Integrated Ocean Drilling Program Expedition 329 Scientific Prospectus

South Pacific Gyre Microbiology

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Abstract

Integrated Ocean Drilling Program Expedition 329 will core and log deep-sea sediment and basement at a series of sites in the South Pacific Gyre. The sites are characterized by different levels of marine productivity and different basement ages (from 6 to 84–124.6 Ma). The primary purposes of the expedition are to (1) document the nature of microbial communities and test the energetic limit to life in the most food poor deep-sea sediment and (2) test the influence of crust age and sediment thickness on microbial communities, availability of electron donors and acceptors, and the hydrologic evolution of crustal basalt. This project will address fundamental questions about the subseafloor biosphere: Are the communities in mid-gyre subseafloor sediments uniquely structured? Do they contain previously unknown kinds of organisms? Is their primary electron donor organic matter from the surface world or hydrogen from in situ radioactive splitting of water? Do their activities and composition vary with properties of the surface world, such as sea-surface chlorophyll concentrations or organic flux to the seafloor? Is microbial activity sustainable in subseafloor basalt by mineral oxidation (e.g., oxidation of iron in the basaltic minerals) or other processes for tens of millions of years after basalt formation? Are microbial communities recognizably present in subseafloor basalts older than 13 Ma?

Expedition schedule

Integrated Ocean Drilling Program (IODP) Expedition 329 is based on IODP drilling Proposal 662-Full3, "Life beneath the seafloor of the South Pacific Gyre" (available at **iodp.tamu.edu/scienceops/expeditions/south_pacific_gyre-microbio.html**). Following ranking by the IODP Scientific Advisory Structure, the expedition was scheduled for the R/V *JOIDES Resolution*, operating under contract with the U.S. Implementing Organization (USIO). The expedition is scheduled to start in Papeete, Tahiti, on 8 October 2010 and to end in Auckland, New Zealand, on 12 December. A total of 52 days will be available for the drilling, coring, and downhole measurements described in this report (for the current detailed schedule, see **iodp.tamu.edu/scienceops/**). Further details about the facilities aboard the *JOIDES Resolution* and the USIO can be found at **www.iodp-usio.org/.** Supporting site survey data for Expedition 329 are archived in the **IODP Site Survey Data Bank.**

Introduction

The nature of life in the sediment beneath mid-ocean gyres is very poorly known. Almost all sites where subseafloor sedimentary life has been studied are on ocean margins (Ocean Drilling Program [ODP] Legs 112, 180, 201, and 204 and IODP Expeditions 301, 307, and 323) or in the equatorial ocean (ODP Legs 138 and 201). Despite those advances, the extent and character of subsurface life throughout most of the ocean remains unknown (Ocean Studies Board, 2003). This absence of knowledge is largely due to ignorance of subseafloor life in the major ocean gyres, which collectively cover most of the area of the open ocean.

The South Pacific Gyre is the ideal region for exploring the nature of subseafloor sedimentary communities and habitats in the low-activity heart of an open-ocean gyre. It is the largest of the ocean gyres. Its center is farther from continents than the center of any other gyre. Surface chlorophyll concentrations and primary productivity are lower in this gyre than in other regions of the world ocean (Behrenfeld and Falkowski, 1997) (Fig. **F1**). Its surface water is the clearest in the world (Morel et al., 2007). The sediment of this region has some of the lowest organic burial rates in the ocean (Jahnke, 1996). Our recent survey cruise demonstrates that shallow sediment of this region contains the lowest cell concentrations and lowest rates of microbial activity ever encountered in shallow marine sediment (Knox-02RR Shipboard Scientific Party, unpubl. data).

The region is also ideal for testing hypotheses of the factors that limit hydrothermal circulation and chemical habitability in aging oceanic crust (sedimentary overburden, basement permeability, and decreasing basal heat flux). It contains a continuous sweep of oceanic crust with thin (1–100 m) sedimentary cover spanning thousands of kilometers and >100 m.y. of seafloor age.

The South Pacific Gyre contains the largest portion of the seafloor that has never been explored with scientific ocean drilling. Consequently, the proposed drilling will advance scientific understanding across a broad front. It will help to constrain the nature of crustal inputs to the subduction factory. It will constrain the origin of the Cretaceous Normal Superchron and the tectonic history of a region as large as Australia. Recovery of sedimentary interstitial waters at several of the proposed sites will provide novel constraint on glacial–interglacial pCO_2 models.

Background

Geological setting

The proposed drilling sites span nearly the entire width of the Pacific plate in the Southern Hemisphere between 20° and 45°S (Fig. F2). This ocean crust was accreted along at least four different plate boundaries (e.g., Pacific/Phoenix, Pacific/Antarctic, Pacific/Farallon, and Pacific/Nazca). Crustal ages range from ~100 Ma (Chron 34n) at proposed Site SPG-1A to ~6 Ma (Chron 3An.1n) at proposed Site SPG-7A. Calculated spreading rates range from slow–intermediate (<20 km/m.y. half-rate) to ultrafast (>80 km/m.y., half-rate).

The site locations cover a relatively wide range of crustal ages, spreading rates, and tectonic/volcanic environments. The depth and crustal age of each coring site correlates well with the predicted depth versus age curve (Stein and Stein, 1994), which suggests the sites are located on representative crust. Calculated spreading rates at each site are somewhat biased toward fast and ultrafast spreading rates (28–95 km/m.y., half-rate). Surprisingly, the 95 km/m.y. value is one of the fastest spreading half-rates measured globally. The abyssal hill fabric is relatively well defined for most coring sites. However, off-axis volcanism at proposed Site SPG-5A and possibly proposed Site SPG-6A masked the original seafloor fabric. Sediment thickness ranges from <3 to 122–130 m and generally increases west and south of our survey area. This sediment thickness trend is consistent with greater sediment cover on older crust and on crust located farther away from the center of the gyre. The notable exception to this trend is along the northern transect on crust accreted along the Pacific-Farallon spreading system and older than ~30 m.y. Sediment at each of the sites generally appears as pelagic drape, with some localized mass wasting deposits. Seismic images also reveal areas of bottom current activity occasionally resulting in localized scouring of all sediment above volcanic basement (e.g., proposed Site SPG-5A).

Microbiological setting

The sedimentary communities and activities of South Pacific Gyre sediment are unlike those in any sediment previously explored by IODP or ODP (D'Hondt et al., 2009). Cell concentrations in the shallow sediment (0–8 meters below seafloor [mbsf]) of proposed Sites SPG-1A–SPG-11B are orders of magnitude lower than concentrations in previously examined sediment of equivalent depth (Kallmeyer et al., 2009) (Fig. F3). Dissolved O_2 penetrates extremely deeply (Fig. F4A) (Fischer et al., 2009). Organic-fueled respiration is much lower in this sediment than in any previously examined deep-sea sediment (D'Hondt et al., 2009).

These results demonstrate that at least in the shallow sediment of the South Pacific Gyre (proposed Sites SPG-1A–SPG-11B) (1) net metabolic activities are low and oxygen (O_2) is the principal net terminal electron acceptor and (2) biomass is substantially different than in any previously examined deep-sea sediment. In contrast, at proposed Site SPG-12A on the southern edge of the gyre, where sea-surface chlorophyll content is much higher, cell concentrations and dissolved chemical concentrations resemble those of ODP Site 1231 (on the northeastern edge of the gyre) (Figs. **F3, F4**), where most of the subseafloor pore water is anoxic and the community may be principally supported by oxidation of organic matter coupled to reduction of Mn(IV), Fe(II), and NO_3^- migrating up from the underlying basaltic aquifer (Shipboard Scientific Party, 2003a; D'Hondt et al., 2004). These results suggest that biomass and microbial activity in subseafloor sediment vary predictably with sea-surface chlorophyll content.

Seismic studies/Site survey data

From 18 December 2006 to 27 January 2007, Cruise KNOX-02RR, aboard the R/V *Revelle,* surveyed all 11 drilling sites (Fig. **F1**). Sediment was geophysically imaged and cored at all 11 sites. Proposed Sites SPG-1A–SPG-11B are in the central portion of the South Pacific Gyre. Proposed Site SPG-12A is below higher productivity water at the gyre's southern edge. There is no proposed Site SPG-8.

All of the sites are at a crossing point of two track lines or on a single track line immediately adjacent to a crossing point. The multibeam and seismic results are provided for each site (see **"Site summaries"**).

Geophysical data collected at each site include SIMRAD EM120 swath map bathymetry and Knudsen digitally recorded 3.5 kHz seismic reflection and multichannel seismic reflection. Data were collected at 4.5–6 kt with continuous Global Positioning System navigation and include at least one set of intersecting track lines. Following each geophysical survey, sediment was recovered using gravity, piston, and multicores. At sites with water depths deeper than 4 km, the sediment is principally abyssal clay capped by manganese nodules. At sites in shallower water (proposed Sites SPG-6A and SPG-7A), the sediment is clayey nannofossil ooze.

All proposed sites, supported by seismic, navigation, and bathymetric data as presented in this report, have been classified as “1Aa” by the Site Survey Panel. The 1Aa classification indicates that all required data are in the data bank and have been reviewed by the SSP and that they image the target adequately and there are no scientific concerns of drill site location and penetration.

Scientific objectives

Our study has the following fundamental objectives:

1. To document the habitats, metabolic activities, genetic composition, and biomass of microbial communities in subseafloor sediment with very low total activity.
2. To test how oceanographic factors (such as surface ocean productivity) control variation in sedimentary habitats, activities, and communities from gyre center to gyre margin.
3. To quantify the extent to which these sedimentary communities may be supplied with electron donors (food) by water radiolysis, a process independent of the surface photosynthetic world.
4. To determine how the basement habitats, potential activities, and, if measurable, communities vary with crust age and hydrologic regime (from ridge crest to abyssal plain).

We propose to meet these objectives by (1) coring the entire sediment column at several sites along two transects in the region of the South Pacific Gyre (Figs. **F1, F2**); (2) coring and logging the upper 100 m of the basaltic basement at three key sites; and (3) undertaking extensive microbiological, biogeochemical, geological, and geophysical analysis of the cores and drill holes.

The project results will address several significant questions. Are the communities in mid-gyre subseafloor sediment uniquely structured? Do they contain previously unknown organisms? What are their principal sources of metabolic energy? Do their principal activities and composition vary with properties of the surface world, such as sea-surface chlorophyll concentrations or organic flux to the seafloor? Is microbial activity is sustainable in subseafloor basalt by mineral oxidation (e.g., oxidation of iron in the basaltic minerals) for tens of millions of years after basalt formation? Are microbial communities recognizably present in subseafloor basalt older than 13 Ma?

These questions can be framed as hypotheses to be tested. For example, we hypothesize that

1. A living community persists in the most organic poor sediment of the world ocean.
2. Organic-fueled metabolic activity is extremely low and oxygen is the principal net terminal electron acceptor in this sediment. Consequently, the diversity of anaerobic activities is far lower here than in previously examined subseafloor sediment.
3. The biomass and composition of this subseafloor sedimentary community is distinctly different from the communities observed in the higher activity anaerobic subseafloor ecosystems that have been examined to date.
4. H_2 from water radiolysis is a significant electron donor for microbes in the most organic-poor subseafloor sediment.
5. Open flow continues in the very old basalt of the western gyre.
6. Basalt oxidation may support microbial activity for 100 m.y. here.
7. Biomass and activity decrease with basement age as electron donor accessibility decreases.

Even if none of the above hypotheses are found to be valid, the results of the proposed expedition will significantly advance understanding of the subsurface world. Scientists will test the extent to which distinct oceanographic and geologic provinces contain distinct subseafloor habitats and distinct subseafloor communities. They will document the extent to which life in the low-activity gyre sediment depends on the surface photosynthetic world—and the extent, if any, to which it is metabolically independent of the surface world. They will place firm constraints on the potential for microbial redox activity in ancient subseafloor basalt and how that potential varies with crust age over 100 m.y. or more. They will place firm constraints on estimates of total subseafloor biomass.

The results of the proposed expedition will also test the factors that control evolution of geothermal circulation and chemical alteration in ocean crust, models of regional tectonic history, geodynamo models, and models of glacial–interglacial ocean-climate change.

Explanation of primary objectives

1. *To document the habitats, metabolic activities, genetic composition, and biomass of microbial communities in subseafloor sediment with very low total activity.*

What are the principal microbial activities in mid-gyre subseafloor sediment? What are the rates of those activities? How dense are the populations in this sediment? What are the communities active here? How do these communities and activities compare to those in subseafloor sediment with much higher levels of activity? How unique are their organisms?

IODP drilling in the South Pacific Gyre will provide a crucial opportunity to document microbial habitats, activities, and community composition in a subseafloor sedimentary ecosystem that is unlike any that IODP or ODP has ever explored.

Metabolic activities

The penetration of high concentrations of O_2 and NO_3^- meters into the sediment at proposed Sites SPG-1A–SPG-10A (Fig. **F4**) suggests that the net rate of electron-accepting activity is even lower in the South Pacific Gyre than at Site 1231 (Fig. **F2**). Along with chemical data from Deep Sea Drilling Project (DSDP) Leg 92, these O_2 and NO_3^- profiles also suggest that the principal electron-accepting activity in the deep subseafloor sediment of this region may be different than at any of the sites where subseafloor sedimentary communities have been previously explored. A sequence of sites was drilled during Leg 92 at ~20°S in the northern portion of the gyre (Leinen, Rea, et al., 1986). At all Leg 92 sites where pore water chemistry was analyzed, dissolved NO_3^- is present throughout the entire sediment column, which is as thick as 50 m (Gieskes and Boulègue, 1986). Because NO_3^- concentrations do not significantly change with depth in the Cruise KNOX-02RR survey cores or the Leg 92 cores, O_2 may be the principal net electron acceptor in subseafloor sediment throughout the South Pacific Gyre. For thermodynamic reasons, iron reduction and SO_4^{2-} reduction cannot yield more energy than NO_3^- reduction at the NO_3^- concentrations of our surveyed sediment and the Leg 92 sites. Consequently, they are very unlikely to be significant electron acceptors in the sediment of this region.

Biomass

Quantification of subseafloor biomass in the South Pacific Gyre will place strong constraints on the size of Earth's subseafloor biomass and total biomass.

On the basis of acridine orange direct counts (AODC) in subseafloor sediment of relatively high activity sites, subseafloor sedimentary biomass has been estimated to constitute 1/10 to 1/3 of Earth's total living biomass (Whitman et al., 1998; Parkes et al., 2000). These estimates make two key assumptions that need to be tested in a range of different geologic settings. First, they assume that the sites where cells were counted are representative of biomass content in the subseafloor sediment of all regions. Second, they assume that all of the cells observed in AODC are alive. Because acridine orange and other deoxyribonucleic acid (DNA) stains (e.g, SybrGreen) bind to DNA and fluoresce whether cells are alive or dead, current estimates of subseafloor biomass place an upper bound on estimates of living biomass.

The results of Cruise KNOX-02RR indicate that the first assumption is wrong because previous sites have not sampled sediment in the major ocean gyres. As described earlier, cell concentrations in the Cruise KNOX-02RR cores are orders of magnitude lower than concentrations at the same depths in all previous IODP/ODP sediment (D'Hondt et al., 2009) (Fig. **F3**).

The fraction of cells alive and active in South Pacific Gyre sediment is not yet known. Catalyzed reporter deposition fluorescence in situ hybridization (CARD-FISH) studies demonstrate that 10% (or more) of the cells in Leg 201 subseafloor sediment are alive and active (Mauclaire et al., 2005). CARD-FISH differentiates active, ribosomal ribonucleic acid (rRNA)-rich cells from rRNA-depleted, inactive, or dead cells by targeting rRNA molecules that occur in high copy number in actively metabolizing cells. If only 10% of subseafloor sedimentary cells are active throughout the world and deep subseafloor gyre sediment contains as few cells per cubic centimeter as in the Cruise KNOX-02RR cores, living subseafloor biomass is much more than a factor of 10 lower than 10%–30% of Earth's total biomass (Kallmeyer et al., 2009).

Community composition

Genetic analyses of bulk sediment samples and cultured bacterial isolates from deep beneath the seafloor demonstrate that similar subseafloor sedimentary environments separated by thousands of kilometers contain similar genetic communities. For example, hydrate-bearing sediment of the Peru margin and the northwestern U.S. margin (Hydrate Ridge) contains similar 16S rRNA gene populations (Inagaki et al., 2006). In contrast, nearby sites with different sedimentary environments contain very different populations (Inagaki et al., 2006).

Genetic analyses of bulk sediment samples and cultured bacterial isolates also demonstrate that previously undiscovered lineages exist in subseafloor sediment, even within a few meters of the seafloor (Sørensen et al., 2004; D'Hondt et al., 2004). For example, 16S rRNA gene sequencing surveys of bulk sediment from a few meters beneath the seafloor at Site 1231 discovered previously unknown archaea with 16S sequences that are deeply rooted in the tree of life (Sørensen et al., 2004).

In combination with the metabolic points described above, these discoveries underscore three important points about potential community composition in subseafloor sediment of the South Pacific Gyre. First, the gyre's subseafloor microbial communities differ from those of any subseafloor sediment explored to date. Second, the very low activity and potentially aerobic communities of the deepest sediment in the South Pacific Gyre are likely to contain unique and previously undiscovered microorganisms. Third, exploration of the gyre sediment may provide deep insight into community composition and structure throughout most of the open ocean, because the genetic communities of subseafloor sediment in the other major ocean gyres may resemble those of the South Pacific Gyre.

2. *To test how oceanographic factors (such as surface ocean productivity) control variation in sedimentary habitats, activities, and communities from gyre center to gyre margin.*

How are subseafloor sedimentary activities and communities affected by oceanographic properties that vary predictably from gyre center to gyre margin?

The proposed expedition will provide a unique opportunity to document how the nature of subseafloor sedimentary life varies with oceanographic properties in the least biologically active region of the world ocean.

Pore water surveys of regions with higher organic fluxes to the seafloor, such as the equatorial Pacific and continental margins, indicate that subseafloor cell concentrations and the principal electron-accepting activity and the net rates of electron-accepting activities vary predictably with sea-surface chlorophyll content and organic flux to the seafloor (D'Hondt et al., 2002, 2004). These relationships appear principally due to reliance of subseafloor sedimentary communities on burial of photosynthesized organic matter for electron donors. Other sedimentary properties that vary with measures of oceanic productivity include sediment composition, which depends on the rate of microfossil production in the overlying water column as well as on the position of the carbonate compensation depth (CCD), and sediment thickness, which largely depends on the rate of calcium carbonate and biosilica production in the overlying water column.

The Cruise KNOX-02RR results (D'Hondt et al., 2009) suggest that the principal electron-accepting activity, net rates of activities, and cell concentrations in the shallow subseafloor sediment of the South Pacific Gyre vary with sea-surface chlorophyll content from within the gyre to outside the gyre and perhaps from site to site within it. If these relationships are shown by IODP drilling to also apply to the deeper subseafloor sediment of this region, then biomass, principal redox activities, and net rates of activity in subseafloor sediment may be largely predicted by surface ocean properties.

3. *To quantify the extent to which these sedimentary communities may be supplied with electron donors (food) by water radiolysis.*

To what extent does the ecosystem of organic-poor sediment depend on in situ radiolysis of pore water?

Expedition 329 will provide an unprecedented opportunity to determine if subseafloor life in very low activity sediment is nourished to a significant extent by H_2 from in situ radiolysis of water.

Buried organic matter appears to be the principal source of electron donors in subseafloor sediment of ocean margins and the equatorial Pacific (D'Hondt et al., 2004). However, the burial flux of organic carbon is so low in the South Pacific Gyre that in situ radiolysis of water may be the principal source of electron donors there (D'Hondt et al., 2009) (Fig. **F5**).

Water radiolysis has been described as a possible source of energy for ecosystems in hard rock far beneath continental surfaces (Pedersen, 1996; Lin et al., 2005a). The longest-lived products of water radiolysis are the electron donor H_2 and the electron acceptor O_2 (Debierne, 1909). H_2 can be supplied by in situ radiolysis or, in theory, by transport of radiolytic H_2 from a much deeper biologically dead environment (e.g., the mantle and oceanic basement deep beneath sediment).

The potential importance of water radiolysis to subseafloor sedimentary communities can be assessed by comparing radiolytic H_2 production to organic-fueled respiration. For this comparison, in situ H_2 production by water radiolysis must be quantified from (1) logging estimates of uranium, thorium, and potassium concentrations

(D'Hondt, Jørgensen, Miller, et al., 2003) or inductively coupled plasma–mass spectrometry (ICP-MS) data (Blair et al., 2007), (2) shipboard physical property measurements (porosity and density) (D'Hondt, Jørgensen, Miller, et al., 2003), and (3) a numerical model of water radiolysis (Blair et al., 2007).

Water radiolysis may provide a higher flux of electron donors than buried organic matter in gyre sediment. Organic-fueled respiration rates are much lower in this sediment than in any previously drilled regions of the world ocean. Clay-rich sediment contains much higher concentrations of radioactive elements than other deep-sea sediment. Furthermore, in fine-grained porous sediment, most alpha and beta production occurs within striking range of pore water. Consequently, clay-rich sediment will yield much higher rates of water radiolysis than other geological environments.

4. *To determine how basement habitats, potential activities, and, if measurable, communities vary with crust age and hydrologic regime (from ridge crest to abyssal plain).*

How does the habitability of subseafloor basalt change with crust age? How does this change depend on basement hydrologic evolution and mineral alteration? Do fractures remain open for flow-in basalt as old as 100 Ma? What is the role of sediment cover in controlling hydrologic flow, alteration, and habitability in subseafloor basalt? Are dissolved oxidants (oxygen and nitrate) available in thinly sedimented subseafloor basalt as old as 100 Ma? Are reduced elements (e.g., iron and sulfur) available for oxidation in basalt as old as 100 Ma? Does mineral oxidation support measurable microbial activity in this basalt?

