OCEAN DRILLING PROGRAM

LEG 201 SCIENTIFIC PROSPECTUS

CONTROLS ON MICROBIAL COMMUNITIES IN DEEPLY BURIED SEDIMENTS, EASTERN EQUATORIAL PACIFIC AND PERU MARGIN

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DISCLAIMER

This Scientific Prospectus is based on precruise JOIDES panel discussions and scientific input from the designated Co-chief Scientists on behalf of the drilling proponents. The operational plans within reflect JOIDES Planning Committee and thematic panel priorities. During the course of the cruise, actual site operations may indicate to the Co-chief Scientists and the Operations Manager that it would be scientifically or operationally advantageous to amend the plan detailed in this prospectus. It should be understood that any proposed changes to the plan presented here are contingent upon approval of the Director of the Ocean Drilling Program in consultation with the Science and Operations Committees (successors to the Planning Committee) and the Pollution Prevention and Safety Panel.

Technical Editors: Karen K. Graber and Lorri Peters

ABSTRACT

During Ocean Drilling Program Leg 201, we will core and recover deep-sea sediments at a series of sites in the eastern equatorial Pacific, the Peru Basin, and the Peru Margin. The purpose of Leg 201 is to explore the distribution, activities, community structure, phylogenetic affinities, and global biogeochemical consequences of microbial communities buried in deep-sea sediments. Subsurface sedimentary environments to be explored during Leg 201 include (1) sulfate reducing, sulfate-depleted methanogenic, and sulfate-reducing methanotrophic zones of the Peru coastal margin; (2) open-ocean sulfate-rich sediments where sulfate reduction, methanogenesis, and methanotrophy apparently co-occur (equatorial Pacific); and (3) open-ocean sulfate-rich sediments where manganese reduction occurs (Peru Basin). The coastal-margin sites will include both shallow-water sites where all of the methane is present as free gas and a deep-water site where methane hydrates occur. This cruise will address several fundamental questions about the deeply buried biosphere. These include: (1) Are different sedimentary geochemical regimes characterized by very different microbial communities—or merely by shifts in the dominating organisms and the level of community activity? (2) How does hydrologic flow through deeply buried sediments and possibly through basement rock affect subsurface microbial communities and their activities? (3) To what extent do past oceanographic conditions affect microbial communities now buried in deep-sea sediments?

INTRODUCTION

The Ocean Drilling Program (ODP) is uniquely positioned to sample one of the least known and potentially strangest ecosystems on Earth—the microbial biosphere of deep marine sediments and the oceanic crust. The growing international interest in the study of this subsurface biosphere is driven by a variety of factors, including the role of subsurface microbial activity in Earth's biogeochemical cycles, the possibility of life on other planets, and sheer fascination with the nature of life on the margin of existence.

Nearly twenty years ago, Deep Sea Drilling Project (DSDP) experiments with methane concentrations and radiotracer uptake first demonstrated active microbial processes in cores recovered from deeply buried marine sediments (Oremland et al., 1982; Whelan et al., 1986; Tarafa

et al., 1987). Over the last fifteen years, studies of ODP cores have extended our understanding of those processes (e.g., Cragg et al., 1992; Getliff et al., 1992) and consistently identified abundant microbes in deeply buried oceanic sediments (e.g., Cragg et al., 1990, 1992; Thierstein and Störrlein, 1991; Parkes et al., 1994, 2000). Microbes have been recovered from burial depths as great as 800 meters below the seafloor (mbsf) (Shipboard Scientific Party, 1999). Recent contamination tracer experiments suggest that most of the microbes reported from ODP cores are inherent to the drilled sediments (Smith et al., 2000).

The number and mass of prokaryotes in the subsurface biosphere of oceanic sediments has been estimated by extrapolation from direct counts of sedimentary microbes at a small number of ODP sites. Based on that extrapolation, the marine subsurface biosphere may compose as much as one-tenth (Parkes et al., 2000) or even one-third (Whitman et al., 1998) of the world's living biomass. In situ metabolic activity by at least a portion of this biosphere is spectacularly demonstrated by hydrates of methane produced by microbes in deep-sea sediments. On a global scale, these hydrates contain 7,500 to 15,000 Gigatons (Gt) of carbon—four to eight times as much carbon as in living surface biosphere and soils combined (Kvenvolden, 1993). Pore water chemical studies (Borowski et al., 1996) and recent microbiological discoveries (Hinrichs et al., 1999; Boetius et al., 2000) suggest that, on an ongoing basis, the CH_4 produced in deep-sea sediments is primarily destroyed by the sulfate-reducing activity of microbes in overlying sediments.

Despite these recent advances, very little is known about the nature and activity of life in deep marine sediments. In particular, we know almost nothing about (1) the continuity of subsurface life from one oceanographic region to another; (2) the specialized metabolic strategies, if any, that are required to survive in deeply buried marine sediments; or (3) the conditions under which subsurface microbes are active or inactive and living or dead.

BACKGROUND

The Quick and the Dead?

There is abundant evidence of both microbial populations and microbial activity in subsurface marine sediments throughout the world ocean. Prokaryotic cells have been found in surprisingly high numbers in buried sediments at every site that has been assayed for their presence (Parkes et

al., 2000). The abundance of those cells varies in a systematic and fairly predictable manner. For example, deeply buried shelf sediments from the Peru Margin (high surface-ocean productivity and shallow-water depth) contain 10^8-10^9 cells/cm^3, and sediments from the eastern equatorial Pacific (low surface-ocean productivity and abyssal water depth) contain only 10^6 cells/cm^3 (Parkes et al., 2000).

Pore water chemical data from hundreds of DSDP and ODP sites documents the occurrence of subsurface catabolic activity in sediments throughout most of the deep ocean (D'Hondt et al, unpubl. data). Microbial sulfate reduction, methane production, and methanotrophy are common processes in deeply buried marine sediments. Other catabolic processes are known to occur in subsurface marine sediments but have been studied very little (such as manganese and iron reduction).

Despite the ubiquity of microbial cells in deeply buried marine sediments and the clear pore water evidence of in situ microbial catabolism, the identity and structure of these communities and the metabolic adaptations of the microbes that constitute them remain largely unknown. Most-probable-number (MPN) experiments have demonstrated that viable cells occur in deeply buried marine sediments (Parkes et al., 2000). However, these viable cells represent only the barest fraction (0.00001% to 0.6%) of the total cells enumerated in the sediments sampled (Parkes et al., 2000). The extent to which this discrepancy between enumerated and viable cells reflects a culturing bias, known also from surface sediments, or the extent to which it reflects a real difference between a small active population and a very large inactive (dormant or dead) population remains to be determined.

The importance of this issue for our understanding of subsurface population structure and metabolic adaptation is underscored by estimates of the mean sulfate reduction per enumerated subsurface cell (D'Hondt et al., unpubl. data). If all of these enumerated cells are alive, their rates of SO_4^{-2} reduction are two to five orders of magnitude lower in the ocean margin anaerobic methanotrophy zone and five to nine or more orders of magnitude lower in open ocean sediments than per-cell rates observed in pure cultures and inferred in surface marine sediments (Jørgensen, 1978; Knoblauch et al., 1999; Ravenschlag et al., 2000; D'Hondt et al., in prep.). In contrast, if subsurface cells actually utilize SO_4^{-2} at the lowest rates measured for cells in laboratory cultures or

inferred for cells in surface marine sediments, fewer than 1 in 100 is actively respiring in the sulfate-reducing methanotrophy zone of the most microbially active sites and fewer than 1 in 1,000,000 is actively respiring at the least microbially active open ocean sites. In short, most of the subsurface microbes enumerated by direct microscopy in marine sediments must be either adapted for extraordinarily low levels of metabolic activity—or dead. This conclusion is supported by available estimates of mean generation times of up to 1 m.y. for deep subsurface microbes (Parkes et al., 2000).

Metabolic Diversity

The metabolic diversity and rates of microbial processes in deep subsurface sediments can be inferred from a broad range of geochemical information, including modeling of pore water profiles of ions, gases, and low molecular weight organic molecules, mass balance calculations of changes in solid phase constituents, and stable isotope fractionation. Basically, the same types and sequences of microbial processes appear to occur deep in the seafloor as are known from the much more active surface sediments of ocean margins. The mechanisms and regulation of the exceedingly slow hydrolytic degradation of macromolecular organic compounds are, however, only poorly understood. So, too, are the fermentative pathways that produce substrates for the terminal mineralization processes such as sulfate reduction or methanogenesis.

The build up of bicarbonate and ammonium are indicators of the diagenesis of organic material in all marine sediments. Sulfate reduction dominates down to the depth of sulfate depletion, many tens or hundreds of meters below the seafloor, where it is followed by methanogenesis. Although methane formation is very slow, the continuous production of a diffusible gas over millions of years results in vast methane accumulations, either dissolved in the pore water or in the condensed form of gas hydrates. In both cases, an upward flux of methane reaches the sulfate zone and supports an interface of enhanced microbial activity based on methane oxidation. In shallow marine sediments, this anaerobic process is catalyzed by a syntrophic community consisting of archaea, which convert methane back to an intermediate such as hydrogen or acetate, and sulfate reducing bacteria, which oxidize the intermediate (e.g. Hoehler et al., 1994; Valentine and Reeburgh 2000; Boetius et al., 2000). Based on pore water modeling of sulfate and methane profiles, the same process appears to drive a significant part of sulfate reduction in the sea bed (e.g., Borowski et al., 1999; D'Hondt et al., unpubl. data). This interface is of particular importance since it constitutes a barrier against the escape of methane up into the ocean water and eventually into the atmosphere.

In deep-sea sediments, such as the Peru Basin sites proposed for Leg 201, manganese oxide may provide an important oxidant of organic material and its reduction can be traced tens of meters into the seafloor (Yeats, Hart, et al., 1976). The reduction of iron oxides expectedly plays a greater quantitative role and iron(III) bound in sheet silicates may provide a slow but continuous source of oxidation potential over 10^5-10^6 years (Raiswell and Canfield, 1996).

H_2 is an important intermediate in the microbial degradation pathways of ocean margin sediments, and its pore water concentration is strictly regulated by the uptake potential of the microbial community and the energy yield of their H_2 metabolism. Thus, the H_2 partial pressure in the sulfate reduction zone is maintained below the threshold level required by archaea to drive methanogenesis (Hoehler et al., 1998). The key role of H_2, known from the metabolic competition among microbial populations in surface sediments, may also be critical for the deep subsurface biosphere. The potential sources for microbial energy metabolism need to be surveyed with an open mind toward new and unexpected types of redox processes and mineral surface reactions.

Microbial Diversity

The phylogenetic and physiological diversity of deep sediment communities remains virtually unknown. Only two isolates of sulfate-reducing bacteria from subsurface sediments (80 and 500 mbsf) have been fully characterized (Bale et al., 1997). These isolates (from a single site in the Japan Sea) are of a new barophilic species, *Desulfovibrio profundus*. Its unusually wide growth temperature range (15°–65°C) has no counterpart in any other known sulfate-reducing bacterium. It is metabolically flexible; it possesses the capability to reduce oxidized sulfur species, nitrate, and Fe(III). Whether deeply buried sulfate-reducing communities throughout the world ocean are dominated by close relatives of *D. profundus* or composed of a host of other organisms remains to tested.

The record of methanogenic isolates from the subsurface is surprisingly spare. MPN enumerations of methanogens in deep marine sediments have yielded cultured methanogens in much smaller numbers than sulfate-reducing bacteria (in the Peru margin; Cragg et al., 1990) or not at all (in the Japan Sea; Cragg et al., 1992). These surveys have, to our knowledge, not lead to the description of

new methanogen species from the marine subsurface. Hence, the phylogenetic composition of marine subsurface methanogenic communities remains essentially unknown.

Organisms responsible for methanotrophy in near nshore surface sediments have been biomarker fingerprinted and phylogenetically identified, but not yet cultured (Hinrichs et al., 1999; Boetius et al., 2000; Teske et al., 2000; Lanoil et al., 2000). Whether or not the same microbial community (composed of a sulfate-reducing bacterium and a previously unknown member of the archeal Methanosarcinales) is responsible for methanotrophy in the more deeply buried biosphere throughout the world ocean (in both ocean-margin and open-ocean environments) remains to be seen.

Novel forms of bacterial metabolism with subsurface potential are constantly being discovered. For example, systematic studies of sulfate- and sulfur-reducing bacteria and archaea have shown that many representatives of these organisms, among them an astonishing set of phylogenetically very deep lineages (Vargas et al. 1998), share an unexpected capacity for Fe(III) reduction (Lonergan et al. 1996). A *Thermus* sp. isolated from a deep South African gold mine used O_2, NO_3^-, Fe(III), S, Mn(IV), Co(III), Cr(VI), and U(VI) as electron acceptors (Kieft et al. 1998). Respiration of metal oxides could allow bacteria and archaea a respiratory mode of life even after other electron acceptors, including oxidized sulfur species, become extinguished with increasing distance from the oxidized biosphere. The metabolic flexibility of *D. profundus* and the South African *Thermus* sp. allow multiple scenarios of subsurface phylogenetic diversity. One possible scenario is that a certain microbial community becomes buried below the sediment surface and basically persists in its phylogenetic diversity and physiological potential over millions of years. This community may be responsible for NO_3^- reduction, manganese reduction, iron reduction, and SO_4^{-2} reduction throughout the vertical expanse of a single sediment column—and even dominate the subsurface respiratory realm throughout the sediments of the world ocean (at least within a broad temperature range, such as 0° to 30°C or 30° to 60°C). A second (and perhaps more likely) scenario is that the phylogenetic composition of subsurface communities may be shaped by variables other than the type of electron acceptors available. For example, it may be controlled by electron donor availability, micronutrient availability, or the ability of well-tuned species or communities to out_compete each other under slightly different local environmental conditions, such as different concentrations of metabolic products and reactants. Also, physical factors such as available pore space, ability to

migrate in the sediment, interactions with mineral surfaces, or distance to solid substrates may be important.

Global Biogeochemical Effects

The subsurface biosphere of marine sediments may affect the surface Earth in a variety of ways. It is now widely recognized that release of CH_4 from marine sediments may affect atmospheric carbon stocks and climate (Dickens et al., 1995; Dickens, 2000; Kennett et al., 2000; Hesselbo et al., 2000; Hinrichs, 2001). It is less widely recognized that SO_4^{-2} reduction by the buried biosphere may also change Earth's surface chemistry and climate. Such reduction is a major sink of SO_4^{-2} from the world ocean (Holland, 1984). Because SO_4^{-2} is the second most abundant anion in seawater (Pilson, 1998) and SO_4^{-2} reduction followed by sulfide precipitation results in the production of two equivalents of alkalinity per mole, subsurface SO_4^{-2} reduction may affect total oceanic alkalinity and, consequently, the partitioning of CO_2 between atmosphere and ocean over geologic time. The ultimate effect of this process on the surface Earth will depend on the extent to which reduced sulfur is fixed in the sediment, rather than diffusing back into the overlying ocean to be oxidized. Furthermore, NO_3^- reduction by the subsurface biosphere may be a net sink of biologically accessible nitrogen from the world ocean (Moore et al., 2001).

Why the Equatorial Pacific and Peru Margin?

In short, we know almost nothing about the population structure, metabolic strategies, community composition, and global biogeochemical influence of the marine deep biosphere. If we are to develop a coherent understanding of the microbial communities that are deeply buried in marine sediments, a focused and interdisciplinary program of deep biosphere study is required. Leg 201 presents such a program.

Sampling of the Leg 201 sedimentary environments will allow us to document the activity, composition, and biogeochemical effects of the subsurface biosphere in environments representative of essentially the entire range of subsurface conditions that can be found in relatively cool (≤~25°C) marine sediments. These include equatorial Pacific sediments typical of the open ocean, Peru Margin sediments typical of a nearshore upwelling regime, and Peru Basin sediments. Much

of the geochemical and sedimentological character of these sediments has been documented during previous ODP and DSDP legs (DSDP Leg 34; Peru Margin ODP Leg 112; Equatorial Pacific ODP Leg 138) (Yeats, Hart, et al., 1976; Suess, von Huene, et al., 1990; Pisias et al., 1995). In short, several widely different marine sedimentary regimes will be explored during this single drilling leg. Few regions in the world contain within a relatively short distance so many marine sedimentary regimes that have been so well characterized.

The environments to be examined include (1) carbonate and siliceous oozes of the equatorial Pacific, (2) clays and nannofossil-rich oozes of the Peru Basin, (3) organic-rich silty sediments of the shallow Peru shelf, and (4) a hydrate-rich deep-water sequence off the continental shelf of Peru (see Fig. 1).

The first two environments are characteristic of open-ocean sedimentary regimes. ODP Leg 138 studies identified abundant subsurface microbes in this equatorial Pacific region (Cragg and Kemp, 1995). Shipboard chemistry from Leg 138 and DSDP Leg 34 (Pisias et al., 1995; Yeats, Hart, et al., 1976) suggest that the deeply buried microbial communities of these two regions, respectively, rely on sulfate and manganese reduction. The subsurface extent of electron acceptors with similar or intermediate standard free energy yields (nitrate, oxygen, and iron oxides) in these regions remains unknown.

The second two environments are characteristic of ocean margin regimes. Studies of ODP Leg 112 samples identified abundant subsurface microbes in Peru Shelf sediments (Cragg et al., 1990). At all sites but one, these shallow-water sediments and the deep-water hydrate-rich sediments are rich in dissolved sulfate at shallow burial depths (down to a few meters below seafloor) and rich in methane at greater burial depths (starting a few meters below seafloor or tens of meters below seafloor) (Suess, von Huene, et al., 1990). The remaining site is sulfate rich and methane poor throughout the targeted drilling interval, thus indicating relatively low microbial activity.

Subsurface flow affects the subsurface environment at both the shallow-water Peru Shelf sediments and the open-ocean equatorial Pacific sites. In the former region, it is brine flow through the sediments. In the latter region, it is seawater flow through the underlying crust and perhaps the deepest sediments.

SCIENTIFIC OBJECTIVES

The overarching objective of Leg 201 is to investigate the nature and extent of microbial activity in several different deeply buried marine sedimentary environments.

During ODP Leg 201, we will address several fundamental questions about the deeply buried biosphere:

1) Are different sedimentary geochemical regimes characterized by completely different microbial communities—or merely by shifts among the dominant species and different levels of community activity?

2) How does hydrologic flow of electron acceptors, electron donors, and potentially of microbes through deep sediments affect community structure and sediment chemistry?

3) To what extent do past oceanographic conditions affect microbial communities now active in deep-sea sediments?

4) How do biogeochemical processes of the deep subsurface biosphere affect the surface world?

It is certain that several aspects of these questions will require extensive postcruise research to fully address. This reliance on postcruise research is necessary for at least two reasons. First, some experiments initiated during the cruise will take months (radiotracer experiments) or years (cultivation experiments) to complete. Second, some key studies, such as genetic assays of the microbiological communities and isotopic studies of biogeochemical fluxes, will be undertaken postcruise because they require technical facilities and expenditures of time beyond those available to an ODP shipboard party during a single cruise.

Despite these limitations, other aspects of the above questions should be successfully addressed during Leg 201. In particular, shipboard biogeochemical studies are expected to provide new understanding of the effects of sediment geochemical regimes, hydrologic flow, and past oceanographic conditions on metabolic diversity, microbial activities, and the nature of metabolic

competition in these subsurface environments. Shipboard studies are also expected to greatly improve understanding of how deep subsurface biogeochemical processes affect the surface world.

SCIENTIFIC APPROACHES

Leg 201 will be unique for ODP through its focus on the subsurface microbial communities and their geochemical activities. Because the samples will be retrieved from very stable sedimentary environments, the microorganisms are expected to be sensitive to chemical and physical change, in particular to oxygen, temperature, and (for the deep-sea sites) pressure. Consequently, all samples for microbiology and for process studies will be transferred from the drilling platform to cooling incubators in the laboratory as quickly as possible and will be kept as whole-round cores until processed. Core segments will be cut under sterile and anoxic conditions and immediately distributed for further processing or conservation. Only sediment from the uncontaminated centers of cores will be used for enumeration, isolation, and identification of microorganisms or for activity analyses and experiments.

While drilling cores for microbiology, contamination tests will be done routinely. Perfluorocarbon tracer (PFT) will be added to the drilling fluid, and the penetration of this tracer solute from the periphery towards the center of cores will be checked. Previous contamination tests combining PFT and bacterial-sized plastic beads have shown this contamination test to be efficient and to provide a useful indicator of potential bacterial contamination from the drilling fluid (Smith et al., 2000).

The study of deep subsurface microorganisms and their activity is a methodological and experimental challenge at the frontiers of modern marine microbiology and biogeochemistry. Many of the studies planned for this cruise are "first time" for ODP and overall for the deep biosphere, and methods and concepts will need to be further developed, refined, or even completely changed. The following selection of scientific approaches was developed on the basis of extensive discussions and experiences of many colleagues and should enable a major step towards our understanding of the deep biosphere. The approaches are, however, still very much in the development phase and will need refinement before they may be recommended for future routine application.

Enumeration, Identification, and Isolation of Microorganisms

Direct cell counts after fluorescent deoxyribonucleic acid (DNA) staining is well established as a method to obtain total numbers of intact cells (e.g., Parkes et al., 2000). The method allows also a description of cell sizes and morphologies and of the spatial structure of the community. Alternative staining techniques (e.g., to discriminate live and dead cells) will be considered.

DNA- and ribonucleic acid (RNA)-based techniques will be applied for the (postcruise) analysis of microbial diversity and activity. Samples will be taken and frozen (liquid N_2 and/or -86°C) for later DNA/RNA extraction and polymerase chain reaction (PCR) amplification and cloning (e.g., Rochelle et al., 1992). Sequence libraries will later be established based on 16S ribosomal DNA (rDNA) to provide a phylogenetic characterization of the microbial populations. This will be supplemented with a denaturing gradient gel electrophoresis (DGGE) separation and sequencing of dominant RNA molecules. Through the amplification and sequencing of selected messenger RNA molecules, the expression of certain key functional genes may be analyzed. Also real-time PCR may be used to quantify selected types of rRNA and messenger RNA (mRNA).

The microbial populations will also be fingerprinted (postcruise) by other molecular techniques, e.g., terminal-restriction fragment length polymorphism (T-RFLP) or membrane lipid biomarkers, including bacterial phospholipid fatty acids (PLFAs) and diverse archaeal lipids. The carbon isotopic composition of these biomarkers will be analyzed to identify the organic substrates of the corresponding microorganisms.

