes. Subsamples were treated according to **Table F3**. Bottom water collected with the pilot core (HFPC-PL02) was subsampled by sterile syringe and tubing, and then a total volume of 100 mL was transferred to a sterile plastic container and stored at +4°C. The detailed onboard treatments and descriptions for each analysis are described in below.

Cell and virus counts

2 cm^3 of the sediment were fixed with 5mL of fixing solution (4% formaldehyde and 3% NaCl) and stored at 4°C. The number of microbial cells in the fixed samples will be counted according to the previously published protocols (Morono et al. 2009; Morono and Inagaki, 2010). 2 cm^3 of sediment samples to be used for virus counts were also directly frozen at -80°C immediately after core recovery.

DNA/RNA-based molecular ecological analyses

30 cm^3 of sediment were placed in a 50 mL Falcon tube and transferred from the sterile

syringe used for sampling to sterile 50 mL falcon tubes. Falcon tubes were capped, parafilmed, and then stored immediately at -80°C in a sealed Ziploc bag. 30 cm^3 of sediment samples to be used for viral community genetic analyses were also frozen at -80°C. Molecular biology analysis including microbial community structure, metagenomics and metatranscriptomics are planned for DNA/RNA samples that will be extracted from the sediments. The extracted DNA will be used for screening of functional gene fragments by substrate-induced gene expression (SIGEX) method (Uchiyama et al., 2004) with discriminative sorting of the positive recombinant cells based on the fluorescence of green fluorescence protein co-expression.

Thermospore analysis

The sediment and bottom water samples obtained during KR18-04 cruise will be used for high temperature (50°C - 70°C) incubation studies of thermophilic endospore-forming bacteria (thermospores), during which amended organic acids will be monitored for depletion in relation to reduction of sulfate. This will complement previous incubation studies of sediment collected during the drilling of Site C0023 in 2016, where sediments from 5 different depth intervals between 206 and 865 mbsf were incubated with organic acids in the presence of sulfate. The bottom water collected from HFPC-PL02 will allow for the comparison of the thermospore community between the water column above Site C0023 and the surface and subsurface sediments of C0023. Those samples were transferred from the sterile syringe used for sampling to sterile 90-mL plastic containers. Containers were capped, parafilmed, and then stored immediately at +4°C in a sealed Ziploc bag. In addition, the metaproteomics analysis will provide insight on metabolic activities of microbial communities at Site C0023. Detected proteins will provide evidence of the environment in which these communities survive, including available energy and nutrient substrates. This information will be particularly useful when paired with metagenomics data.

Microstructure observation

To visualize microbial cells colonizing narrow habitable space in sediments, 3% of low melting agarose were placed at both end of 3-5 cm^3 of sediments sampled in tip-cut syringe. The syringe with agarose plug were placed directly into the fixing solution (4% formaldehyde and 3% NaCl) and stored at 4°C. The samples will be embedded in resin and visualized with micro-scale X-ray CT scan image analysis (Uramoto et al., 2014).

Intact polar lipids and dipicolinic acids

Sediment cores obtained during KR18-04 were subsampled to investigate the abundance and composition of microbial communities using biomarker approaches, such as intact polar lipids (IPLs) and dipicolinic acid (DPA) which are encoding information on active microbial cells and spores, respectively (Lipp et al., 2008; Lomstein et al., 2012). The obtained sample complements the depth profile obtained for Hole C0023A during post-cruise research of IODP Exp. 370. For the planned biomarker analyses, 30 cm^3 of sediments were placed in 50-mL Falcon tubes and stored at -80°C. In addition, a large volume solid phase sample (~1 liter) was obtained by pooling parts of the push cores and piston cores that remained after sampling. This sample is stored at +4°C and will serve to study the response of the microbial community to temperature in incubation experiments, including e.g. change of microbial community, formation of spores and metabolites.

Biopolymer analysis

20 cm^3 of sediments were placed in 50-mL Falcon tube and placed in -80°C freezer. The samples will be characterized for biopolymers produced at the seafloor.

Future Prospectus

During the KR18-04 cruise with RV *Kairei* and ROV *Kaiko*, though only a day each for the *Kaiko* dive (Dive #377) and the piston coring could be conducted because of the stormy weather, we could retrieve 1.5-year record of in-situ borehole temperatures from Hole C0023A, shallow sediment core samples, and heat flow data on the seafloor. Those data and samples, including the follow-up shore-based studies, are essential to meet scientific objectives of the T-Limit project, and therefore we think that KR18-04 cruise was successful. Nevertheless, some important data and samples remain still missing from Sites 808 and 1173, where long-term observatory data for the borehole pressure need to be recovered. Site 1173, which locates in the incoming, undeformed sediment of the Shikoku Basin prior to the subduction, is a particularly important site and further scientific drilling of this site has already been approved as an original site of IODP T-Limit proposal (IODP 865-Full). A future return to Site 1173 would provide corresponding data for Site C0023 and the comparison of both sites would help to refine our insights into the temperature limit of the deep subseafloor sedimentary biosphere.