The proposed expedition will provide a unique opportunity to determine how basement habitability and communities vary with crust age, sediment cover, and hydrologic conditions over 100 m.y. or more of basement history in the most thinly sedimented region of the world ocean.

Subseafloor basalt has been described as the largest potential microbial habitat on Earth (Fisk et al., 1998). Glass alteration textures have been interpreted as evidence of microbial colonization in subseafloor basalt as old as 145 Ma (Fisk et al., 1998; Furnes and Staudigel, 1999). Laboratory experiments indicate that microbial communities may play an important role in basalt weathering (Staudigel et al., 1998). Indeed, that microbes can derive their energy from rock weathering (e.g., oxidation of iron and sulfur in minerals) has been demonstrated with seafloor cultured isolates (Edwards et al., 2003). Fluid samples from a CORKed borehole show that microbes exist in the formation fluid of 3.5 Ma subseafloor basalt (Cowen et al., 2003).

Despite this evidence, the nature and extent of subseafloor basaltic communities remain largely unknown and the factors that control the "redox habitability" of the basalt (the ability of the basalt to fuel microbial reactions) are relatively unexplored. The biological significance of the textural features and the factors that control alteration and its timing in crust are not well constrained.

Metabolic habitability of subseafloor basalt ultimately depends on the supply of electron donors (principally, reduced Fe and S) from minerals in the basalt and the supply of electron acceptors (O_2 and NO_3^-) from seawater flowing into and through the basalt. Processes that change these supplies over the basalt's lifetime include mineral alteration, which changes the supply of electron donors, and the evolution of hydrologic flow through the basalt, which changes the supply of electron acceptors.

Evolution of crust alteration and metabolic habitability

Compilation of DSDP/ODP geochemical data, mostly from the North Atlantic, suggests that oxidation of Fe and S in the upper few hundred meters of subseafloor basalt occurs principally during the first 10 m.y. after basalt formation (Bach and Edwards, 2003). During this interval, the Fe(III)/ΣFe ratio of the bulk basalt in the database increases from ~0.15 to 0.35 and most of the sulfur is oxidized (Bach and Edwards, 2003). Whether this alteration is microbially mediated or not, it changes the redox habitability of the basalt. Alteration patterns are heterogeneous within and between cores, with greatest alteration in permeable zones, such as brecciated pillows (Bach and Edwards, 2003).

Recent studies of altered basalt at ODP Site 801 (~170 Ma) showed that alteration characteristics of this ancient oceanic crust are generally similar to those observed in much younger crust (e.g., ODP Hole 504B at ~6 Ma), suggesting that most alteration takes place when oceanic crust is young (Alt et al., 1992). If oxidative alteration ceases in 10–15 m.y. after crust formation, then the oceanic crust inhabitable by mineral-oxidizing microbes is limited to crust younger than 10–15 Ma.

Other evidence suggests that oxidative alteration need not be limited to crust younger than 10–15 Ma. Older crust has the potential for continued redox alteration, and it probably occurs in some geochemical/hydrological regimes. Geophysical measures of matrix density suggest that about half of all intergrain-scale crustal alteration in the uppermost basalt occurs in crust older than 10–15 Ma (Jarrard et al., 2003). In the equatorial Pacific, dissolved O_2 and NO_3^- are present in basement as old as 35 Ma (D'Hondt et al., 2004). As much as 65% of the Fe in the Bach and Edwards (2003) remains as Fe(II) in shallow oceanic basalt older than 10 Ma. Whether this Fe(II) continues to be oxidized at very slow rates where exposed to dissolved O_2 and NO_3^- or is physically inaccessible to oxidation remains to be determined.

Sediment thickness provides one explanation for the average change in redox alteration at 10–15 Ma. Once sedimentary cover is thick enough and anoxic enough to seal basement from contact with oxidized seawater, redox disequilibria (habitability) disappears and oxidative alteration ceases. Drilling basement of different ages beneath the unusually thin sediment cover in this region of the Pacific presents a unique test of this hypothesis. If a critical sediment thickness is necessary to curtail oxidative alteration, then the young (13.5 Ma), moderate (33.5 Ma), and old (84–125 Ma) basement sites of the proposed drilling effort will exhibit more intense or differing styles of alteration relative to other fast-spreading sites of comparable age and greater sediment cover (e.g., Site 801, ~400 m of sediment at ~170 Ma; ODP Site 1256, ~200 m of sediment at 15 Ma; and Hole 504B, ~250 m of sediment at ~6 Ma). If this is the case, the basalt of the South Pacific Gyre are redox habitable for many tens of millions of years after basalt formation.

Evolution of crustal hydrology and chemical habitability

The timing and distribution of shallow crustal alteration is intriguingly linked to evidence of the hydrologic evolution of subseafloor basalt. Seismic velocity and macroporosity data suggest that porosity and bulk permeability of subseafloor basalt decrease rapidly during the first 10–15 m.y. after basalt formation (Jarrard et al., 2003). These decreases are thought to derive from secondary mineralization in the basalt. Mineralization reduces surface area within the crust and may limit electron donor and nutrient availability in the basaltic aquifer.

Global compilations of heat flow data indicate that advective heat loss is high in young seafloor and decreases with increasing age until ~65 Ma, where, on average, advective heat loss ceases (Stein and Stein, 1994) (Fig. **F6**). Fisher and Becker (2000) explain these observations by invoking closure of small-scale permeability in basement within 10–15 m.y. but ongoing flow for 65 m.y. in large fractures and faults that fill a relatively small volume of the rock. Three mechanisms are thought responsible for limiting hydrothermal circulation in old crust: (1) buildup of low-permeability sediment cover, which reduces basement relief and isolates the oceanic crust from overlying seawater; (2) ongoing mineralization, which decreases basement permeability with age; and (3) decreasing basal heat flux with age, which reduces the driving force for buoyant fluid flow. Basement permeability is commonly thought to ultimately control the cessation of hydrothermal circulation (Stein and Stein, 1994; Jarrard et al., 2003).

However, a growing body of evidence suggests that oceanic crust remains permeable enough to sustain advective heat loss through its life. This evidence includes the significant variance in age-dependent heat flow averages (Stein and Stein, 1994) (Fig. **F6**), large variations in heat flow survey data of Cretaceous-aged crust (Von Herzen, 2004; Fisher and Von Herzen, 2005) velocity logs, macroporosity data, matrix data (Jarrard et al., 2003), present-day fluid flow within ~132 Ma basement at ODP Site 1149 (Shipboard Scientific Party, 2000), and celadonite precipitation ages that indicate low-temperature fluid circulation at the Trodos ophiolite (Gallahan and Duncan, 1994). Given this evidence, the termination of the average heat flow deficit at ~65 Ma probably signifies that much of the open circulation between crust and ocean has largely stopped by then, rather than that hydrothermal flow has ended (Anderson and Skilbeck, 1981; Jacobson, 1992; Stein and Stein, 1994).

We hypothesize that advective fluid flow remains relatively vigorous even on old seafloor. If this is true, the apparent waning of hydrothermal circulation at ~65 Ma is controlled by sediment, rather than by basement permeability. The South Pacific Gyre offers a unique opportunity to test this hypothesis because its sediment is thin and discontinuous and its basement relief is relatively variable. If the hypothesis is true, large-scale permeability will be high regardless of basement age and heat flow data at the proposed sites will tend to deviate significantly from conductive values. Surface heat flow data from our recent survey (Fig. **F6**) and from scattered older measurements within 200 km of proposed Sites SPG-1A and SPG-4A suggest that an active flow system is present in the basement throughout the region of proposed drilling.

If this hypothesis is correct, the supply of the dissolved electron acceptors O_2 and NO_3^- to the upper basement of the South Pacific Gyre may remain high long past the first 10–15 m.y. after basalt formation. If drilling of the lowermost sediment and the basalt shows the occurrence of these electron acceptors in the upper basement at our proposed sites, either (1) the metabolic habitability of South Pacific Gyre basalt remains sufficient to support life for as long as 100 m.y. or (2) the metabolic habitability of this basalt is ultimately controlled by the inaccessibility of electron donors in the basalt rather than by access to electron acceptors. If the first case applies, increased oxidative alteration will be evident in crust of increasingly greater age (e.g., proposed Site SPG-1A alteration will be greater than proposed Site SPG-4A alteration, which will be greater than proposed Site SPG-6A alteration). If the second case applies, oxidative alteration will be similar at all three sites.

Basement community composition

IODP drilling of the basalt at sites will provide a direct opportunity to test the existence and composition of microbial communities in oceanic crust of three very different ages (13.5, 33.5, and 80–125 Ma). Molecular and microscopic analysis of uncontaminated basalt from the site with youngest crust will test whether or not microbes take full advantage of the redox habitability of relatively young crust in open exchange with the overlying ocean. Molecular and microscopic analysis of uncontaminated basalt from the sites with older crust will test whether or not ongoing oxidation of the subseafloor basalt (by occurrence of reduced Fe and supply of dissolved O_2 and NO_3^- by ongoing advection and diffusion through the sediment) sustains similar (or different) communities, albeit at slower rates of redox activity. Collectively, the study of these sites will track the compositional and functional evolution of basement communities >100 m.y. in a thinly sedimented region of fast-spreading crust, where we hypothesize supply of dissolved oxidants to continue for as long as 125 m.y. (or more).

Explanation of secondary objectives

Oceanic crust inputs to the subduction factory

The altered oceanic crust at our oldest basement sites (proposed Sites SPG-1A and SPG-2A) is outboard of the Tonga-Kermadec subduction zone. Characterizing the style and extent of alteration in this subducting plate will provide an important reference for assessing the role of altered oceanic crust in the subduction process. Of global arcs, the Tonga arc has perhaps the smallest sediment flux (Plank and Langmuir, 1998) and the altered oceanic crust in this system is proportionally more influential in the subduction process than at most other arcs. If the low sedimentation rate at this site has resulted in unusual alteration characteristics in the subducting oceanic crust, these differences may translate into geochemically distinct signatures in the magmas produced at the Tonga arc.

Regional tectonics

Proposed Sites SPG-1A and SPG-2A are centrally located in ocean crust accreted during the Cretaceous Normal Superchron (CNS). The tectonic history of this Australia-sized area is poorly constrained because correlatable magnetic seafloor anomalies are not present. Although limited bathymetry data suggest a general north–south spreading direction, the actual direction(s) and the presence of a failed rift within the region are poorly constrained. Drilling and logging ocean crust at either of these sites would address these questions. Radiometric dating of plagioclase within the recovered basalt would provide important age constraints (Koppers et al., 2003) and structural analysis of dipping flow units with Formation MicroScanner (FMS) logging data would provide a spreading direction (Pockalny and Larson, 2003). Resolving the tectonic history of this region is critical to understanding the effect of large igneous provinces (LIPs) on tectonic processes and whether the Ontong Java, Manihiki, and Hikurangi plateaus were created by one or multiple mantle plumes (Taylor, 2006).

Geodynamo

The causal mechanisms for the Cocos-Nazca spreading center are still debated. Some authors have proposed the presence of a strong magnetic field during superchrons (Larson and Olson, 1991; Tarduno et al., 2001), which would argue for an efficient geodynamo (i.e., large intensities during low reversal frequency). Others argue for a weak field (Loper and McCartney, 1986; Pick and Tauxe, 1993), which suggests increased convective vigor in the core would increase the reversal rate by generating frequent instabilities. If the latter model applies, the Cocos-Nazca spreading center record is low in paleointensity but may contain frequent reversals. To add further controversy, the limited number of paleointensity measurements from the Cocos-Nazca spreading center yield very different results for different methods (Pick and Tauxe, 1993; Tarduno et al., 2001). Drilling basement at proposed Site SPG-1A will provide important data and samples to test these models and methods. The paleointensity methods could be compared and the measurements would provide important data (in conjunction with the radiometric age) for the origin of the Cocos-Nazca spreading center and its relationship to the geodynamo.

Paleoceanography

Interstitial waters of the South Pacific Gyre represent a unique archive of glacial-aged water where relict NO_3^- can be used to test hypotheses of glacial–interglacial ocean-climate change with significantly lower uncertainty than through proxy measurements.

It has been hypothesized that changes in strength of the biological carbon pump caused the dominant variation in Earth's climate and atmospheric CO_2 over the last 1 m.y. (Broecker, 1982). We will test the two principal models of this variation by reconstructing the preformed NO_3^- content and deep ocean $\delta^{15}NO_3^-$ of the last glacial maximum (LGM) through measurement of O_2, NO_3^-, and $\delta^{15}NO_3^-$ in the interstitial fluid of gyre sediment. Our tests utilize the fact that pore fluids from depths greater than ~30–50 mbsf (e.g., at proposed Sites SPG-1A and SPG-11B) are samples of paleo-bottom water that have effectively been out of diffusive contact with the ocean since the LGM.

Relationship to previous drilling

Proposed Site SPG-1A is located in the western portion of the gyre, near DSDP Sites 595 and 596 (Menard, Natland, Jordan, Orcutt, et al., 1987). There are no DSDP/ODP/IODP sites near any of the other proposed sites. The closest sites were cored during Leg 92, which recovered Oligocene and younger sediment from a series of sites at 20°S (Leinen, Rea, et al., 1986). The Leg 92 sites are located beneath higher productivity waters than the central gyre (Fig. **F1**). The entire sediment column was cored at the Leg 92 sites; basement was encountered between 1 and 50 mbsf (with sediment depth increasing westward).

Coring-drilling strategy

The objectives of this proposal require an IODP riserless drilling expedition. The sediment and basalt that are necessary to meet our objectives cannot be collected without a drilling platform capable of recovering cores from as deep as 171 mbsf in 3170–5700 mbsl water depth. The duration of the proposed expedition is 65 days (Tables **T1, T2, T3**).

Our general strategy will be to core the entire sediment column multiple times at seven sites and to core the upper basement at three sites. The sites collectively underlie the full range of surface-ocean productivity conditions present in the South Pacific Gyre, ranging from the extremely low productivity conditions of the gyre center (proposed Sites SPG-6A and SPG-7A) to the moderately high (for open ocean) productivity at the southern edge of the gyre (proposed Site SPG-12A at the northern edge of the Antarctic Convergence) (Figs. **F1, F2**). This series of sites is composed of two transects (Fig. **F1**), with the first transect centered at ~26°S, beneath the heart of the South Pacific Gyre, and the second transect centered at ~42°S in the southern portion of the gyre.

The sites in the northern sequence have been continuously far from shore and beneath the low-productivity gyre waters for many tens of millions of years (Figs. F1, F2). They provide an ideal opportunity to meet our first objective (to document the nature of life in subseafloor sediment with very low biomass and very low rates of activity). In combination with the southern transect, the northern transect is also necessary to meet our second objective (to determine how subseafloor sedimentary activities and communities vary from gyre center to gyre margin).

Proposed Sites SPG-1A–SPG-11B are necessary for our third objective (to quantify the extent to which subseafloor communities in organic-poor sediment are sustained by H_2 from radiolysis of water). Proposed Sites SPG-1A and SPG-11B are particularly crucial for this objective because their sediment columns are thick enough that their dissolved He-4 (alpha particle) concentrations and fluxes will be measurable.

The sites in the second transect have been in the southern portion of the present gyre (proposed Sites SPG-9A–SPG-11B) or south of the gyre (proposed Site SPG-12A) for tens of millions of years. Particularly at proposed Site SPG-12A, chlorophyll-a concentrations and primary productivity are much higher than at all of the sites in the northern transect (Figs. F1, F2). This transect is necessary to meet our second objective (to document how subseafloor sedimentary activities and communities vary from gyre center to gyre margin). Because proposed Site SPG-12A provides an anoxic standard of comparison for the other sites, it is also crucial for documenting the potential uniqueness (or ubiquity) of the communities and activities that persist in the low-activity, low-biomass sediment beneath the gyre center.

The northern sequence of sites (proposed Sites SPG-1A–SPG-7A) is placed on crust of steadily increasing age from east to west (Fig. F2). They range in age from 7 to as much as 125 Ma (proposed Site SPG-1A). Crust age of the southern sites ranges from 39 to 73 Ma. Their water depths generally follow the classic curve (Parsons and Sclater, 1977) of increasing water depth with increasing basement age (Fig. F2). These sites are necessary to meet our fourth objective (to document the evolution of basalt hydrology and its implications for metabolic habitability and microbial communities in ocean crust under very thin sediment cover).

We propose to drill three or four sediment holes with the advanced piston corer (APC) at each site. We also propose to drill and log a single hole into the upper basement at three select sites (to at least 100 m below the sediment/basalt interface). In the first sediment hole, we will sample for microbiological and geochemical analyses at regular intervals in each core. This sampling will include O_2 measurements immediately after core recovery. This record will be immediately used to (1) define broad downhole microbiological and biogeochemical trends, (2) identify horizons of special interest (including, but not limited to, the sediment/basalt interface, redox interfaces, metalliferous layers), and (3) identify diagnostic properties of those horizons. Cores from the second hole will be used for (1) key biogeochemical and physical property measurements that rely on optodes or electrodes inserted into whole cores (e.g., dissolved O_2 concentrations and electrical resistivity) and (2) close sampling of key horizons identified from the first hole. The third hole will be used for high-resolution sampling of special horizons identified in the first and second holes. The fourth hole will provide a continuous record of sediment appropriate for high-resolution paleoceanographic sampling. Where circumstances only allow coring of three holes, the cores from the final hole will generally be retained as a continuous record; they may be used for high-resolution electrode or optode analyses. This multihole strategy is necessary because (1) microbiological and biogeochemical studies are time- and labor-intensive and (2) microbiological and biogeochemical analyses require immediate sampling, whereas optode measurements require cores to thermally equilibrate for several hours before analysis (Fischer et al., 2009). Shipboard core flow will be arranged to maintain maximal sample integrity for microbiological and biogeochemical analyses, as successfully executed during Leg 201.

This drilling strategy will require the shipboard scientists to rapidly generate data from each sediment hole in order to guide sampling at the next hole. Key redox species, particularly dissolved O_2 (but also including NO_3^-, Fe(II), Mn(II), and SO_4^{2-} to the extent possible) will be immediately measured from samples taken from freshly cut ends of cores as they arrive on the catwalk. Cut-off syringe samples will be taken on the catwalk from cut core ends for contamination tracing (perflourocarbon), cell counts, and volatile hydrocarbons (methane, etc.). The gas chromatograph for perfluorocarbon analysis will be operated continuously, so recovered cores can be immediately assayed for potential microbial contamination (Smith et al., 2000a, 2000b; House et al., 2003). Rapid turnaround on these assays (<10 min/sample) will allow us to focus resources on uncontaminated samples while working in a microbiologically relevant time frame.

Logging/Downhole measurements plan

The main objectives of the downhole measurement program are to document crustal physical properties, define structural and lithologic boundaries as a function of depth, and identify alteration in the basaltic basement from the passage of fluids. In addition, wireline logging data can be compared to results of laboratory analyses of discrete samples and should help delineate alteration patterns, fracture densities, and structural orientations and determine how these correlate with current and paleostress environments. These measurements will complement core measurements by determining the thickness of lithologic units in intervals where core recovery is poor.

Borehole logging and core-log integration are invaluable for reconstructing recovery gaps and estimating bulk geochemical and structural characteristics of deep basement drill sites. Logging of the South Pacific Gyre will help characterize current physical properties that may set important constraints on the downhole microbial community.

Two wireline tool string deployments are planned for four sites drilled during Expedition 329 (Fig. **F7**). These tool strings will provide measurements including natural gamma radiation, density, sonic velocities, resistivity, and borehole images. The operational time estimates for all deployments are given in Table **T1.** Detailed descriptions of all wireline tools and applications are provided at **iodp.ldeo.columbia.edu/TOOLS_LABS/tools.html.**

The first tool string deployment in each hole will consist of at least total and spectral gamma ray, density, caliper, and resistivity measurements. These measurements will be used for characterization of stratigraphic sequences, assessment of alteration, and reconstruction of the basement stratigraphy. Depending on borehole conditions, the second tool string will consist of a FMS, Dipole Sonic Imager (DSI), and natural gamma ray sensor. The FMS will provide high-resolution borehole images of lithostratigraphic sequences and boundaries, oriented fracture patterns, and structural features that could be related to current and paleostress environments. These images can also be used for reconstruction and reorientation of core pieces.

A check shot survey has not been included in the current operations plan; it is possible based on scientific need and time that a third tool string consisting of the Versatile Seismic Imager (VSI) and a natural gamma ray tool may be deployed.

Operational risks

There are no geologic or man-made hazards to drilling near any of the proposed sites. No hydrocarbons are expected at proposed Sites SPG-1A–SPG-11B because sediment drilling targets are <94 m thick, the flux of organic matter to the seafloor is extraordinarily low (Jahnke, 1996), the geothermal gradient is far too low to reach the thermal maturation window at any site (heat flow = 18–93 mW/m^2), and the sediment temperature is <5°C at all sites and sediment depths (R. Harris, pers. comm., 2007). Additionally, the sediment is likely to be oxic throughout the entire column at proposed Sites SPG-1A–SPG-11B. At proposed Site SPG-12A, very low hydrocarbon concentrations are expected. Subseafloor respiration at this site is predominantly anaerobic, and we predict methane concentrations similar to those at Site 1231 (<2 ppm headspace concentration after 20 min at 60°C), which is characterized by similarly low rates of subseafloor respiration (D'Hondt et al., 2003). As at Site 1231, the flux of organic matter to the seafloor is relatively low at proposed Site SPG-12A (Jahnke, 1996), and the geothermal gradient is far too low to reach the thermal maturation window in the 122–130 m thick seddiment.

At all sites, we will follow the standard shipboard safety protocols of measuring methane/ethane ratios and monitoring total dissolved gas content.

