International Ocean Discovery Program (IODP)
Expedition 370 Scientific Prospectus Summary

T-Limit of the Deep Biosphere off Muroto (T-Limit)

Deciphering factors that constrain the extent of the deep-biosphere in a subduction zone

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Introduction

Determining the vertical extent of the habitable zone on Earth and by inference the factors that limit life's maximum depths is a central goal pursued by the international marine and continental deep-biosphere community (IODP New Science Plan, Challenge 6). Ranges of environmental factors such as temperature, pH, salinity, availability of potential metabolic energy, nutrients, fluid movement, and pore space are known to exert controls on habitability with temperature commonly used as the variable defining the deepest boundary of the deep biosphere in estimates of its size.

The currently known upper temperature limit of life for microorganisms inhabiting comparatively energy-rich hydrothermal vent environments is in the range of 113–122°C (Blöchl et al., 1997; Kashefi and Lovley, 2003; Takai et al., 2008). On the other hand, the estimates relevant for the energy-limited marine sedimentary biosphere are derived from studies of petroleum biodegradation, suggesting that sterilization takes place at formation temperatures in the range of 80–90°C (Head et al., 2003; Wilhelms et al., 2001). The lower temperature limit in subsurface relative to hydrothermal settings is possibly linked to the enhanced requirement of metabolic energy at elevated temperatures for the repair of degraded biomolecules (cf. Head et al., 2003); the potential supply of metabolic energy is low in sedimentary settings that tend to be lean in electron acceptors and/or donors compared to hydrothermal vents, at which reduced fluids are mixed with oxygenated seawater. The response of subseafloor communities to temperature might be complex. Experimental studies with surface sediments demonstrate a non-linear effect of temperature on biogeochemistry and prokaryotes, with rapid changes occurring over small temperature intervals, and reveal that substrate addition and increase of metabolic energy result in a stimulation of microbial activities at elevated temperatures (Roussel et al., 2015). The influence of temperature on microbial communities was one of the central questions during the IODP Expedition 331 at the Iheya North hydrothermal field in the Okinawa Trough, but this task was severely complicated by extremely high geothermal gradients of 0.6–7°C m^{-1}. Consequently, microbial life seemed to cease within a few tens of meters below seafloor and strongly condensed temperature zones prevented in-depth examination of critical depth intervals. To date, the influence of temperature on the amount, activity and taxonomic composition of deep subseafloor sedimentary biomass has not been rigorously studied through scientific ocean drilling combined with state-of-the-art investigative techniques.

During the IODP T-Limit project, we will revisit the Muroto Transect in the Nankai Trough off the Kochi Prefecture, Japan, to explore the limits of subseafloor life and the conditions conducive to its sustenance in the vicinity of Ocean Drilling Program (ODP) Sites 1173, 1174, and 808 (Fig. F1). In this area of the Nankai Trough, the heat flow is exceptionally high and results in temperatures of ~110–130°C at the sediment-basement interface at around 0.7–1.2 kmbsf (Moore et al., 1991; Moore and Saffer, 2001). This particular geological setting will enable in-depth examination of the temperature-dependent biotic–abiotic transition zone; 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 (20-45°C), thermophilic (45-80°C) and hyperthermophilic (>80°C) microorganisms.

The IODP T-Limit project will build on data sets resulting from ODP Legs 190 and 196. For Sites 1173 and 1174, depth profiles of microbial cell concentrations (Fig. F2) were obtained by manual microscopic cell count with a minimal quantification limit of ~10^5 cells cm^{-3}. Cell concentrations appeared to drop abruptly to non-detectable levels at sediment depths around 500 and 800 meters below seafloor (mbsf) at Sites 1173 and 1174, respectively, where estimated *in situ* temperatures exceed 85–90°C (Shipboard Scientific Party, 2001). Our recent findings for deep subseafloor sediments drilled using the *Chikyu*'s riser drilling system during the IODP Expedition 337 in the northwestern Pacific coast off the Shimokita Peninsula, Japan, underline the relevance of our research question and highlight the advancement of scientific technologies since Leg 190 in 2000. Cell concentrations dropped drastically in ~40–60°C sediments at depths below ~1.5 kmbsf, but indigenous microbial cells were detected down to 2.4 kmbsf with concentrations ranging from <10 to ~10^4 cells cm^{-3} and peaking in sediments associated with lignite coalbed layers (Inagaki et al, 2015).