Acknowledgements

The authors thank all support provided by the captain and crews of the RV *Kairei* and the ROV *Kaiko* team, and shipboard technicians aboard the RV *Kairei* during the KR18-04 cruise. We also thank the science support team at the Kochi Core Center. The KR18-04 cruise is supported by Japan Agency for Marine-Earth Science and Technology. The authors sincerely acknowledge all members supporting IODP Expedition 370, and the technical support provided by the Center for Deep-Earth Exploration, JAMSTEC. The present study was supported in part by the Centre for Global Change Science (to M.-Y.T.), the Japan Society of Promotion Sciences (JSPS) KAKENHI Start-up Grant (no. 16H07489 to D.P.), the Deutsche Forschungsgemeinschaft (Grant HE 8034/1-1 to V.B.H.) and the Alfred P. Sloan Foundation for the Deep Carbon Observatory (Deep Life Community: to F.I.).

References

Blöchl, E., Rachel, R., Burggraf, S., Hafenbradl, D., Jannasch, H.W., and Stetter, K.O., 1997. *Pyrolobus fumarii*, gen. and sp. nov., represents a novel group of archaea, extending the upper temperature limit for life to 113°C. *Extremophiles*, 1(1):14–21.

D'Hondt, S., Rutherford, S., and Spivack., A.J., 2002. Metabolic activity of subsurface life in deep-sea sediments. *Science*, 295(5562):2067–2070.

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.

Head, I.M., Jones, D.M., and Larter, S.R., 2003. Biological activity in the deep subsurface and the origin of heavy oil. *Nature*, 426(6964):344–352.

Heberling, C., Lowell, R.P., Liu, L., and Fisk, M.R., 2010. Extent of the microbial biosphere in the oceanic crust. *Geochemistry, Geophysics, Geosystems*, 11(8):Q08003.

Heuer, V.B., Inagaki, F., Morono, Y., Kubo, Y., Maeda, L., and the Expedition 370 Scientists, 2017. *Expedition 370 Preliminary Report: Temperature Limit of the Deep Biosphere off Muroto*. International Ocean Discovery Program. http://dx.doi.org/10.14379/iodp.pr.370.2017

Hinrichs, K.-U., and Inagaki, F., 2012. Downsizing the deep biosphere. *Science*, 338(6104):204–205.

Hoehler, T. M., and Jørgensen, B. B., 2013. Microbial life under extreme energy limitation. *Nature Reviews Microbiology*, 11(2):88-94.

Ijiri, A., Inagaki, F., Kubo, Y., Adhikari, R. R., Hattori, S., Hoshino, T., Imachi, H., Kawagucci, S., Morono, Y., Ohtomo, Y., Ono, S., Sakai, S., Takai, K., Toki, T., Wang, D. T., Yoshinaga, M. Y., Arnold, G. L., Ashi, J., Case, D. H., Fesker, T., Hinrichs, K.-U., Ikenaga, Y., Ikehara, M., Kallmeyer, J., Kumagai, H., Lever, M. A., Morita, S., Nakamura, K., Nakamura, Y., Nishizawa, M., Orphan, V. J., Røy, H., Schmidt, F., Tani, A., Tanikawa, W., Terada, T., Tomaru, H., Tsuji, T., Tsunogai, U., Yamaguchi, Y. T., and Yoshida, N., 2018. Deep-biosphere methane production stimulated by geofluids in the Nankai accretionary complex. *Science Advances*, 4(6):eaao4631.

Inagaki, F., Hinrichs, K.-U., Kubo, Y., Bowles, M.W., Heuer, V.B., Long, W.-L., Hoshino, T., Ijiri, A., Imachi, H., Ito, M., Kaneko, M., Lever, M.A., Lin, Y.-S., Methé, B.A., Morita, S., Morono, Y., Tanikawa, W., Bihan, M., Bowden, S.A., Elvert, M., Glombitza, C., Gross, D., Harrington, G.J., Hori, T., Li, K., Limmer, D., Liu, C.-H., Murayama, M., Ohkouchi, N., Ono, S., Park, Y.-S., Phillips, S.C., Prieto-Mollar, X., Purkey, M., Riedinger, N., Sanada, Y., Sauvage, J., Snyder, G., Susilawati, R., Takano, Y., Tasumi, E., Terada, T., Tomaru, H., Trembath-Reichert, E., Wang, D.T., and Yamada, Y., 2015. Exploring deep microbial life in coal-bearing sediment down to ~2.5 km below the ocean floor. *Science*, 349(6246):420–424.