Weather and sea hazards

Expedition 329 has been scheduled to take place during the early part of the Southern Hemisphere summer, when weather and sea state are calmest. Significant wave height varies considerably from summer to winter and increases greatly with latitude (Fig. **F8A**) (National Imagery and Mapping Agency [NIMA], 1998). The northern limit of Antarctic icebergs approaches proposed Site SPG-12A in Southern Hemisphere spring (September–November) (NIMA, 1998).

Wind force (Beaufort scale) is ~4 (11–15 kt) at proposed Sites SPG-1A–SPG-7A, 4–5 (11–20 kt) at proposed Sites SPG-9A and SPG-10A, and 5–6 (16–26 kt) at proposed Sites SPG-11B and SPG 12A (Fig. **F8B**) (NIMA, 1998). Particularly at the southern sites, wind force is greater in Southern Hemisphere winter than summer.

Maximum drift is higher at poleward proposed Sites SPG-9A–SPG-12A (10–15 nmi/day) than at equatorward proposed Sites SPG-1A–SPG-7A (5–10 nmi/day) (Fig. **F8C**) (NIMA, 1998).

High-wind tropical storms occur occasionally in the region of our most northwesterly sites (proposed Sites SPG-1A–SPG-4A) (Fig. F9) (Hurricanes, Typhoons, and Tropical Cyclones Worldwide, **www.solar.ifa.hawaii.edu/Tropical**). In 15 years (1994–present), 20 named storms passed through the region of proposed Sites SPG-1A–SPG-4A in the January–March window and 1 passed through the region of proposed Sites SPG-1A–4A in the November–December window. During these events, wind speed was typically 20–40 kt in this region and only very rarely exceeded 40–60 kt. Storm incidence rapidly decreases eastward along the line of drill sites; in 15 y, most high-wind storms occurred west of all the drill sites and only a couple occurred east of proposed Sites SPG-1A and SPG-2A.

Given the seasonal variation in significant wave height, wind force, and high-wind tropical storms, the ideal drilling window is November–December.

Sampling strategy

All standard IODP shipboard analyses will be performed during the proposed expedition. Shipboard procedures that are not standard for IODP are discussed here. Many of the shipboard microbiological and geochemical analyses will follow those of Leg 201 (D'Hondt, Jørgensen, Miller, et al., 2003). Many analyses that will be performed postcruise are also described here.

Chemistry and microbial activities

Documentation of microbial processes in South Pacific Gyre sediment requires a wide range of biogeochemical analyses. Transport-reaction modeling of dissolved metabolite concentrations will allow us to quantify rates of principal net activities in the sediment (e.g., reductions of O_2, NO_3^-, SO_4^{2-}, Mn[IV], and Fe[II]) (Jørgensen, 2000). Chemical data (e.g., the position of the chloride maximum from the LGM) and thermal data will be used to account for advective transport in this modeling (D'Hondt et al., 2004). Concentration data will also allow us to calculate mineral stabilities and, in anoxic sediment, to test thermodynamic models of metabolic competition (Hoehler et al., 1998; Wang et al., 2004). Mineralogical, textural, and isotopic studies will be used to evaluate the extent of microbial mineral alteration (Boyd and Scott, 2001; Rouxel et al., 2003).

We will undertake key biogeochemical analyses and radiotracer incubations on the ship and collect appropriate samples for critical categories of postcruise biogeochemical studies. Particular shipboard effort will be placed on obtaining rapid, high-resolution concentrations of key dissolved chemicals that we expect to be present in the sediment at each site. In oxic sediment, these species include O_2, NO_3^-, H_2, SO_4^{2-}, and many other ions. In anoxic sediment, they include NO_3^-, H_2, SO_4^{2-}, and many other dissolved metabolites, including, but not limited to, Fe(II), Mn(II), sulfide, acetate, formate, lactate, methane, ethane, and propane. Dissolved O_2 concentrations will be measured by using micro-electrodes or micro-optodes on thermally equilibrated core sections (using methodology fine-tuned on recent coring cruises to the South Pacific Gyre, North Pond, and North Pacific Gyre). Concentrations of other dissolved species will be determined by ion chromatography (NO_3^-, SO_4^{2-}, acetate, formate, and lactate), gas chromatography (dissolved gases), and ICP (dissolved Fe and Mn).

Gross rates of activities in sediment and basalt will be quantified by radiotracer incubations (Soffientino et al., 2006). The potential for autotrophic activity in basalt and sediment will be assessed by addition of $Na^{14}CO_2/NaH^{14}CO_3$ followed by short incubations to quantify ^{14}C incorporation into cellular biomass (method adapted from Wirsen et al. [1993], who used it to demonstrate autotrophy associated with hydrothermal sulfides). This shipboard assay has been successfully used on young basalt from Loihi Seamount (Clement, Tebo, and Edwards, unpubl. data, 2006). The ^{14}C-incorporated cells can be phylogenetically identified and visualized by micro-autoradiography–fluorescence in situ hybridization (MAR-FISH) technique (Lee et al., 1999; Ouverney and Fuhrman, 1999). Alternatively, assimilation ratio of ^{13}C- and/or ^{15}N-labeled substrates will be determined by nano-scale secondary mass spectrometry (NanoSIMS) at single-cell level (Dekas et al., 2009; Morono and Kallmeyer, pers. comm., 2010). Stimulation of in situ communities by lithotrophic substrates (sulfide, Fe[II], and Mn[II]) has also been successful with young basalt.

Uncontaminated samples of sediment and basalt will be collected and frozen for (1) shore-based solid-phase and isotopic analyses, (2) microbial biomarker studies, and (3) analyses of mineralogy and solid-phase geochemistry. These analyses will include studies of Fe and S isotopes and elemental composition.

Community composition

To document community composition in uncontaminated sediment and basalt, a large number of samples will be taken for postcruise molecular studies, including, but not limited to, FISH and 16S rRNA gene-tagged sequencing with 454 technology (e.g., Sogin et al., 2006). The molecular studies will allow us to determine (1) the microbial community structure (i.e., diversity richness and community evenness) and (2) their phylogenetic compositions. The RNA sequencing studies, targeting both 16S rRNA and messenger RNA (mRNA), will document the genetic and functional taxonomy of the actively growing and metabolizing fraction of the microbial community (Fukui et al., 1996; Poulsen et al., 1993). If the extracted DNA or RNA concentration is not enough for subsequent molecular and metagenomic analyses, whole genomes will be amplified by multiple displacement amplification with phi29 polymerase under strict experimental condition. These high-throughput molecular studies will quantify bacterial and archaeal diversity in these subseafloor environments and will enable detection of rare members that are not able to be sampled using conventional capillary sequencing approaches.

To determine the physiology, potential biogeochemical function, diversity, and habitat range of low-activity subseafloor microorganisms we will initiate a very broad range of cultivations during the expedition. As during Leg 201 (Shipboard Scientific Party, 2003b), many of these cultivations will use complex substrates, diversified electron acceptors, gradient media, and helper cultures. Cultivations will also allow ground-truthing of molecular analyses and provide estimates of minimum cultivable populations.

Biomass estimates

To document microbial biomass in the sediment and basement, we will use general nucleic acid stains [SybrGreen] for shipboard counts of total cell concentrations (Morono et al., 2009). The cell concentrations observed on expedition KNOX-02RR are well below the detection limit for the standard technique of counting cells in a slurried sediment (Kallmeyer et al., 2009). To undertake counts at this concentration requires separation and concentration of the cells from their sedimentary matrix (Kallmeyer et al., 2008). The accuracy of this approach has been quantified by parallel standard counts and cell-separation counts on samples from previous IODP sites (Kallmeyer et al., 2008) and Cruise KNOX-02RR (A. Puschell, unpubl. data, 2007). By adding autofluorescent microspheres to cell count slurries as a dilution standard, we will quantify the dilution/concentration factor of the cell-separation step and improve estimates of total cell density.

In addition to routine cell counts, we will take uncontaminated samples of both sediment and basalt for FISH counting to determine the extent to which counted cells are truly living, dormant, or dead. Quantifications of phylogenetic groups of active cells using FISH probes will be checked against DNA-based quantification using quantitative polymerase chain reaction (Schippers et al., 2005).

Potential for radiolysis

We will directly assess the potential of water radiolysis for supporting life in the organic-poor sediment and underlying basalt of the gyre. To estimate water radiolysis rates, we will determine uranium, thorium, and potassium concentrations by (1) natural gamma ray logging of representative cores and (2) analysis of selected samples by ICP-MS.

To verify rates of water radiation, we will determine the flux of He-4 to interstitial water. In subseafloor environments, radiolytic H_2 production will principally result from production of He-4 (alpha particles) (Blair et al., 2007). Consequently, measurement and transport modeling of He-4 concentration profiles will constrain estimates of radiolysis rates independently of estimates based on abundances of radioactive elements.

To verify rates of H_2 production by water radiolysis, we will undertake postcruise experiments with killed samples (of the cored sediment and basalt) plus tritium-labeled water and quantify the appearance of labeled H_2 over the course of the experiments. Similar (unlabeled) experiments with artificial radiation sources were done by Lin et al. (2005b).

To quantify rates of microbial uptake of radiolytic H_2, we will measure dissolved H_2 concentrations in the cored sediment and at the sediment/basalt interface and compare them to the concentrations expected from the water radiolysis rates with no uptake (Fig. **F10**). The expected rate of radiolytic H_2 production is so high (Blair et al., 2007) that in situ H_2 concentrations will be measurable shipboard if the H_2 is not biologically utilized. If H_2 is biologically utilized and held to a thermodynamic minimum, its in situ concentrations will be below detection.

Finally, we will compare radiolysis rates to measured hydrogen turnover in sediment incubations with known numbers of microbial cells, using an array of relevant electron acceptors (O_2, NO_3^-, and oxidized metals). In this way, the microbial rates of hydrogen oxidation that are thermodynamically possible in the subsurface ecosystem can be constrained. Bacterially catalyzed redox reactions are many orders of magnitude faster than abiotic reactions. Because of its high activation enthalpy, the recombination of O_2 and H_2 will not occur at all with measurable rates at temperatures below 400°C; bacterial catalysis allows this reaction on a timescale of minutes.

Contamination tracing

To evaluate the extent to which contaminating cells may have penetrated a sample, we will use (1) perfluorocarbon tracer in the drilling fluid (in sediment and basalt), (2) fluorescent microspheres injected at the drill bit (in basalt), and (3) genomic comparison of the basalt or sediment sample with drilling fluid from the time of coring. The first two techniques have been successfully used on multiple ODP legs and Expedition 301 (Smith et al., 2000a, 2000b; House et al., 2003; Lever et al., 2006). The third technique is common for studies of seafloor samples, where contamination is ubiquitous and genomic signatures of the contaminating material are subtracted from those of the seafloor sample. Previous results have consistently shown that core centers are much less contaminated than core peripheries (by factors of 3–300) and that APC cores are generally not significantly contaminated (House et al., 2003). The uppermost 1.5 m section of APC cores tends to be more contaminated than deeper sections (Lever et al., 2006). Contamination tends be much greater in RCB cores (e.g., on surfaces of cored basalt) and extended core barrel (XCB) cores than in APC cores. In all categories of core, potential contamination varies considerably from sample to sample. Consequently, to avoid contamination of microbiological results, contamination tests must be conducted on each core or hard rock sample that is used for a microbiological experiment (D'Hondt, Jørgensen, Miller, et al., 2003).

Geophysical properties

Physical properties will be used to quantify chemical fluxes through the sediment and to quantify the extent of basement alteration as a function of basement age and fluid flow. Formation factor will be measured for calculating fluxes of chemical species through the sediment. At all sites, sediment temperatures will be logged with the advanced piston corer temperature tool (APCT-3) and analyzed for active fluid flow. Basement holes will be logged with a suite of geophysical tools, including velocity (DSI), density, porosity, resistivity, magnetometer, natural gamma radiation, and FMS logs. For both sediment and basalt, core-based physical properties (bulk density, matrix [or grain] density, porosity, thermal conductivity, core temperature, *P*-wave velocity, and light absorption spectroscopy [LAS]) will be measured.

Alteration of the basaltic oceanic crust

Assessment of the extent and relative importance of secondary alteration to the basaltic crust will require an integrated program of petrographic, geochemical, and borehole analyses. At hand-sample and thin section scales, we will carefully describe general alteration textures and characteristics (e.g., veins, halos, vesicle filling, mineral/matrix replacement, and glass palagonitization), principal secondary mineralogy (e.g., saponite, celadonite, calcite, Fe oxyhydroxide, etc.), and the size, distribution, and orientation of veins and other structural features in the crust. Discrete samples of the core, representing "fresh" (e.g., pristine glass, if recovered), average, and end-member altered domains, will be powdered and analyzed for bulk major, trace, and volatile element chemistry, as a means of characterizing the bulk crustal composition and geochemical effects of alteration. Borehole logging and core-log integration are invaluable for reconstructing recovery gaps and estimating bulk geochemical and structural characteristics of deep basement drill sites (Barr et al., 2002; Révillon et al., 2002; Kelley et al., 2003; Pockalny and Larson, 2003). Radiogenic isotope measurements will place important constraints on the timing of alteration at each site. For example, calcite formed during crust alteration often contains high concentrations of uranium but little to no lead, making the lead isotopic system a potentially useful calcite precipitation geochronometer, especially in old oceanic crust (Hauff et al., 2003).

Research plan proposals (sample and data requests) and sample allocation committee

Shipboard and shore-based researchers should refer to the IODP Sample, Data, and Obligations Policy (**www.iodp.org/program-policies**). Every member of the scientific party is obligated to carry out scientific research for the expedition and publish it. For this purpose each scientist must submit a detailed research plan and a sample and/or data request prior to the expedition, using the Sample/Data Request form available at **smcs.iodp.org.** A sample/data request is also required for individuals not requesting samples but working on shipboard data only. The sampling plan should be limited to samples needed to fulfill the expedition scientific objectives within 36 months postexpedition. All sample and data requests must be submitted 3 months prior to the expedition to ensure the coordinated preparation of an integrated sampling plan. Based on research requests submitted (sample and data), the Sample Allocation Committee (SAC; composed of the Co-Chief Scientists, the Staff Scientist, and the IODP-USIO Curator) will work with the scientific party to formulate a formal sampling and data-sharing plan for shipboard and postcruise activities.

The sampling strategy will be subject to modification depending upon the actual material/data recovered, expertise of the science participants, and collaborations that may evolve between scientists before, during, and soon after the expedition. Substantial collaboration and cooperation are highly encouraged. Modifications to the sampling plan and access to samples and data during the expedition and the 1 year postexpedition moratorium period require SAC approval.

All sample frequencies, types, and sizes must be justified on a scientific basis and will depend on core recovery, the full spectrum of other requests, and the expedition objectives. Success will require collaboration, integration of complementary data sets, and consistent methods of analysis.

We anticipate taking the bulk of the samples for everyone's personal research during the expedition, but a small shore-based sampling effort might be necessary roughly 3–5 months after the expedition to complete the sampling of the cores that will be dedicated to stratigraphic and paleoenvironmental studies. All sampling necessary to acquire ephemeral data types, contamination testing, chronostratigraphic information, or to achieve essential sample preservation will be conducted during the expedition. Small intervals of high-resolution sampling may be sampled at sea with SAC approval to provide initial material for study prior to the postcruise sampling party. Critical intervals may require special handling, a higher sampling density, reduced sample size, or continuous core sampling for a set of particular high-priority research objectives. The SAC may require an additional formal sampling plan before critical intervals are sampled.

Cores will be delivered to the IODP Gulf Coast Core Repository at Texas A&M University in College Station, Texas. The 1 y moratorium period will begin at the end of the expedition or at the end of the postexpedition sampling party if one is held.

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Table T1. Expedition 329 primary site operations plan and time estimate.

South Pacific Gyre
Operations Plan and Time Estimate
(Midgley, 08 February 2010)

Site number	Location (latitude longitude)	Seafloor depth (mbrf)	Operations description		Transit (days)	Drilling/ Coring (days)	LWD/MWD log (days)
Papeete, Tahiti			Begin expedition	4.0	Port call days		
			Transit ~978 nmi to SPG-1A @ 10.5 kt		3.9		
SPG-1A	23.8508°S	5708	Hole A: APC to ref. ~71 mbsf.			1.4	
EPSP	165.6442°W		Hole B: APC to ref. ~71 mbsf. APCT-3 measurements			0.7	
to 271 mbsf			Hole C: APC to ref. ~71 mbsf.			0.6	
Pending			Hole D: APC to ref. ~71 mbsf.			1.0	
			Hole E: RCB to ref. ~171 mbsf. Logging with triple combo and FMS-sonic			4.4	1.1
			Subtotal days on site:	9.2			
			Transit ~494 nmi to SPG-2A @ 10.5 kt		2.0		
SPG-2A	26.0516°S	5138	Hole A: APC to ref. ~17 mbsf.			0.7	
EPSP	156.8943°W		Hole B: APC to ref. ~17 mbsf. APCT-3 measurements			0.3	
to ~17 mbsf			Hole C: APC to ref. ~17 mbsf.			0.2	
(basement)			Hole D: APC to ref. ~17 mbsf.			0.7	
			Subtotal days on site:	1.9			
			Transit ~1019 nmi to SPG-4A @ 10.5 kt		4.0		
SPG-4A	26.4816°S	4295	Hole A: APC to ref. ~20 mbsf.			0.7	
EPSP	137.9394°W		Hole B: APC to ref. ~20 mbsf. APCT-3 measurements			0.3	
to 220 mbsf			Hole C: APC to ref. ~20 mbsf.			0.2	
Pending			Hole D: APC to ref. ~20 mbsf.			0.6	
			Hole E: RCB to ref. ~120 mbsf. Logging with triple combo and FMS-sonic			3.7	1.0
			Subtotal days on site:	6.4			
			Transit ~793 nmi to SPG-6A @ 10.5 kt		3.1		
SPG-6A	27.9167°S	3749	Hole A: APC to ref. ~15–23 mbsf.			0.6	
EPSP	123.1609°W		Hole B: APC to ref. ~15–23 mbsf. APCT-3 measurements			0.3	
to 223 mbsf			Hole C: APC to ref. ~15–23 mbsf.			0.2	
Pending			Hole D: RCB to ref. ~115–123 mbsf. Logging with triple combo and FMS-sonic			3.4	1.0
			Subtotal days on site:	5.5			
			Transit ~1073 nmi to SPG-10A @ 10.5 kt		4.3		
SPG-10A	39.3103°S	5294	Hole A: APC to ref. ~21 mbsf.			0.8	
EPSP	139.8006°W		Hole B: APC to ref. ~21 mbsf. APCT-3 measurements			0.3	
to 21 mbsf			Hole C: APC to ref. ~21 mbsf.			0.8	
(basement)							
			Subtotal days on site:	1.9			
			Transit ~625 nmi to SPG-11B @ 10.5 kt		2.5		
SPG-11B	41.8571°S	5103	Hole A: APC to ref. ~94–100 mbsf.			1.2	
EPSP	153.1192°W		Hole B: APC to ref. ~94–100 mbsf. APCT-3 measurements			0.9	
to 100 mbsf			Hole C: APC to ref. ~94–100 mbsf.			1.4	
(basement)							
			Subtotal days on site:	3.5			
			Transit ~499 nmi to SPG-12A @ 10.5 kt		2.0		
SPG-12A	45.9642°S	5320	Hole A: APC to ref. ~122–130 mbsf.			1.5	
EPSP	163.1842°W		Hole B: APC to ref. ~122–130 mbsf. APCT-3 measurements			1.1	
to 130 mbsf			Hole C: APC to ref. ~122–130 mbsf. Logging with triple combo and FMS-sonic			1.6	1.3
(basement)							
			Subtotal days on site:	5.5			
			Transit ~1127 nmi to Auckland, NZ @ 10.5 kt		5.2		
Auckland, NZ			End expedition		27.0	29.6	4.4

Port call days:	4.0	Total operating days:	61.0
Subtotal days on site:	34.0	Total expedition days:	65.0

Note(s): Transit from proposed Site SPG-12A to Auckland will cross two time zones and the international dateline. Time changes reflected in time estimate. LWD = logging while drilling, MWD = measurement while drilling, EPSP = Environmental Protection and Safety Panel, APC = advanced piston corer, APCT-3 = advanced piston corer temperature tool, triple combo = triple combination, FMS = Formation MicroScanner.

Table T2. Expedition 329 alternate site operations plan and time estimate.

South Pacific Gyre (Alternate Sites)
Operations Plan and Time Estimate
(Midgley, 18 February 2010)

Site number	Location (latitude longitude)	Seafloor depth (mbrf)	Operations description	Transit (days)	Drilling/ Coring (days)	LWD/MWD log (days)
SPG-3A	27.9420°S	4863	Hole A: APC to ref. ~6 mbsf.		0.6	
EPSP	148.5899° W		Hole B: APC to ref. ~6 mbsf.		0.2	
to 206 mbsf			Hole C: APC to ref. ~6 mbsf.		0.2	
Pending			Hole D: RCB to ref. ~106 mbsf.		4.2	
			Subtotal days on site: 5.1			
SPG-5A	28.4464°S	4232	Hole A: APC to ref. ~22 mbsf.		0.7	
EPSP	131.3904° W		Hole B: APC to ref. ~22 mbsf. APCT-3 measurements		0.3	
to ~22 mbsf			Hole C: APC to ref. ~22 mbsf.		0.2	
(basement)			Hole D: APC to ref. ~22 mbsf.		0.7	
			Subtotal days on site: 1.8			
SPG-7A	27.7379°S	3699	Hole A: APC to ref. ~<3 mbsf.		0.5	
EPSP	117.6197° W		Hole B: APC to ref. ~<3 mbsf.		0.1	
to 203 mbsf			Hole C: APC to ref. ~<3 mbsf.		0.1	
Pending			Hole D: RCB to ref. ~103 mbsf.		3.8	
			Subtotal days on site: 4.5			
SPG-9A	38.0615°S	4936	Hole A: APC to ref. ~20 mbsf.		0.7	
EPSP	133.0917° W		Hole B: APC to ref. ~20 mbsf. APCT-3 measurements		0.3	
to ~20 mbsf			Hole C: APC to ref. ~20 mbsf.		0.2	
(basement)			Hole D: APC to ref. ~20 mbsf.		0.7	
			Subtotal days on site: 2.0			

Note(s): LWD = logging while drilling, MWD = measurement while drilling, EPSP = Environmental Protection and Safety Panel, APC = advanced piston corer, RCB = rotary core barrel, APCT-3 = advanced piston corer temperature tool.