To obtain pure cultures of important microbial representatives, different methods of viable counts, enrichment, and isolation will be applied (and initiated on board) (e.g., Parkes et al., 1995). Through decimal dilutions in different growth media, MPN viable counts of the corresponding microorganisms will be made. Although such counts may greatly underestimate the true population sizes, the highest positive dilutions provide optimal material for a subsequent isolation of dominant members of the microbial communities. Because the subsurface microorganisms expectedly grow extremely slowly, the isolation of truly indigenous species may take several years. Alternative techniques, such as single cell isolation by laser tweezers, will also be considered.

The physiological types of microorganisms targeted for isolation will include sulfate reducers, methanogens, methane oxidizing consortia, fermenters, manganese reducers, and iron reducers.

Activity of Subsurface Microorganisms

Pathways and rates of microbial processes will be analyzed by different approaches. Among these, direct experimental measurements of specific processes will be done based on incubation of samples with radioactively labeled substrates. The application of radiotracers on the *JOIDES Resolution* is unique for Leg 201, and the prerequisite laboratory facilities, experimental procedures, and handling protocols are therefore described in some detail in a following section.

Experimental Estimation of In Situ Activities

Because of very low process rates and correspondingly slow turnover of electron acceptors or donors, it is not possible to experimentally determine the metabolic activity in subsurface sediments simply from changes in chemical concentrations over time. Instead, radioactively labeled substrates may be added in trace amounts to sediment samples, upon which the bacteria metabolize the radiotracer with the same relative turnover as the indigenous substrate. The sensitivity of process rate measurements may thereby be increased many thousand fold (Jørgensen, 1978; Whelan et al., 1986; Tarafa et al., 1987; Cragg et al., 1992).

Microbial processes that will be analyzed experimentally include sulfate reduction, methanogenesis, methane oxidation, and the turnover of selected small organic molecules such as acetate. Because of its universal and regulatory importance for anaerobic sediment metabolism, the turnover of tritiated H_2 may also be studied. The radiotracer incubation experiments will be initiated as soon as possible after retrieval of core samples and will run during the duration of the cruise. Further experiments on the effect of temperature, pressure, or certain substrates will be continued postcruise. The processing of samples and determination of metabolic rates will be done postcruise because of the requirement for special radioisotope laboratory facilities.

Additional indicators of microbial activity will include the measurement of adenosine triphosphate (ATP). The concentration of ATP in individual cells is related to their metabolic rate.

Modeling of In Situ Activities

Major pore water constituents involved in biological processes include bicarbonate, ammonium, sulfate, methane, hydrogen sulfide, and manganese ions. Through the downcore analysis of these chemical species and diffusion diagenesis modeling of their production or consumption, the depth

distribution of several primary microbial processes can be quantified. This approach, however, is dependent on the correct determination of transport coefficients of pore fluid and solutes. Some of the sites to be drilled on the Peruvian shelf are known to include a subsurface brine intrusion advecting offshore through a porous sediment interval. Also, the basement rock underlying the equatorial Pacific sites may be porous enough to allow significant fluid transport. The physical properties of cored sediments will therefore be closely related to modeled solute transports. A comparison of modeled and experimentally determined processes will improve the confidence in the resulting rates.

Geochemistry of the Deep Biosphere

Many dissolved constituents in the pore water are substrates or products of microbial metabolism and will be analyzed to determine these metabolic processes and their downcore changes. The analytical program will include a broad spectrum of inorganic anions and cations, dissolved gases such as CH_4, H_2S, and H_2, small organic molecules such as volatile fatty acids, as well as the total dissolved organic carbon (DOC).

The interactions between microbial processes in different sediment layers and the ambient sediment composition and geochemistry will be studied in particular for the biologically relevant elements of carbon, nitrogen, phosphorous, sulfur, iron, and manganese. The solid phase analyses will include the quantity and quality of organic material, organic carbon, and nitrogen, organic and inorganic phosphate, carbonates, sulfur-iron minerals, and metal oxides. Stable isotope analyses of carbon, nitrogen, and sulfur species will provide additional information on their original sources and their transformations at depth in the sediment. This information will be used to differentiate ongoing processes and to determine the role of depositional environment in the geological past for the present-day subsurface microbial activity.

DRILLING STRATEGY

Drilling Operations

To optimize the recovery and scientific analysis of microbiologically critical horizons from each environment, we will drill at least two holes at each site (Table 1). In general, we will use advanced hydraulic piston core (APC) until refusal and then shift to extended core barrel (XCB). In intervals

where XCB coring yields exceptionally poor recovery, we may attempt motor driven core barrel (MDCB) or rotary core barrel (RCB) coring.

Downhole chemical transitions are sharp in sediments that are rich in organic matter and microbial activity. Under such conditions, critical transitions in microbial activity and community composition are also expected to be sharp and difficult to identify, anaerobically sample, and geochemically and microbiologically analyze, particularly considering the requirement of rapid processing prior to equilibration of the cores with shipboard temperature resulting in degradation of their microbiological community and its environment. These difficulties will be further complicated by the rapid pace of core recovery that is typical of relatively shallow-water environments, such as the Peru Margin (at the site nearest to shore, a 10-m core will probably be recovered every 20 to 30 min). If a third hole is necessary at any site to sample a microbiologically critical transition interval (e.g., an anaerobic methanotrophy zone or a hydrate interface), we will drill a third hole to the shallow target depth (mbsf) of that critical interval.

In the first hole at each site, we will undertake paired microbiological and geochemical analyses. The sampling frequency will be dictated by recovery rate and core handling and processing time. The resultant data will be immediately used to (1) define broad downhole microbiological and geochemical trends (on 10-m scales), (2) geochemically focus on stratigraphic horizons of special interest, and (3) identify diagnostic sedimentological or physical properties of those horizons. The second (and possibly third) hole will be used for high-resolution microbiological, geochemical, and geophysical studies of special stratigraphic horizons identified in the previous holes.

Special Downhole Operations

Downhole temperature gradients will be measured with the APC temperature shoe when piston coring, and with the Davis-Villinger temperature probe when coring with the XCB assembly. Recent improvements to the ODP pressure coring system (PCS) cutting shoe will be tested in a series of operations currently set for three sites, allowing sufficient time between deployments to allow evaluation of tool performance. In the event the PCS performs well, we may chose to deploy it more often and at more than three sites. We also plan to deploy the APC methane tool. This device will be used to monitor the effects of gas loss in cores from the time the core is cut until it reaches the deck by recording temperature, pressure, and conductivity with sensors mounted in the APC piston. Testing of hydrate autoclave coring equipment (HYACE, HYACINTH) has been endorsed by the

ODP advisory structure. As much as one day of operations may include HYACE/HYACINTH testing.

PRIMARY PROPOSED SITES

Proposed Site	Previous Site	Latitude	Longitude	Water depth (m)
EQP-2A	(ODP Site 851)	2°46.22'N	110°34.31'W	3761
EQP-1A	(ODP Site 846)	3°05.70'S	90°49.08'W	3296
PRU-3A	(ODP Site 684)	8°59.49'S	79°54.35'W	426
PRU-2A	(ODP Site 680)	11°03.90'S	78°04.67'W	252
PRU-1A	(ODP Site 681)	10°58.60'S	77°57.46'W	150
PRU-4A	(ODP Site 685)	9°06.78'S	80°35.01'W	5071
PRB-2A	(DSDP Site 321)	12°01.29'S	81°54.24'W	4827

Site EQP-2A (ODP Site 851)

Owing to the ship's track, our first site will be the open-ocean proposed Site EQP-2A (Fig. 1). The extremely low sedimentary organic carbon concentrations (≤0.1%) (Fig. 2) and relatively high burial depths (>300 mbsf) expected for this site render it ideal for testing whether the microbial communities, activities, and survival strategies necessary to survive in very deeply buried organic-poor marine sediments are similar to or different from those in open-ocean sediments with an order of magnitude more organic matter (e.g., proposed Site EQP-1A) or distant locations with shallower burial depths, lower mean rates of subsurface catabolic activity, and perhaps different principal electron donors (MnO_2) (e.g., proposed Sites PRB-1A and PRB-2A). Because Site EQP-2A contains a deeply buried interval of subsurface (basement) hydrologic flow, coring this site will also enable Leg 201 scientists to test how subsurface hydrologic flow affects community structure and sediment chemistry in organic-poor sediments with sulfate-rich pore waters. Our drilling target is to penetrate a few meters into basement.

Site EQP-1A (ODP Site 846)

Coring at proposed Site EQP-1A will allow Leg 201 scientists to document the environmental and microbial circumstances under which the standard microbial paradigm (of catabolic control by standard free energy) is violated by methanogenesis occurring in sulfate-rich open-ocean sediments

(CH_4 exceeds 120 µL/L at depth here). Because Site EQP-1A contains a deeply buried interval of subsurface (basement) hydrologic flow, drilling this site will also enable Leg 201 scientists to test how subsurface hydrologic flow affects community structure and sediment chemistry in relatively organic-poor sediments with sulfate-rich pore waters.

Site PRU-3A (ODP Site 684)

Coring at proposed Site PRU-3A will provide a critical standard of comparison for the other shallow-water Peru Margin sites (proposed Sites PRU-1A and PRU-2A) (Fig. 3) because Site PRU-3A contains the same near-surface sulfate/methane transition as Site PRU-1A, but may lack the subsurface brine flow of Sites PRU-1A and PRU-2A. In short, Site PRU-3A is the only "normal" upwelling zone methanogenic sedimentary sequence proposed for coring during Leg 201. Coring this site will also provide an opportunity for identifying the methanotrophic communities of deeply buried marine sediments.

Site PRU-2A (ODP Site 680)

Coring at proposed Site PRU-2A will provide a critical standard of comparison for Site PRU-1A because the same subsurface brine flow as at Site PRU-1A introduces sulfate into sulfate-rich sediments at Site PRU-2A. Consequently, we expect that the effect of that flow on microbial communities and activity is likely to be very different at the two sites.

Site PRU-1A (ODP Site 681)

Coring at proposed Site PRU-1A will enable Leg 201 scientists to test how introduction of an electron acceptor by subsurface hydrologic flow affects community structure and sediment chemistry in organic-rich (methanogenic) sediments. Coring this site will also provide multiple opportunities for identifying the methanotrophic communities of deeply buried marine sediments.

Site PRU-4A (ODP Site 685)

Coring at proposed Site PRU-4A will allow Leg 201 scientists to determine if and how hydrate-bearing sequences differ in microbial activity and community structure from nearby methane-rich sequences that lack hydrates (Sites PRU-1A and PRU-3A) and nearby sulfate-rich sequences with low methane concentrations. It will also provide a Peru-Margin microbial and biogeochemical counterpoint to the hydrate-rich sites targeted for coring during Leg 204 (Hydrate Ridge).

Site PRB-2A (DSDP Site 321)

Because Mn concentrations are expected to peak unusually deep in this sequence (at 50 mbsf or deeper, based on DSDP Site 321) (Fig. 4), coring at proposed Site PRB-2A will provide an excellent opportunity to sample Mn-reducing microbial communities in very organic-poor relatively deeply buried marine sediments. Because of its extremely low electron donor (organic matter) concentrations, Site PRB-2A will provide a challenging opportunity for (1) determining conditions under which subsurface microbes may be active, inactive, or dead and (2) assessing metabolic strategies that are necessary for survival in deeply buried marine sediments.

ALTERNATE SITES

Alternate sites are listed as discussed below, not necessarily in priority order except for Site PRB-1A, which remains the top priority alternate site.

Proposed Site	Previous Site	Latitude	Longitude	Water Depth (m)
PRB-1A	DSDP Site 320	9°00.40'S	83°31.80'W	4487
	ODP Site 679	11°03.76'S	78°16.25'W	450
	ODP Site 682	11°15.99'S	79°03.73'W	3789
	ODP Site 686	13°28.81'S	76°53.49'W	446
	ODP Site 687	12°51.78'S	76°59.43'W	307
	ODP Site 688	11°32.28'S	78°56.65'W	3826
	ODP Site 683	9°01.69'S	80°24.40'W	3072
	ODP Site 845	9°34.95'N	94°35.45'W	3704
	ODP Site 849	0°10.98'N	110°31.18'W	3839
	ODP Site 850	1°17.83'N	110°31.29'W	3786
	ODP Site 844	7°55.28'N	90°28.85'W	3414
	ODP Site 847	0°11.59'N	95°19.23'W	3334
	ODP Site 848	2°59.63'S	110°28.79'W	3853
	ODP Site 852	5°17.55'N	110°04.58'W	3861
	ODP Site 853	7°12.66'N	109°45.08'W	3715

Because time constraints appear to limit our operations strategy to a less ambitious schedule than

outlined in the proposal for this expedition, we have relegated one of the original primary sites to alternate status. However, recognizing that unforeseen circumstances may require us to alter our operations schedule once we are at sea, we have included several other alternate sites in our planning as well. In each case, as with our primary sites, these alternate sites are all previously occupied ODP sites. Since we envision the opportunity to occupy these sites as being vanishingly small, we have not accorded them new prospectus site numbers, but refer instead to the original ODP nomenclature. None of these alternate sites are intended to be occupied in lieu of primary sites, but only in case of unexpected circumstances; whereby, the primary scientific objectives outlined in this prospectus cannot be achieved at our primary sites, or in the unlikely event we find ourselves with additional time at the conclusion of operations at our primary sites. A brief summary of the scientific objectives that might be addressed at each of these sites follows. Depth of penetration requested for all sites is the same depth as approved for previous expedition.

Site PRB-1A (DSDP Site 320)

Coring at proposed Site PRB-1A will allow Leg 201 scientists to (1) determine conditions under which subsurface microbes may be active, inactive but capable of resuscitation, or dead; (2) begin to assess the specialized metabolic strategies, if any, that are required to survive in deeply buried marine sediments; and (3) explore phylogenetic affinities and differences between microbes of the organic-poor Peru Basin sediments and nearby organic-rich Peru Margin sites.

ODP Site 679

ODP Site 679 has a very deeply buried anaerobic-methanotrophy zone (~170 mbsf) and would consequently provide an interesting record for comparing to the primary proposed sites. For example, it would allow us to test whether or not there is any significant difference between the microbial communities in relatively high-activity methanogenic zones that lie ~20 mbsf, as at our primary sites, and the microbial community in the much lower-activity methanogenic zone of Site 679. Heat flow data suggest that there may be flow in the ~250-mbsf interval at this site, but pore water chemistry data is not available in that depth interval. If there is flow at depth, sulfate-based methanotrophy along the flow boundary could be what is keeping the anaerobic-methanotrophy zone deep at this site (because less methane can diffuse upward if it's being oxidized at a deep flow boundary).

ODP Sites 682, 686, and 687

These sites appear to be fairly standard continental-margin ODP sites (e.g., they have abundant methane and no sulfate beginning a little below seafloor). Because they do not contain the intervals of subsurface flow that we targeted as a coring objective, recoring the positions of one or more of these sites could provide a control site for comparison to Sites PRU-1A, PRU-2A, PRU-3A, and PRU-4A.

ODP Site 688

This site would be interesting for recoring because it is probably about as high in microbial activity as we are likely to see on an ODP cruise (its sediments contain 2% to 9% organic carbon and very high levels of dissolved ammonium down to 500 mbsf). However, this site does not optimally fulfill our flow and hydrate-focused objectives, and the anaerobic-methanotrophy zone is very near to the sediment surface.

ODP Site 683

Cores recovered from ODP Site 683 (and Site 682) might contain hydrates (as inferred by the shipboard Scientific Party from ODP Leg 112). However, no hydrates were recovered from any cores at these sites and pore water chemical evidence for hydrates at these sites is weak (for example, chloride varies smoothly downhole instead of the erratic behavior commonly observed in sediments recovered from hydrate-bearing sites).

ODP Site 845—Potential Alternate for Site EQP-2A

This site exhibits clear pore water geochemical evidence of basement flow. Although pore water Sr concentrations indicate less carbonate diagenesis at this site than at ODP Site 851 (proposed Site EQP-2A), the geochemical records of the two sites otherwise resemble each other closely.

Nonideal Alternates for Site EQP-1A

ODP Sites 849 and 850 are nonideal alternates for Site EQP-1A (ODP Site 846). Although both Site 849 and Site 850 contain co-occurring methane and sulfate and geochemical evidence of basement hydrologic flow, their downhole methane concentrations are a factor of two to three lower than at Site 846 (Site EQP-1A). Pore water communication between their upper sediment columns and lowermost sediment columns is more greatly hindered by chert than at Site 846.

ODP Site 849

Methane and high sulfate co-occur here (methane approaches 50 µL/L). Pore water profiles indicate basement flow. A pronounced chert layer at 237 mbsf greatly hinders pore water geochemical (and microbiological?) communication between the shallower sediments in diffusional contact with the sediment/water interface and the deeper sediments in diffusional contact with the basement.

ODP Site 850

Methane and high sulfate co-occur here also (methane approaches 40 µL/L). Pore water profiles indicate basement flow. Three chert layers in the Site 850 interval from Core 138-850C-36X to 41X (~360 to 390 mbsf) limit pore water geochemical communication between the shallower sediments in diffusional contact with the sediment/water interface and the deeper sediments in diffusional contact with the basement.

Other Equatorial Pacific Sites

ODP Sites 844 and 847

Sites 844 and 847 are normal open-ocean sites. Their sulfate concentrations decline 1 to 2 mmol downhole and they contain no geochemical evidence of subsurface flow. They could provide appropriate control sites for comparison to Sites EQP-1A and EQP-2A.

ODP Sites 848, 852, and 853

ODP Sites 848, 852, and 853 are open-ocean sites with very low accumulation rates. All exhibit little or no downhole change in mean sulfate concentrations. They might provide an equatorial Pacific counterpart to the low-activity Peru Basin Sites PRB-1A and PRB-2A. ODP Site 848 is a fairly normal open-ocean site, with only 100 m of sediment overlying basement and very little sulfate reduction (sulfate concentrations vary slightly but do not decline significantly downhole). At ODP Site 852, only ~125 m of sediment overlies basement. Leg 138 scientists inferred the Site 852 sediments to be oxidized throughout, with early diagenesis limited to the top few meters of the sediment. Although sulfate concentrations do not decline significantly downcore, there is some pore water geochemical evidence of basement interaction at Site 852. ODP Site 853 is similar to Site 852, but with only ~70 m of sediment thickness and even less downhole variation in pore water geochemical composition.

SAMPLING STRATEGY

General Philosophy

The drilling strategy outlined above will require the shipboard scientists to rapidly generate data from each hole to guide sampling at the next hole. Shipboard scientific procedures will be followed in a manner designed to aid and profit from such rapid data generation. For example, the gas chromatograph for headspace methane analyses is already operated in a continuous mode by which samples are added as soon as cores are recovered. The ion chromatograph (used for measurements of dissolved SO_4) can be precalibrated and similarly operated in a continuous mode. The gas chromatograph for analyzing perfluorocarbon tracers can also be operated continuously, so recovered cores can be immediately assayed for potential microbial contamination (Smith et al., 2000). The rapid turnaround on these assays (<10 min per sample) will allow shipboard microbiologists and geochemists to focus their resources on uncontaminated samples while still working in a microbiologically relevant time frame. Whereas sporadic and short-term (a few days) continuous perflurocarbon tracer deployments have been successful, our plan requires longer-term continuous operation. This operation will require automation of the currently labor intensive addition of perfluorocarbons to the drilling fluid.

To ensure as little damage as possible to the microbiological community present in these cores, a unique sampling strategy is required. Since microorganisms existing at deep-water seafloor temperatures (2°-4°C) can be particularly sensitive to elevated temperature (>10°C), there is a critically acute need to prevent thermal equilibration of the cores after recovery and prior to processing. The detailed example of a shipboard sampling plan and core flow outlined below may be possible to complete before thermal equilibration where core recovery is slow; however, rapid core recovery will likely require refrigeration of cores immediately after recovery until they can be processed.

Sampling guidelines and policy are available at the following World Wide Web site: http://www odp.tamu.edu/publications/policy.html. The Sample Allocation Committee (SAC), which consists of the two co-chiefs, staff scientist, and ODP curator onshore or curatorial representative aboard ship, will work with the entire science party to formulate a formal Leg 201 specific sampling plan for shipboard and postcruise sampling.

Archives

Because all sites have been previously occupied, a permanent archive for these sites already exists in the ODP repository. With the exception of the whole-round sampling required for microbiological experiments, however, the permanent archive for this cruise will be the ODP-defined "minimum permanent archive" and will be reconstructed postcruise. We will limit all routine and personal shipboard sampling, outside of contamination-free microbiological sampling, to the working halves of the cores.

Example Core Flow and Shipboard Sampling Strategy

Because the outer part of the core will have been bathed in surface seawater (our drilling fluid) and exposed to oxygen during handling, only the inner part of the core is appropriate for many microbiological samples. This necessitates removing whole-round cores (WRC), so that the exterior contaminated part of the core can be pared away in a sterile, anoxic environment to preserve the more pristine interior. We expect that on the order of 1.5 m of WRC will be required to accommodate all the experiments outlined for each sample in the "Scientific Approaches" section above. Ideally, these will all come from the same 1.5-m interval to accumulate as many measurements as possible on the same interval, but poor or disturbed recovery may require sampling over a longer interval to ensure or at least optimize the chance of sampling uncontaminated core.

As soon as the core arrives on deck and is carried to the catwalk, it will be marked by the ODP technical staff into 1.5-m sections. To minimize equilibration of the cores, it may be necessary to modify the standard ODP practice of shelving a recovered core barrel on the rig floor while a new core barrel is deployed and a joint of pipe is added. At deep water sites, we will probably ask the rig floor to deliver the core liner to the catwalk as soon as it can be removed from the core barrel, recognizing this may slow the coring operation. One of the shipboard microbiology contingent will be charged to identify one 1.5-m section for microbiology (MBIO) for rapid microbiological processing (Fig. 5). Once the MBIO section is selected, it and an adjacent 1.5-m section (either above or below, according to placement of the MBIO section) will be wiped with ethanol, labeled with a red permanent marker with orientation and section number, and removed from the core (total 3.0-m section), before any other cut is made. The ends will be covered with plastic caps but not sealed with acetone, and the 3.0-m section will be carried (using clean, ethanol-swabbed gloves) into the core lab. This section will be taken immediately to a sterile, oxygen-free subsampler, and the

remainder of the core will be processed according to standard ODP protocol. Routine samples for measurements of ephemeral properties will be collected from the non-MBIO core sections. These measurements include those for organic geochemistry for safety monitoring (free gas and 20-cm^3 sediment samples), biostratigraphy (core catcher samples), and physical properties.