Kallmeyer, J., Pockalny, R., Adhikari, R.R., Smith, D.C., and D'Hondt, S., 2012. Global distribution of microbial abundance and biomass in subseafloor sediment. *Proceedings of the National Academy of Sciences of the United States of America*, 109(40):16213–16216.

Kashefi, K., and Lovley, D.R., 2003. Extending the upper temperature limit for life. *Science*, 301(5635):934.

Lomstein, B.A., Langerhuus, A.T., D'Hondt, S., Jørgensen, B.B., and Spivack, A., 2012. Endospore abundance, microbial growth and necromass turnover in deep sub-seafloor sediment. *Nature*, 484(7392):101–104.

Lipp, J.S., Morono, Y., Inagaki, F., and Hinrichs, K.-U., 2008. Significant contribution of Archaea to extant biomass in marine subsurface sediments. *Nature*, 454(7207):991–994.

Moore, G.F., Shipley, T.H., Stoffa, P.L., Karig, D.E., Taira, A., Kuramoto, S., Tokuyama, H., and Suyehiro, K., 1990. Structure of the Nankai Trough accretionary zone from multichannel seismic reflection data. *Journal of Geophysical Research: Solid Earth*, 95(B6):8753–8765.

Moore, G.F., Taira, A., Klaus, A., et al., 2001. *Proceedings of the Ocean Drilling Program, Initial Reports*, 190: College Station, TX (Ocean Drilling Program). http://dx.doi.org/10.2973/odp.proc.ir.190.2001

Moore, J.C., and Saffer, D., 2001. Updip limit of the seismogenic zone beneath the accretionary prism of southwest Japan: an effect of diagenetic to low-grade metamorphic processes and increasing effective stress. *Geology*, 29(2):183–186.

Morono, Y., Terada, T., Masui, N., and Inagaki, F., 2009. Discriminative detection and enumeration of microbial life in marine subsurface sediments. *The ISME Journal*, 3(5):503–511.

Morono, Y., and Inagaki, F., 2010. Automatic slide-loader fluorescent microscope for discriminative enumeration of subseafloor life. *Scientific Drilling*, 9:32–36.

Morono, Y., Terada, T., Nishizawa, M., Ito, M., Hillion, F., Takahata, N., Sano, Y., and Inagaki, F., 2011. Carbon and nitrogen assimilation in deep subseafloor microbial cells. *Proceedings of the National Academy of Sciences of the United States of America*, 108(45):18295–18300.

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*, 371(6496):410–413.

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

Parkes, R.J., Cragg, B., Roussel, E., Webster, G., Weightman, A., and Sass, H., 2014. A review of prokaryotic populations and processes in sub-seafloor sediments, including biosphere:geosphere interactions. *Marine Geology*, 352:409–425.

Takai, K., Nakamura, K., Toki, T., Tsunogai, U., Miyazaki, M., Miyazaki, J., Hirayama, H., Nakagawa, S., Nunoura, T., and Horikoshi, K., 2008. Cell proliferation at 122°C and isotopically heavy CH_4 production by a hyperthermophilic methanogen under high-pressure cultivation. *Proceedings of the National Academy of Sciences of the United States of America*, 105(31):10949–10954.

Trembath-Reichert, E., Morono, Y., Ijiri, A., Hoshino, T., Dawson, K. S., Inagaki, F., and Orphan, V. J.

(2017) Methyl-compound use and slow growth characterize microbial life in 2 km-deep subseafloor coal and shale beds. *Proceedings of the National Academy of Sciences of the United States of America*, **114**: E9206-E9215.

Uchiyama, T., Abe, T., Ikemura, T., and Watanabe, K., 2004. Substrate-induced gene-expression screening of environmental metagenome libraries for isolation of catabolic genes. *Nature Biotechnology*, 23(1):88-93.

Uramoto, G.-I., Morono, Y., Uematsu, K., and Inagaki, F., 2014. An improved sample preparation method for imaging microstructures of fine-grained marine sediment using microfocus X-ray computed tomography and scanning electron microscopy. *Limnology and Oceanography: Methods*, 12:469-483.