Table T3. Projected timeline of operations. (**See table notes.**)

Location	Transit distance (nmi)	Transit time (days)	Water depth (mbsl)	Sediment depth (mbsf)	Total coring depth (mbsf)	Coring time (days)	Logging time (days)	Expedition duration (days)
Primary sites								
Papeete, Tahiti (4 days)								
SPG-1A	978	3.9	5697	71	171	8.1	1.1	
SPG-2A	494	2.0	5127	17	17	1.9		
SPG-4A	1019	4.0	4284	20	120	5.5	1.0	
SPG-6A	793	3.1	3738	15–23	123	4.5	1.0	
SPG-10A	1073	4.3	5283	21	21	1.9		
SPG-11B	625	2.5	5092	94–100	100	3.5		
SPG-12A	499	2.0	5309	122–130	130	4.2	1.3	
Auckland, NZ	1127	5.2						
Totals:	6608	27.0				29.6	4.4	65.0
Secondary sites								
SPG-3A			4852	6	106	5.1		
SPG-5A			4221	22	22	1.8		
SPG-7A			3688	<3	103	4.5		
SPG-9A			4925	20	20	2.0		

Notes: Transit time is at 10.5 kt. Coring depths and times for proposed Sites SPG-1A, SPG-4A, and SPG-6A assume 100 m of basement penetration. Coring time estimates assume four advanced piston corer (APC) holes for the first three primary sites. The remaining four sites are scheduled for four APC holes, but an extra hole will be piston cored if time becomes available in the schedule. Any additional time will be used to deepen the basement holes. Coring time estimates also include one complete pipe trip per site (down and back) for the APC/sediment only sites and, at basement sites, two complete pipe trips per site (down and back), one for the APC holes and one for the rotary core barrel (RCB) hole. Logging time estimates assume two complete logging runs per site at ~12 h/trip for sites selected for logging. Proposed Sites SPG-3A and SPG-7A are alternate sites for basement objectives. Proposed Sites SPG-5A and SPG-9A are alternate sites for sediment objectives. Proposed Site SPG-8 is not included in the drilling plan because it was not surveyed during Cruise Knox-02RR.

Figure F1. Map of annual chlorophyll-a (Chl-a) concentrations overlain by proposed site locations (circled numbers; corresponds to site number). White circled numbers = primary sites, gray circled numbers = secondary sites. White lines = basement age in 10 m.y. increments. As illustrated by paleoposition histories of proposed Sites SPG-1 and SPG-12 (black lines), paleopositions determined with a fixed hotspot reference frame indicate that proposed Sites SPG-1–SPG-10 have been in the gyre for tens of millions of years and proposed Site SPG-12 has been at the gyre margin for tens of millions of years.

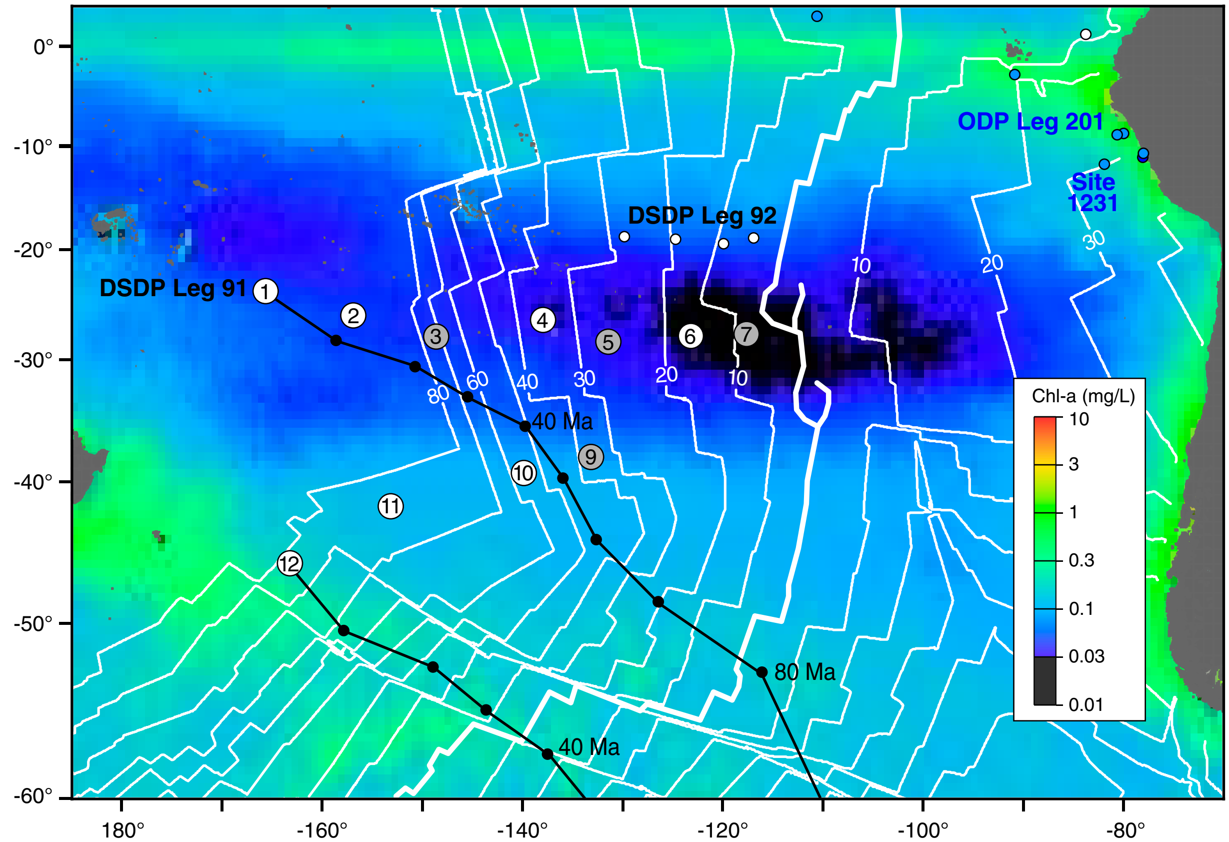


Figure F2. South Pacific seafloor bathymetry map (Smith and Sandwell, 1997) illustrating tectonic setting and location of proposed sites (circled numbers; corresponds to site number). White circled numbers = primary sites, gray circled numbers = secondary sites. White circles = nearest DSDP drill sites; blue circles = nearest ODP drill sites.

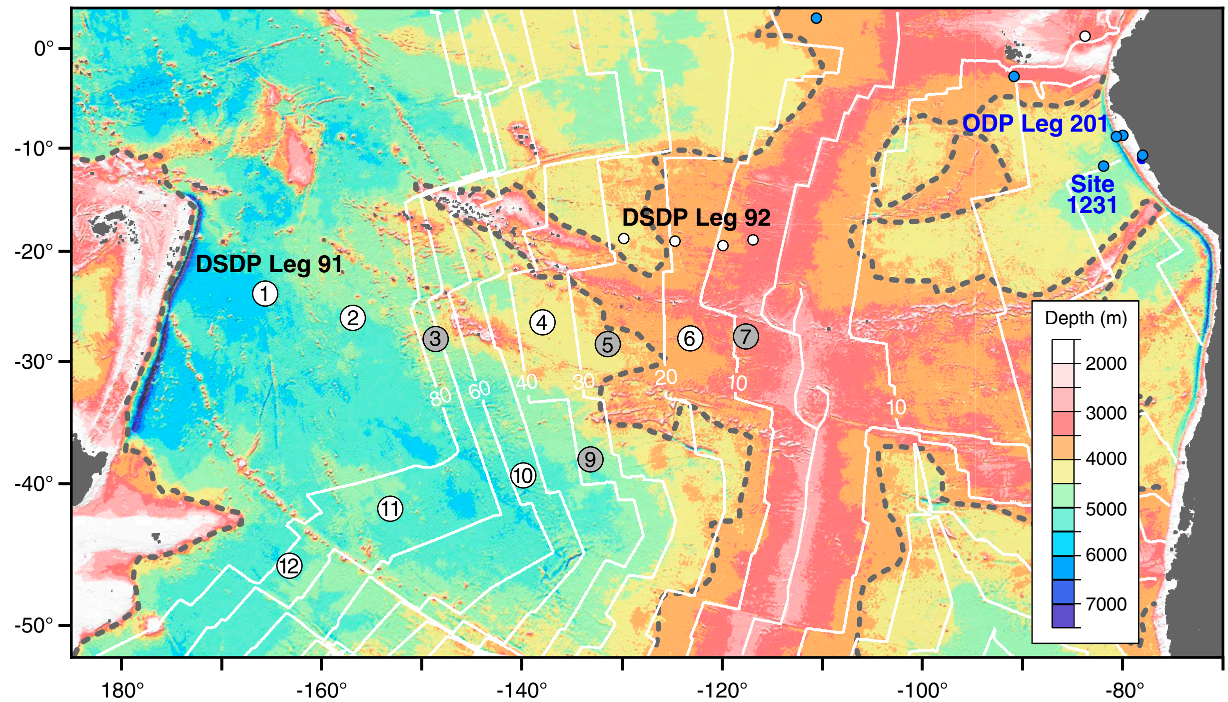


Figure F3. Subseafloor cell concentrations in shallow sediment at proposed sites (open circles = data from proposed Sites SPG-1–SPG-11, open triangles = data from proposed Site SPG-12, just outside the gyre) and at all previously counted ODP/IODP sites (solid circles) (D'Hondt et al., 2009). Proposed Site SPG-12 is the only Cruise KNOX-02RR site at which concentrations approach previous ODP/IODP values.

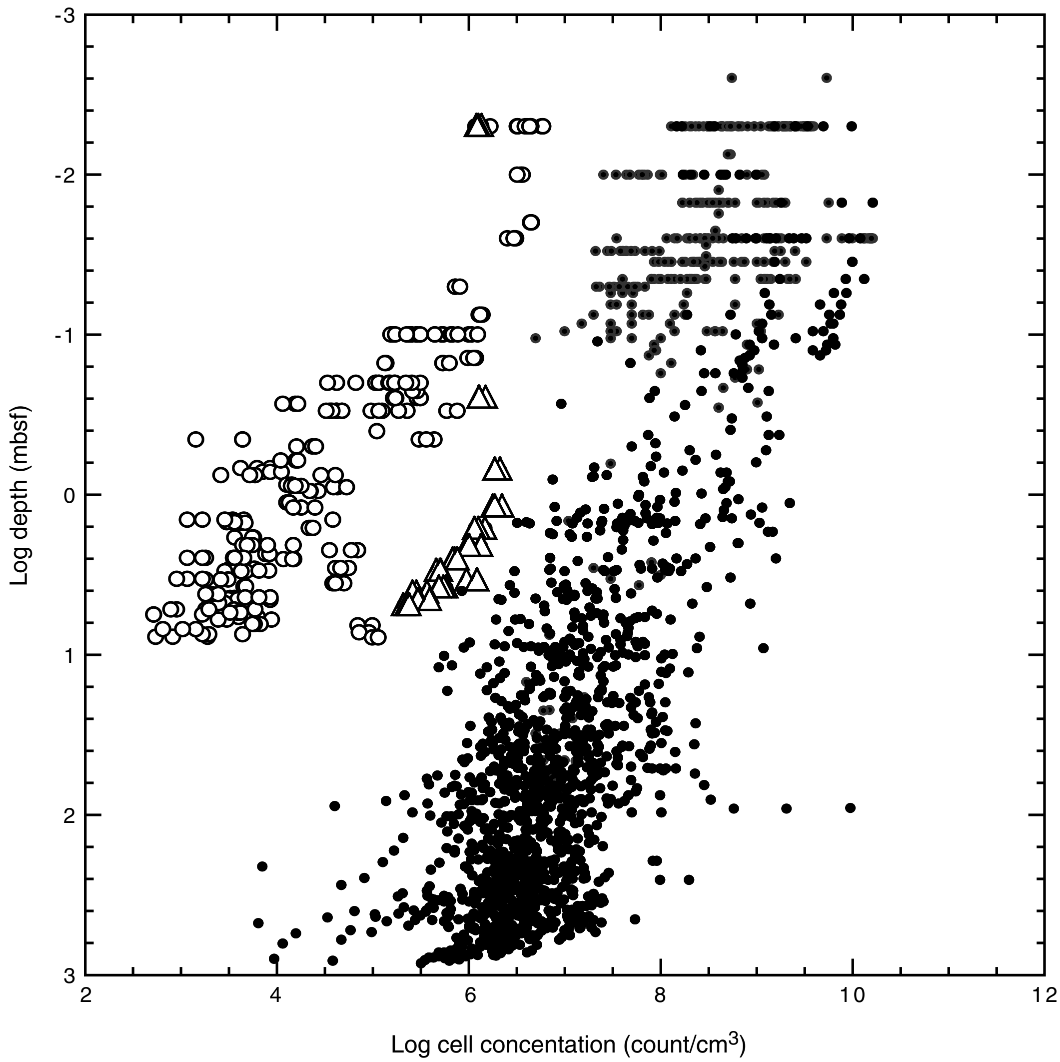


Figure F4. Dissolved (**A**) oxygen (O_2) and (**B**) nitrate (NO_3^-) profiles in the shallow sediment at the proposed sites (D'Hondt et al., 2009).

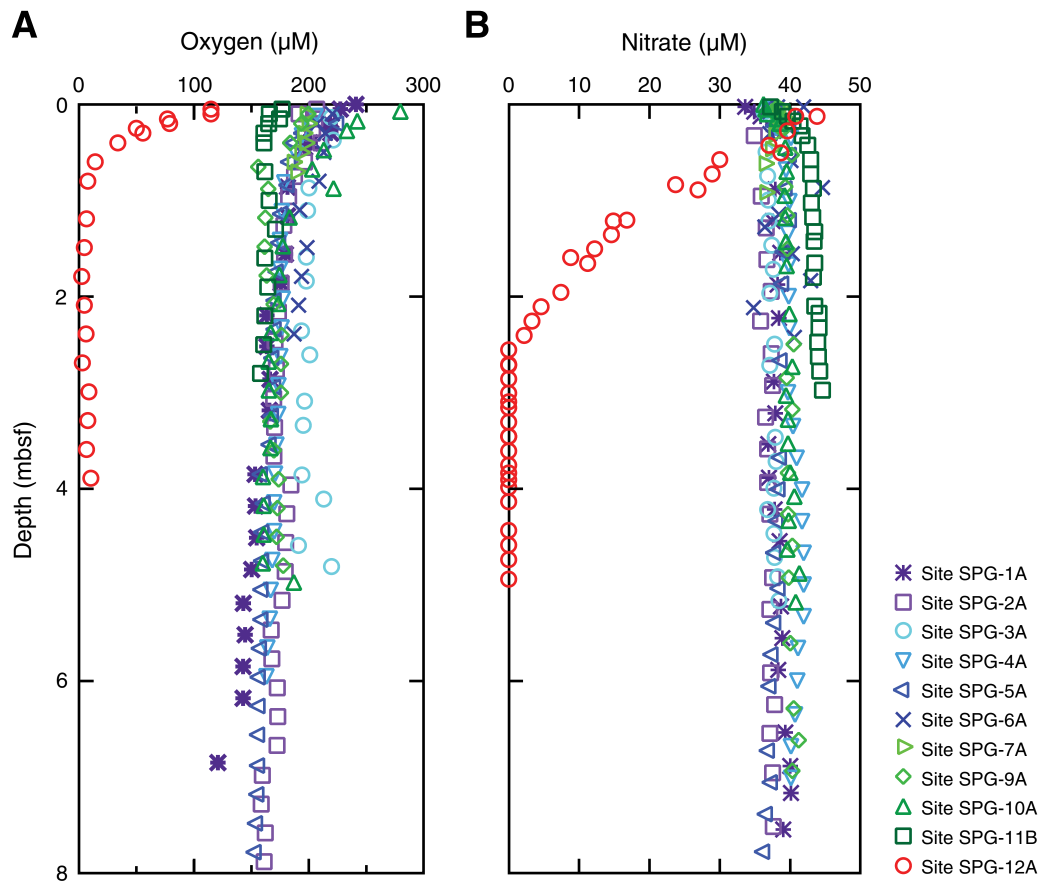


Figure F5. Model for relative rates of organic carbon (OC) oxidation and radiolytic H_2 oxidation with depth. Dotted pink line = maximum depth below seafloor cored curing Cruise KNOX-02RR. Radiolytic H_2 may provide more than half of the electron donors at some proposed sites (D'Hondt et al., 2009). Because organic carbon is oxidized at highest rates in the youngest sediment, at some depth below seafloor (dashed blue line), microbial oxidation of radiolytic H_2 may exceed oxidation of buried organic carbon. At still greater depth (solid green line), reactive organic matter may be depleted and radiolytic H_2 may be the sole electron donor. These predictions can only be tested by drilling the entire sediment column.

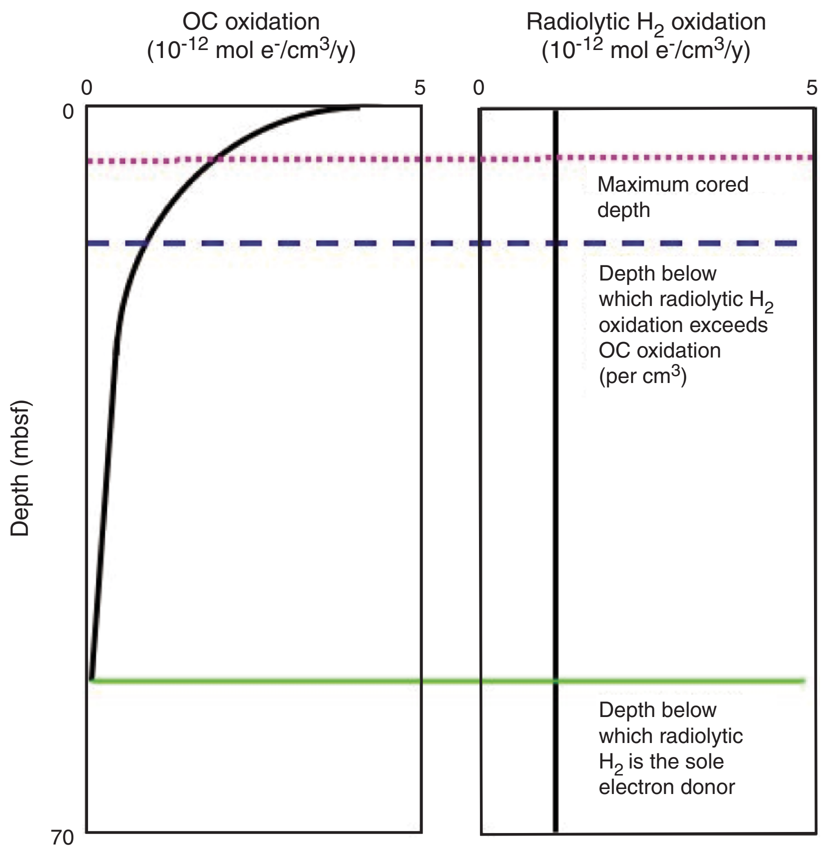


Figure F6. Surface heat flow data from Cruise Knox-02RR survey sites [Cruise Knox-02RR Shipboard Science Party, unpubl. data] superimposed on the compilation data of Stein and Stein (1994). Red circles = primary drilling sites, light blue circles = secondary sites, red arrows = primary sites for basement drilling. Heavy black line = expected heat flow for conductive crust in the absence of advection. No Cruise Knox-02RR data are available for proposed Sites SPG-11B or SPG-12A because of technical failure and thermistor loss.

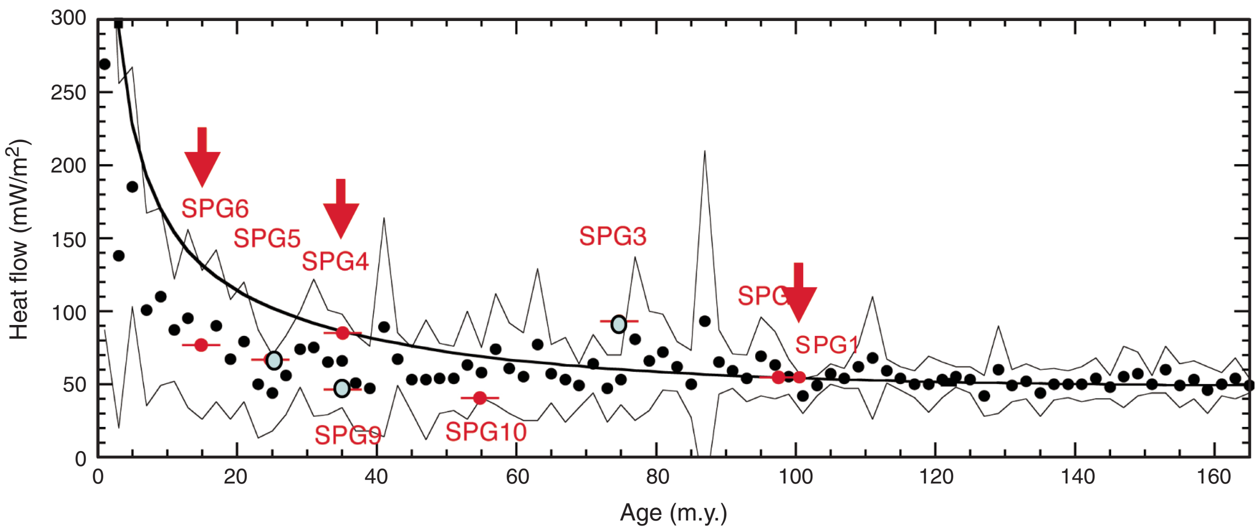


Figure F7. Proposed tool strings for downhole logging, Expedition 329.