Given the recent technological progresses, the IODP T-Limit project aims (1) to study the factors that control biomass, activity and diversity of microbial communities in a subseafloor environment where temperatures increase from ~30°C to 130°C 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.

In order to fully exploit the technological potential available to us today, the scientific work program onboard the *Chikyu* will be complemented by simultaneous analytical work at the Kochi Core Center (KCC), which is located within reach of a helicopter shuttle for transportation of freshly cored samples. The investigation of microbial communities and processes close to the limits of subseafloor life and the deep biosphere will in particular benefit from super-clean room facilities, aseptic core sampling techniques, and the state-of-the-art analytical infrastructures at KCC, such as nanoscale secondary ion mass spectrometry (NanoSIMS). Scientists onboard the *Chikyu* and at KCC will process samples, share data, and report results together as one team of expedition scientists according to IODP policies.

Due to the schedule of the *Chikyu*, the scientific objectives of the IODP T-Limit project, as approved by IODP's Science Evaluation Panel recommendations on proposal 865-Full, cannot be achieved within a single expedition. In order to increase flexibility, we have split up the complete work program into work packages that we aim to accomplish with two expeditions as ship time becomes available during suitable seasons. Expedition 370 is the first expedition of the IODP T-Limit project. It will drill one of two proposed sites (i.e., ODP11-74B) in the vicinity of ODP Site 1174 down to the basement in order to (1) retrieve high quality sediment and basalt core samples from critical temperature intervals, (2) conduct *in situ* temperature measurements during drilling, and (3) deploy a temporary temperature observatory (TTO) into the borehole.

IODP Expedition 370 scientific objectives

Expedition 370, as the first expedition of the IODP T-Limit project, will specifically address the following questions:

- Do subseafloor sedimentary microbial communities populate sediments that lie within the temperature range close to the putative limit of microbial life?
- Is the temperature increase with depth accompanied by substantial changes in the composition, function, and activity of microbial communities?
- Does the population density of microbial communities decrease gradually or abruptly with increasing temperature? And do features like porosity or fluid movement and the introduction of electron acceptors influence the location of the limit to life such that the biotic fringe "flickers out" over a certain depth range rather than "blinks out abruptly" at a specific depth? For example, is microbial activity stimulated by fluid flow along the décollement?
- Are ubiquitous thermophilic spores abundant, and do they germinate when temperatures increase with sediment burial?
- Which microorganisms and processes occur in the deep sulfate-methane transition zone in the depth interval of ~600–700 mbsf (~75°C)?

Characterizing the chemical and physical environment in marine sediments and the underlying basaltic basement will allow us to address questions such as:

- Which mechanisms guide the selection of microorganisms at elevated temperatures?
- Are there other adaptations evident in cells present at the biotic fringe besides tolerance to high temperatures?
- Do pairs of electron acceptors and donors accumulate that would normally be consumed by biologically mediated redox reactions?
- Is there a measurable impact of the smectite-illite transition on subseafloor microbial communities?
- Does the presumed availability of electron-acceptor-rich seawater in the basement

stimulate microbial activity in the overlying sediments? If there are active (or survived) microbial communities in the high-temperature crustal habitat, is there evidence for the inoculation of overlying sediments with basement-derived microbes?

- What is the extent and distribution of hydrothermal veining and crustal alteration related to circulation of seawater? How much of this alteration may be related to microbial activity?