Whitman, W.B., Coleman, D.C., and Wiebe, W.J., 1998. Prokaryotes: the unseen majority. *Proceedings of the National Academy of Sciences of the United States of America*, 95(12):6578–6583.

Wilhelms, A., Larter, S.R., Head, I., Farrimond, P., di-Primio, R., and Zwach, C., 2001. Biodegradation of oil in uplifted basins prevented by deep-burial sterilization. *Nature*, 411(6841):1034–1037.

Table F1. Sediment samples corrected at Site C0023 during KR18-04 cruise.

Core number	Core type	Coordinates	Water depth (m)	Penetrations (cm)
C0023-Dive773-BY	Push core	32°22.0297’N, 134°58.0847’E	4769	30
C0023-Dive773-Y	Push core	32°22.0273’N, 134°58.0690’E	4769	28.5
C0023-Dive773-R	Push core	32°22.0273’N, 134°58.0690’E	4769	0 (lost)
C0023-HFPC-01	Piston core	32°21.7898’N, 134°57.8374’E	4791	565
C0023-HFPC-PL01	Pilot core	32°21.7898’N, 134°57.8374’E	4791	141
C0023-HFPC-02	Piston core	32°21.7939’N, 134°57.8682’E	4790	550
C0023-HFPC-PL02	Pilot core	32°21.7939’N, 134°57.8682’E	4790	165

Table F2. In situ temperature data of heat flow measurement at Site C0023 during the KR18-04 cruise and IODP Expedition 370 (Heuer et al., 2017).

Core/Hole	Type	Number of sensors	Temperature gradient (mK/m)	Thermal conductivity (W/m/K)	Heat flow (mW/m^2)
C0023-HFPC-01	SAHF	5	222	0.78	173
C0023-HFPC-02	SAHF	4	198	0.78	154
C0023-HFPC-02	MTL	5	220	0.78	172
IODP C0023 Hole A	APCT-3	5	111	1.28	140-145

Table F3. Microbiological sub-sampling for sediment cores during KR18-04 cruise.

Sub-sample	Volume (cm^3)	Storage condition
IPL/DPA	30	Frozen at -80°C
T-gradient incubation	125	Stored at +4°C
HPT cultivation	30	Stored at +4°C
Cell counts	2	Fixed and stored at +4°C
DNA	2	Frozen at -80°C
Microstructure	5	Fixed and stored at +4°C
Virus community	30	Frozen at -80°C
Virus counts	2	Frozen at -80°C
Incubations	50	Stored at +4°C
Metaproteomics	50	Frozen at -80°C
Polymer	20	Frozen at -80°C
SIGEX	30	Frozen at -80°C