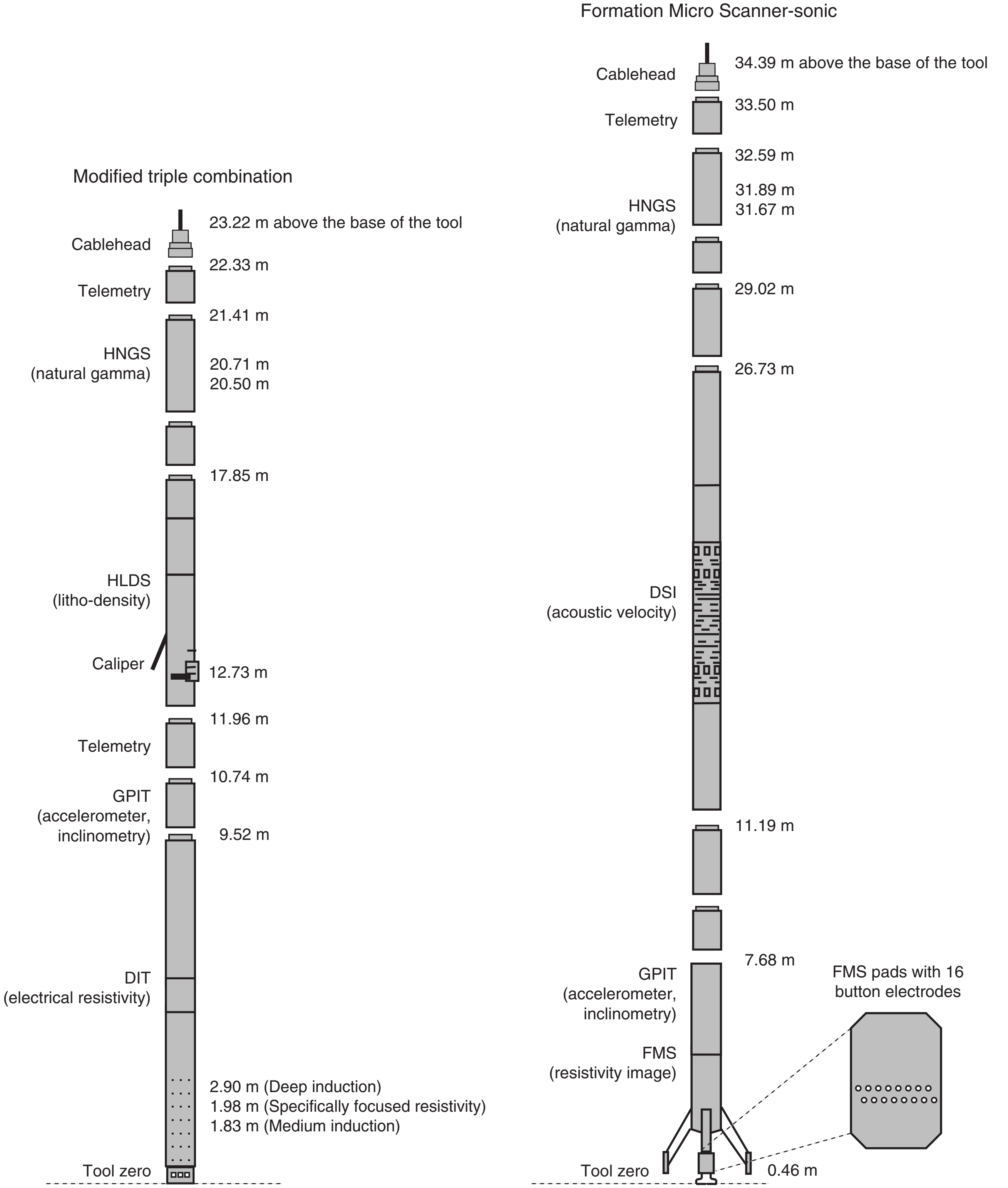


Figure F8. Monthly progression of (**A**) significant wave height, (**B**) wind force, and (**C**) maximum drift at the proposed sites.

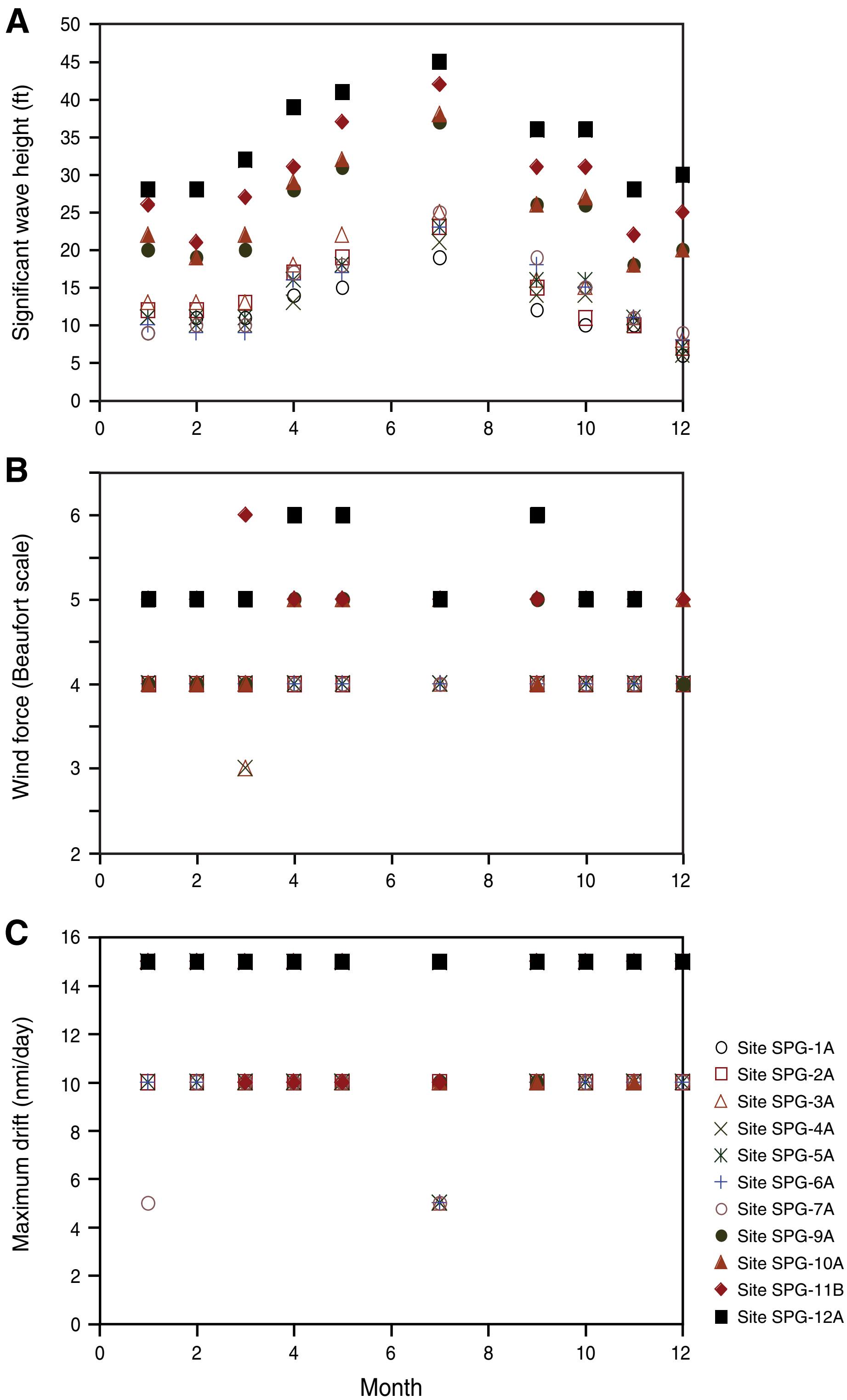


Figure F9. Locations and wind speeds of high-wind tropical storms in (**A**) October–December and (**B**) January–March (1994–present) (**www.solar.ifa.hawaii.edu/Tropical/**). Stars = site locations.

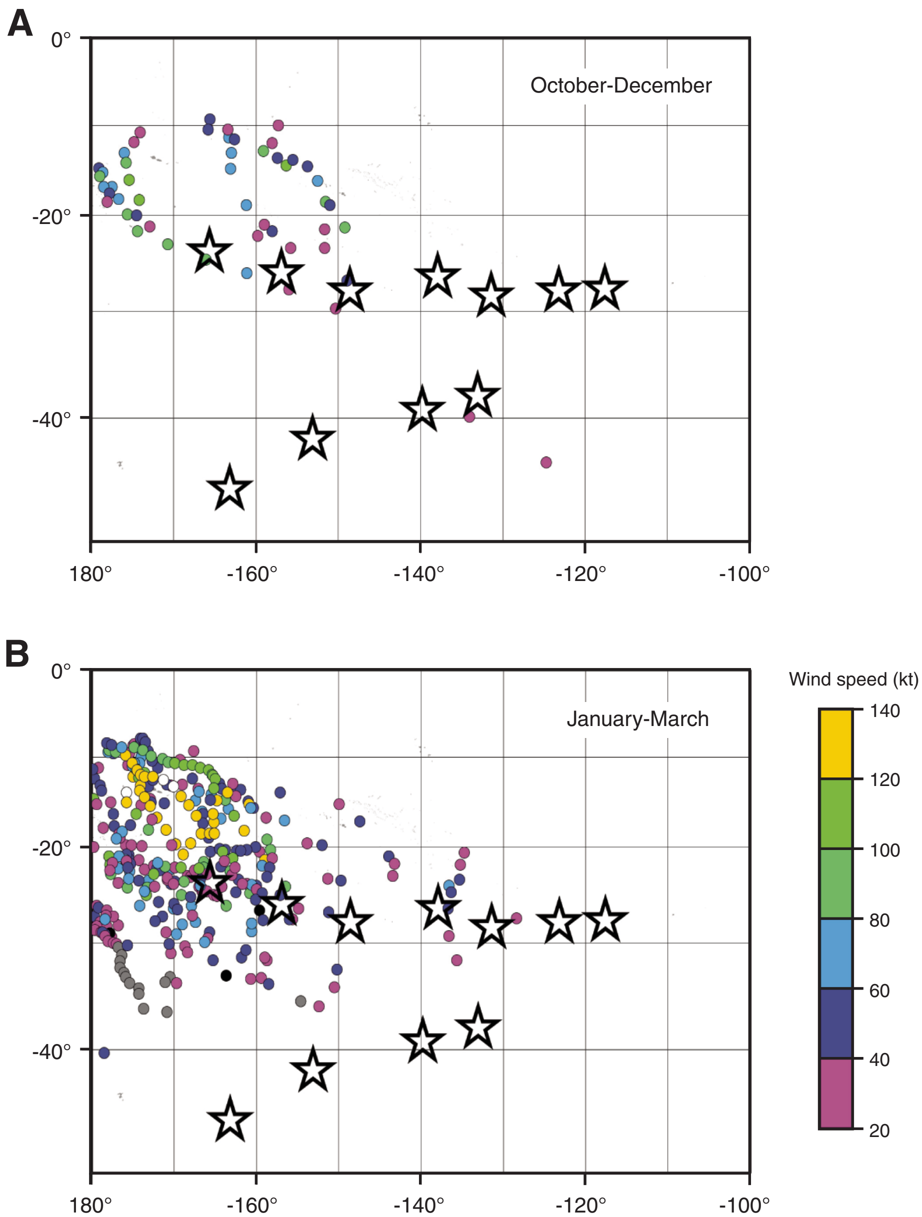
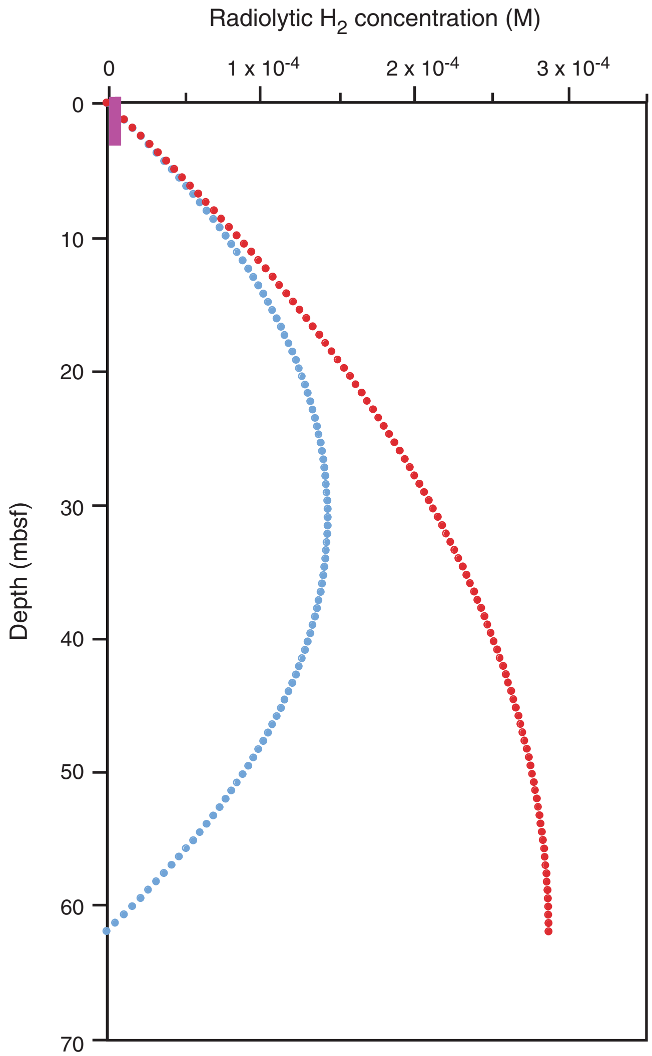


Figure F10. Models of radiolytic H_2 concentrations with depth. If H_2 is microbially oxidized, its concentration will be below our detection limit. Pink line = concentrations measured at proposed Site SPG-11B during Cruise KNOX-02RR (below detection limit of 3.5×10^{-8} M). Other lines indicate predicted concentrations if the H_2 is not oxidized (e.g., if sediment is sterile), but radioactivity and porosity are constant throughout the sediment: blue line = diffusive loss of H_2 to both overlying ocean and underlying basement aquifer, red line = diffusive loss to the overlying ocean but basement is impermeable to chemical exchange. The constancy of radioactivity and porosity with depth and the concentrations of dissolved H_2 at depth can only be tested by drilling to basement.



Site summaries

Site SPG-1A

Priority:	Primary
Position:	23°51.0508′S, 165°38.6538′W (WGS 84) (23.8508°S, 165.6442°W)
Water depth (m)	5697
Target drilling depth (mbsf):	171 (including 100 m of basement)
Approved maximum penetration (mbsf):	Up to 100 m basement penetration
Previous drilling in area:	DSDP Leg 91 Site 596, <1 nmi away
Comments:	International waters; 365 nmi off Palmerston Island, Cook Islands Preferred weather window: November–December
Survey coverage (track map, seismic profile):	High-resolution seismics: • Primary line(s): MCS Line 1 - Shotpoint 1167 (Fig. **AF1**) • Crossing line(s): MCS Line 3 - Shotpoint 2280 (Fig. **AF2**) • Subbottom profile, 3.5 kHz: Line 1 - JDay 354 at 19:48 GMT • Detailed bathymetry and site location (swath bathymetry: collected with SIMRAD EM120) (Fig. **AF3**)
Sediment cores:	Two piston cores, two trigger cores, one gravity core, and one eight-barrel multicore taken at site location
Objectives (see text for complete details):	• Document the habitats, metabolic activities, genetic composition, and biomass of microbial communities in subseafloor sediments with very low total activity. • Test how oceanographic factors (such as surface ocean productivity) control variation in sedimentary habitats, activities, and communities from gyre center to gyre margin. • Quantify the extent to which these sedimentary communities may be supplied with electron donors by water radiolysis, a process independent of the surface photosynthetic world. • Determine how the basement habitats, potential activities, and communities vary with crust age and hydrologic regime (from ridge crest to abyssal plain).
Drilling/Coring program:	• Quadruple APC to basement, RCB to 100 m of basement • Temperature measurements: APCT-3
Downhole logging program:	Wireline logging: triple combo, FMS-sonic
Anticipated lithology:	Dark brown to dark yellowish brown clay; manganese nodules present at top

Site summaries (continued)

Site SPG-2A

Priority:	Primary
Position:	26°03.0953′S, 156°53.6566′W (WGS 84) (26.0516°S, 156.8943°W)
Water depth (m)	5127
Target drilling depth (mbsf):	17 m
Approved maximum penetration (mbsf):	Until basement is encountered
Previous drilling in area:	DSDP Leg 91 Site 596, 500 nmi away
Comments:	International waters; 255 nmi from Mangaia Island, Cook Islands Preferred weather window: November–December
Survey coverage (track map, seismic profile):	High-resolution seismics: • Primary line(s): MCS Line 1 - Shotpoint 2911 (Fig. **AF4**) • Crossing line(s): MCS Line 3 - Shotpoint 3608 (Fig. **AF5**) • Subbottom profile, 3.5 kHz: Line 1 - JDay 358 at 02:20 GMT • Detailed bathymetry and site location (swath bathymetry: collected with SIMRAD EM120) (Fig. **AF6**)
Sediment cores:	Two piston cores, two trigger cores, one gravity core, and five cores of an eight-barrel multicore taken at site location
Objectives (see text for complete details):	• Document the habitats, metabolic activities, genetic composition, and biomass of microbial communities in subseafloor sediments with very low total activity. • Test how oceanographic factors (such as surface ocean productivity) control variation in sedimentary habitats, activities, and communities from gyre center to margin. • Quantify the extent to which these sedimentary communities may be supplied with electron donors by water radiolysis, a process independent of the surface photosynthetic world.
Drilling/Coring program:	• Quadruple APC to basement • Temperature measurements: APCT-3
Downhole logging program:	None specified
Anticipated lithology:	Homogeneous dark brown clay; manganese nodules recovered from core tops

Site summaries (continued)

Site SPG-3A

Priority:	Alternate
Position:	27°56.5189′S, 148°35.3969′W (WGS 84) (27.9420°S, 148.5899°W)
Water depth (m)	4852
Target drilling depth (mbsf):	106 m, including 100 into basement
Approved maximum penetration (mbsf):	Up to 100 m basement penetration. EPSP requested that TAMU considers the steepness of the slope.
Previous drilling in area:	Closest drilling: • IODP Expedition 310 Site 15, 610 nmi away (drilling of corals on Tahiti Platform) • DSDP Leg 33 Site 318, 800 nmi away (Tuomotu Ridge volcanics)
Comments:	International waters; 230 nmi from Rapa, French Polynesia Preferred weather window: November–December
Survey coverage (track map, seismic profile):	High-resolution seismics: • Primary line(s): MCS Line 3 - Shotpoint 5072 (Fig. **AF7**) • Crossing line(s): None (Fig. **AF8**) • Subbottom profile, 3.5 kHz: Line 3 - JDay 360 at 22:08 GMT • Detailed bathymetry and site location (swath bathymetry: collected with SIMRAD EM120) (Fig. **AF9**)
Sediment cores:	Two piston cores, two trigger cores, one gravity core, and five cores of an eight-barrel multicore
Objectives (see text for complete details):	• Document the habitats, metabolic activities, genetic composition, and biomass of microbial communities in subseafloor sediments with very low total activity. • Test how oceanographic factors (such as surface ocean productivity) control variation in sedimentary habitats, activities, and communities from gyre center to gyre margin. • Quantify the extent to which these sedimentary communities may be supplied with electron donors by water radiolysis, a process independent of the surface photosynthetic world. • Determine how the basement habitats, potential activities, and communities vary with crust age and hydrologic regime (from ridge crest to abyssal plain).
Drilling/Coring program:	• Quadruple APC to basement • RCB upper 100 m of basement
Downhole logging program:	None specified
Anticipated lithology:	Homogeneous dark brown to very dark brown clay; manganese nodules recovered from core tops

Site summaries (continued)

Site SPG-4A

Priority:	Primary
Position:	26°28.8932′S, 137°56.3625′W (WGS 84) (26.4816°S, 137.9394°W)
Water depth (m)	4284
Target drilling depth (mbsf):	120 m, including 100 into basement
Approved maximum penetration (mbsf):	Up to 100 m basement penetration
Previous drilling in area:	Closest drilling: • DSDP Leg 92 Site 597, 640 nmi away
Comments:	International waters; 250 nmi from Gambier Island, French Polynesia Preferred weather window: November–December
Survey coverage (track map, seismic profile):	High-resolution seismics: • Primary line(s): MCS Line 3 - Shotpoint 6896 (Fig. **AF10**) • Crossing line(s): MCS Line 1 - Shotpoint 6125 (Fig. **AF11**) • Subbottom profile, 3.5 kHz: Line 3 - JDay 364 at 04:29 GMT • Detailed bathymetry and site location (swath bathymetry: collected with SIMRAD EM120) (Fig. **AF12**)
Sediment cores:	Two piston cores, two trigger cores, one gravity core, and one eight-barrel multicore
Objectives (see text for complete details):	• Document the habitats, metabolic activities, genetic composition, and biomass of microbial communities in subseafloor sediments with very low total activity. • Test how oceanographic factors (such as surface ocean productivity) control variation in sedimentary habitats, activities, and communities from gyre center to gyre margin. • Quantify the extent to which these sedimentary communities may be supplied with electron donors by water radiolysis, a process independent of the surface photosynthetic world. • Determine how the basement habitats, potential activities, and communities vary with crust age and hydrologic regime (from ridge crest to abyssal plain).
Drilling/Coring program:	• Quadruple APC to basement; RCB upper 100 m of basement • Temperature measurements: APCT-3
Downhole logging program:	Wireline logging: triple combo, FMS-sonic
Anticipated lithology:	Homogeneous very dark brown clay with some mottling to very dark gray clay