Drilling and coring operations

The main operations to be completed during Expedition 370 are to drill, core and install a TTO at a single location, Site ODP11-74B. During the expedition, selected core samples will be transported to KCC via helicopter. The sequencing of operations (Fig. F3) reflects the prioritization of the activities and engineering constraints. The sequence of operations consist of:

1. Drilling and running casing to the top 140 m.
2. Continuous coring with Hydraulic Piston Coring System (HPCS) and Extended Shoe Coring System (ESCS) from ~200 mbsf to the top of basement basalt (~1210 mbsf). The modified HPCS will also be used at key intervals. Formation temperature measurement with a probe at a regular interval of 100 m.
3. Casing set to 1210 mbsf.
4. Continues coring ~50 m into the basement with Rotary Core Barrel (RCB).
5. Running tubing and completion assembly (including *in situ* temperature sensor-loggers with the autonomous string) to ~1210 mbsf.

Observatory plan

The installation plan and technical details of the observatory are not yet finalized. The following is the most likely scenario: After coring operation is completed, the 4.5 inch-diameter casing will be extended to the top of basement (~1210 mbsf). A string will be constructed by attaching multiple temperature sensor-loggers to a Vectran rope. The string of ~50 sensors will have a TTO-hangar attachment on the top and will be fixed to the wellhead for later retrieval. The sensors will be spaced following lithological and structural changes through the borehole. The exact length of the temperature string and location of the sensor loggers will be decided on board after looking at the results of coring. To retrieve the data, the string of sensor loggers will be recovered by either the *Chikyu* or other ROV-deployed research vessel (e.g., R/V *Kaiko*). If budget allows, the 4.5 inch-tubing will be comprised of an outside string of several thermistors with data telemetered to a data logger at the wellhead.

Analytical research plans

Since ODP Leg 190 in 2000, analytical methods have advanced tremendously, particularly in molecular microbiology, cell quantification, and molecular and isotopic geochemical techniques. A return to Site 1174 with a multidisciplinary team of scientists tackling novel questions and equipped with an innovative toolbox for the interrogation of microbial life, and geomicrobial processes will therefore provide new insights into the composition and functioning of the deep biosphere at this site. In order to meet the scientific objectives of Expedition 370, we seek to combine expertise in subsurface microbiology and biogeochemistry, in organic geochemistry, isotope geochemistry, mineralogy and petrology, physical properties, and local geology. In our research plans for shipboard, shore-based and post-cruise research, we seek to combine standard core descriptions with critical investigative strategies including (but not limited to):

- Culture-independent techniques targeting microbial communities and for detection of life signatures, e.g.:
  quantification of cellular biomass, spores, and viruses; DNA extraction and gene quantification, 16S rDNA amplicon sequencing, omics analyses (e.g., shotgun metagenomics, metatranscriptomics, metaproteomics), biomarker analyses (e.g., intact polar membrane lipids, menaquinones, ubiquinones and coenzyme F430), determination of aspartic acid racemization, analysis of adenosine triphosphate as proxy for metabolic energy availability
- Cultivation-based approaches for measuring activity, substrate specificity, and for isolation of microbes, e.g.:
  radiotracer-based measurements of activity, cultivation and enrichment, spore germination experiments, single-cell microbiology, stable isotope and radioisotope probing targeting biomolecules and single cells (e.g., NanoSIMS), temperature-gradient incubation experiments
- Organic matter, potential substrates and metabolites, e.g.:
  chemical kerogen analysis, kerogen maturation experiments, analysis of molecular constituents of dissolved organic matter (DOM), analysis of low-molecular-weight microbial metabolites and substrates, methane isotopologues
- Biogeochemistry of electron acceptors, nutrients, and major anions and cations
- Mineral biosignatures, e.g.;
  identification of diagnostic signatures of microbial metabolism on the surface of sediment particles (e.g., vertical scanning interferometry with a coupled RAMAN probe, fast scanning atomic force microscope, scanning electron microscope equipped with secondary-electron, backscattered and cathodoluminescence detectors, powerful EDX system)
- Thermodynamic modeling
- Quality assurance/quality control (QA/QC) for microbiological samples

Science party

To achieve the scientific objectives of the IODP T-Limit project, Expedition 370 will invite a total of 25–30 scientists, who will be assigned to shipboard team and shore-based team.