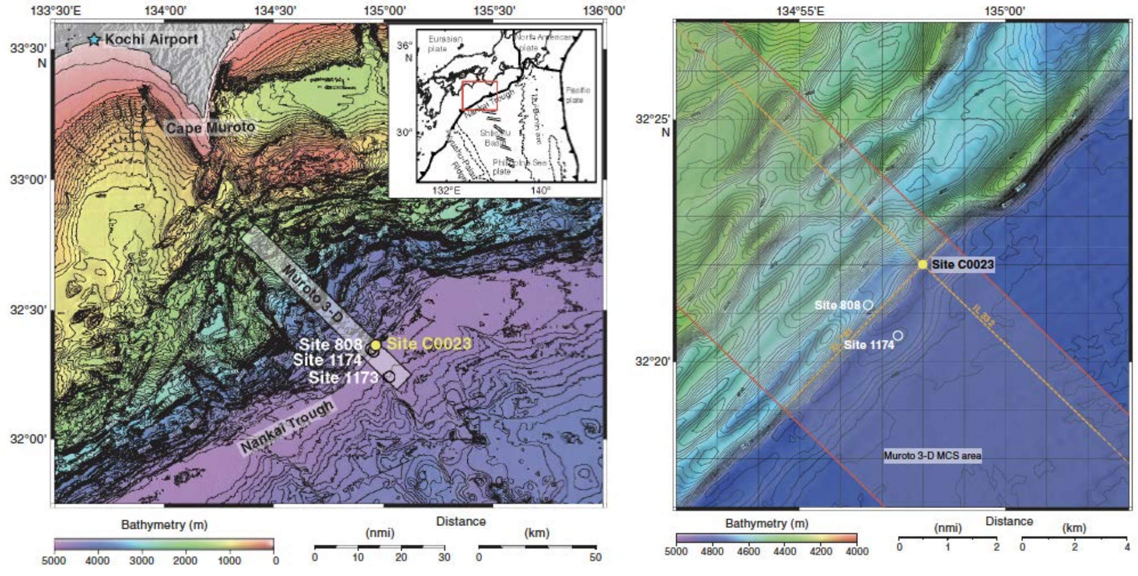

Figure F1. Regional bathymetry map with drilling site locations off Cape Muroto (right). Inset: general tectonic configuration of Japanese Island system. Close-up bathymetry map showing IODP Site C0023 (T-Limit), ODP Sites 808, and 1174 (left). Yellow circle = Site C0023, open white circles = existing nearby ODP drill sites. Red outline = area of 3-D seismic reflection volume of the Muroto Transect, yellow thick dashed lines = cropped seismic sections of In-line (IL) 332 and Cross-line (XL) 781. MCS = multichannel seismic.

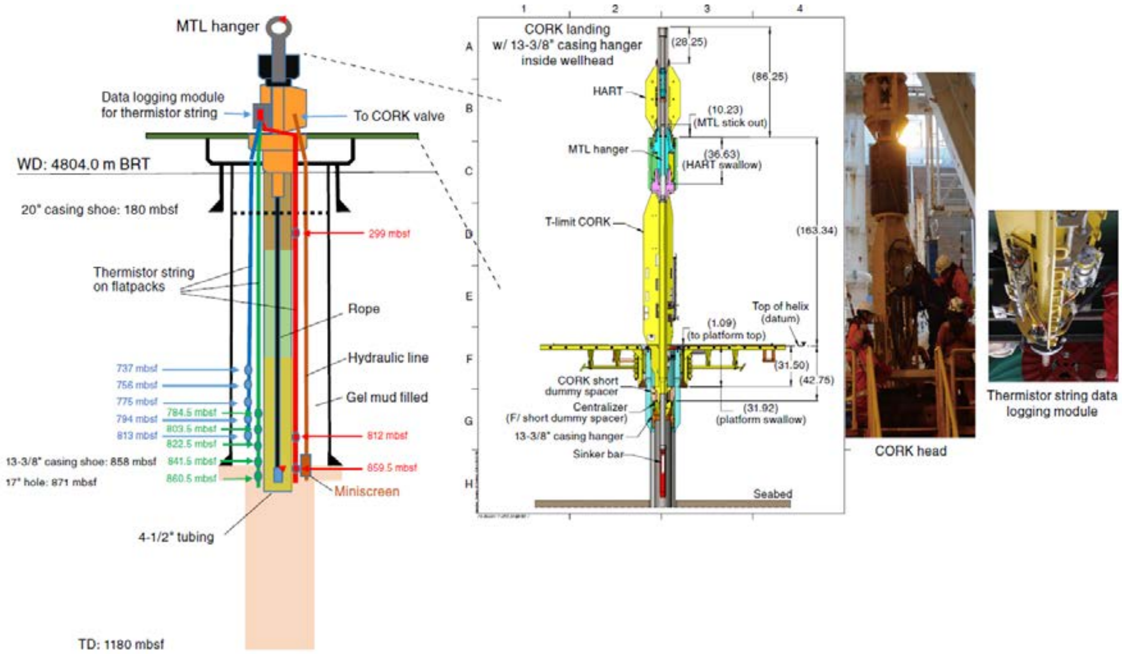

Figure F2. Overall configuration of T-Limit temperature observatory system at Site C0023 Hole A.