The MBIO section will be subsampled in a cutting rig continuously bathed with sterile oxygen-free nitrogen. First, the section adjacent to the MBIO section will be removed and the non-MBIO section returned to the core lab for standard processing. The remaining nominally 1.5-m section will be cut into a series of WRCs and distributed to various parts of the shipboard laboratory. Although we may not envision all the possible types of samples that could be accommodated, what follows is an example of how the 1.5-m section might be distributed (see Fig. 5).

First, two 10-cm WRCs would be removed from the end of the section cut on the catwalk for PFT contamination and interstitial water (IW) analysis. These WRCs would be removed from the end in case the exterior cut was not sterile (not a requirement for the PFT or IW analysis). The next 60 cm would be removed and transported immediately to the radioisotope van (see "Radioisotope Protocols" section below). Ten-centimeter WRCs would be cut for MPN, enrichment cultures, and fluorescent in situ hybridization (FISH) analysis. These, along with 5-cm whole rounds for cell counts, ATP analysis, and additional pore water analysis, would be transported to the anaerobic chamber in the microbiology laboratory for further processing. Two 10-cm WRCs would be removed and placed in the ultra low temperature freezer (-86°C) for postcruise DNA and biomarker analyses and a third would be deep frozen as a residual sample. The outline above is provided to give interested scientists an idea of our core flow strategy and may require modification at sea based on recovery rate, completeness, or disturbance.

Radioisotope Protocols

Since radioisotope studies have never been carried out on board the *JOIDES Resolution*, we include the following section as our current plan of action for these experiments. The low-energy beta emitters planned for the cruise do not constitute a health hazard, but radioactive contamination could be a potential problem for other research, in particular for the sensitive analyses of natural radioisotopes. A van produced specifically for and dedicated to radioisotope experiments should be on the vessel prior to departure from port call. Dr. Bo Jørgenson, Co-Chief Scientist, is responsible for overseeing radioisotope work at the Max Planck Institute for Marine Microbiology and has

agreed to accept senior responsibility for radioisotope work during Leg 201. In addition, any scientists working in the radioisotope facility will be experienced in performing these experiments, and a scientist on each shift will be assigned active responsibility for radioisotope handling and safety.

Once a section has been subsampled for radioisotope studies, the subsection will be hand carried to the van and passed to the scientist in the van. We intend to keep traffic in and out of the van to a minimum, and only personnel trained in radioisotope work should be in the van. Sample processing will be done within a plastic basin with the surrounding bench covered with plastic-backed absorbent paper. All solutions will be stored in tightly capped containers and routine contamination wipe tests will be performed. Any personnel in the van will need to wear gloves, a lab coat, and safety glasses at all times. Radioisotope stock solutions, radiolabeled samples, and contaminated laboratory products do not leave the isotope van at any time during the cruise.

Three types of experiments are planned at this time: (1) determining the rates of sulfate reduction using the radiotracer $^{35}SO_4^{2-}$, (2) rates of methanogenesis with ^{14}C-labeled HCO_3^- or acetate and anaerobic oxidation of methane with $^{14}CH_4$, and (3) a tritiated (^3H) amino acid mixture will be used to determine the number of active cells via microautoradiography. The general procedure will be to put an aliquot of uncontaminated sediment into 12-mL serum vials and store them for a few hours in an incubator at in situ temperature (or potentially some other temperature for experimental purposes). Ten microliters of carrier-free radioisotope will be injected by microsyringe into each sample. At the conclusion of the incubation period, the bacterial activity will be fixed and the sample will be frozen. Samples will then be transferred into 50-mL screw-capped plastic centrifuge vials for safe handling and transport. These samples should be sent by air freight, packed in sturdy containers, and maintained in a frozen condition.

Radiotracers expected to be on board at the beginning of the expedition are stock solutions of 500 MBq of $^{35}SO_4^{2-}$ in distilled water, 500 MBq of ^{14}C-labeled HCO_3^-, 250 MBq of ^{14}C-labeled acetate, 100 MBq of ^{14}C-labeled methane, and a total of 10 MBq of a mixture of 15 tritiated amino acids. The total amount of contaminated laboratory products we expect to generate will be <2 L of liquid and as much as 10 kg of solid material. Solid and liquid contaminated laboratory products will be returned to Texas A&M University.

Special Requirements

Special requirements include (1) radioisotope training/advisor for ODP/TAMU, (2) shipping supplies for large frozen shipment, (3) modification of PFT delivery system, and (4) three times the current –86°C freezer space (minimum). Radioisotope van requirements include (1) meets University-National Oceanographic Laboratory System (UNOLS) specifications, (2) additional refrigerator, freezer, and incubators, (3) supplies and safety gear.

LOGGING STRATEGY

Downhole logging provides continuous in situ measurements of geophysical properties such as resistivity, porosity, natural radioactivity, or sonic velocity, which can be used to assess the physical, chemical, and structural characteristics of the formation. Logging of the equatorial Pacific sites (proposed Sites EQP-1A and EQP-2A) and Peru Margin sites (proposed Sites PRU-1A, PRU-2A, PRU-3A, and PRU-4A) will help characterize current physical properties that may set important constraints on the downhole microbial community. For example, at Sites PRU-1A and PRU-2A, downhole logs will enable an assessment of the depth extent of high porosity/flow zones for the deep brine incursion. Comparison of the logs between the sites will allow some parameterization of the horizontal flow gradient. Similarly, at Sites EQP-1A and EQP-2A, logs may allow assessment of the flow regime at the base of the sediment column. In addition, logging will allow us to assess the occurrence of hydrates at Site PRU-4A (ODP Site 685), where the zone of gas hydrate stability extends down to >600 mbsf. Intervals containing hydrate are best characterized in situ by increases in resistivity and velocity (Vp and Vs) logs. These may be the only direct indicators of the presence of hydrate at Site PRU-4A. Logs might be particularly useful to draw a complete characterization of the subseafloor environment of the microbial communities at some sites visited during ODP Leg 112, where XCB core recovery was very poor (often <5%).

The logging program will consist of at least one logging run with the triple combination (triple combo) tool string (porosity, resistivity, density, natural gamma, and temperature) at all sites in the Equatorial Pacific (EQP-1A and EQP-2A) and at the sites on the Peru margin with sufficient penetration (>200 m) (PRU-1A, PRU-2A, and PRU-4A). Hole conditions and time permitting, the Formation MicroScanner (FMS)/sonic tool string might be run at any of these sites, where high resolution electrical images from the FMS could help refine our characterization of the downhole environment.

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FIGURES

Figure 1. A. Map showing general locations of drill sites occupied during Legs 138 (rectangle B) and 112 (rectangle C). **B.** Detail map of equatorial Pacific primary sites with nomenclature. Previous ODP designations are in parentheses. **C.** Detail map of Peru Margin primary sites and nomenclature. Previous DSDP/ODP site designations are in parentheses.

Figure 2. Pore water sulfate (open circles) and headspace methane (solid squares) for ODP Sites (a) 846 and (b) 851 (proposed Sites EQP-1A and EQP-2A, respectively; data from Mayer, Pisias, Janecek, et al., 1992).

Figure 3. Pore water sulfate (open circles) and headspace methane (solid squares) for ODP Sites (a) 680 , (b) 681, and (c) 684 (proposed Sites PRU-2A, PRU-1A, and PRU-3A, respectively; data from Suess, von Huene, et al., 1990).

Figure 4. Pore water sulfate (open circles) and manganese (solid squares) for DSDP Site 321 (proposed Site PRB-2A; data from Yeats, Hart, et al., 1976).

Figure 5. Example of subsampling strategy for microbiological studies (DNA = deoxyribonucleic acid, ATP = adenosine triphosphate, FISH = fluoresence in situ hybridization, MPN = most probable number, IW = interstitial water, PFT = perfluorocarbon tracer, MBIO = microbiology).

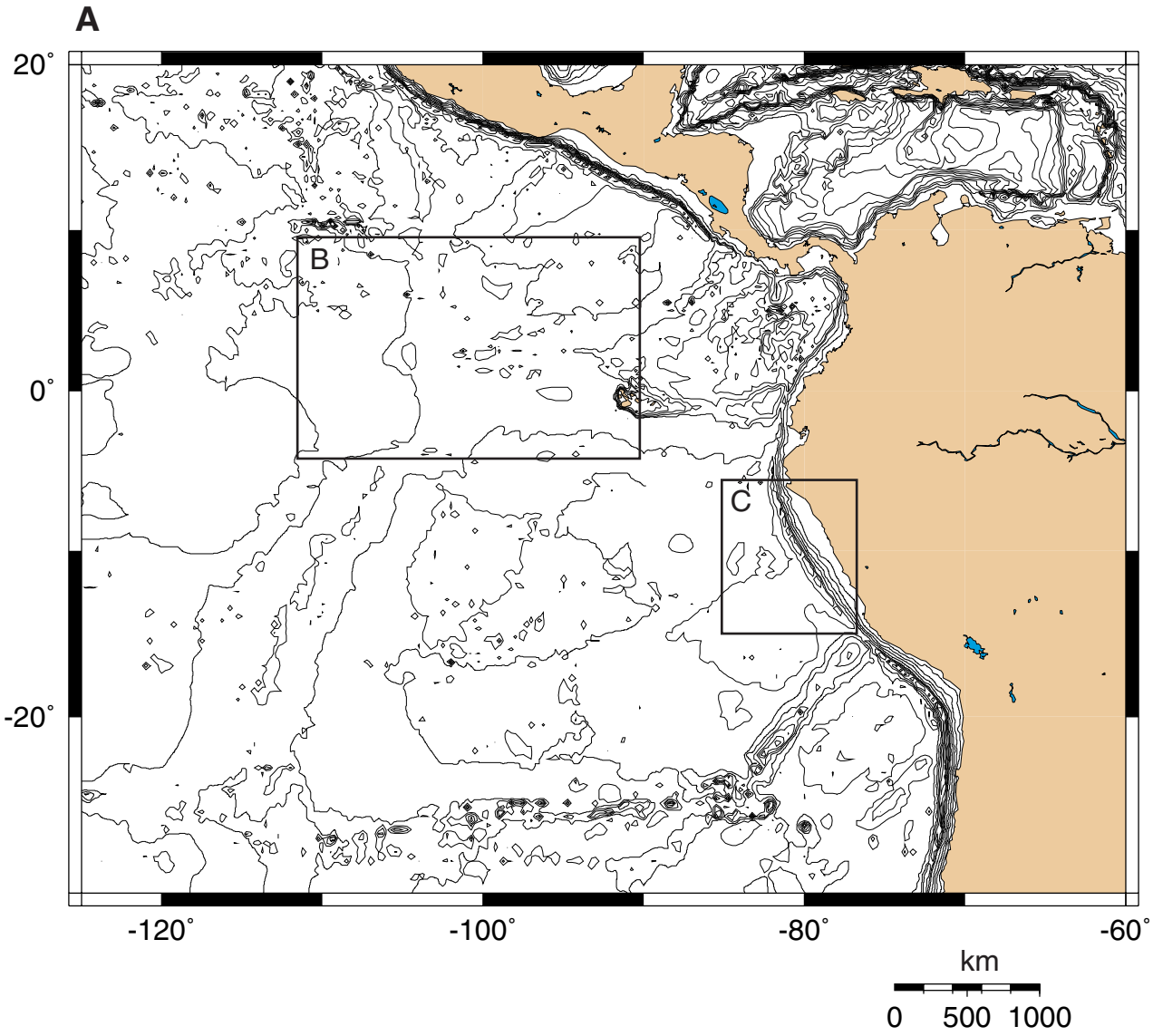
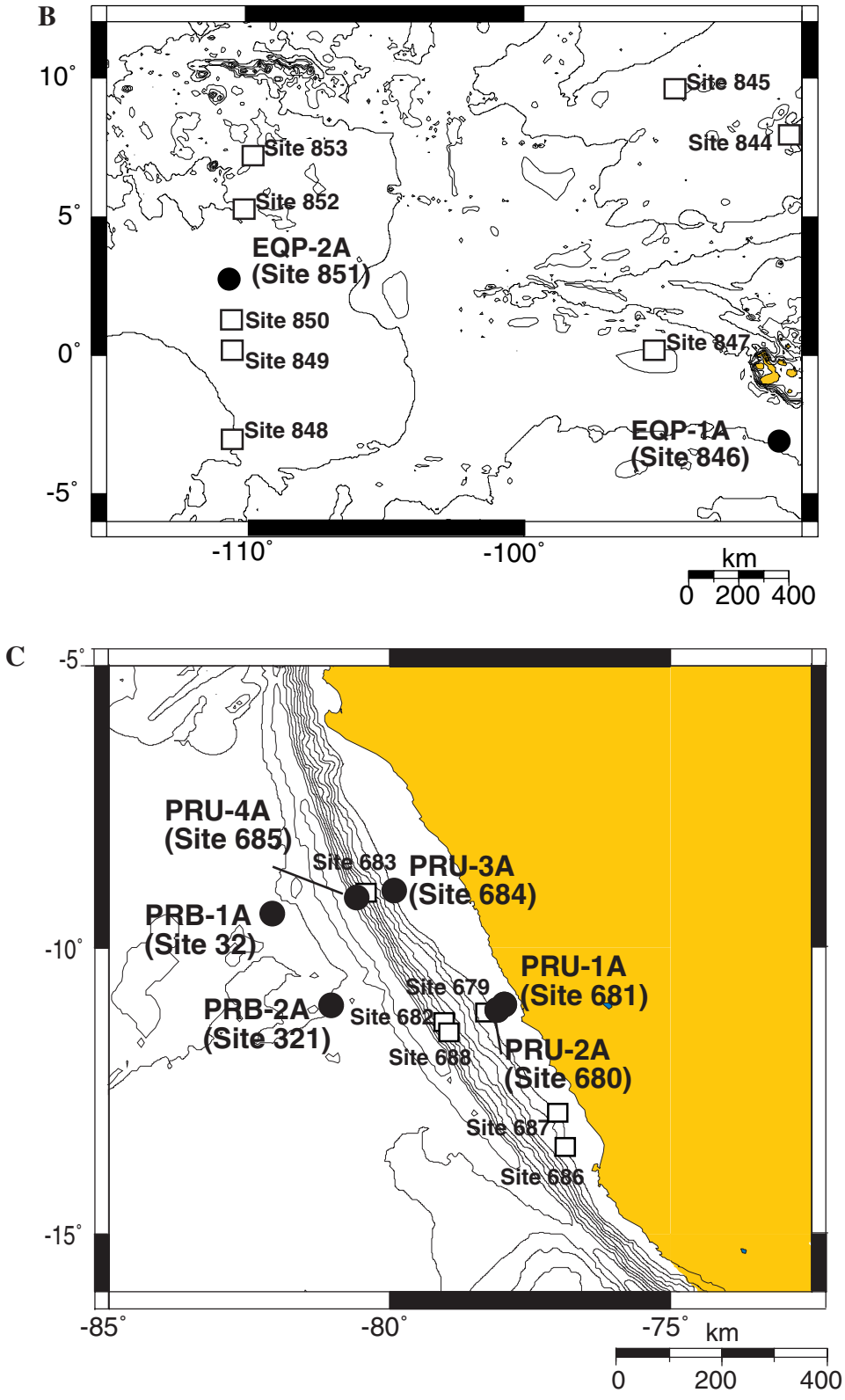


Figure 1


B
10˚
5˚
0˚
-5˚
-110˚
-100˚
Site 845
Site 844
Site 853
Site 852
EQP-2A
(Site 851)
Site 850
Site 849
Site 847
Site 848
EQP-1A
(Site 846)
km
0 200 400
C
-5˚
-10˚
-15˚
-85˚
-80˚
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km
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PRU-4A
(Site 685)
Site 683
PRU-3A
(Site 684)
PRB-1A
(Site 32)
PRU-1A
(Site 681)
Site 679
PRB-2A
(Site 321)
Site 682
Site 688
PRU-2A
(Site 680)
Site 687
Site 686

Figure 1

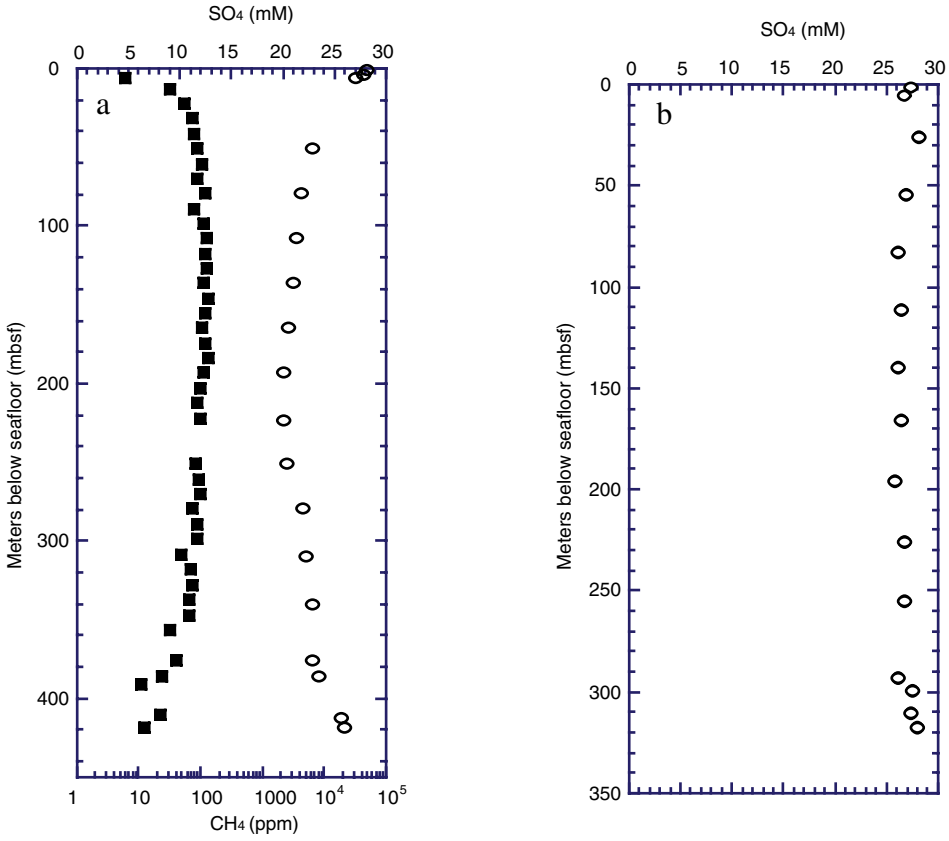

SO4 (mM)
0 5 10 15 20 25 30
a
Meters below seafloor (mbsf)
0 100 200 300 400
1 10 100 1000 10^4 10^5
CH4 (ppm)
SO4 (mM)
0 5 10 15 20 25 30
b
Meters below seafloor (mbsf)
0 50 100 150 200 250 300 350

Figure 2

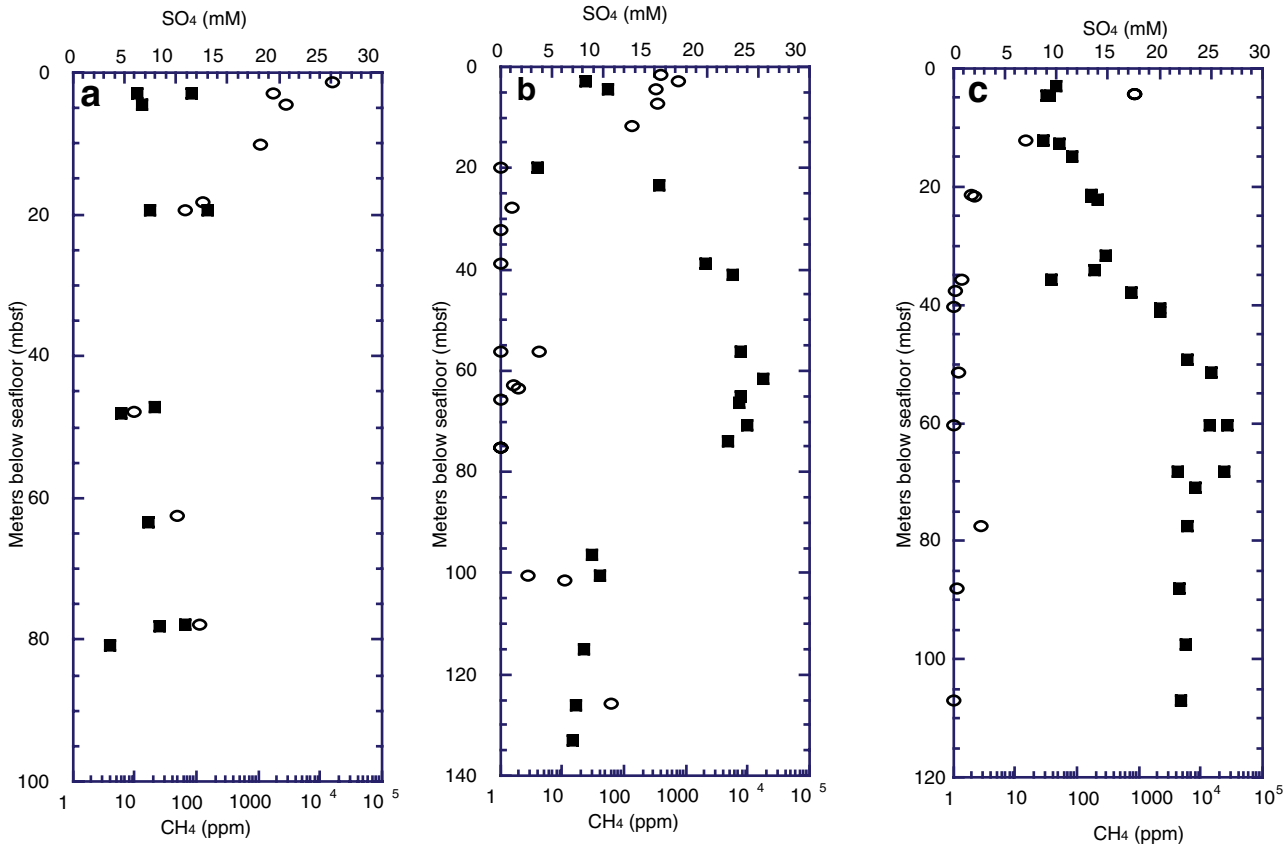


Figure 3

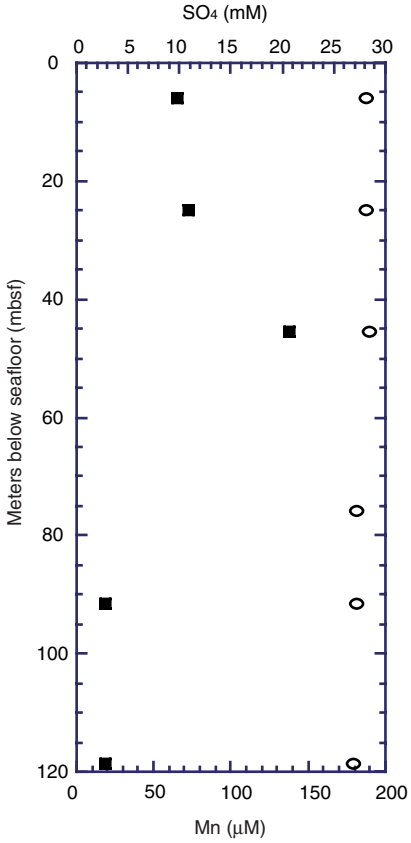

SO4 (mM)
0
5
10
15
20
25
30
Meters below seafloor (mbsf)
0
20
40
60
80
100
120
0
50
100
150
200
Mn (µM)