Shipboard team

The shipboard team will be in charge of sampling, QA/QC including contamination assessments, time-sensitive (bio-)geochemical and microbiological analyses, and IODP standard measurements in onboard *Chikyu* laboratories. For the shipboard team, Expedition 370 will invite 20–25 scientists, including two of the three Co-Chief Scientists, sedimentologists, organic and inorganic geochemists/biogeochemists, (geo-)microbiologists, physical property specialists, paleomagnetologists/biostratigraphers, petrologists, structural geologists, and hydrogeologists (temperature monitoring specialists). In addition to typical requirements in each of these specialties, particular needs in Expedition 370 include, but are not limited to, the following specific expertise:

- Biogeochemists and geochemists with experience/background in pore water analysis of biologically relevant chemical species, in rate measurements using radioactive tracers, in hydrogen and hydrocarbon gas analysis, and in geochemical modeling of transport and reaction of dissolved constituents;
- Microbial ecologists and molecular biologists with experience in shipboard cell enumeration, in shipboard anaerobic and aseptic sampling of sediment and rock core samples, in shipboard contamination assessment using tracers such as perfluorocarbon tracer (PFT), in cultivation or cultivation-based molecular approaches, and/or in single cell biology and (meta)genomics;
- Paleomagnetologists and biostratigraphers with experience in age determination and hole correlations in the northeastern Pacific; and
- Temperature monitoring specialists with experience in borehole observatory and *in situ* temperature measurement.

<u>Shore-based team</u>

The shore-based team will conduct an advanced set of measurements, which requires shore-based facilities in KCC, on fresh samples directly sent from the *Chikyu*. The team's activity will mainly focus on microbiological and (bio-)geochemical analyses, but analytical work of other expertise will also be considered if proposed.

For the shore-based team, Expedition 370 will invite 5–10 scientists, including one of three Co-Chief Scientists. Particular needs include, but are not limited to, the following specific expertise:

- Geochemists with experience in quantitative and compositional analysis of dissolved organic matter;
- Microbial ecologists and molecular biologists with experience in shore-based microbiological sub-sampling, in anaerobic cultivation techniques (e.g., using high-pressure chamber, flow-through bioreactor), in DNA/RNA-based molecular analyses, and/or in single cell biology and omics approaches;

The shore-based team's activity will start two weeks after the start of the expedition, and is expected to continue for the same length of time as shipboard work. The activity will be closed two weeks after the end of expedition, when the team prepares a draft report, which will be merged into the IODP Expedition Report.