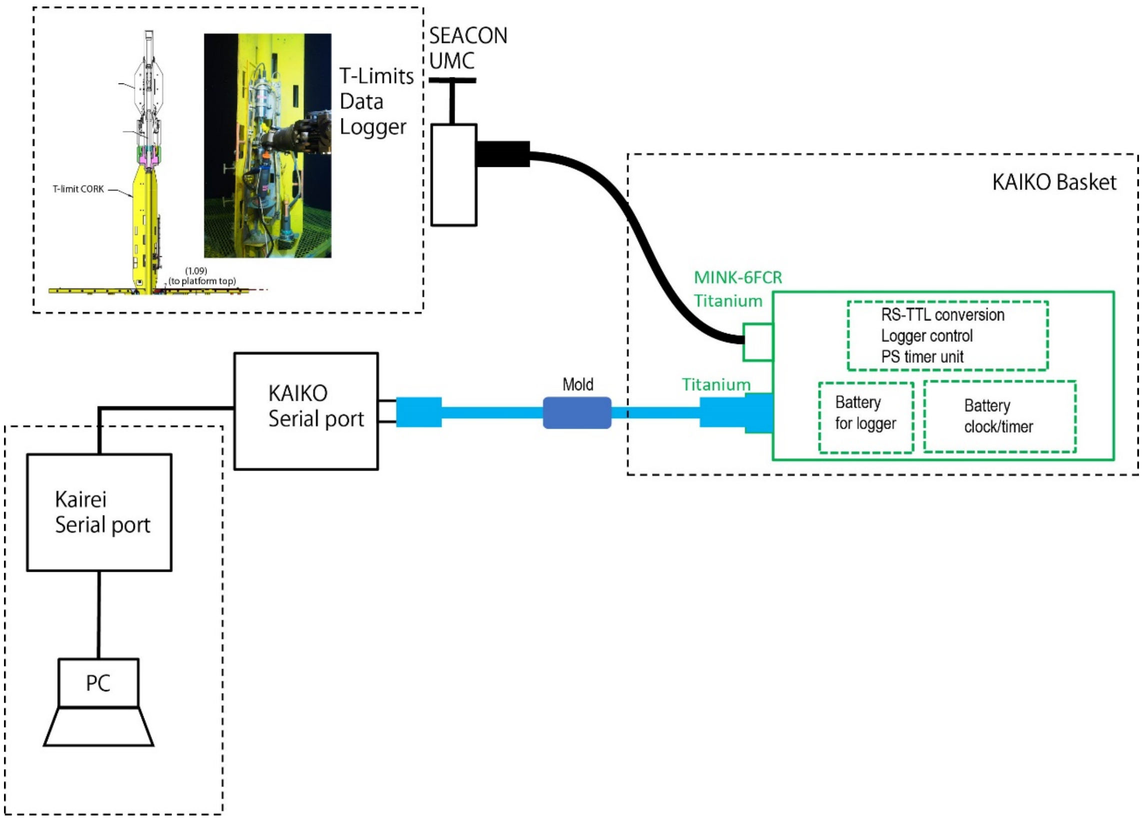

Figure F3. Schematic figure of the configuration of data retrieval and battery module used during KR18-04 cruise.

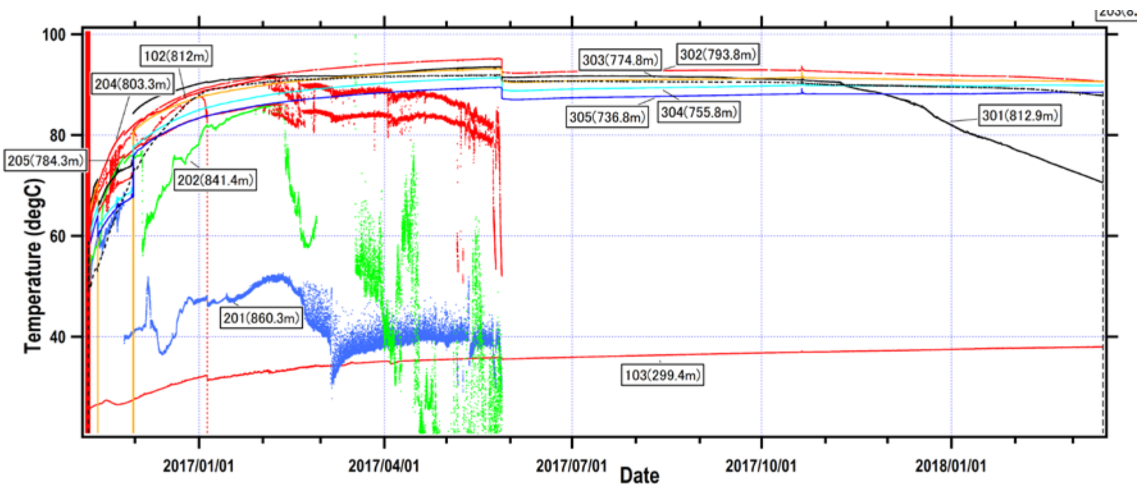

Figure F4. Temperature data recovered from thermistor sensors deployed in Hole C0023A.