Figure 4

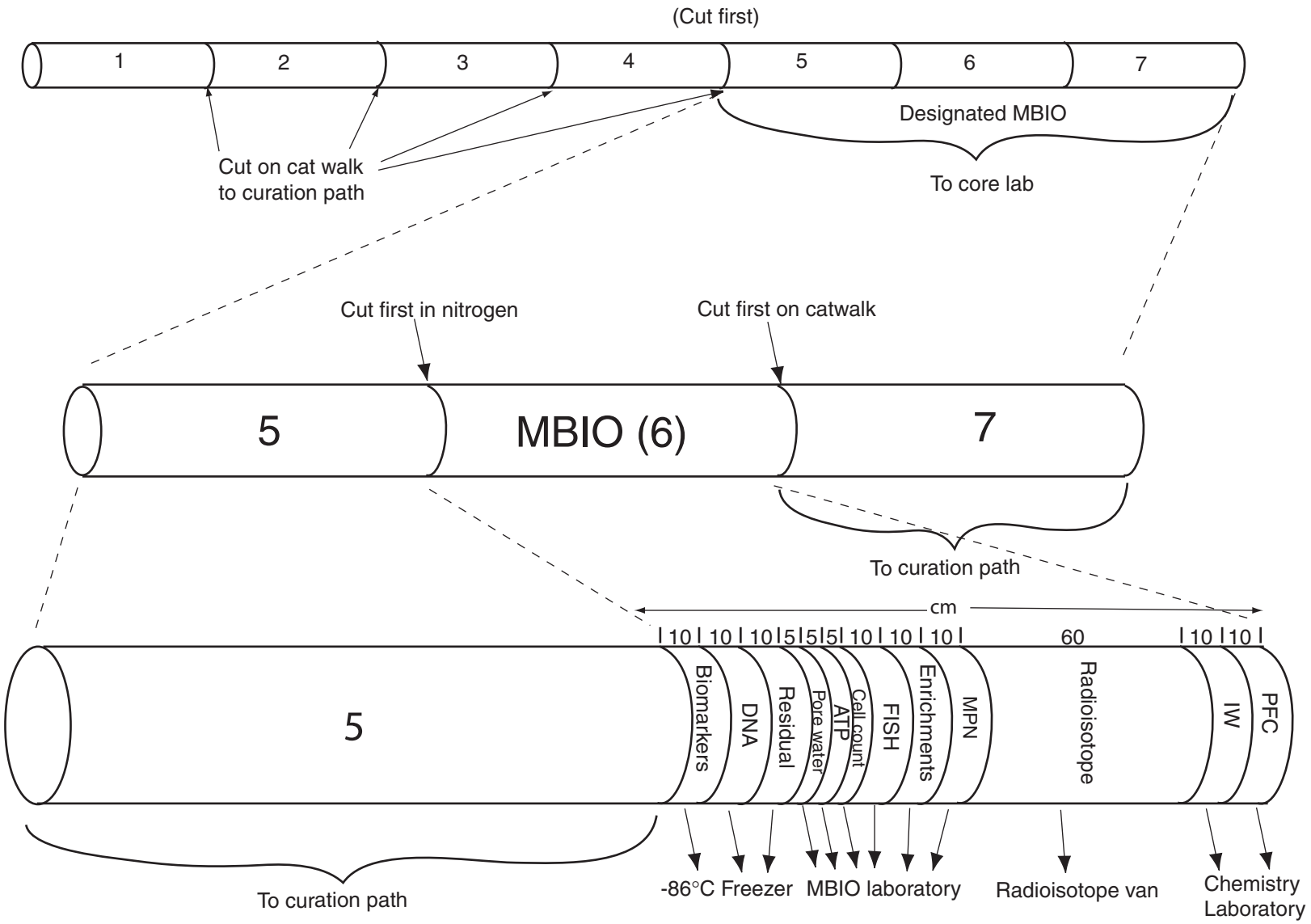

(Cut first)
1
2
3
4
5
6
7
Designated MBIO
Cut on cat walk
to curation path
To core lab
Cut first in nitrogen
Cut first on catwalk
5
MBIO (6)
7
To curation path
cm
10 10 10 5 5 5 10 10 10 60 10 10
5
Biomarkers
DNA
Residual
Pore water
ATP
Cell count
FISH
Enrichments
MPN
Radioisotope
IW
PFC
To curation path
-86°C Freezer
MBIO laboratory
Radioisotope van
Chemistry
Laboratory

Figure 5

Table 1. Leg 201 Operations Plan and Time Estimate for Primary Sites

Site No.	Location (Lat/Long)	Water Depth (mbrf)	Operations Description	(hrs)	Total Transit (days)	Total Drilling (days)	Total Logging (days)	Total On-site (days)
San Diego	32.45°N, 117.10°W		In Port San Diego - Refuel/resupply/crew change	120.0				
			Transit 1837 nmi from San Diego to EQP-2A @ 10.5 kt	175.0	7.3			
EQP-2A	2°46.2'N	3791	Hole A: APC 120m/XCB to 318m, APCT, APCM, DVTP-P, PCS	70.6		2.9		
(ODP 851-	110°34.3'W		Hole B: TBD (est 36.9 hrs/1.5 days)					
Leg 138)			Hole C: APC 120m/XCB to 318m (10 hr to log w/triple combo)	79.5		2.9	0.4	6.2
			Transit from EQP-2A to EQP-1A: 1243 nmi @ 10.5 kts	118.4	4.9			
EQP-1A	3°5.7'S	3325	Hole A: APC 200m/XCB to 400m, APCT, APCM, DVTP-P, PCS	73.2		3.1		
(ODP 846-	90°49.1'W		Hole B: TBD (est 42.5 hrs/1.8 days)					
Leg 138)	(methane expected)		Hole C: APC 200m/XCB to 400m (10 hr to log w/triple combo)	83.6		3.1	0.4	6.6
			Transit from EQP-1A to PRU-3A: 697 nmi @ 10.5 kts	66.4	2.8			
PRU-03A	8°59.5'S	437	Hole A: APC 50m/XCB to 160m, APCT, APCM, DVTP-P	35.0		1.4		
(ODP 684-	79°54.4'W		Hole B: TBD (est 13.4 hrs/0.6 days)					
Leg 112)			Hole C: APC 50m/XCB to 160m (no wireline logging)	31.6		1.3	0.0	2.7
			Transit from PRU-03A to PRU-2A: 259 nmi @ 10.5 kts	24.7	1.0			
PRU-02A	11°3.9'S	264	Hole A: APC 100m/XCB to 200m, APCT, APCM, DVTP-P	36.7		1.5		
(ODP 680-	78°4.7'W		Hole B: TBD (estimate: 14.8 hrs/0.6 days)					
Leg 112)			Hole C: APC 100m/XCB to 200m (7.5 hr to log w/triple combo)	44.8		1.6	0.3	3.4
			Transit from PRU-2A to PRU-1A: 9 nmi @ 10.5 kts	0.9	0.1			
PRU-01A	10°58.6'S	162	Hole A: APC 110m/XCB to 200m, APCT, APCM, DVTP-P	39.4		1.6		
(ODP 681-	77°57.5'W		Hole B: TBD (estimate: 18.7 hrs/0.8 days)					
Leg 112)	(shallow gas ???)		Hole C: APC 110m/XCB to 200m (7.5 hr to log w/triple combo)	48.5		1.7	0.3	3.6
			Transit from PRU-1A to PRU-4A: 156 nmi @ 10.5 kts	14.8	0.6			
PRU-04A	9°6.8'S	5081	Hole A: APC 40m/XCB to 200m, APCT, APCM, DVTP-P, PCS	90.3		3.8		
(ODP 685-	80°35.0'W		Hole B: TBD (estimate: 55.7 hrs/2.3 days)					
Leg 112)	(possible gas hydrates		Hole C: APC 40m/XCB to 200m (11.0 hr to log w/triple combo)	104.1		3.8	0.5	8.1
			Transit from PRU-4A to PRB-2A: 201 nmi @ 10.5 kts	19.2	0.8			
PRB-02A	12°1.3'S	4838	Hole A: APC to 124m, APCT, APCM, DVTP-P	54.2		2.3		
(DSDP 321-	81°54.2'W		Hole B: TBD (estimate: 22.1 hrs/0.9 days)					
Leg 34)			Hole C: APC to 124m (no wireline logging)	49.4		2.1	0.0	4.4
			Transit 1390 nmi from PRB-2A to Valparaiso @ 10.5 kt	131.7	5.5			
Valparaiso	33.05°S, 71.4°W		End of Leg - In port Valparaiso, Chile	0.0				

SUBTOTAL:	**23.0**	**33.1**	**1.9**	**35.0**
ESTIMATED OPERATING DAYS (including 5.0 day port call):	**63.0**			

Time Estimate File: leg201.dat **M. Storms 12-Jun-01**

SITE SUMMARIES

Site: EQP-2A

Priority: Primary
Position: 2°46.22'N, 110°34.31'W
Water Depth: 3761 m
Sediment Thickness: 318 m
Target Drilling Depth: Through sediment–basement transition
Approved Maximum Penetration: 318 m (To 350 m requested from PPSP)
Seismic Coverage: *Thomas Washington* Venture I line O, same position as Site 851.

Objectives:

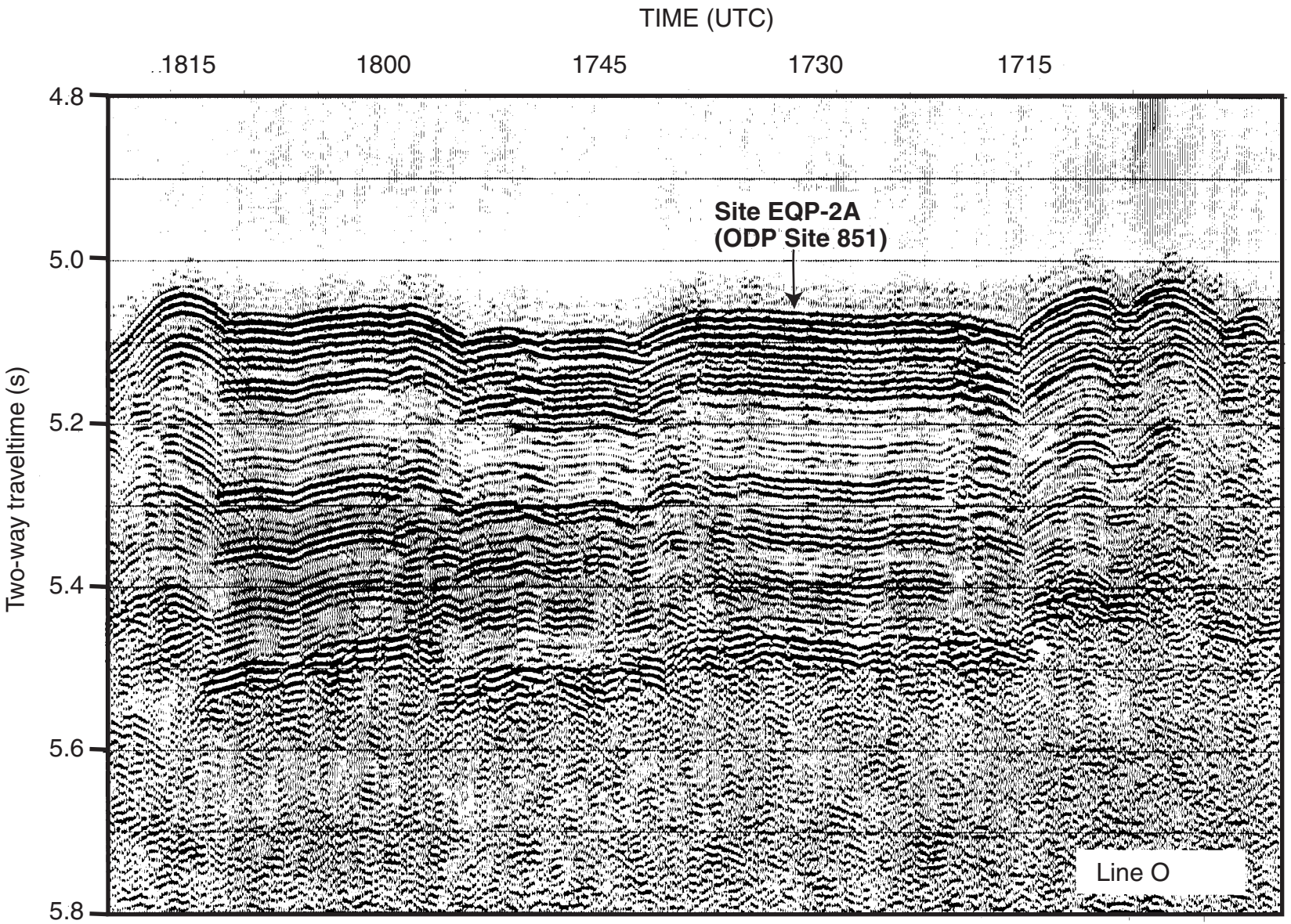
1) Test by comparison with other sites during this expedition whether microbial communities, activity, and survival strategies are different in this deeply buried, organic-poor environment than those in open-ocean sediments with more organic matter or shallower burial.

2) Examine how subsurface (up from basement) hydrologic flow affects microbiological community structure and sediment chemistry in organic-poor sediments with sulfate-rich pore waters.

Drilling Program: APC to refusal, XCB to and a couple of meters into basement. At least two holes, possibly three. Test site for PCS. May require additional mudline cores.

Logging and Downhole Program: Triple combo, possible FMS, APC-temperature, DVTP, and MBARI methane tool.

Nature of Rock Anticipated: Foraminifer nannofossil and diatom nannofossil ooze, basaltic basement.



Single-channel seismic profile over ODP Site 851 (proposed Site EQP-2A). Profile collected during VENTURE 01 cruise of the *Thomas Washington*.

Site: EQP-1A

Priority: Primary
Position: 3°05.70'S, 90°49.08'W
Water Depth: 3296 m
Sediment Thickness: 420 m
Target Drilling Depth: Through sediment–basement transition
Approved Maximum Penetration: 400 m (to 450 m requested from PPSP)
Seismic Coverage: *Thomas Washington* Venture I line G, same position as ODP Site 846.

Objectives:

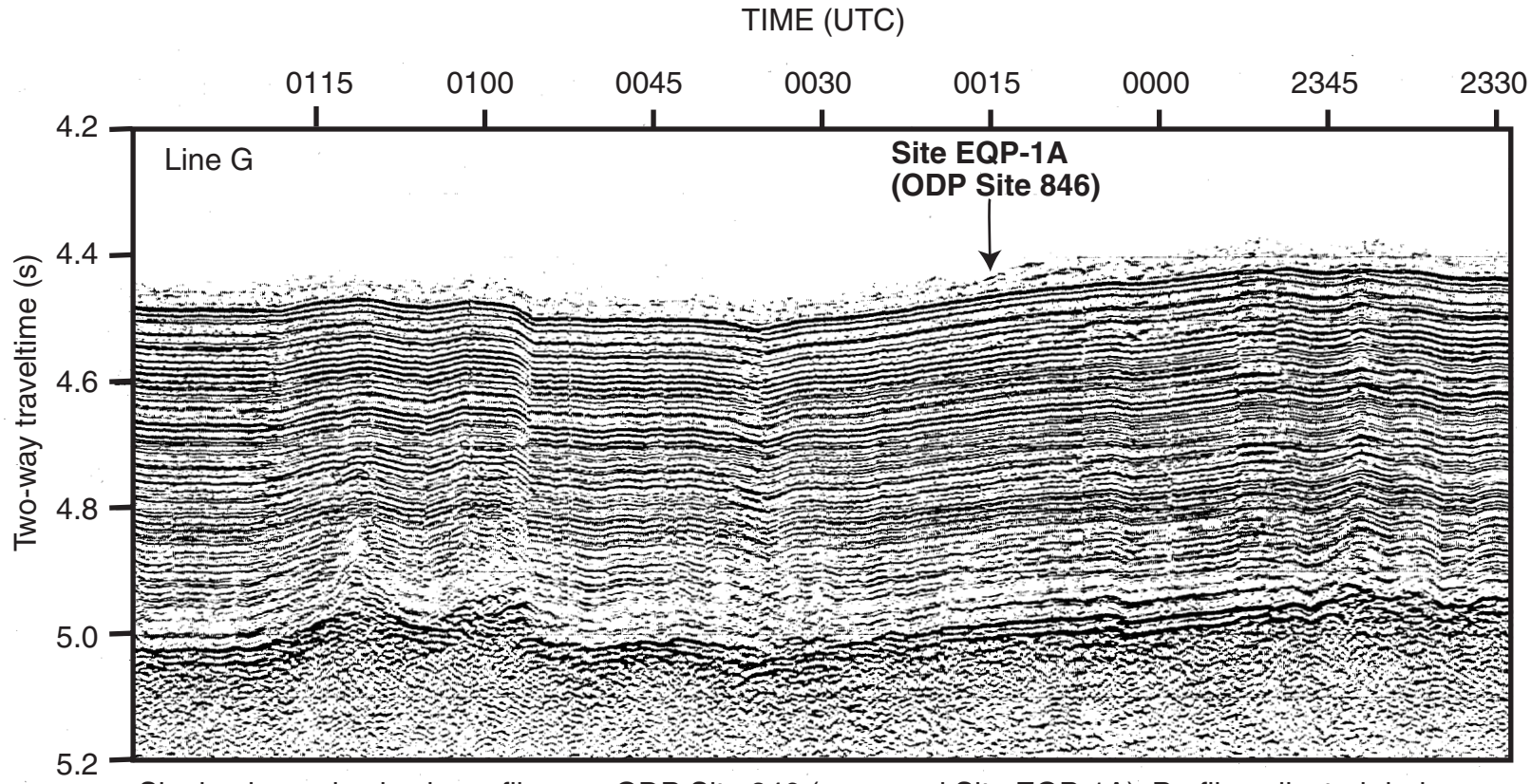
1) Test if the accepted microbial paradigm (of respiration control by standard free energy) is violated by methanogenesis in sulfate-rich open-ocean sediments.

2) Examine how subsurface (up from basement) hydrologic flow affects microbiological community structure and sediment chemistry in relatively organic-poor sediments with sulfate-rich pore waters.

Drilling Program: APC to refusal, XCB to and a couple of meters into basement. At least two holes, possibly three. Test site for PCS. May require additional mudline cores.

Logging and Downhole Program: Triple combo, possible FMS, APC-temperature, DVTP, and APC methane tool.

Nature of Rock Anticipated: Carbonate (mostly nannofossil) ooze and siliceous (both diatom and radiolarian) ooze, basaltic basement.



Single-channel seismic profile over ODP Site 846 (proposed Site EQP-1A). Profile collected during VENTURE 01 cruise of the *Thomas Washington*.

Site: PRU-3A

Priority: Primary
Position: 8°59.49'S, 79°54.35'W
Water Depth: 426 m
Sediment Thickness: >160 m
Target Drilling Depth: 160 m
Approved Maximum Penetration: 160 m
Seismic Coverage: Line H from the Yaquina Yaloc survey and multichannel line 2025, which intersects the site and line 1900 which crosses Line 2025 ~7 km southwest of the site, same position as ODP Site 684.