Site summaries (continued)

Site SPG-5A

Priority:	Alternate
Position:	28°26.7840′S, 131°23.4215′W (WGS 84) (28.4464°S, 131.3904°W)
Water depth (m)	4221
Target drilling depth (mbsf):	22
Approved maximum penetration (mbsf):	Until basement is encountered
Previous drilling in area:	Closest drilling: • DSDP Leg 92 Site 597, 600 nmi away
Comments:	International waters; 240 nmi from Pitcairn Island, United Kingdom Preferred weather window: November–December
Survey coverage (track map, seismic profile):	High-resolution seismics: • Primary line(s): MCS Line 3 - Shotpoint 8705 (Fig. **AF13**) • Crossing line(s): MCS Line 1 - Shotpoint 7871 (Fig. **AF14**) • Subbottom profile, 3.5 kHz: Line 3 - JDay 001 at 14:12 GMT • Detailed bathymetry and site location (swath bathymetry: collected with SIMRAD EM120) (Fig. **AF15**)
Sediment cores:	Two piston cores, two trigger cores, one gravity core, and one eight-barrel multicore
Objectives (see text for complete details):	• Document the habitats, metabolic activities, genetic composition, and biomass of microbial communities in subseafloor sediments with very low total activity. • Test how oceanographic factors (such as surface ocean productivity) control variation in sedimentary habitats, activities, and communities from gyre center to gyre margin. • Quantify the extent to which these sedimentary communities may be supplied with electron donors by water radiolysis, a process independent of the surface photosynthetic world. • Determine how the basement habitats, potential activities, and communities vary with crust age and hydrologic regime (from ridge crest to abyssal plain).
Drilling/Coring program:	• Quadruple APC to basement • Temperature measurements: APCT-3
Downhole logging program:	None specified
Anticipated lithology:	Very dark brown pelagic clay with mottles appearing in top meter, increasing downcore; changes to dark yellowish brown with depth and composition changes to nannofossil-bearing clay

Site summaries (continued)

Site SPG-6A

Priority:	Primary
Position:	27°55.0017′S, 123°9.6568′W (WGS 84) (27.9167°S, 123.1609°W)
Water depth (m)	3738
Target drilling depth (mbsf):	115–123 (including 100 m into basement)
Approved maximum penetration (mbsf):	Up to 100 m basement penetration
Previous drilling in area:	Closest drilling: • DSDP Leg 92 Site 598, 550 nmi away
Comments:	International waters; 210 nmi from Ducie Island, United Kingdom Preferred weather window: November–December
Survey coverage (track map, seismic profile):	High-resolution seismics: • Primary line(s): MCS Line 2 - Shotpoint 10020 (Fig. **AF16**) • Crossing line(s): MCS Line 4 - Shotpoint 10708 (Fig. **AF17**) • Subbottom profile, 3.5 kHz: Line 2 - JDay 004 at 05:37 GMT • Detailed bathymetry and site location (swath bathymetry: collected with SIMRAD EM120) (Fig. **AF18**)
Sediment cores:	Two piston cores, two trigger cores, one gravity core, and six cores of an eight-barrel multicore
Objectives (see text for complete details):	• Document the habitats, metabolic activities, genetic composition, and biomass of microbial communities in subseafloor sediments with very low total activity. • Test how oceanographic factors (such as surface ocean productivity) control variation in sedimentary habitats, activities, and communities from gyre center to gyre margin. • Quantify the extent to which these sedimentary communities may be supplied with electron donors by water radiolysis, a process independent of the surface photosynthetic world. • Determine how the basement habitats, potential activities, and communities vary with crust age and hydrologic regime (from ridge crest to abyssal plain).
Drilling/Coring program:	• Triple to quadruple APC to basement; RCB upper 100 m of basement • Temperature measurements: APCT-3
Downhole logging program:	Wireline logging: triple combo, FMS-sonic
Anticipated lithology:	Stiff dark yellowish brown clay grading to yellowish brown foraminifer-bearing clayey nannofossil ooze

Site summaries (continued)

Site SPG-7A

Priority:	Alternate
Position:	27°44.2713′S, 117°37.1815′W (WGS 84) (27.7379°S, 117.6197°W)
Water depth (m)	3688
Target drilling depth (mbsf):	<103 (including 100 m into basement)
Approved maximum penetration (mbsf):	Up to 100 m basement penetration. EPSP requested that TAMU considers the steepness of the slope.
Previous drilling in area:	Closest drilling: • DSDP Leg 92 Site 599, 500 nmi away
Comments:	International waters; 450 nmi from Ducie Island, United Kingdom Preferred weather window: November–December
Survey coverage (track map, seismic profile):	High-resolution seismics: • Primary line(s): MCS Line 3 - Shotpoint 11865 (Fig. **AF19**) • Crossing line(s): MCS Line 1 - Shotpoint 11264 (Fig. **AF20**) • Subbottom profile, 3.5 kHz: Line 3 - JDay 006 at 12:29 GMT • Detailed bathymetry and site location (swath bathymetry: collected with SIMRAD EM120) (Fig. **AF21**)
Sediment cores:	One piston core, one trigger core, one gravity core, one 4 inch jumbo gravity core, and six cores of an eight-barrel multicore
Objectives (see text for complete details):	• Document the habitats, metabolic activities, genetic composition, and biomass of microbial communities in subseafloor sediments with very low total activity. • Test how oceanographic factors (such as surface ocean productivity) control variation in sedimentary habitats, activities, and communities from gyre center to gyre margin. • Determine how the basement habitats, potential activities, and communities vary with crust age and hydrologic regime (from ridge crest to abyssal plain).
Drilling/Coring program:	Quadruple APC to basement; RCB upper 100 m of basement
Downhole logging program:	None specified
Anticipated lithology:	Successions of brown foraminifer-bearing clayey nannofossil ooze grading to dark brown clay

Site summaries (continued)

Site SPG-9A

Priority:	Alternate
Position:	38°03.6871′S, 133°05.5024′W (WGS 84) (38.0615°S, 133.0917°W)
Water depth (m)	4925
Target drilling depth (mbsf):	20
Approved maximum penetration (mbsf):	Until basement is encountered
Previous drilling in area:	Closest drilling: • DSDP Leg 92 Site 597, 1100 nmi away
Comments:	International waters; 800 nmi from Pitcairn Island, United Kingdom Preferred weather window: November–December
Survey coverage (track map, seismic profile):	High-resolution seismics: • Primary line(s): MCS Line 4 - Shotpoint 12423 (Fig. AF22) • Crossing line(s): MCS Line 1 - Shotpoint 13715 (Fig. AF23) • Subbottom 3.5 kHz: Line 1 - JDay 011 at 03:49 GMT • Detailed bathymetry and site location (swath bathymetry: collected with SIMRAD EM120) (Fig. AF24)
Sediment cores:	Two piston cores, two trigger cores, one gravity core, and one eight-barrel multicore
Objectives (see text for complete details):	• Document the habitats, metabolic activities, genetic composition, and biomass of microbial communities in subseafloor sediments with very low total activity. • Test how oceanographic factors (such as surface ocean productivity) control variation in sedimentary habitats, activities, and communities from gyre center to gyre margin. • Quantify the extent to which these sedimentary communities may be supplied with electron donors by water radiolysis, a process independent of the surface photosynthetic world.
Drilling/Coring program:	• Quadruple APC to basement • Temperature measurements: APCT-3
Downhole logging program:	None specified
Anticipated lithology:	Brown pelagic clay with rare foraminifers

Site summaries (continued)

Site SPG-10A

Priority:	Primary
Position:	39°18.6158′S, 139°48.0381′W (WGS 84) (39.3103°S, 139.8006°W)
Water depth (m)	5283
Target drilling depth (mbsf):	21
Approved maximum penetration (mbsf):	Until basement is encountered
Previous drilling in area:	Closest drilling: • DSDP Leg 92 Site 597, 1350 nmi away
Comments:	International waters; 750 nmi from Rapa, French Polynesia Preferred weather window: November–December
Survey coverage (track map, seismic profile):	High-resolution seismics: • Primary Line(s): MCS Line 6 - Shotpoint 15516 (Fig. **AF25**) • Crossing line(s): MCS Line 4 - Shotpoint 14861 (Fig. **AF26**) • Subbottom profile, 3.5 kHz: Line 6 - JDay 013 at 16:03 GMT • Detailed bathymetry and site location (swath bathymetry: collected with SIMRAD EM120) (Fig. **AF27**)
Sediment cores:	Two piston cores, three trigger cores, one gravity core, and seven cores of an eight-barrel multicore
Objectives (see text for complete details):	• Document the habitats, metabolic activities, genetic composition, and biomass of microbial communities in subseafloor sediments with very low total activity. • Test how oceanographic factors (such as surface ocean productivity) control variation in sedimentary habitats, activities, and communities from gyre center to gyre margin. • Quantify the extent to which these sedimentary communities may be supplied with electron donors by water radiolysis, a process independent of the surface photosynthetic world.
Drilling/Coring program:	• Triple to quadruple APC to basement • Temperature measurements: APCT-3
Downhole logging program:	None specified
Anticipated lithology:	Homogeneous brown clay with yellowish brown circular mottles; baseball-sized manganese nodules present at top

Site summaries (continued)

Site SPG-11B

Priority:	Primary
Position:	41°51.4268′S, 153°07.1519′W (WGS 84) (41.8571°S, 153.1192°W)
Water depth (m)	5092
Target drilling depth (mbsf):	94–100
Approved maximum penetration (mbsf):	Until basement is encountered
Previous drilling in area:	Closest drilling: • ODP Leg 181 Site 1123, 810 nmi away
Comments:	International waters; 950 nmi from Rapa, French Polynesia Preferred weather window: November–December
Survey coverage (track map, seismic profile):	High-resolution seismics: • Primary line(s): MCS Line 3 - Shotpoint 16524 (Fig. **AF28**) • Crossing line(s): MCS Line 1 - Shotpoint 15990 (Fig. **AF29**) • Subbottom profile, 3.5 kHz: Line 3 - JDay 017 at 06:08 GMT • Detailed bathymetry and site location (swath bathymetry: collected with SIMRAD EM120) (Fig. **AF30**)
Sediment cores:	Two trigger cores, one gravity core, and six cores of an eight-barrel multicore
Objectives (see text for complete details):	• Document the habitats, metabolic activities, genetic composition, and biomass of microbial communities in subseafloor sediments with very low total activity. • Test how oceanographic factors (such as surface ocean productivity) control variation in sedimentary habitats, activities, and communities from gyre center to gyre margin. • Quantify the extent to which these sedimentary communities may be supplied with electron donors by water radiolysis, a process independent of the surface photosynthetic world.
Drilling/Coring program:	• Triple to quadruple APC to basement • Temperature measurements: APCT-3
Downhole logging program:	None specified
Anticipated lithology:	Homogeneous brown clay with yellowish brown circular mottles; baseball-sized manganese nodules present at top

Site summaries (continued)

Site SPG-12A

Priority:	Primary
Position:	45°57.8539′S, 163°11.0531′W (WGS 84) (45.9642°S, 163.1842°W)
Water depth (m)	5309
Target drilling depth (mbsf):	122-130
Approved maximum penetration (mbsf):	Until basement is encountered
Previous drilling in area:	Closest drilling: • ODP Leg 181Site 1123, 400 nmi away
Comments:	International waters; 560 nmi from Chatham Island, New Zealand Preferred weather window: November–December
Survey coverage (track map, seismic profile):	High-resolution seismics: • Primary line(s): MCS Line 3 - Shotpoint 17828 (Fig. **AF31**) • Crossing line(s): MCS Line 1 - Shotpoint 17196 (Fig. **AF32**) • Subbottom profile, 3.5 kHz: Line 3 - JDay 020 at 10:12 GMT • Detailed bathymetry and site location (swath bathymetry: collected with SIMRAD EM120) (Fig. **AF33**)
Sediment cores:	Four gravity cores
Objectives (see text for complete details):	• Document the habitats, metabolic activities, genetic composition, and biomass of microbial communities in subseafloor sediments with very low total activity. • Test how oceanographic factors (such as surface ocean productivity) control variation in sedimentary habitats, activities, and communities from gyre center to gyre margin. • Quantify the extent to which these sedimentary communities may be supplied with electron donors by water radiolysis, a process independent of the surface photosynthetic world.
Drilling/Coring program:	• Triple to quadruple APC to basement • Temperature measurements: APCT-3
Downhole logging program:	Wireline logging: triple combo, FMS-sonic
Anticipated lithology:	Siliceous ooze

Figure AF1. A. Single-channel seismic (SCS) Line 1 from Cruise KNOX-02RR, proposed Site SPG-1A. TD = total depth, MORB = mid-ocean-ridge basalt, SP = shotpoint, WD = water depth, MCS = multichannel seismic, BP = band-pass, AGC = automatic gain control, VE = vertical exaggeration. **B.** Portion of 3.5 Hz seismic Line 1. **C.** Survey track chart, proposed Site SPG-1A (seismic Lines 1–3). Start of line (sol) and end of line (eol) indicated with time (z) and shotpoint (sp). Tick marks are every 10 min (50 shots); selected tick marks labeled with shotpoint. Red dot = proposed Site SPG-1A. (**Figure shown on next page.**)

Figure AF1 (continued). (Caption shown on previous page.)

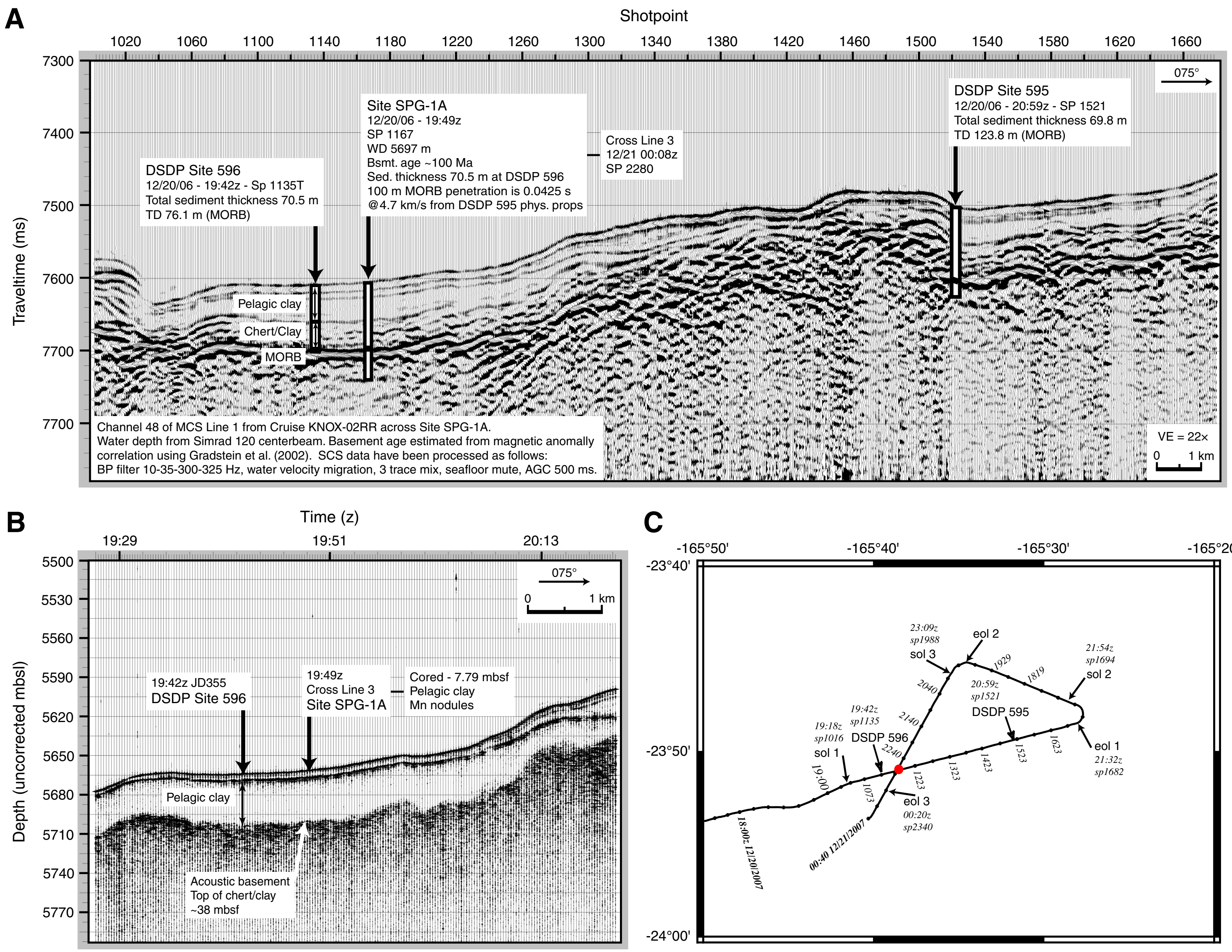


Figure AF2. A. Single-channel seismic (SCS) Line 3 from Cruise KNOX-02RR, proposed Site SPG-1A. SP = shotpoint, WD = water depth, MORB = mid-ocean-ridge basalt, MCS = multichannel seismic, BP = band-pass, AGC = automatic gain control, VE = vertical exaggeration. **B.** Portion of 3.5 Hz seismic Line 3. **C.** Survey track chart, proposed Site SPG-1A (seismic Lines 1–3). Start of line (sol) and end of line (eol) indicated with time (z) and shotpoint (sp). Tick marks are every 10 min (50 shots); selected tick marks labeled with shotpoint. Red dot = proposed Site SPG-1A. (**Figure shown on next page.**)

Figure AF2 (continued). (Caption shown on previous page.)

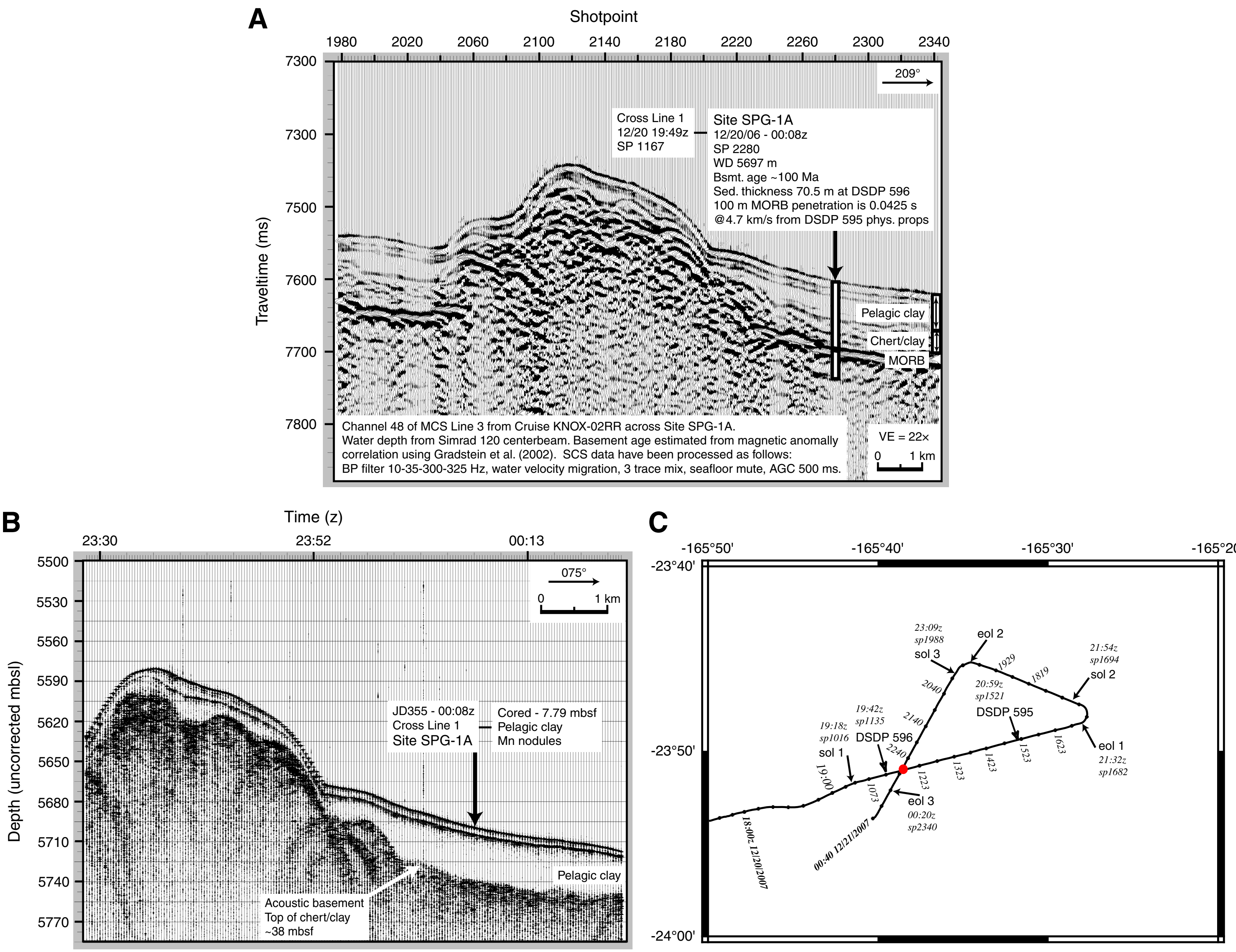


Figure AF3. Bathymetry and track chart, proposed Site SPG-1A. sol = start of seismic line, eol = end of seismic line, z = time, sp = shotpoint.