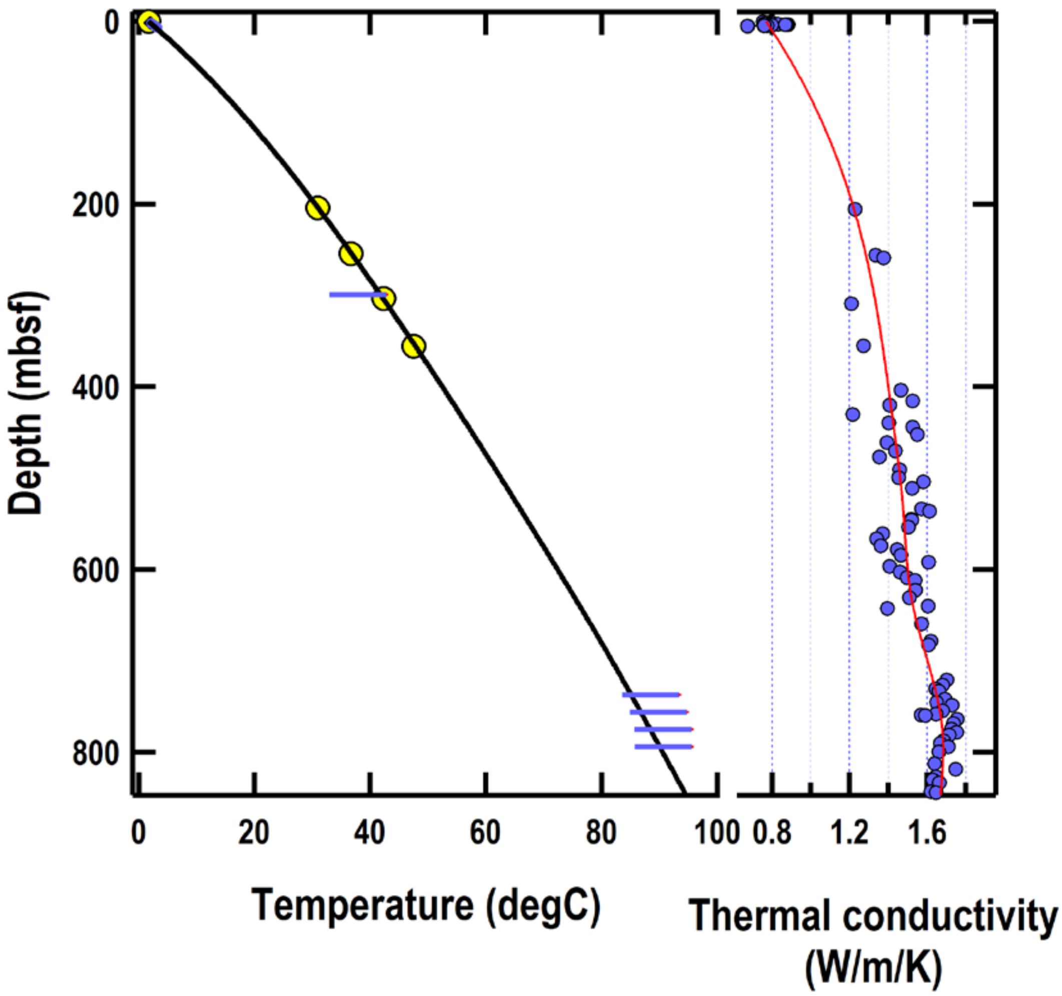

Figure F5. Temperature data versus sediment depth at Site C0023. Yellow dots represent APCT-3 data. Black curve shows a prediction based on in situ temperature (APCT-3, yellow dots) data and thermal conductivity data (blue dots in right panel) measured during IODP Expedition 370 (Heuer et al., 2017). Blue bars represent preliminary temperature data obtained from thermistor sensors during KR18-04 cruise.

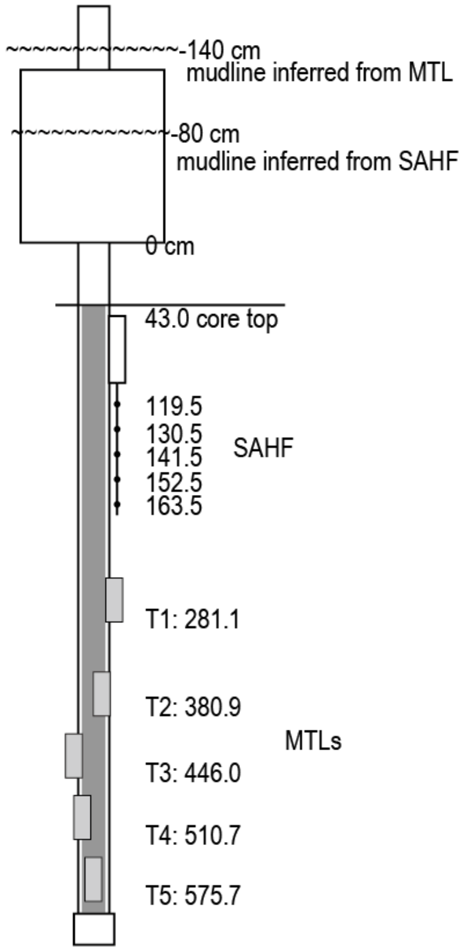

Figure F6. Position of heat flow sensors on a stand-alone heat flow probe (SAHF) and five temperature loggers (MTLs: Multiple-Temperature Loggers) in the temperature observatory of Site C0023.