Objectives:

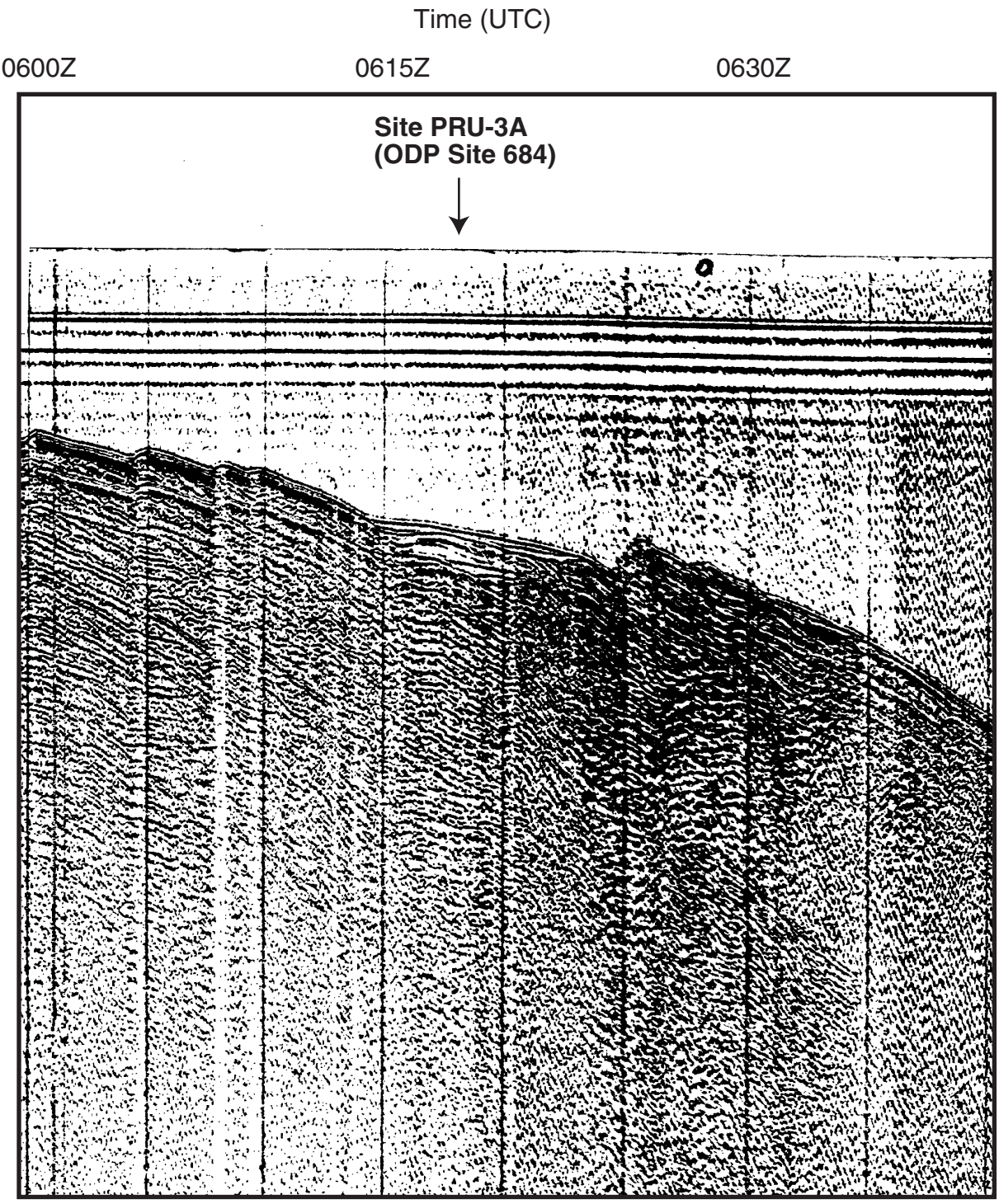
1) Examine microbial communities, activity, and survival strategies in a "normal" upwelling-zone methanogenic sedimentary sequence for comparison with other shallow water sites where a subsurface brine flow appears to disrupt the pore water chemistry. This site shows no evidence of the brine incursion.

2) Identify the methanotrophic communities of deeply buried marine sediments.

Drilling Program: APC to refusal, XCB to total depth (TD). At least two holes, possibly three. Possible MDCB/RCB coring to improve recovery. Possible PCS deployment. May require additional mudline cores

Logging and Downhole Program: APC-temperature, DVTP, and APC methane tool.

Nature of Rock Anticipated: Diatomaceous and terrigenous mud, silt, and sand.



Single-channel seismic profile over ODP Site 684 (proposed Site PRU-3A). Profile is from OSU SCS line Yaloc 740322.

Site: PRU-2A

Priority: Primary
Position: 11°03.90'S, 78°04.67'W
Water Depth: 252 m
Sediment Thickness: >2000 m
Target Drilling Depth: 300 m
Approved Maximum Penetration: 300 m
Seismic Coverage: Yaloc 20-03-74, additional single-channel and 3.5-kHz data were also collected during ODP Leg 112, same position as ODP Site 680.

Objectives: Test how microbial communities and activity differs between a "normal" upwelling-zone methanogenic sedimentary sequence (proposed Site PRU-3A) and at this site where a subsurface brine flow introduces sulfate into a sulfate-bearing sediment. This will also be compared with a third site where the subsurface brine flow effects and organic-rich (methanogenic) sediments and the microbiological communities they host.

Drilling Program: APC to refusal, XCB to TD. At least two holes, possibly three. Possible MDCB/RCB coring to improve recovery. Possible PCS deployment. May require additional mudline cores.

Logging and Downhole Program: Triple combo, possible FMS, APC-temperature, DVTP, and APC methane tool.

Nature of Rock Anticipated: Foraminifer diatomaceous mud and ooze, sand.

See seismic line for Site PRU-1A.

Site: PRU-1A

Priority: Primary
Position: 10°58.60'S, 77°57.46'W
Water Depth: 150 m
Sediment Thickness: >2000 m
Target Drilling Depth: 300 m
Approved Maximum Penetration: 200 m (to 300 m requested to PPSP)
Seismic Coverage: Yaloc 20-03-74, additional single-channel and 3.5-kHz data were also collected during ODP Leg 112, same position as ODP Site 681.

Objectives:

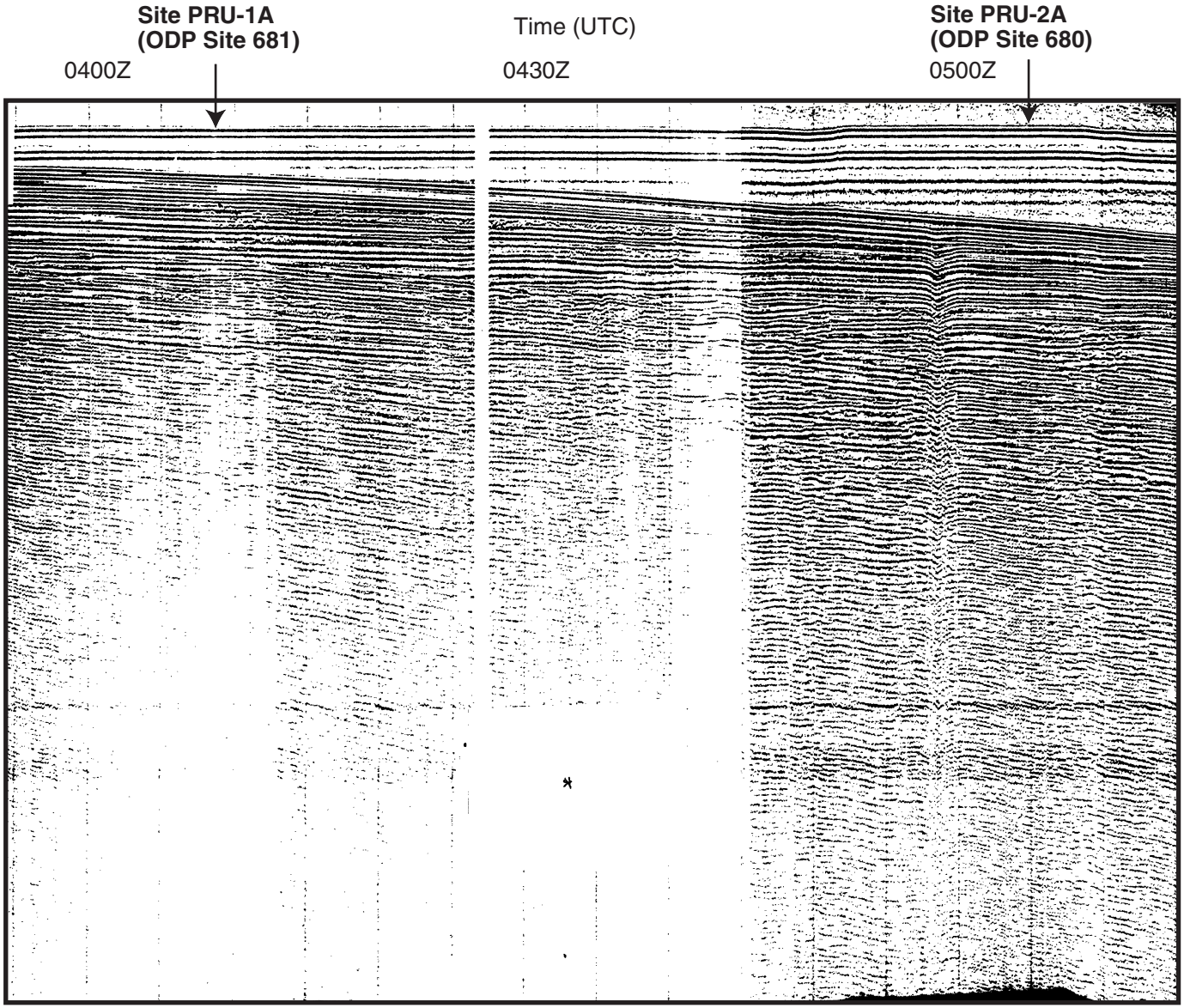
1) Test how introduction of an electron acceptor by subsurface hydrologic flow affects community structure and sediment chemistry in organic-rich (methanogenic) sediments.

2) Identify the methanotrophic communities of deeply buried marine sediments.

Drilling Program: APC to refusal, XCB to TD. At least two holes, possibly three. Possible MDCB/RCB coring to improve recovery. Possible PCS deployment. May require additional mudline cores.

Logging and Downhole Program: Triple combo, possible FMS, APC-temperature, DVTP, and APC methane tool.

Nature of Rock Anticipated: Foraminifer diatomaceous mud and ooze, sand.



Single-channel seismic profile over ODP Sites 681 and 680 (proposed Sites PRU-1A and PRU-2A, respectively). Profile is from OSU SCS line Yaloc 740320.

Site: PRU-4A

Priority: Primary
Position: 9° 06.78'S, 80°35.01'W
Water Depth: 5071 m
Sediment Thickness: >468 m
Target Drilling Depth: 300 m
Approved Maximum Penetration: 200 m (to 300 m requested to PPSP)
Seismic Coverage: Single-channel seismic line CDP-2. Charcot line 17 crosses common depth point (CDP) 2 ~2.5 km southwest of Site PRU-4A, same position as ODP Site 685.

Objectives:

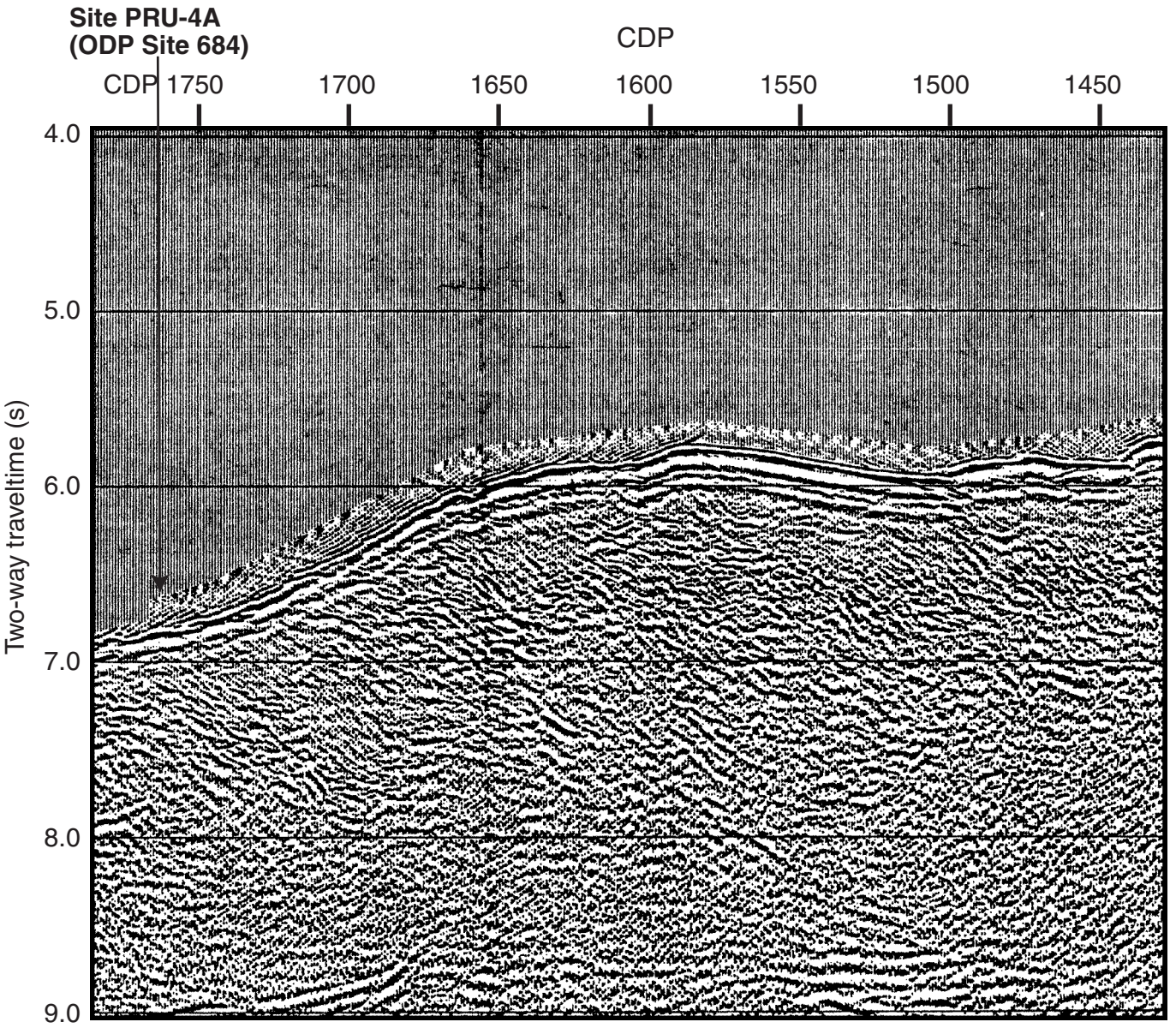
1) Test how hydrate-bearing sequences differ in microbial activity and community structure from nearby methane-rich sequences that lack hydrates (proposed Sites PRU-1A and PRU-3A).

2) Provide a Peru Margin microbial and biogeochemical counterpoint to the hydrate-rich sites targeted for coring during Leg 204 (Hydrate Ridge).

Drilling Program: APC to refusal, XCB to TD. At least two holes, possibly three. Possible MDCB/RCB coring to improve recovery. PCS test site. May require additional mudline cores.

Logging and Downhole Program: Triple combo, possible FMS, APC-temperature, DVTP, and APC methane tool.

Nature of Rock Anticipated: Diatomaceous mud and mudstone.



Multichannel seismic profile over ODP Site 685 (proposed Site PRU-4A). Site is at CDP 1763 on MCS line Peru-2.

Site: PRB-2A

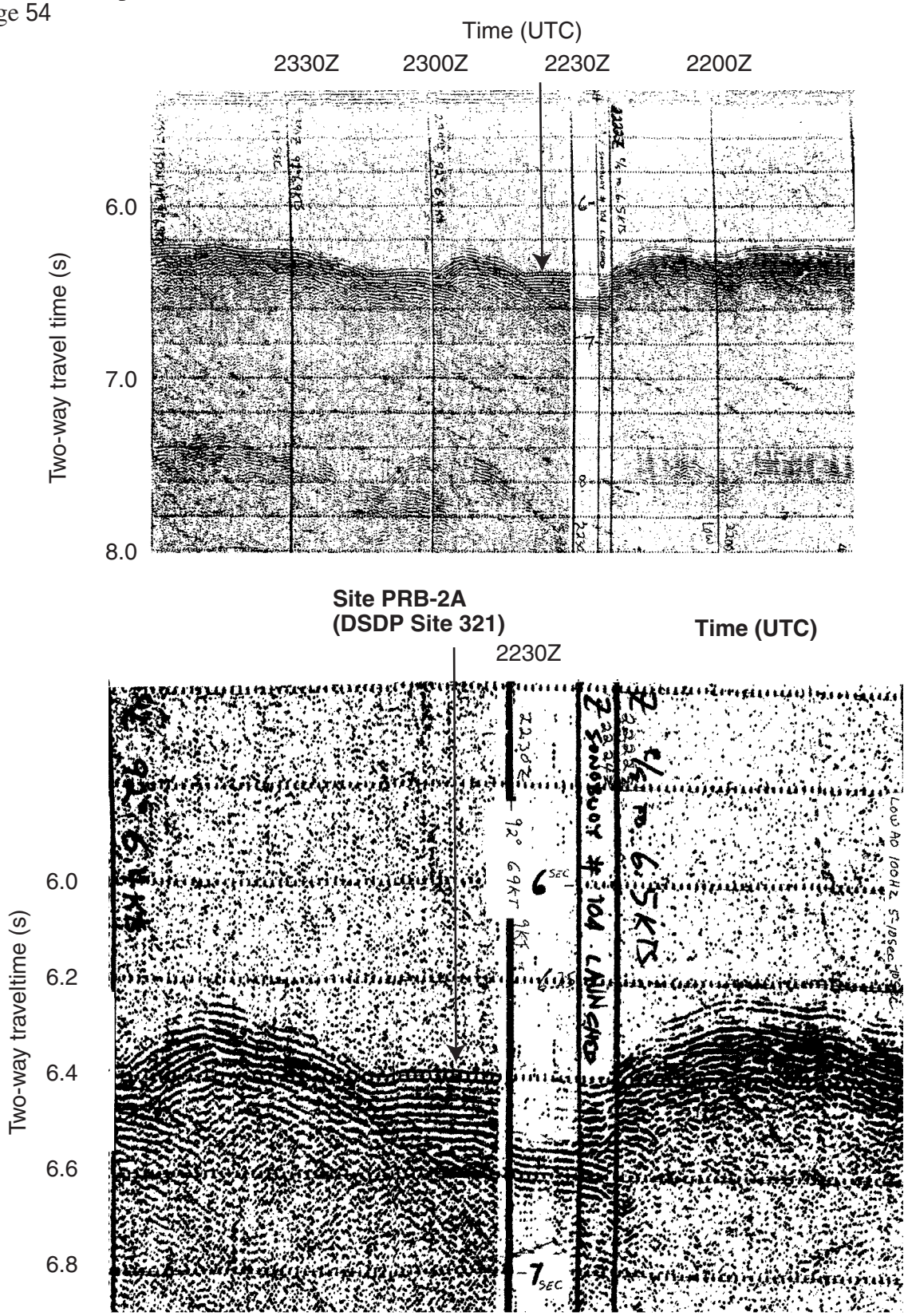
Priority: Primary
Position: 12°01.29'S, 81°54.24'W
Water Depth: 4827 m
Sediment Thickness: 124 m
Target Drilling Depth: Through sediment–basement transition
Approved Maximum Penetration: 124 m (to 150 m requested to PPSP)
Seismic Coverage: Kana Keoki Leg 7 single-channel seismic profiles and single-channel and 3.5kHz profiles collected during DSDP Leg 34, same position as DSDP Site 321.

Objective: Test how Mn-reducing microbial communities in very organic-poor relatively deeply buried marine sediments differ from other environments studied during this expedition.

Drilling Program: APC to refusal, XCB to TD. At least two holes, possibly three. Possible MDCB/RCB coring to improve recovery. Possible PCS deployment. May require additional mudline cores.

Logging and Downhole Program: APC-temperature, DVTP, and APC methane tool.

Nature of Rock Anticipated: Siliceous clay, nannofossil ooze, basaltic basement.



Seismic profile over DSDP Site 321 (proposed Site PRB-2A). Profile is from *Kana Keoki*, 1971, Leg 7.

Site: PRB-1A

Priority: Alternate
Position: 9°00.40'S, 83°31.80'W
Water Depth: 4487 m
Sediment Thickness: 155 m
Target Drilling Depth: Through sediment–basement transition
Approved Maximum Penetration: 155 m (to 175 m requested to PPSP)
Seismic Coverage: Kana Keoki Leg 8 single-channel and 3.5-kHz seismic data. The site was also surveyed during DSDP Leg 34, for which single-channel seismic data are also available, same position as DSDP Site 320.

Objectives:

1) Determine conditions under which subsurface microbes may be active, inactive but capable of resuscitation, or dead.

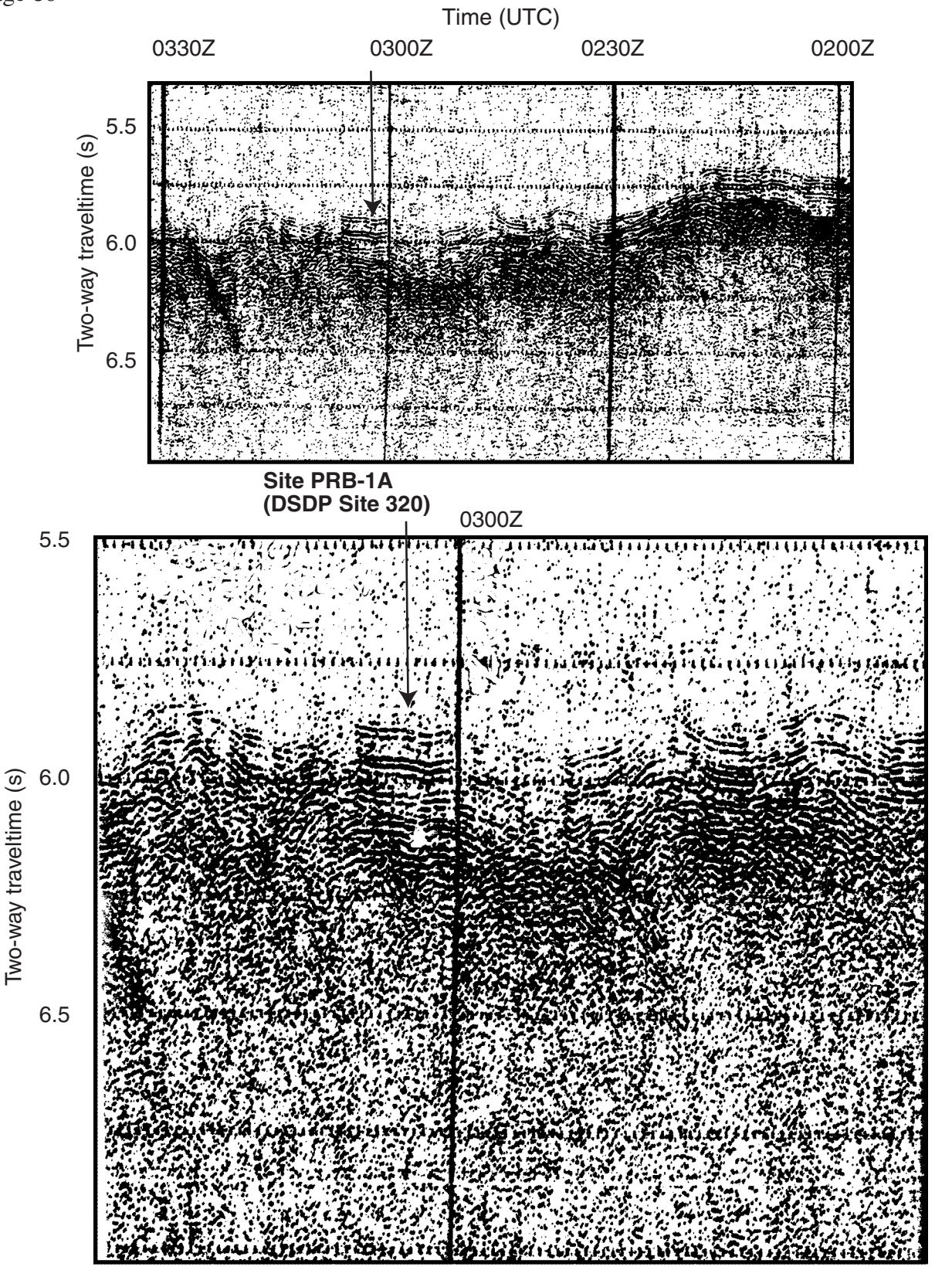
2) Begin to assess the specialized metabolic strategies, if any, that are required to survive in deeply buried marine sediments.