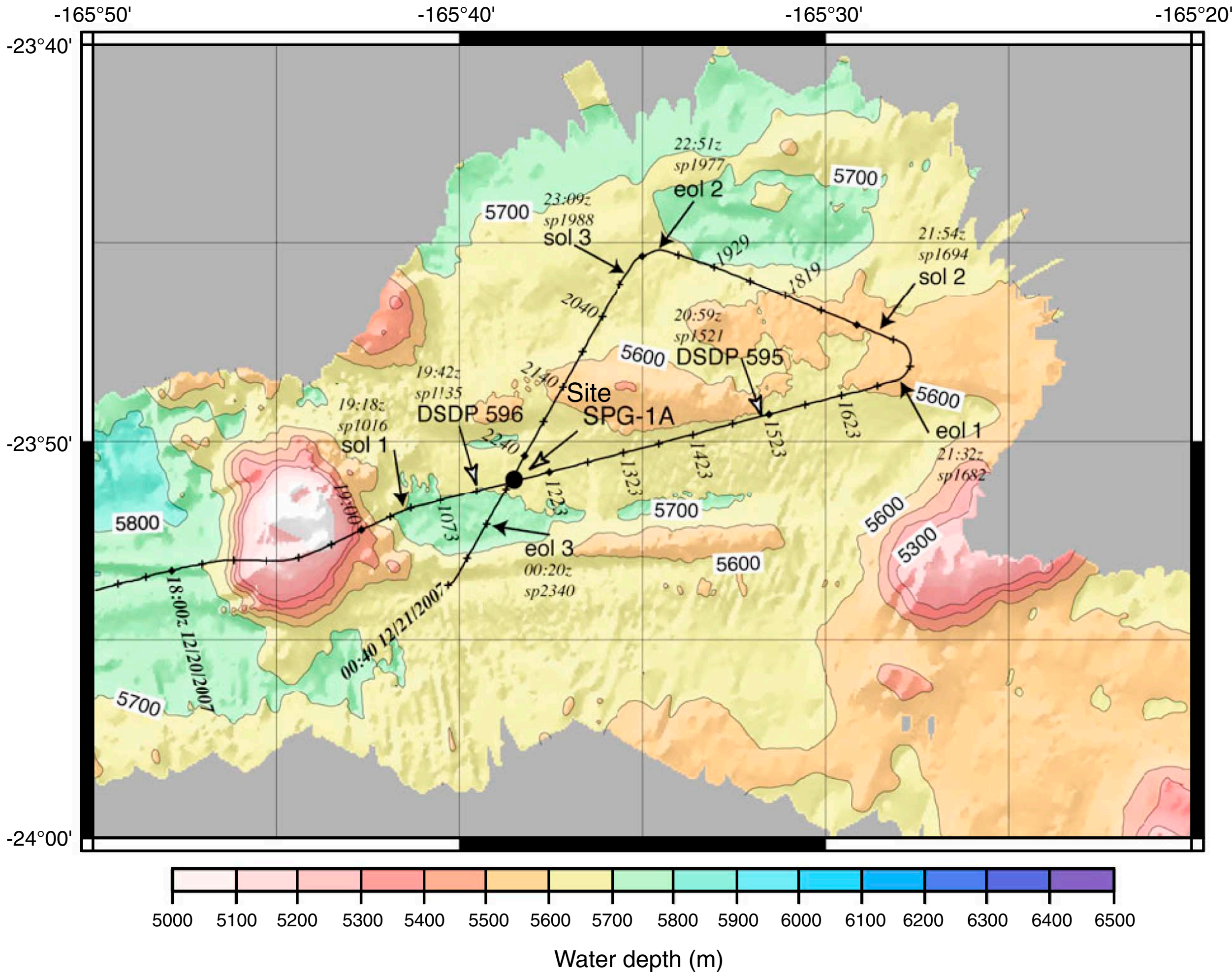


Figure AF4. Single-channel seismic (SCS) Line 1 from Cruise KNOX-02RR, proposed Site SPG-2A. SP = shotpoint, CPA = closest point of approach, WD = water depth, MORB = mid-ocean-ridge basalt, MCS = multichannel seismic, BP = band-pass, AGC = automatic gain control, VE = vertical exaggeration. **B.** Portion of 3.5 Hz seismic Line 1. **C.** Survey track chart, proposed Site SPG-2A (seismic Lines 1–3). Start of line (sol) and end of line (eol) indicated with time (z) and shotpoint (sp). Tick marks are every 10 min (50 shots); selected tick marks labeled with shotpoint. Red dot = proposed Site SPG-2A. (**Figure shown on next page.**)

Figure AF4 (continued). (Caption shown on previous page.)

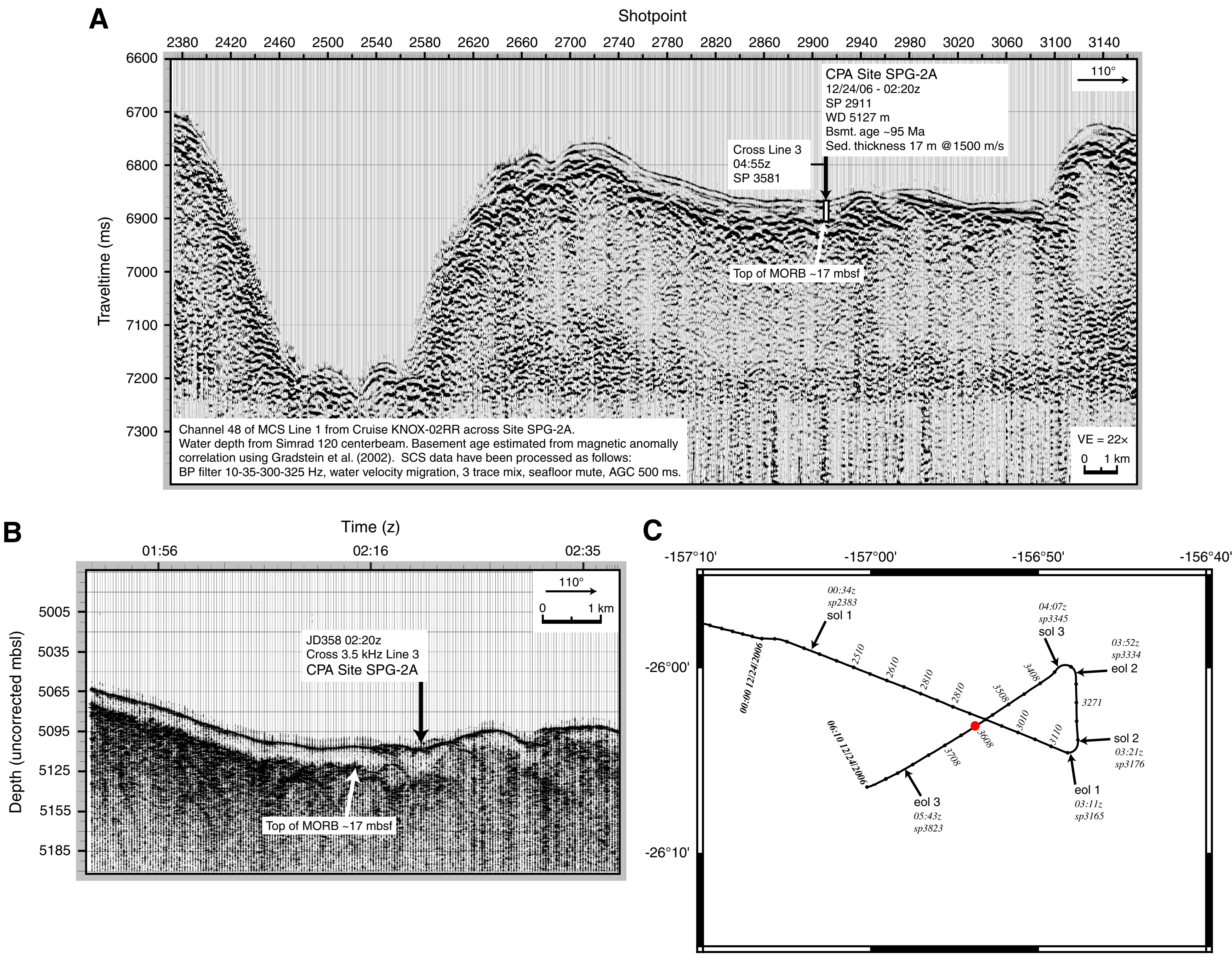


Figure AF5. A. Single-channel seismic (SCS) Line 3 from Cruise KNOX-02RR, proposed Site SPG-2A. MCS = multichannel seismic, BP = band-pass, TD = total depth, WD = water depth, SP = shotpoint, MORB = mid-ocean-ridge basalt, VE = vertical exaggeration. **B.** Portion of 3.5 Hz seismic Line 3. **C.** Survey track chart, proposed Site SPG-2A (seismic Lines 1–3). Start of line (sol) and end of line (eol) indicated with time (z) and shotpoint (sp). Tick marks are every 10 min (50 shots); selected tick marks labeled with shotpoint. Red dot = proposed Site SPG-2A. (**Figure shown on next page.**)

Figure AF5 (continued). (Caption shown on previous page.)

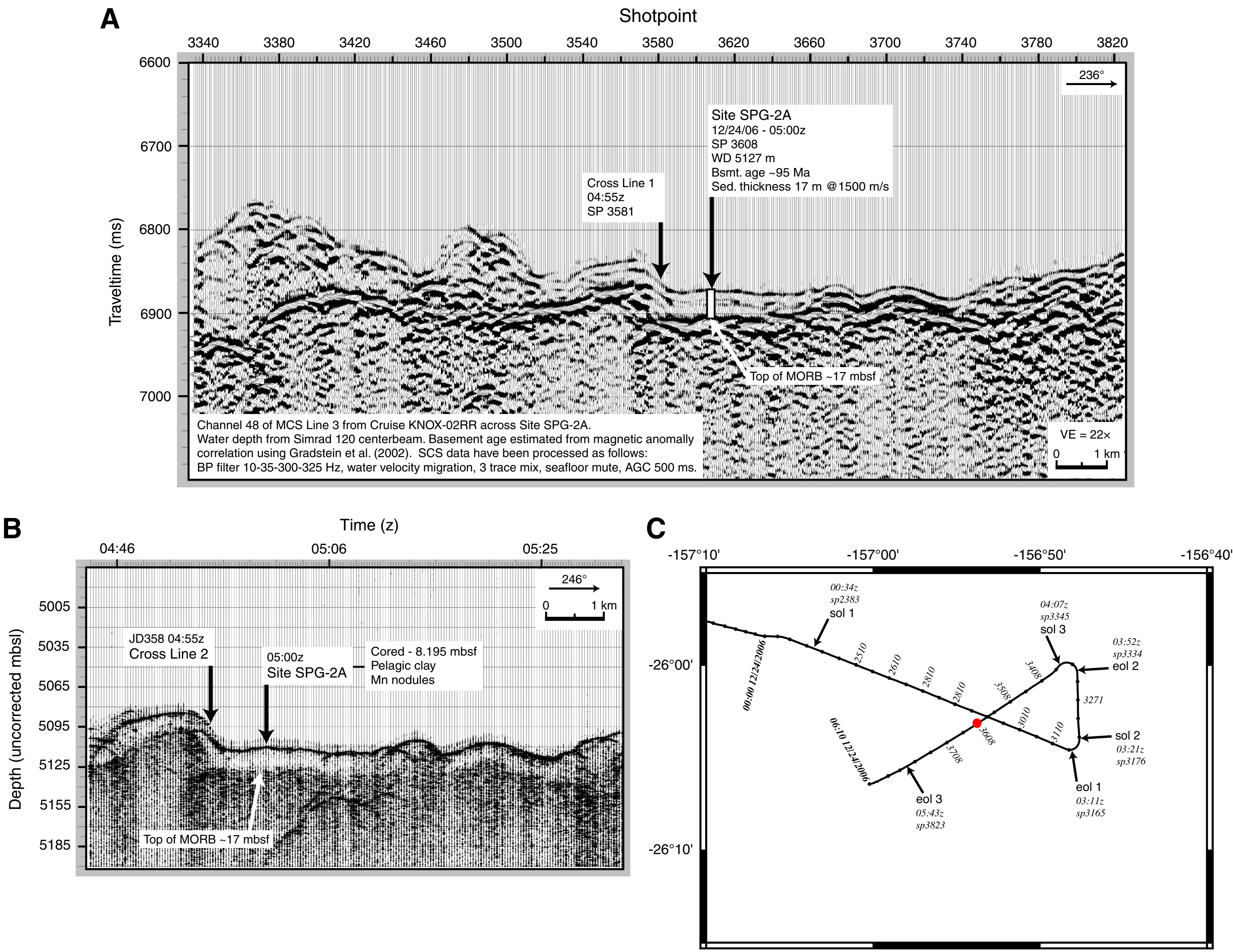


Figure AF6. Bathymetry and track chart, proposed Site SPG-2A. sol = start of seismic line, eol = end of seismic line, z = time, sp = shotpoint.

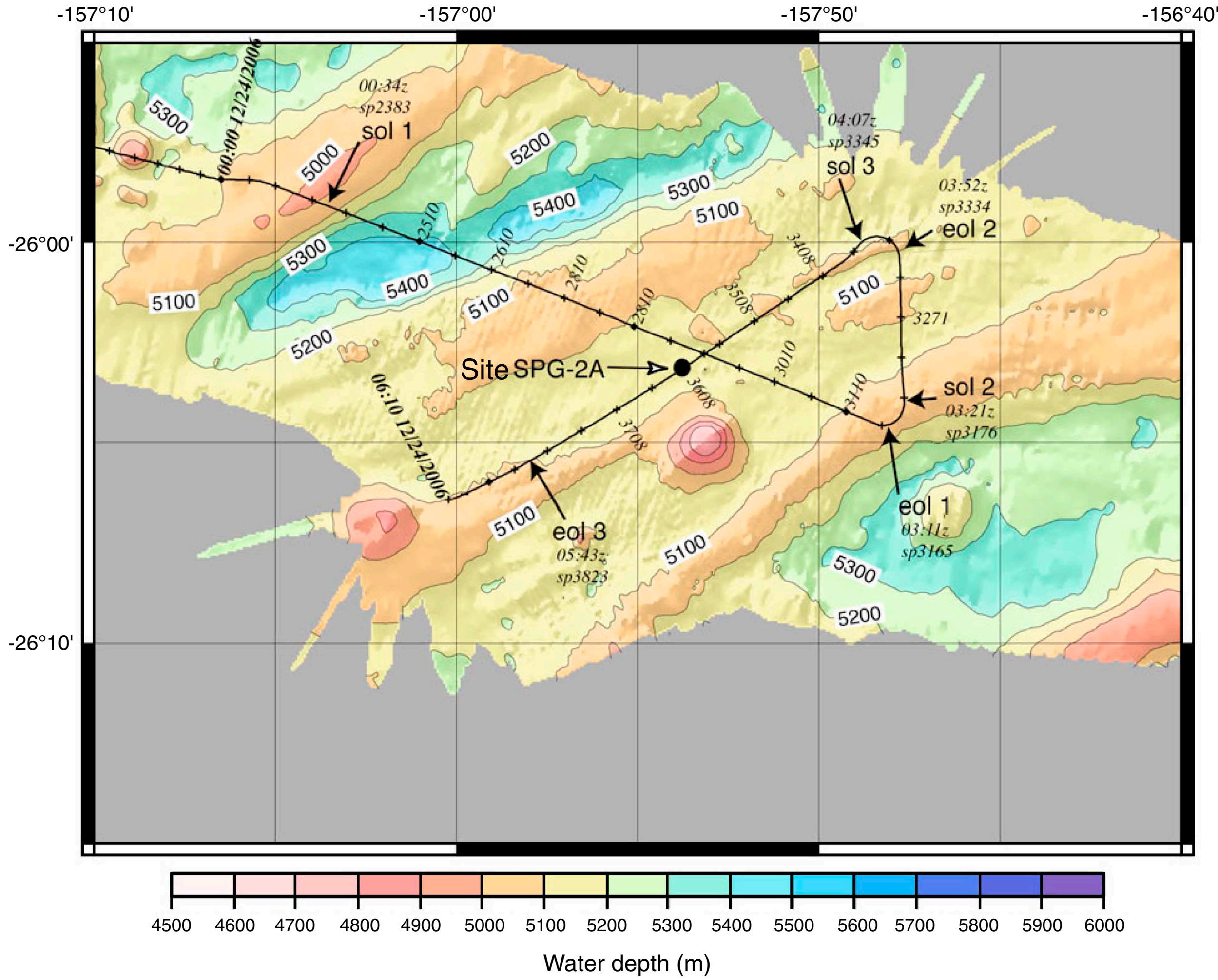


Figure AF7. Single-channel seismic (SCS) Line 3 from Cruise KNOX-02RR, proposed Site SPG-3A. SP = shotpoint, WD = water depth, MORB = mid-ocean-ridge basalt, MCS = multichannel seismic, BP = band-pass, AGC = automatic gain control, VE = vertical exaggeration. **B.** Portion of 3.5 Hz seismic Line 3. **C.** Survey track chart, proposed Site SPG-3A (seismic Lines 1–3). Start of line (sol) and end of line (eol) indicated with time (z) and shotpoint (sp). Tick marks are every 10 min (50 shots); selected tick marks labeled with shotpoint. Red dot = proposed Site SPG-3A. (**Figure shown on next page.**)

Figure AF7 (continued). (Caption shown on previous page.)

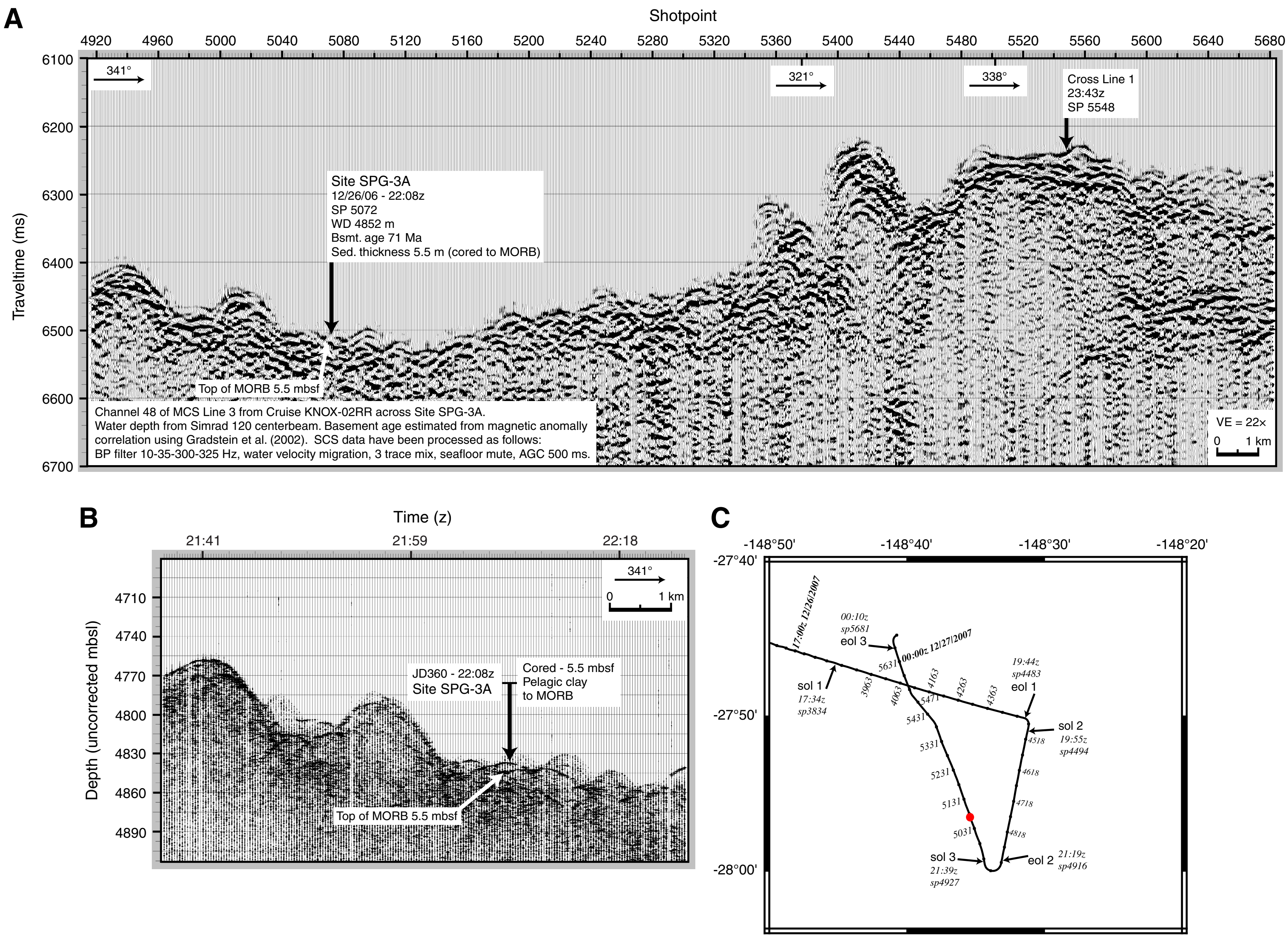


Figure AF8. Single-channel seismic (SCS) Line 1 from Cruise KNOX-02RR, proposed Site SPG-3A. SP = shotpoint, MCS = multichannel seismic, BP = band-pass, AGC = automatic gain control, VE = vertical exaggeration. **B.** Portion of 3.5 Hz seismic Line 1. **C.** Survey track chart, proposed Site SPG-3A (seismic Lines 1–3). Start of line (sol) and end of line (eol) indicated with time (z) and shotpoint (sp). Tick marks are every 10 min (50 shots); selected tick marks labeled with shotpoint. Red dot = proposed Site SPG-3A. (**Figure shown on next page.**)

Figure AF8 (continued). (Caption shown on previous page.)