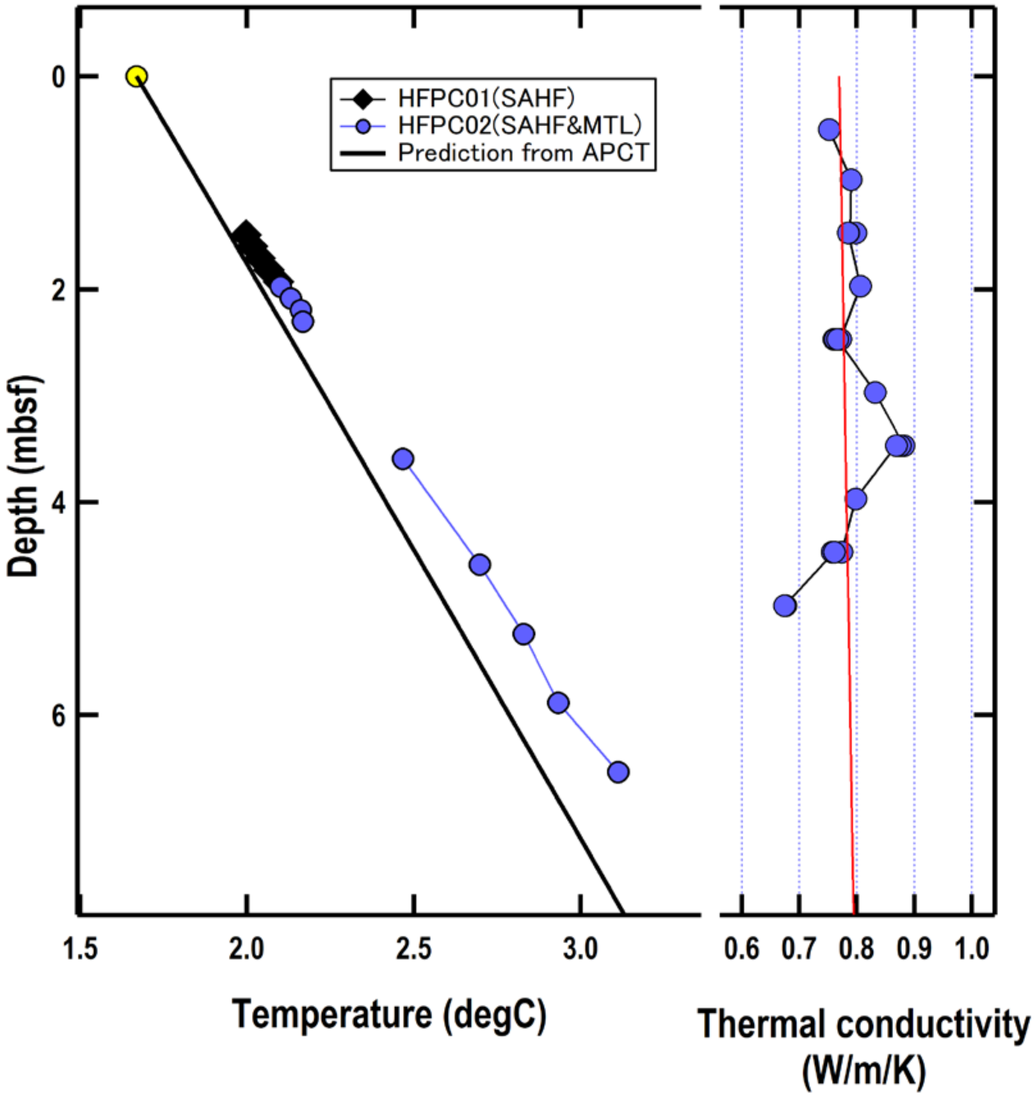

Figure F7. Temperature and thermal conductivity versus depth profiles obtained during piston coring operations for C0023-HFPC-01 and C0023-HFPC-02 during KR18-04 cruise. Note that the depth values for temperature are preliminary and subject to change, and the depths for thermal conductivity were measured from the core top.