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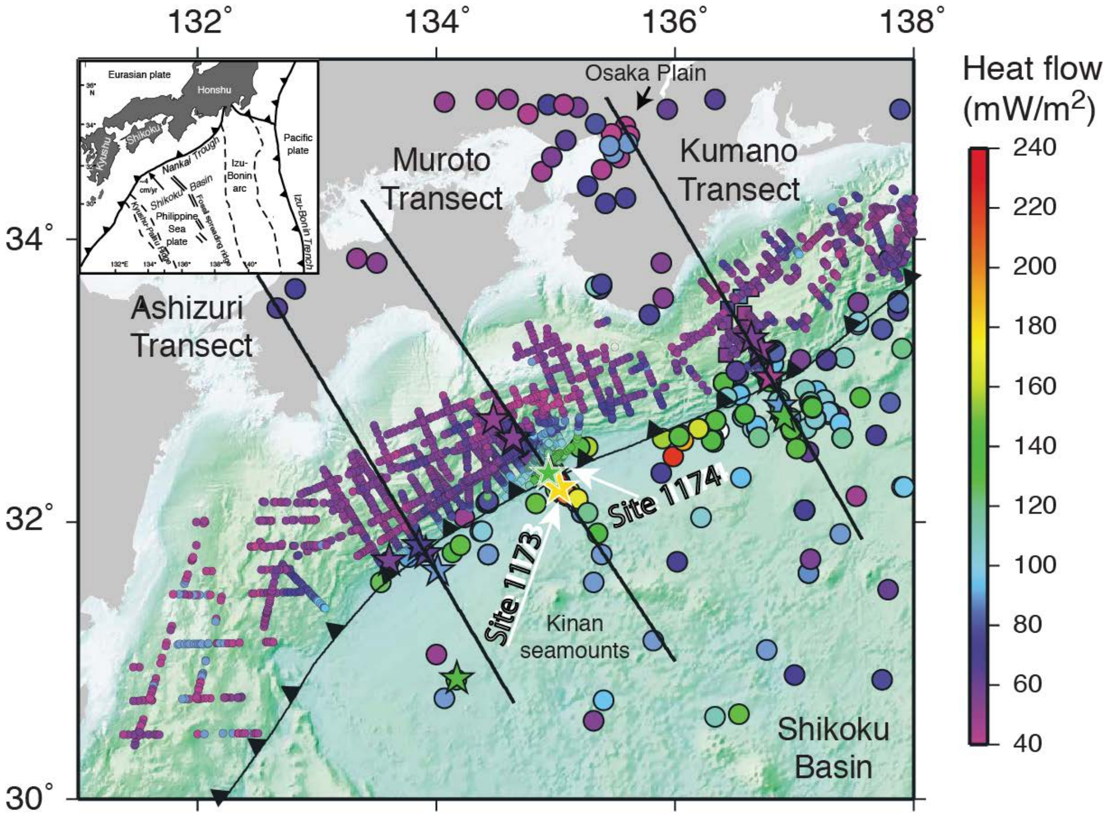

Figure F1. Map showing heat flow data and ODP/IODP Transects and Sites in the Nankai Trough (modified from Harris et al., 2013). Marine probe (circles), boreholes (stars), and bottom-simulating reflector (BSR) (small circles) are color coded by heat flow. On land, circles show borehole values of heat flow.