3) Explore phylogenetic affinities and differences between microbes of the organic-poor Peru Basin sediments and nearby organic-rich Peru Margin sites.

Drilling Program: APC to refusal, XCB to TD. At least two holes, possibly three. Possible MDCB/RCB coring to improve recovery. Possible PCS deployment. May require additional mudline cores.

Logging and Downhole Program: APC-temperature, DVTP, and APC methane tool.

Nature of Rock Anticipated: Siliceous clay, nannofossil ooze, basaltic basement.



Seismic profile over DSDP Site 320 (proposed Site PRB-1A). Profile is from *Kana Keoki*, 1971, Leg 8.

Site: ODP Site 679

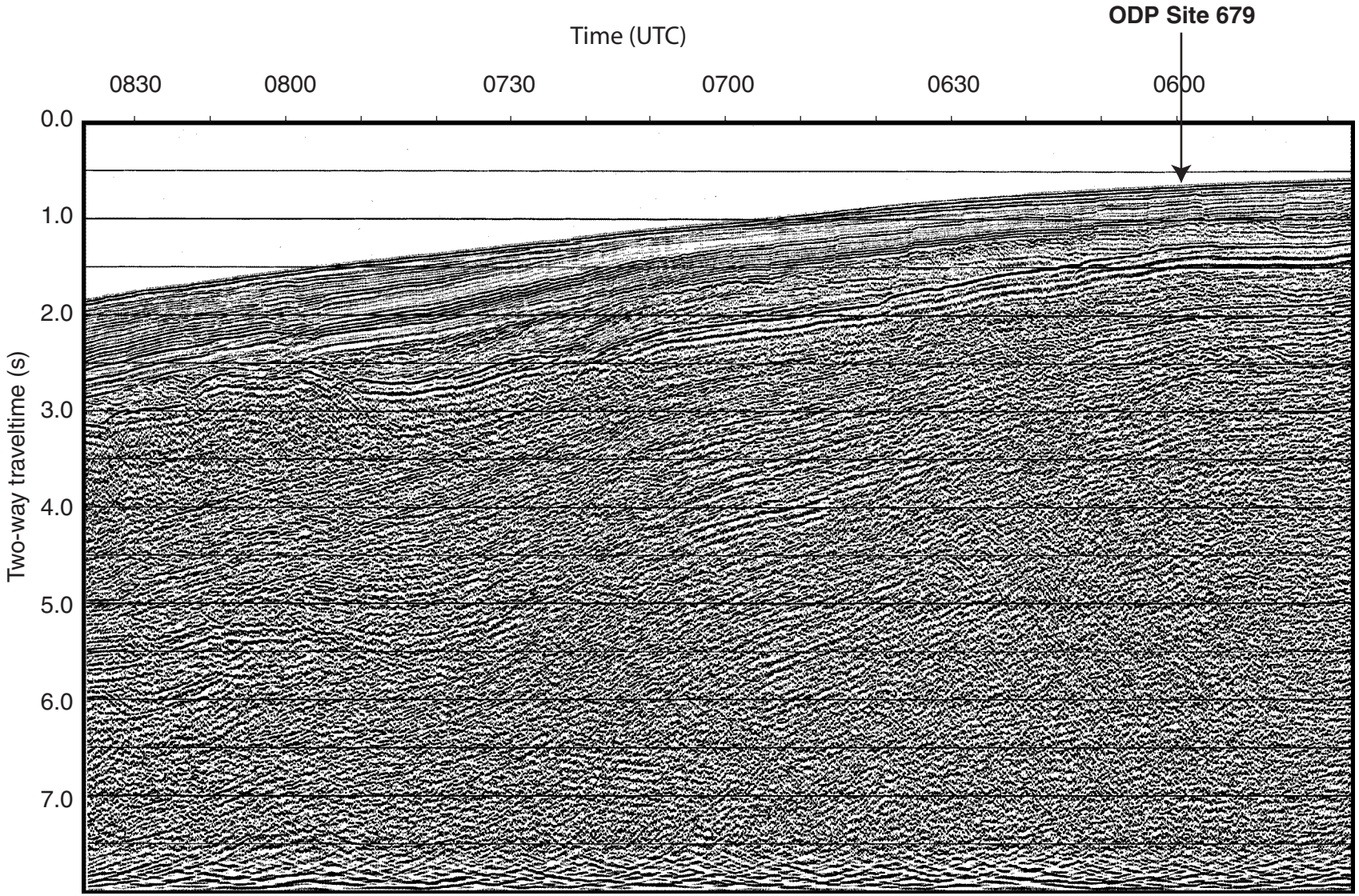
Priority: Alternate
Position: 11°03.76'S, 78°16.25'W
Water Depth: 450 m
Sediment Thickness: >356 m
Target Drilling Depth: 356 m
Approved Maximum Penetration: Requested same maximum penetration as for Leg 112.
Seismic Coverage: HIG MCS line 14 (8505) at 0603Z.

Objective: Compare deeply buried anaerobic-methanotrophy zone to other sites occupied during this expedition.

Drilling Program: TBD

Logging and Downhole Program: TBD

Nature of Rock Anticipated: Diatomaceous mud, sand, and sandy mud.



Multichannel seismic profile over ODP Site 679, HIG MCS line 14.

Site: ODP Site 682

Priority: Alternate
Position: 11°15.99'S, 79°03.73'W
Water Depth: 3789 m
Sediment Thickness: >436 m
Target Drilling Depth: 436 m
Approved Maximum Penetration: Requested same maximum penetration as for Leg 112.
Seismic Coverage: HIG MCS line 13.

Objectives:

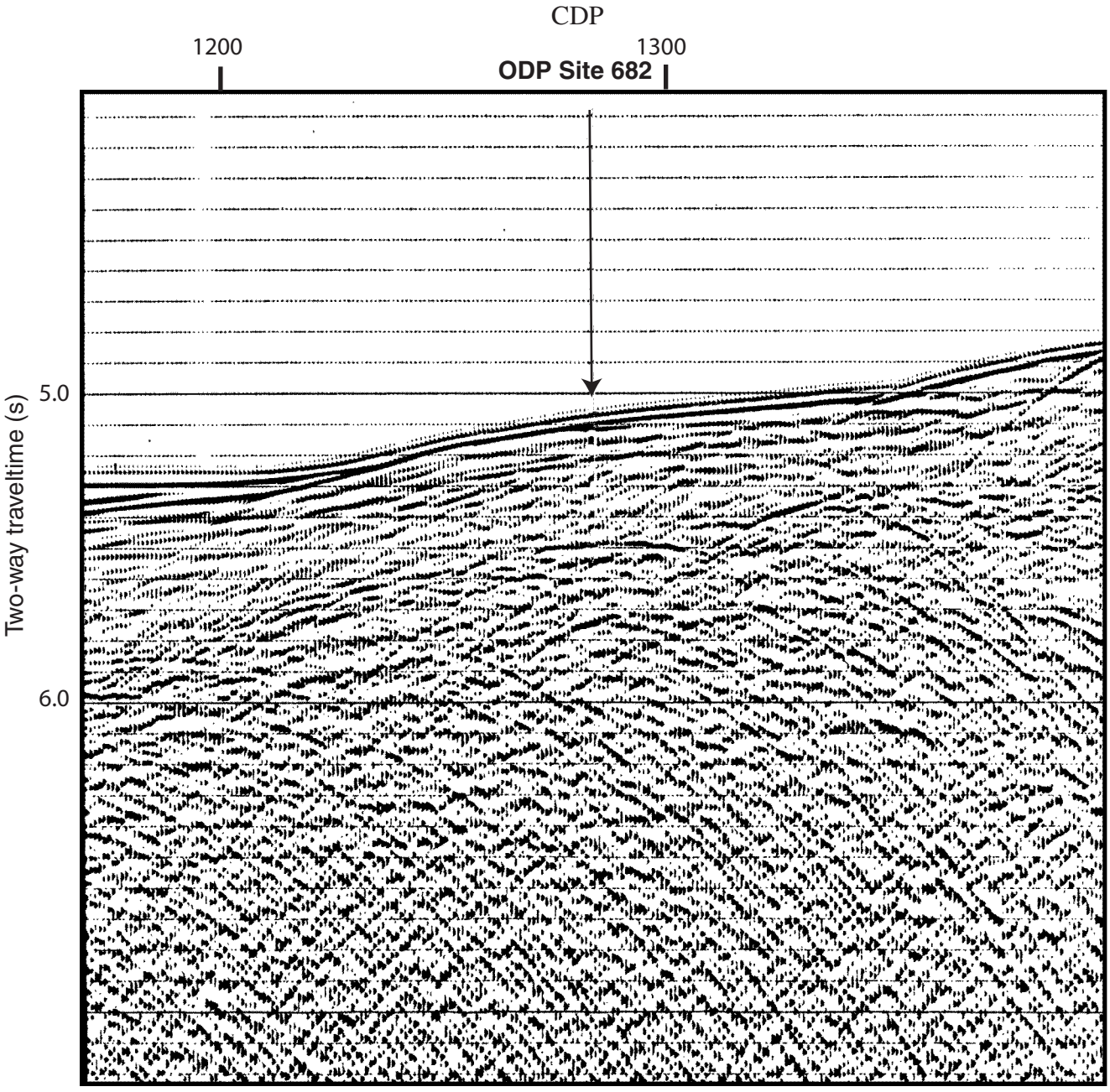
1) Compare standard continental margin site without subsurface brine flow to other sites occupied during this expedition.

2) Examine microbial communities and activity in a possible hydrate-bearing environment.

Drilling Program: TBD

Logging and Downhole Program: TBD

Nature of Rock Anticipated: Diatomaceous mud, mudstone, siliceous ooze, silt, and siltstone.



Multichannel seismic profile over ODP Site 682. Site is on HIG MCS line 13.

Site: ODP Site 686

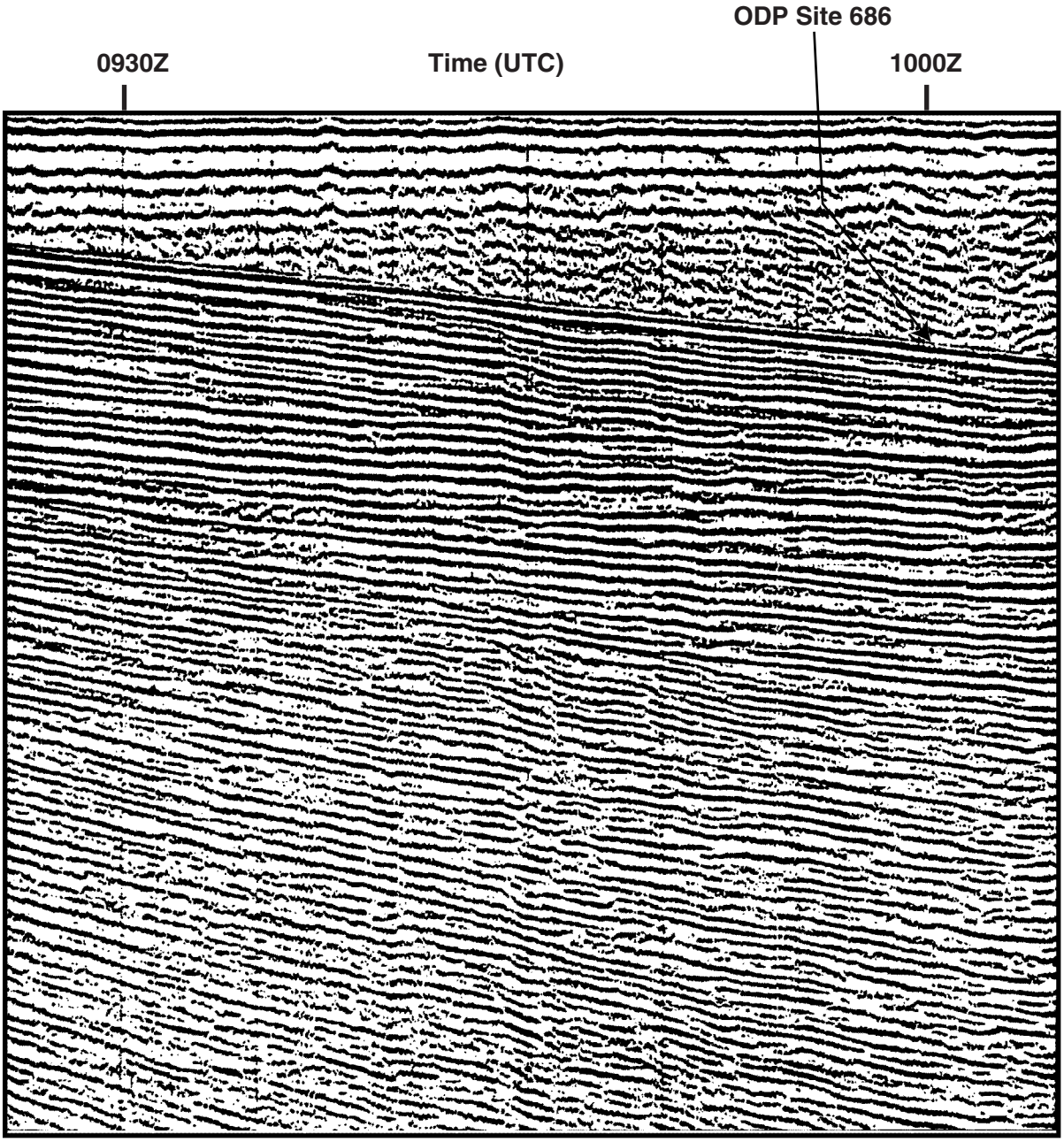
Priority: Alternate
Position: 13°28.81'S, 76°53.49'W
Water Depth: 446 m
Sediment Thickness: >303 m
Target Drilling Depth: 303 m
Approved Maximum Penetration: Requested same maximum penetration as for Leg 112.
Seismic Coverage: OSU SCS line Yaloc 12-03-74 at 1000Z.

Objective: Compare standard continental margin site without subsurface brine flow to other sites occupied during this expedition.

Drilling Program: TBD

Logging and Downhole Program: TBD

Nature of Rock Anticipated: Siltstone, diatomaceous mud, mudstone, siliceous ooze, and sand.



Single-channel seismic profile over ODP Site 686. Site is on line Yaloc 12-03-74 at 1000Z.

Site: ODP Site 687

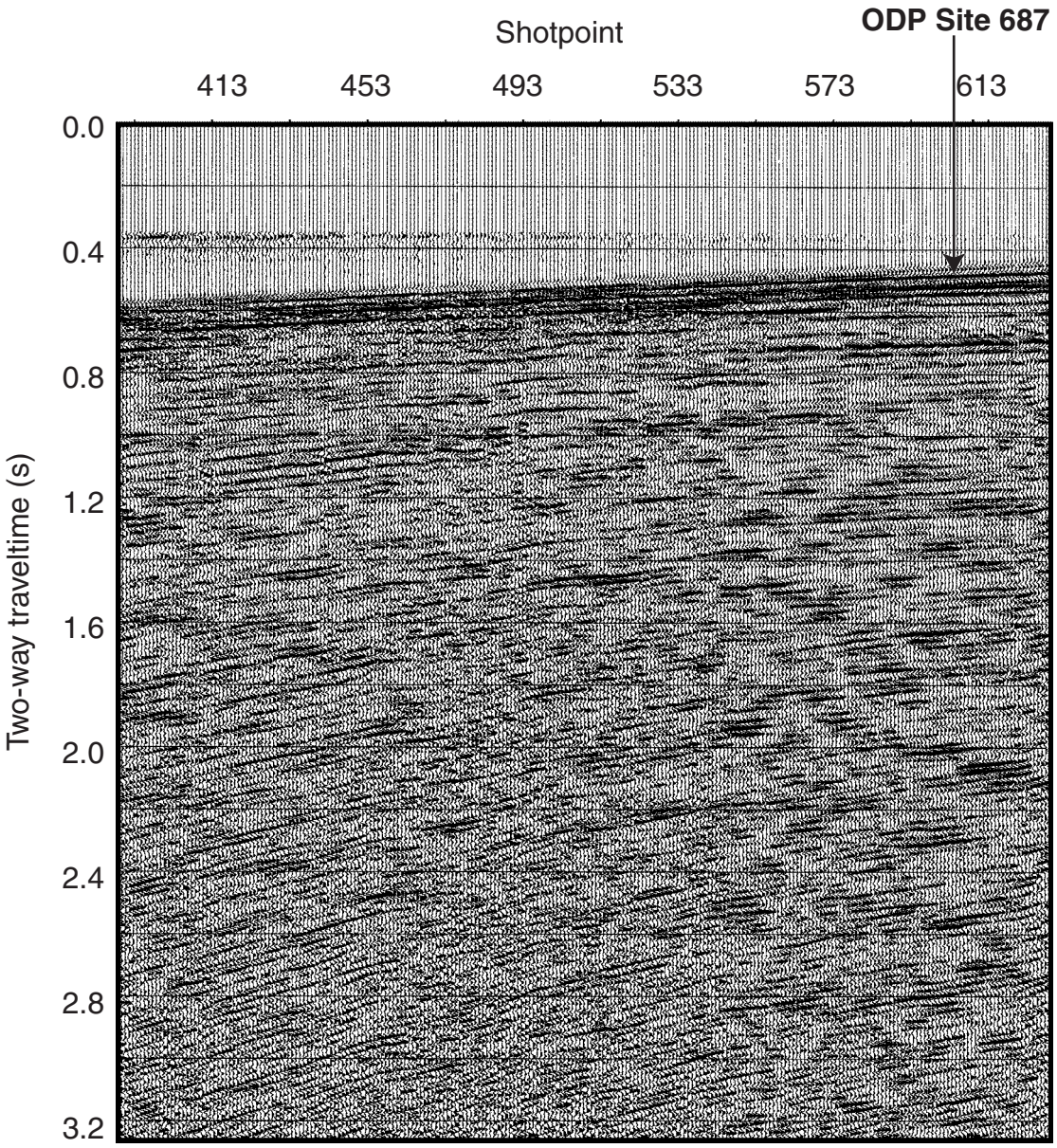
Priority: Alternate
Position: 12°51.78'S, 76°59.43'W
Water Depth: 307 m
Sediment Thickness: >207 m
Target Drilling Depth: 207 m
Approved Maximum Penetration: Requested same maximum penetration as for Leg 112.
Seismic Coverage: OSU SCS line Yaloc 13-03-74 at 1945Z.

Objective: Compare standard continental margin site without subsurface brine flow to other sites occupied during this expedition.

Drilling Program: TBD

Logging and Downhole Program: TBD

Nature of Rock Anticipated: Diatomaceous mud, silt, siltstone, sand, and siliceous ooze.



Single-channel seismic profile collected on Leg 112 over ODP Site 687 (Leg 112, line 9, shotpoint 609).

Site: ODP Site 688

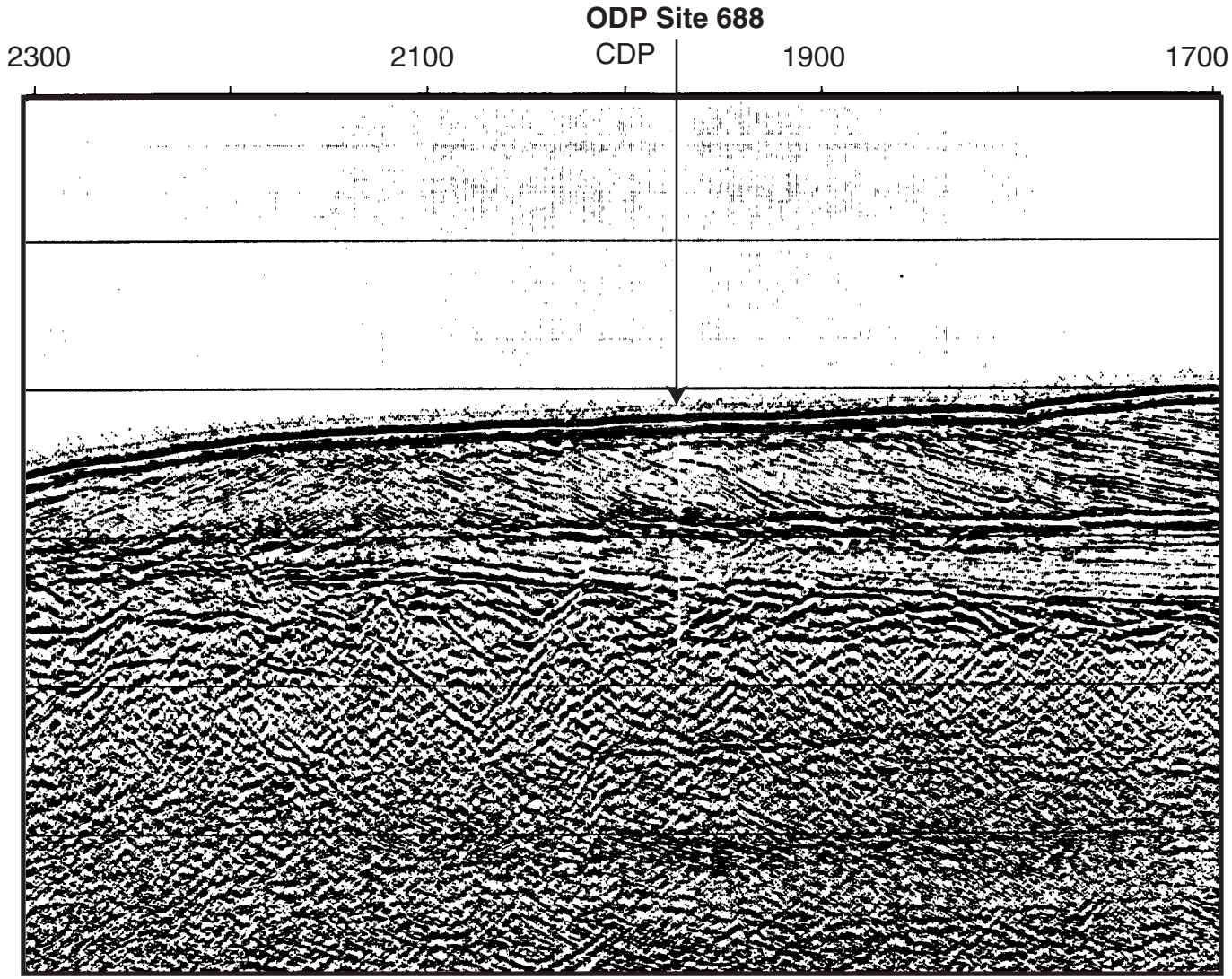
Priority: Alternate
Position: 11°32.28'S, 78°56.65'W
Water Depth: 3826 m
Sediment Thickness: >429 m
Target Drilling Depth: 429 m
Approved Maximum Penetration: Requested same maximum penetration as for Leg 112.
Seismic Coverage: Shell MCS line P1017 at CDP 1975.