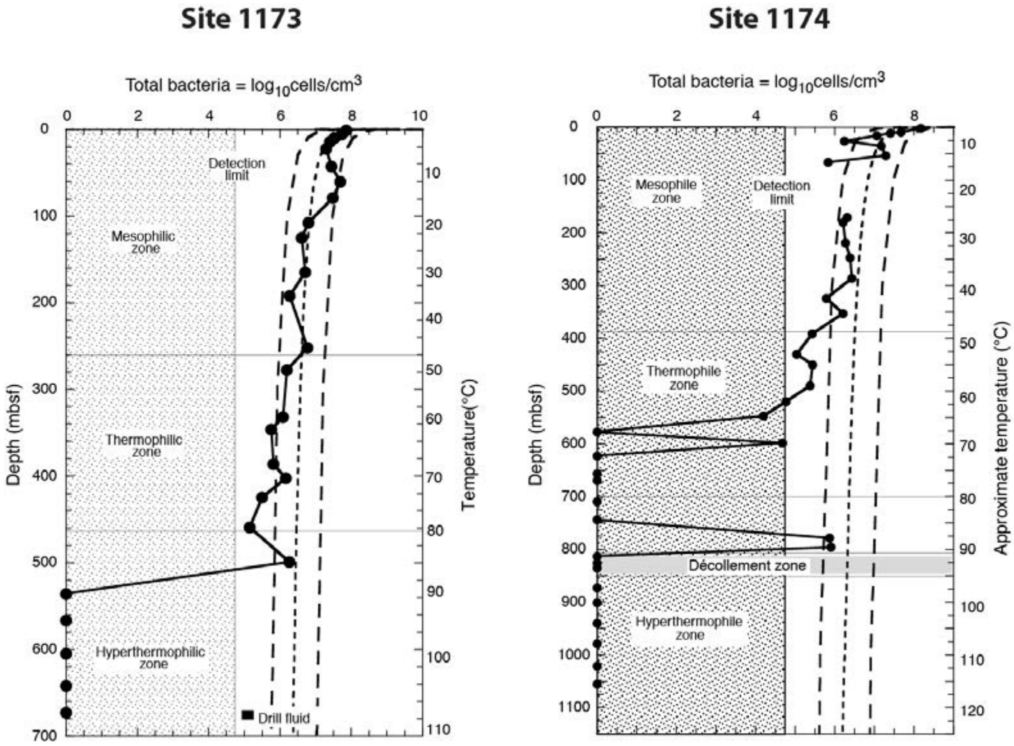

Figure F2. Cell count plots for Sites 1173 and 1174 with depth and temperature axes. At Site 1174, the first drop of cell counts below the detection limit was observed at ~550 mbsf, which corresponds to a temperature of ~65°C. Interestingly, counts exceeded the detection limit was observed again in deeper sediments just above the décollement at ~800 mbsf where temperatures reached ~90°C. Cell concentrations at Site 1173 were in a range typical for the Pacific margin sediment until ~500 mbsf (~85°C) but dropped to non-detectable levels below that depth. With improved cell separation and enumeration protocols, we expect to extend the cell concentration profiles well beyond the depths where no cells were reported during Leg 190 in 2000.

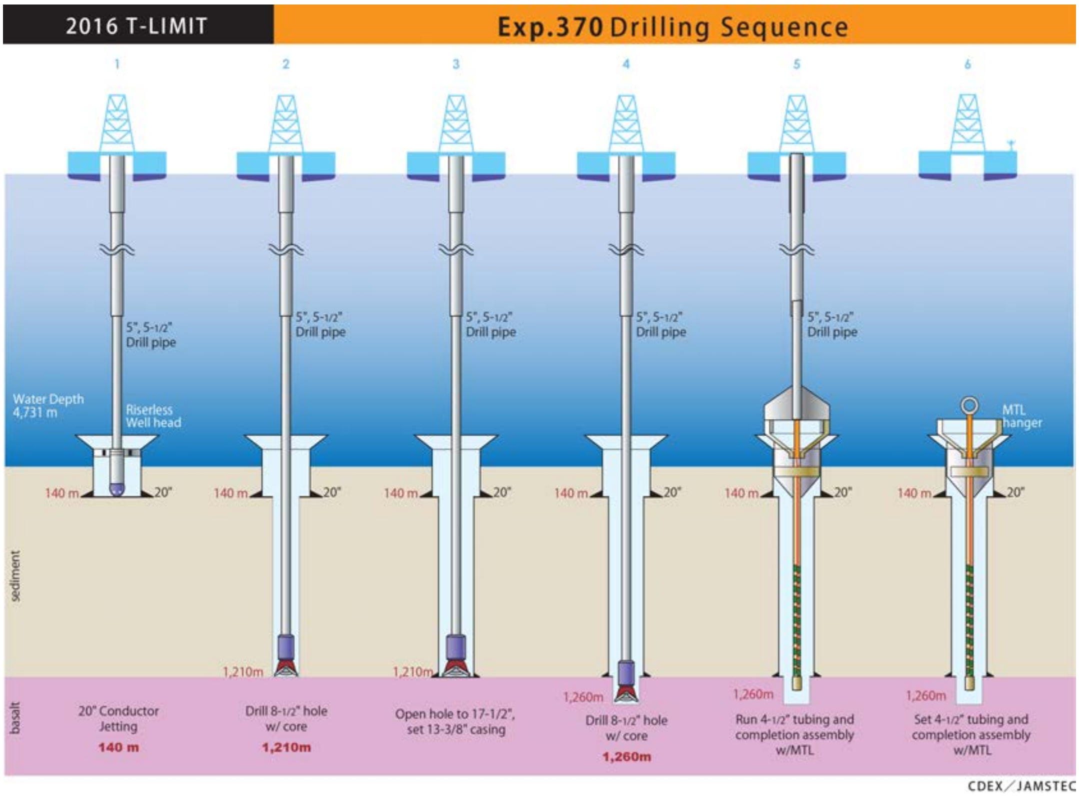

Figure F3. General sequence of Expedition 370 drilling operation.