Objective: Compare this site with 2% to 9% organic carbon and very high levels of dissolved ammonium, which is probably the most microbiologically active we are likely to encounter with an ODP expedition with other continental margin sites occupied during this expedition.

Drilling Program: TBD

Logging and Downhole Program: TBD

Nature of Rock Anticipated: Diatomaceous mud, mudstone, siliceous ooze, calcareous ooze, sand, silt, and siltstone.



Multichannel seismic profile over ODP Site 688, MCS line P 1017, CDP 1975.

Site: ODP Site 683

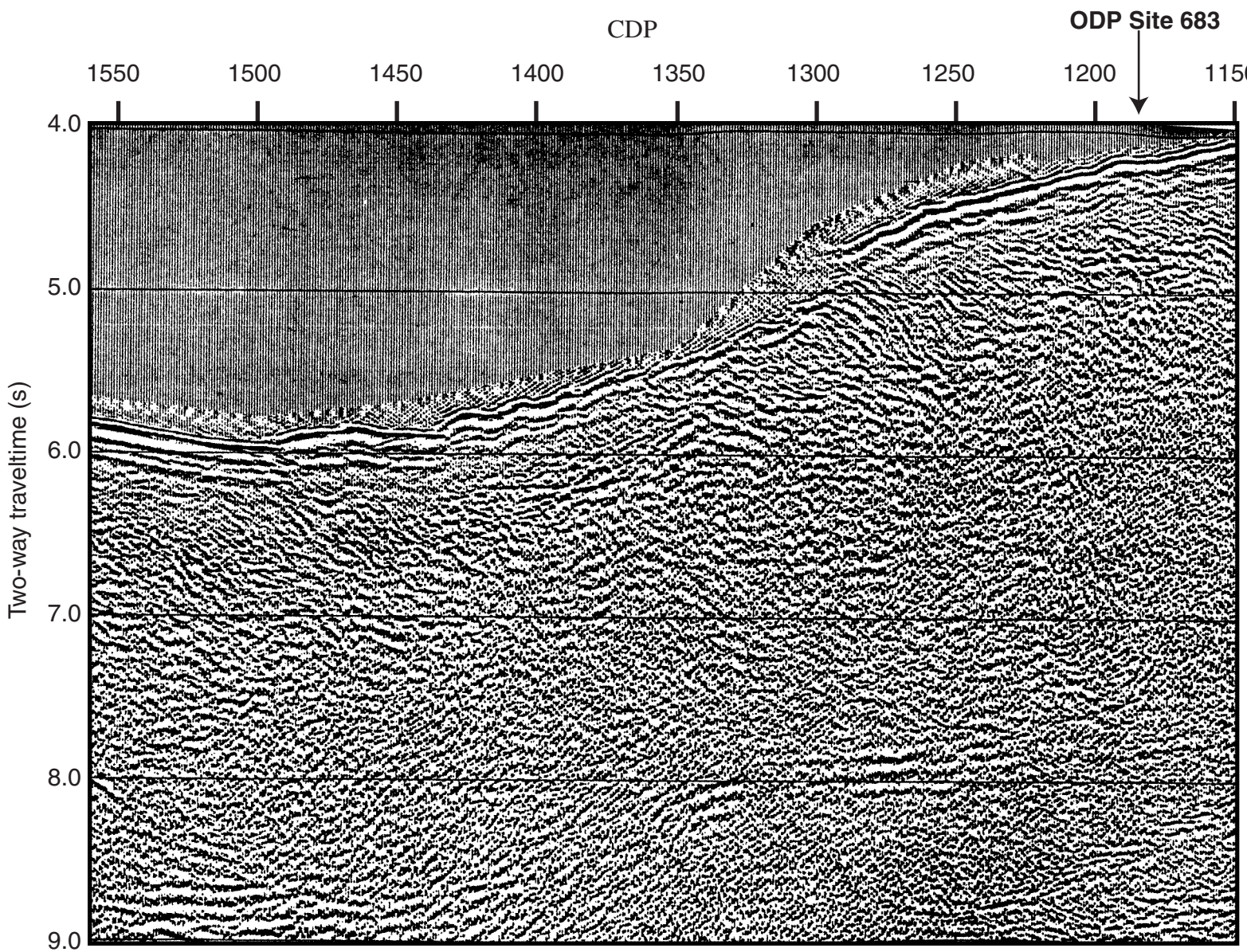
Priority: Alternate
Position: 9°01.69'S, 80°24.40'W
Water Depth: 3072 m
Sediment Thickness: >419 m
Target Drilling Depth: 419 m
Approved Maximum Penetration: Requested same maximum penetration as for Leg 112.
Seismic Coverage: MCS line Peru-2 at CDP 1183.

Objective: Examine microbial communities and activity in a possible hydrate-bearing environment.

Drilling Program: TBD

Logging and Downhole Program: TBD

Nature of Rock Anticipated: Diatomaceous mud, mudstone, siliceous ooze, siltstone, and sand.



Multichannel seismic profile over ODP Site 683. Site is at CDP 1183 on MCS line Peru-2.

Site: ODP Site 845

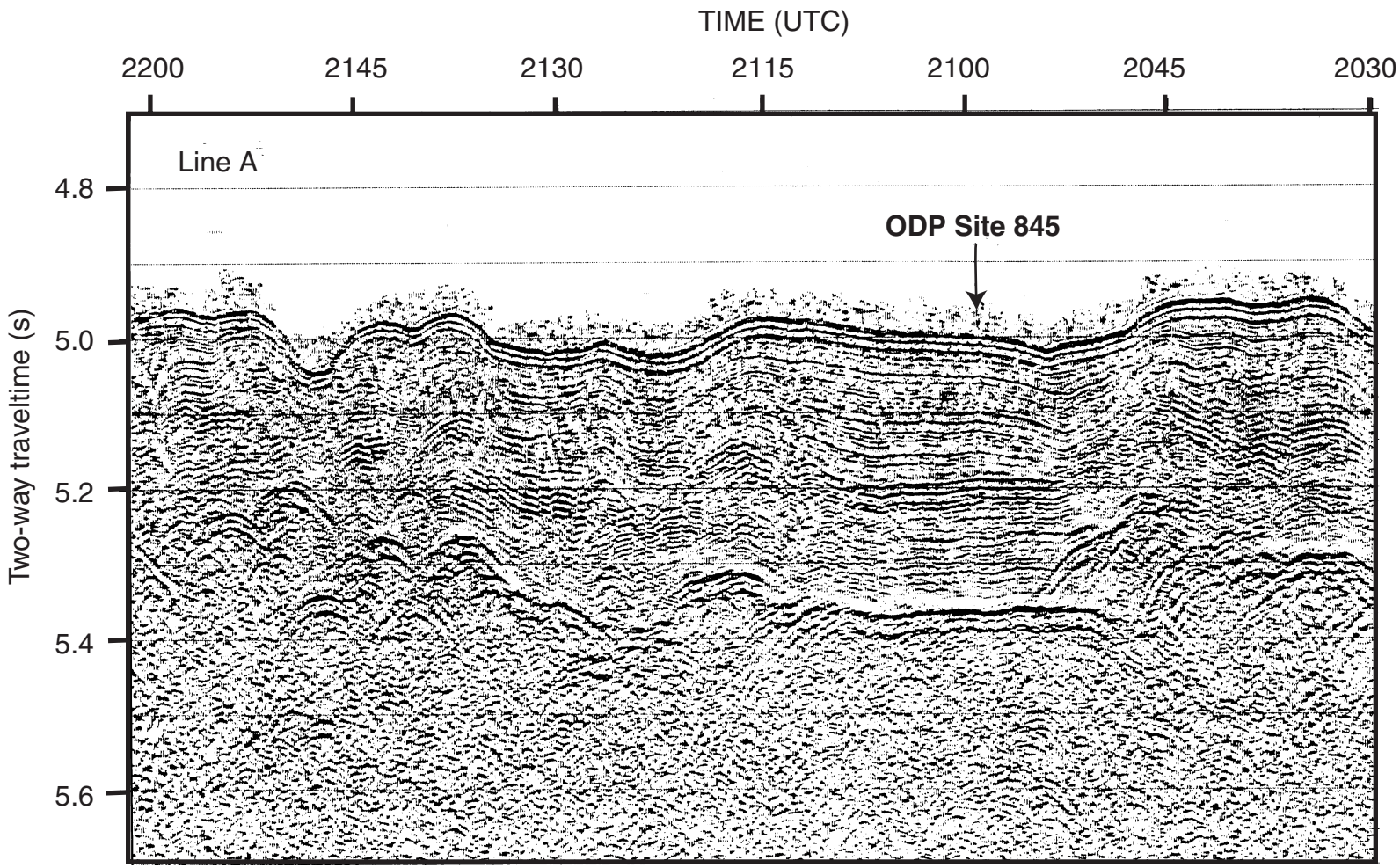
Priority: Alternate
Position: 9°34.95'N, 94°35.45'W
Water Depth: 3704 m
Sediment Thickness: 292 m
Target Drilling Depth: Through sediment–basement transition (>292 m)
Approved Maximum Penetration: Requested same maximum penetration as for Leg 138.
Seismic Coverage: *Thomas Washington* station 15, 2059UTC, 28 September 1989.

Objective: Alternate for Site EQP-2A. This site exhibits clear pore water geochemical evidence of basement flow, although pore water Sr concentrations indicate less carbonate diagenesis at this site than at ODP Site 851.

Drilling Program: TBD

Logging and Downhole Program: TBD

Nature of Rock Anticipated: Diatom and radiolarian clay, nannofossil ooze, and pelagic carbonate.



Single-channel seismic profile over ODP Site 845. Profile collected during VENTURE 01 cruise of the *Thomas Washington.*

Site: ODP Site 849

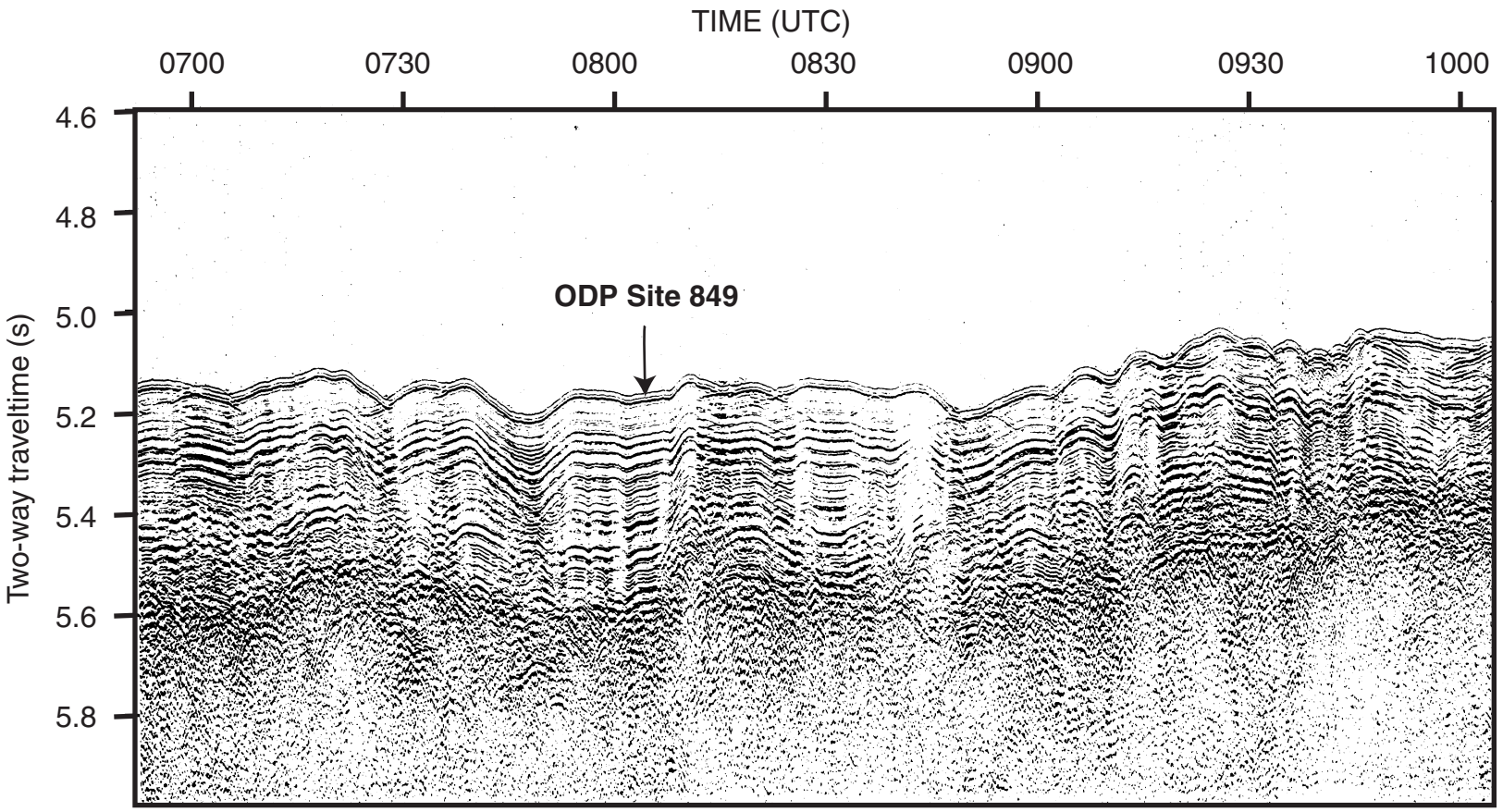
Priority: Alternate
Position: 0°10.98'N, 110°31.18'W
Water Depth: 3839 m
Sediment Thickness: 350 m
Target Drilling Depth: Through sediment–basement transition (>350 m)
Approved Maximum Penetration: Requested same maximum penetration as for Leg 138.
Seismic Coverage: *Thomas Washington* station 6, 0805UTC, 09 September 1989.

Objectives: Nonideal alternate for Site EQP-2A. Methane and high sulfate co-occur here (methane approaches 50 µL/L). Pore water profiles indicate basement flow. A pronounced chert layer at 237 mbsf greatly hinders pore water geochemical (and microbiological?) communication between the shallower sediments in diffusional contact with the sediment/water interface and the deeper sediments in diffusional contact with the basement.

Drilling Program: TBD

Logging and Downhole Program: TBD

Nature of Rock Anticipated: Diatom nannofossil ooze.



Single-channel seismic profile over ODP Site 849. Profile collected during VENTURE 01 cruise of the *Thomas Washington*.

Site: ODP Site 850

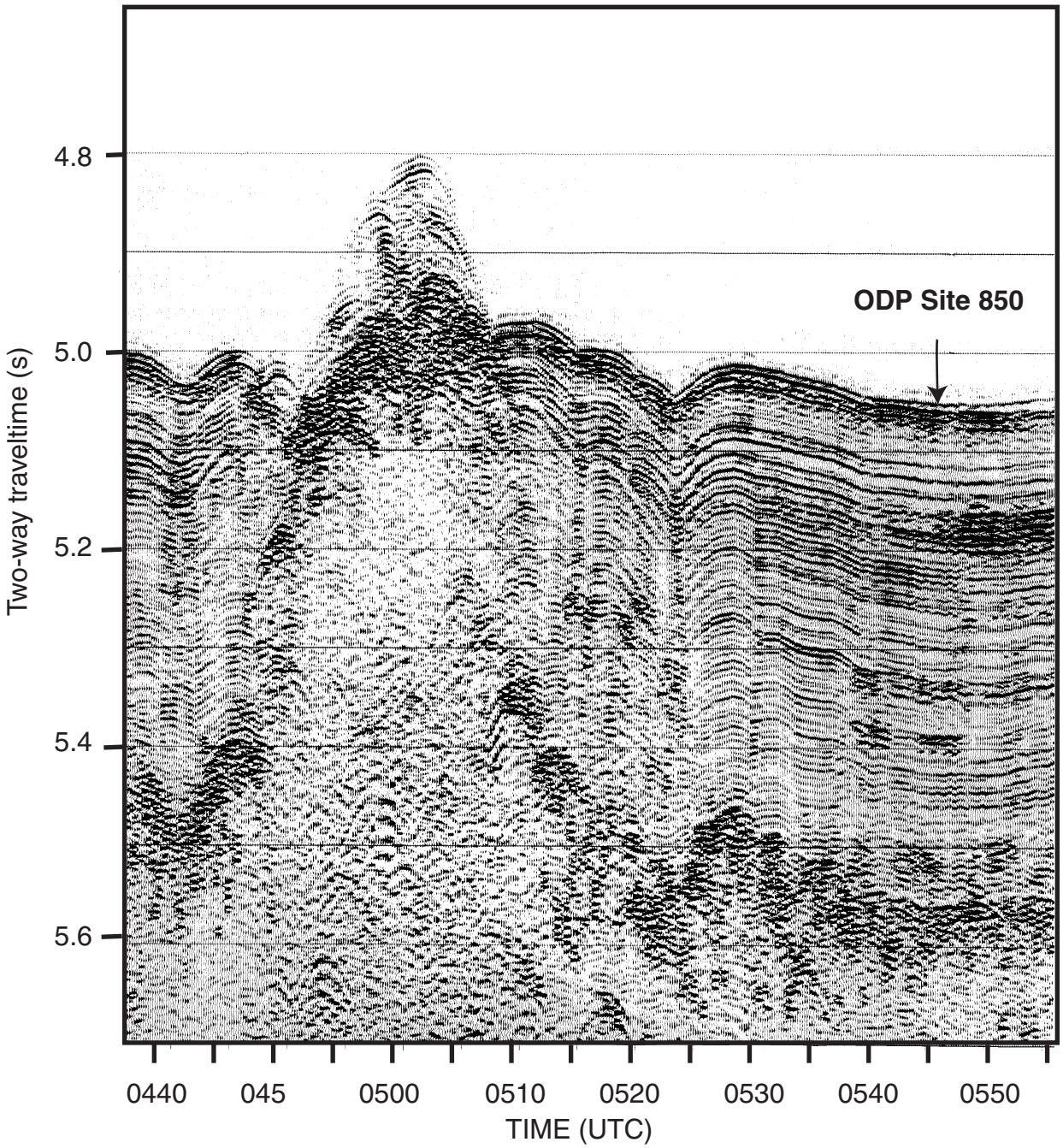
Priority: Alternate
Position: 1°17.83'N, 110°31.29'W
Water Depth: 3786 m
Sediment Thickness: 400 m
Target Drilling Depth: Through sediment–basement transition (>400 m)
Approved Maximum Penetration: Requested same maximum penetration as for Leg 138.
Seismic Coverage: Single-channel seismic collected onboard *JOIDES Resolution* on approach to site.

Objectives: Nonideal alternate for Site EQP-2A. Methane and high sulfate co-occur (methane approaches 40 µL/L). Pore water profiles indicate basement flow. Three chert layers in the Site 850 interval from Core 138-850C-36X to Core 138-850C-41X (~360 to 390 mbsf) limit pore water geochemical communication between the shallower sediments in diffusional contact with the sediment/water interface and the deeper sediments in diffusional contact with the basement.

Drilling Program: TBD

Logging and Downhole Program: TBD

Nature of Rock Anticipated: Nannofossil ooze and chert.



Single-channel seismic profile over ODP Site 850. Profile collected during site survey on ODP Leg 138.

Site: ODP Site 844

Priority: Alternate
Position: 7°55.28'N, 90°28.85'W
Water Depth: 3414 m
Sediment Thickness: 291 m
Target Drilling Depth: Through sediment–basement transition (>291 m)
Approved Maximum Penetration: Requested same maximum penetration as for Leg 138.
Seismic Coverage: *Thomas Washington* station 13, 0655UTC, 25 September 1989.

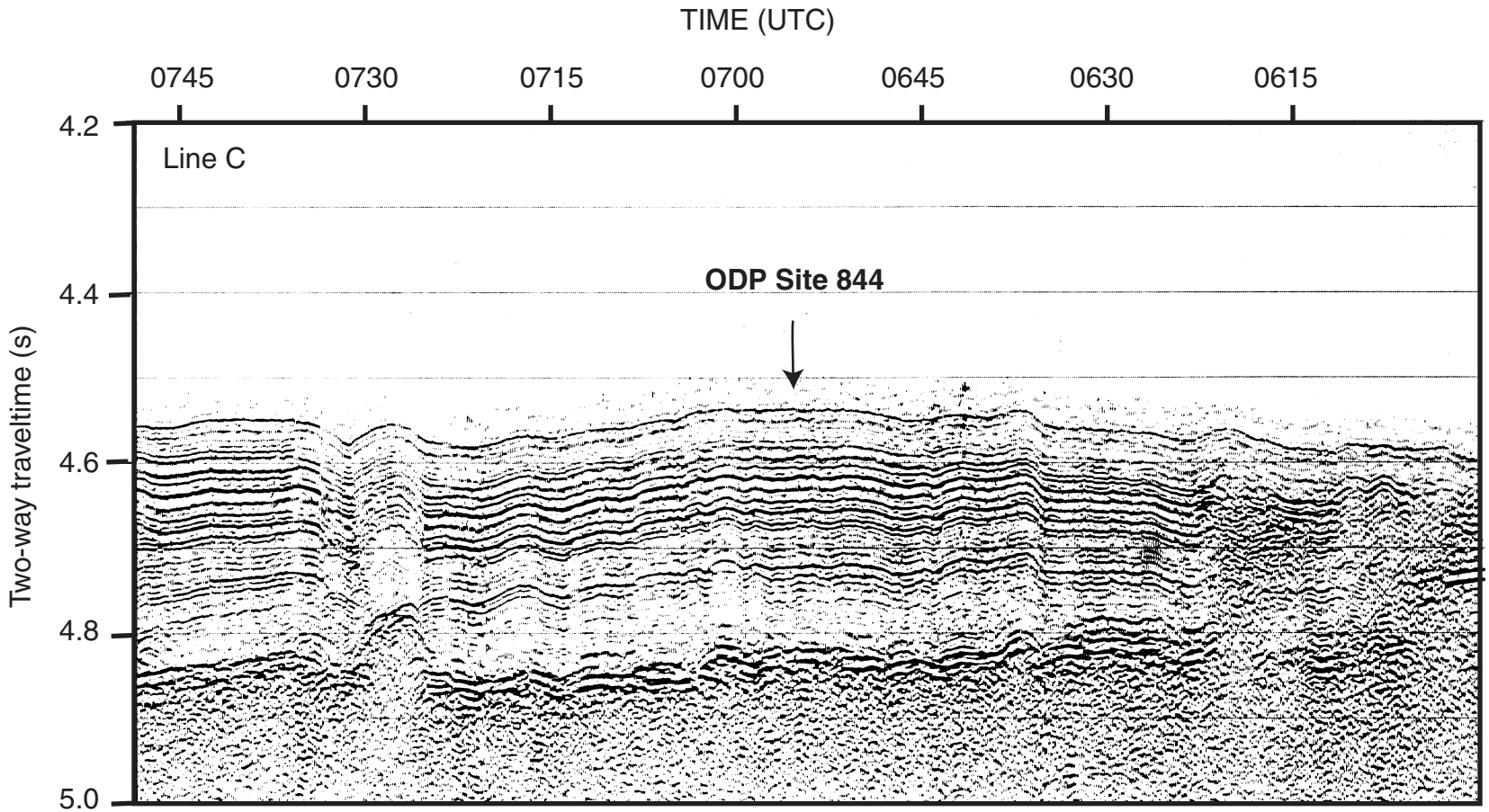
Objectives:

Control site for comparison to proposed Sites EQP-1A and EQP-2A. Normal open-ocean site, sulfate concentrations decline 1 to 2 mmol downhole and they contain no geochemical evidence of subsurface flow.

Drilling Program: TBD

Logging and Downhole Program: TBD

Nature of Rock Anticipated: Diatomaceous ooze, diatomaceous clay, radiolarian ooze, and nannofossil ooze.



Single-channel seismic profile over ODP Site 844. Profile collected during VENTURE 01 cruise of the *Thomas Washington.*

Site: ODP Site 847

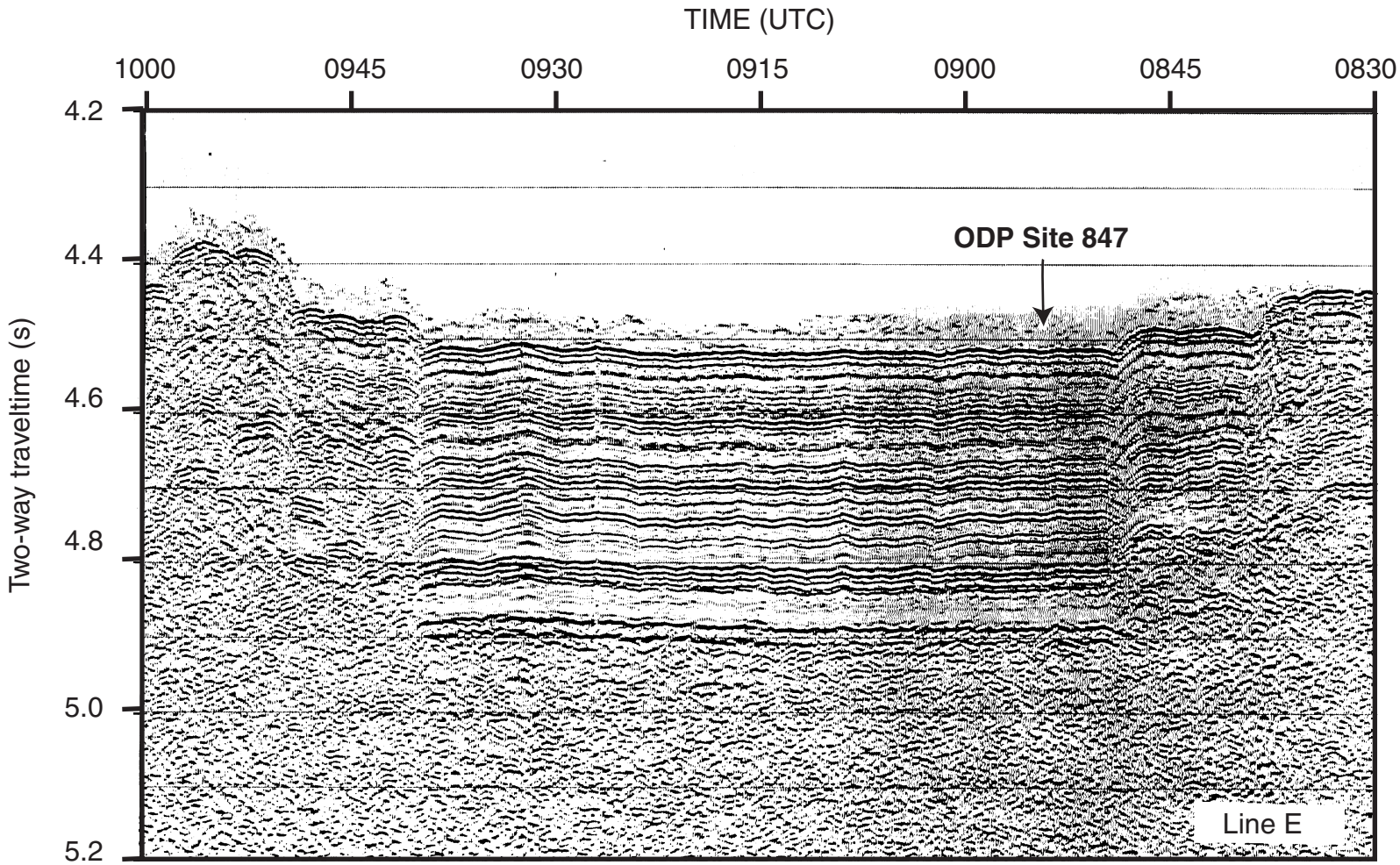
Priority: Alternate
Position: 0°11.59'N, 95°19.23'W
Water Depth: 3334 m
Sediment Thickness: 295 m
Target Drilling Depth: 295 m
Approved Maximum Penetration: Requested same penetration as requested for Leg 138.
Seismic Coverage: *Thomas Washington* station 11, 0854UTC, 16 September 1989.

Objective: Control site for comparison to proposed Sites EQP-1A and EQP-2A. Normal open-ocean site, sulfate concentrations decline 1 to 2 mmol downhole and they contain no geochemical evidence of subsurface flow.

Drilling Program: TBD

Logging and Downhole Program: TBD

Nature of Rock Anticipated: Nannofossil ooze and diatom nannofossil ooze.



Single-channel seismic profile over ODP Site 847. Profile collected during VENTURE 01 cruise of the *Thomas Washington*.

Site: ODP Site 848

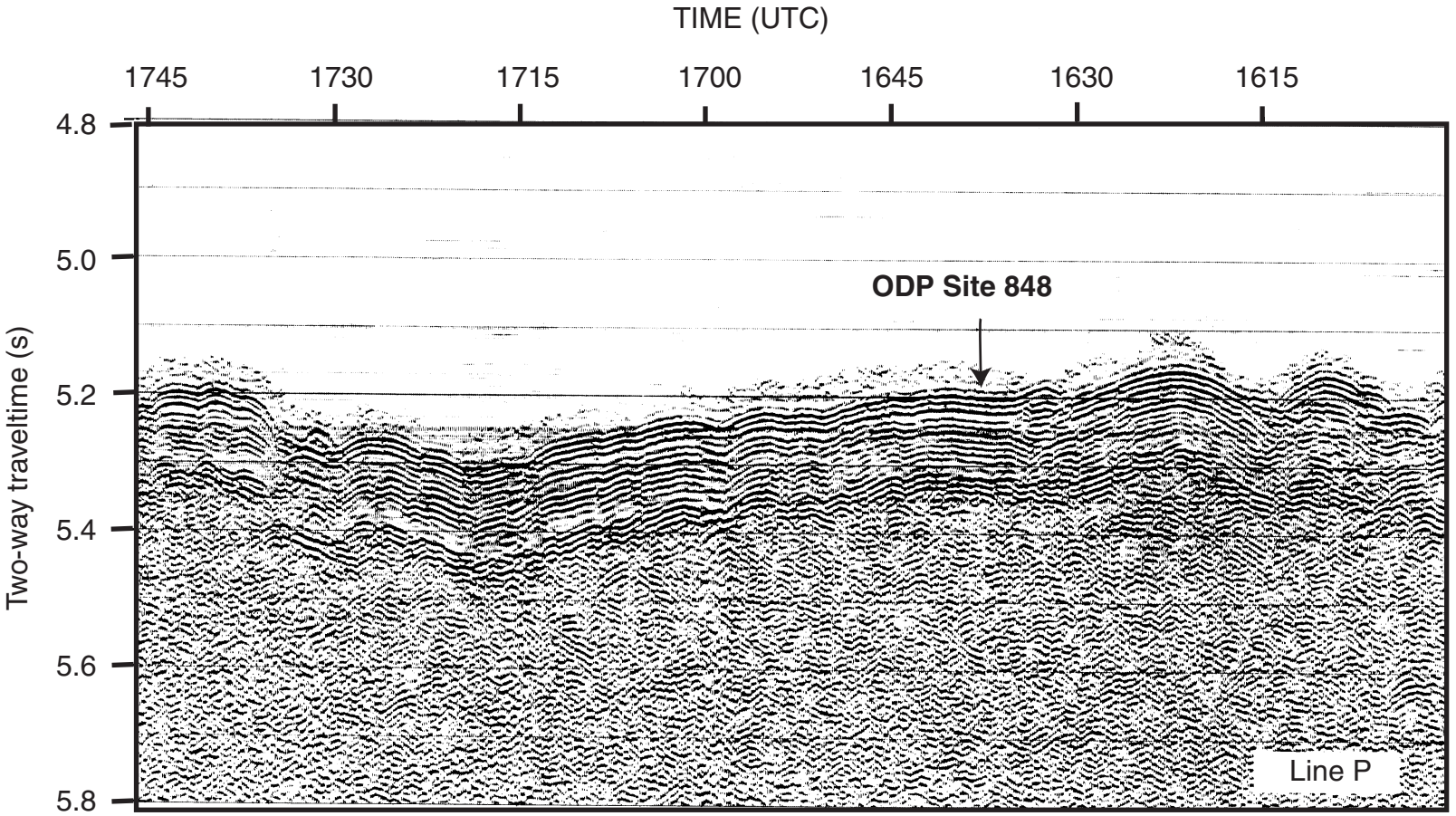
Priority: Alternate
Position: 2°59.63'S, 110°28.79'W
Water Depth: 3853 m
Sediment Thickness: 100 m
Target Drilling Depth: Through sediment–basement transition
Approved Maximum Penetration: Requested same maximum penetration as for Leg 138.
Seismic Coverage: *R/V Thomas Washington* station 7, 1638UTC, 10 September 1989.

Objectives: Equatorial Pacific, low accumulation rate counterpart to the low-activity Peru Basin proposed Sites PRB-1A and PRB-2A. Little or no change in mean sulfate concentration downsection. Normal open-ocean site, with only 100 m of sediment overlying basement and very little sulfate reduction.

Drilling Program: TBD

Logging and Downhole Program: TBD

Nature of Rock Anticipated: Foraminifer nannofossil ooze and siliceous nannofossil ooze.



Single-channel seismic profile over ODP Site 848. Profile collected during VENTURE 01 cruise of the *Thomas Washington.*

Site: ODP Site 852

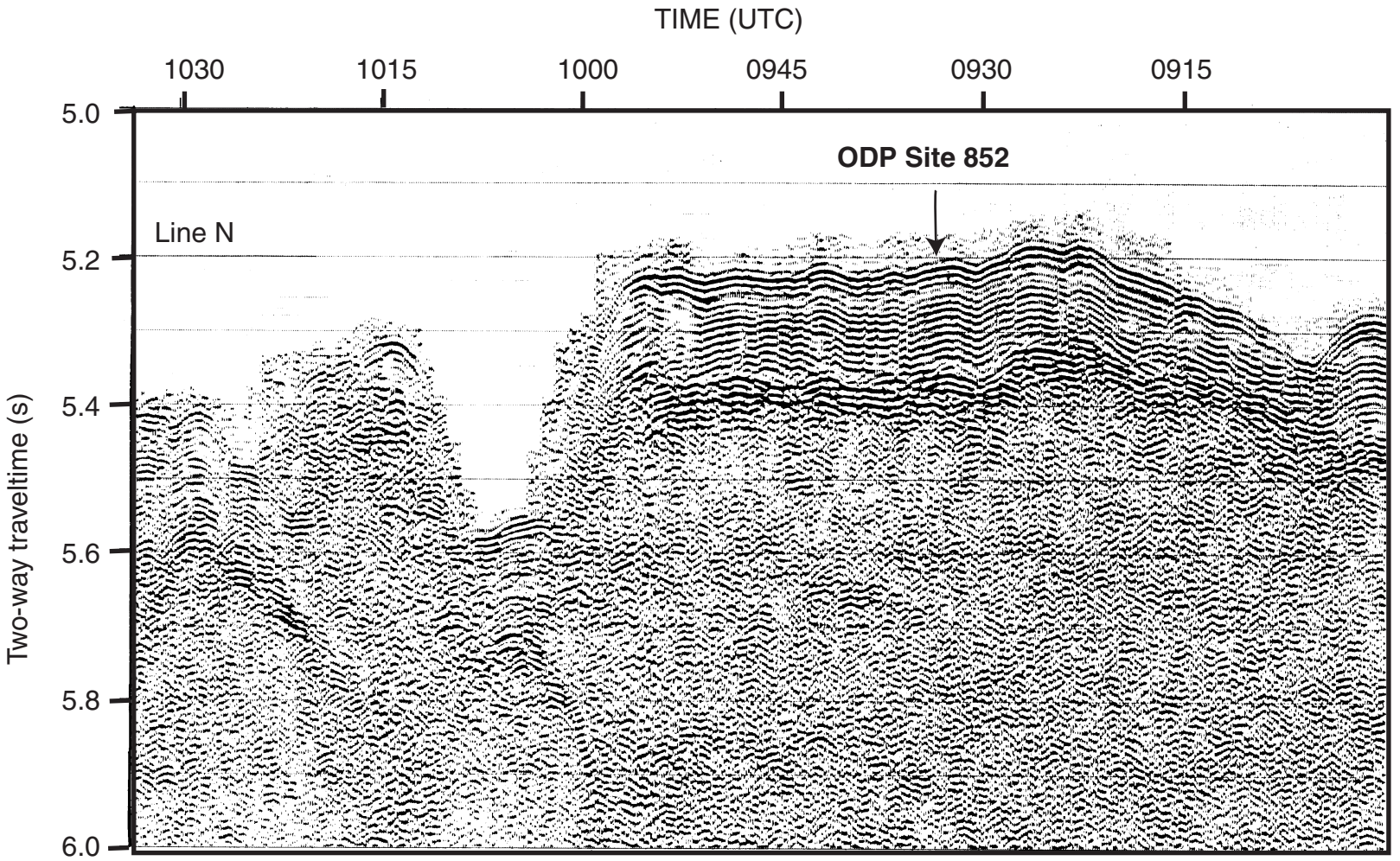
Priority: Alternate
Position: 5°17.55'N, 110°04.58'W
Water Depth: 3861 m
Sediment Thickness: 125 m
Target Drilling Depth: Through sediment–basement transition
Approved Maximum Penetration: Requested same maximum penetration as for Leg 138.
Seismic Coverage: *Thomas Washington* station 5a, 0933UTC, 06 September 1989.

Objectives: Equatorial Pacific, low accumulation rate counterpart to the low-activity Peru Basin proposed Sites PRB-1A and PRB-2A. Little or no change in mean sulfate concentration downsection. Possibly oxidized throughout, with early diagenesis limited to the top few meters of the sediment.

Drilling Program: TBD

Logging and Downhole Program: TBD

Nature of Rock Anticipated: Foraminifer nannofossil ooze and nannofossil foraminifer ooze, and radiolarian nannofosil ooze.



Single-channel seismic profile over ODP Site 852. Profile collected during VENTURE 01 cruise of the *Thomas Washington*.

Site: ODP Site 853

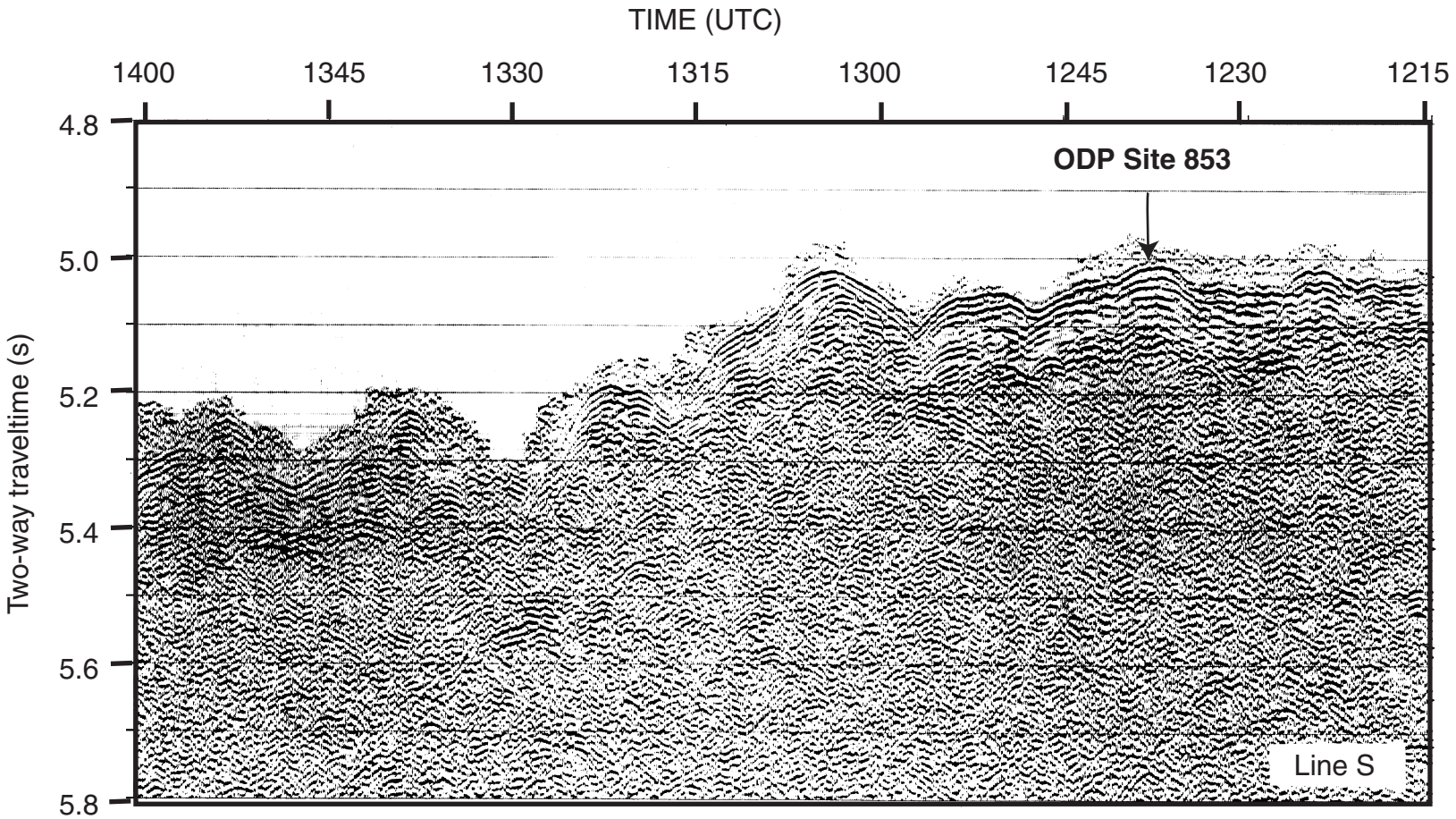
Priority: Alternate
Position: 7°12.66'N, 109°45.08'W
Water Depth: 3715 m
Sediment Thickness: 70 m
Target Drilling Depth: Through sediment–basement transition
Approved Maximum Penetration: Requested same maximum penetration as for Leg 138.
Seismic Coverage: *Thomas Washington* station 5, 1238UTC, 05 September 1989.

Objectives: Equatorial Pacific, low accumulation rate counterpart to the low-activity Peru Basin proposed Sites PRB-1A and PRB-2A. Little or no change in mean sulfate concentration downsection. Similar to Site 852, but with only about 70 m of sediment thickness and even less downhole variation in pore water geochemical composition.

Drilling Program: TBD

Logging and Downhole Program: TBD

Nature of Rock Anticipated: Nannofossil foraminifer ooze and foraminifer clayey nannofossil ooze.



Single-channel seismic profile over ODP Site 853. Profile collected during VENTURE 01 cruise of the *Thomas Washington*.

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