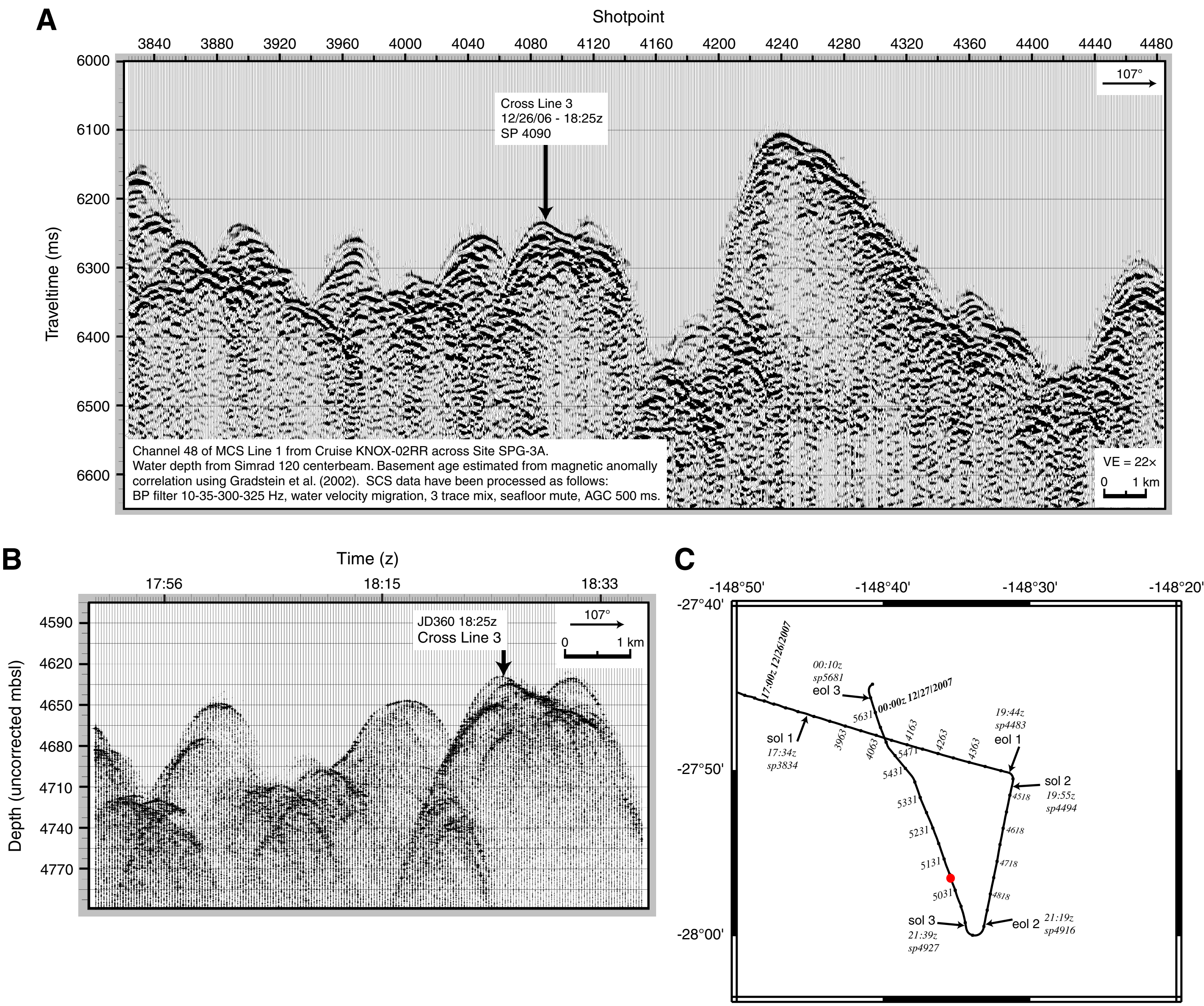


Figure AF9. Bathymetry and track chart, proposed Site SPG-3A. sol = start of seismic line, eol = end of seismic line, z = time, sp = shotpoint.

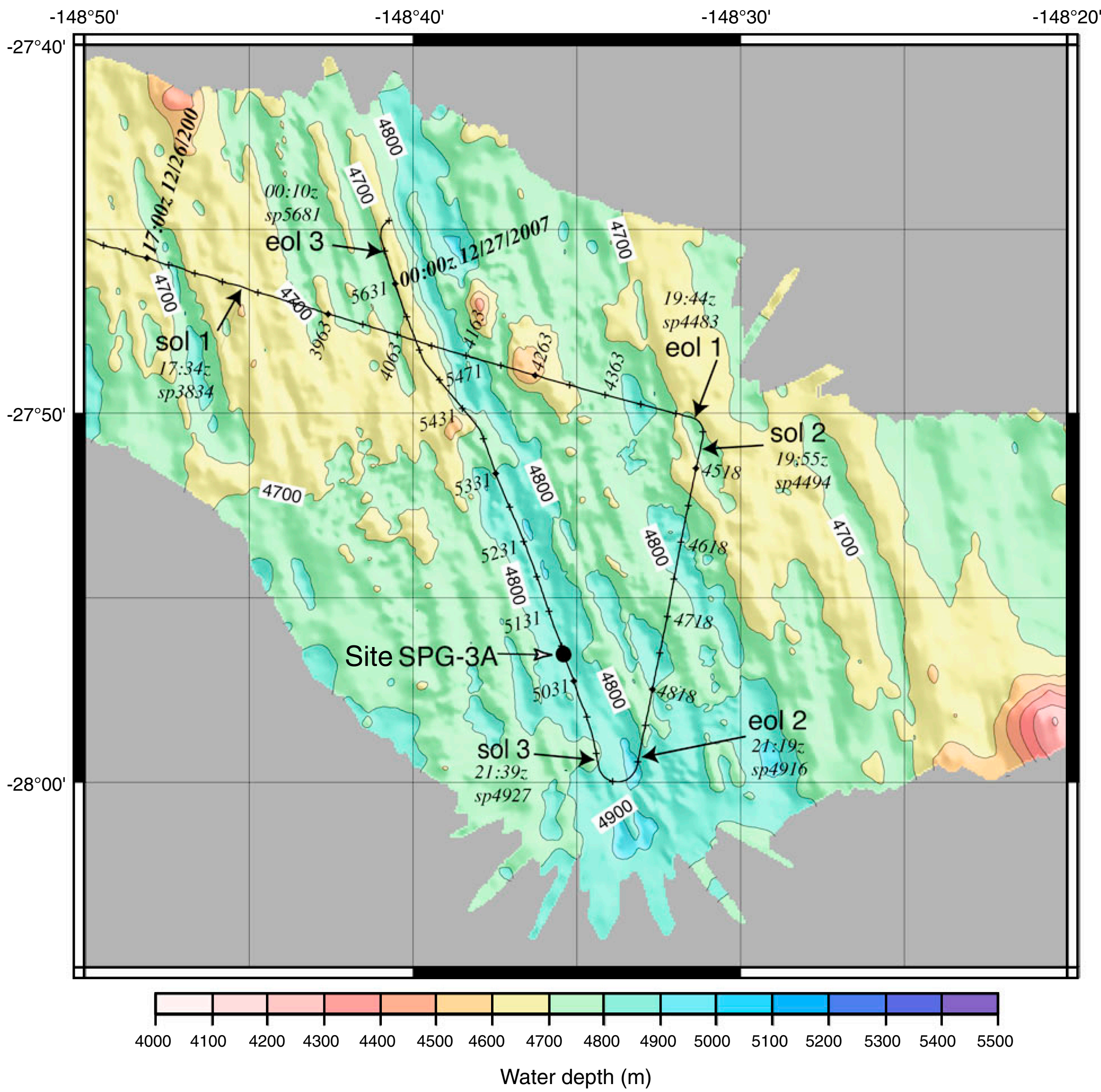


Figure AF10. Single-channel seismic (SCS) Line 3 from Cruise KNOX-02RR, proposed Site SPG-4A. SP = shotpoint, WD = water depth, MORB = mid-ocean-ridge basalt, MCS = multichannel seismic, BP = band-pass, AGC = automatic gain control, VE = vertical exaggeration. **B.** Portion of 3.5 Hz seismic Line 3. **C.** Survey track chart, proposed Site SPG-4A (seismic Lines 1–3). Start of line (sol) and end of line (eol) indicated with time (z) and shotpoint (sp). Tick marks are every 10 min (50 shots); selected tick marks labeled with shotpoint. Red dot = proposed Site SPG-4A. (**Figure shown on next page.**)

Figure AF10 (continued). (Caption shown on previous page.)

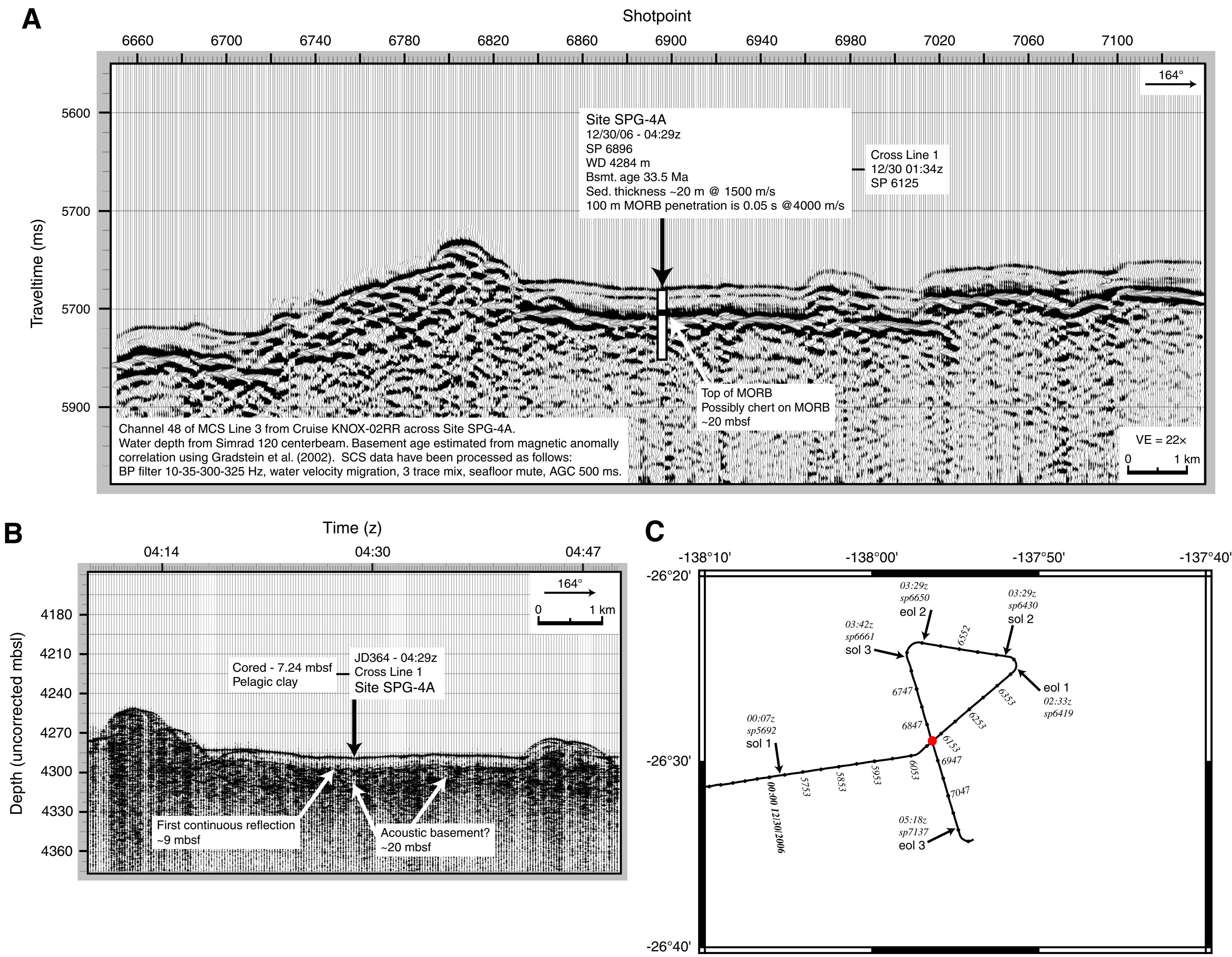


Figure AF11. Single-channel seismic (SCS) Line 1 from Cruise KNOX-02RR, proposed Site SPG-4A. SP = shotpoint, WD = water depth, MORB = mid-ocean-ridge basalt, MCS = multichannel seismic, BP = band-pass, AGC = automatic gain control, VE = vertical exaggeration. **B.** Portion of 3.5 Hz seismic Line 1. **C.** Survey track chart, proposed Site SPG-4A (seismic Lines 1–3). Start of line (sol) and end of line (eol) indicated with time (z) and shotpoint (sp). Tick marks are every 10 min (50 shots); selected tick marks labeled with shotpoint. Red dot = proposed Site SPG-4A. (**Figure shown on next page.**)

Figure AF11 (continued). (Caption shown on previous page.)

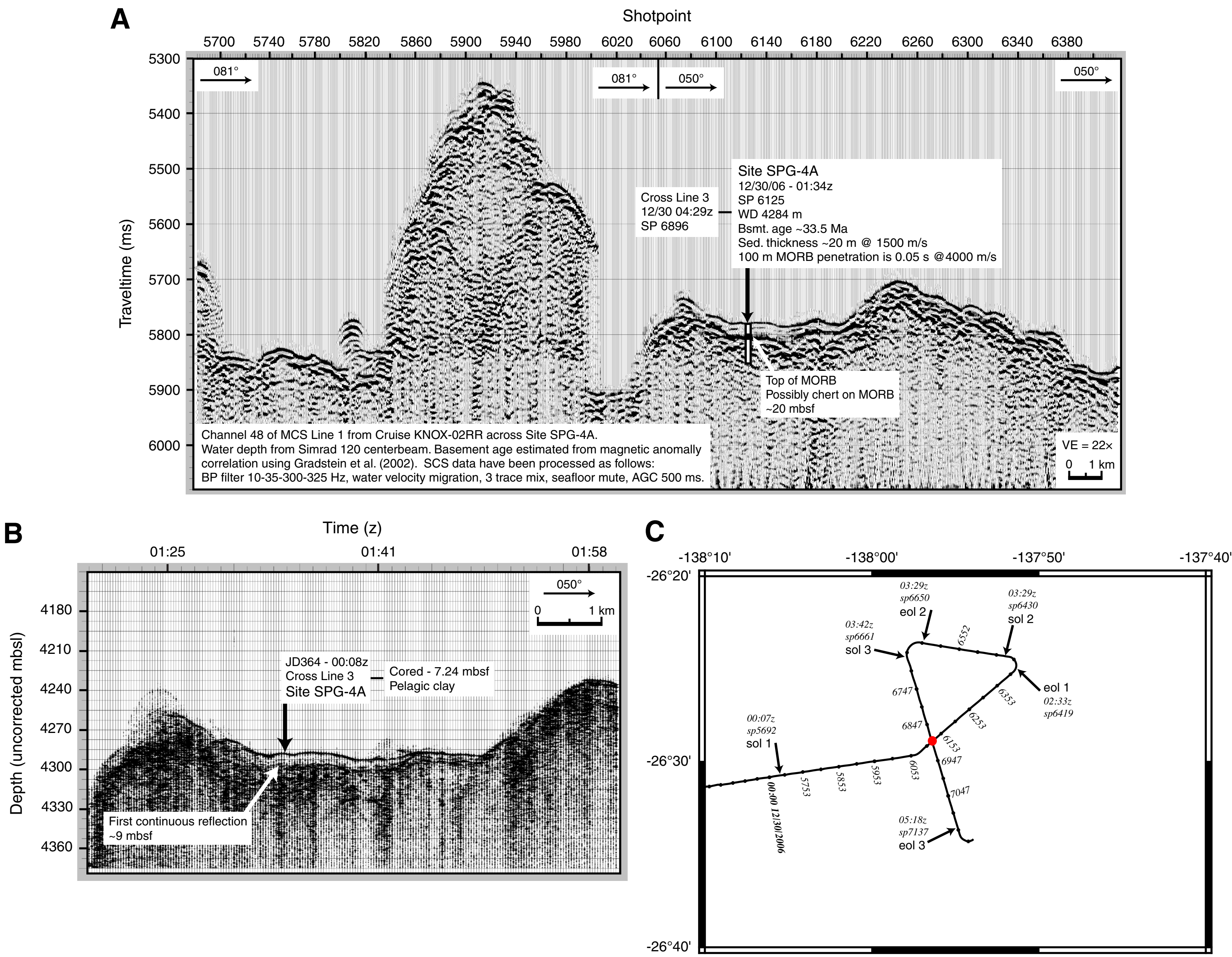


Figure AF12. Bathymetry and track chart, proposed Site SPG-4A. sol = start of seismic line, eol = end of seismic line, z = time, sp = shotpoint.

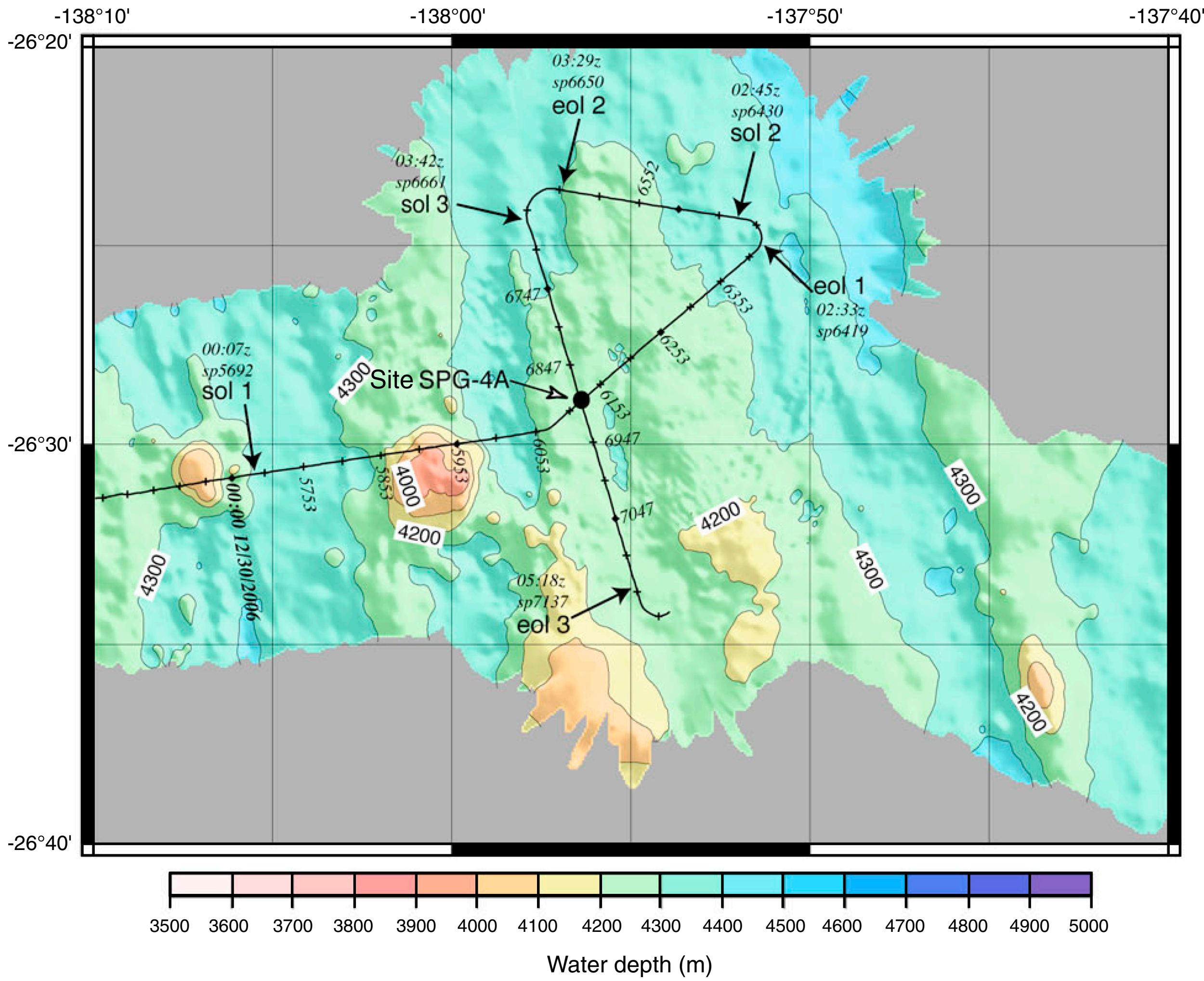


Figure AF13. Single-channel seismic (SCS) Line 3 from Cruise KNOX-02RR, proposed Site SPG-5A. SP = shotpoint, WD = water depth, MORB = mid-ocean-ridge basalt, MCS = multichannel seismic, BP = band-pass, AGC = automatic gain control, VE = vertical exaggeration. **B.** Portion of 3.5 Hz seismic Line 3. **C.** Survey track chart, proposed Site SPG-5A (seismic Lines 1–3). Start of line (sol) and end of line (eol) indicated with time (z) and shotpoint (sp). Tick marks are every 10 min (50 shots); selected tick marks labeled with shotpoint. Red dot = proposed Site SPG-5A. (**Figure shown on next page.**)

Figure AF13 (continued). (Caption shown on previous page.)

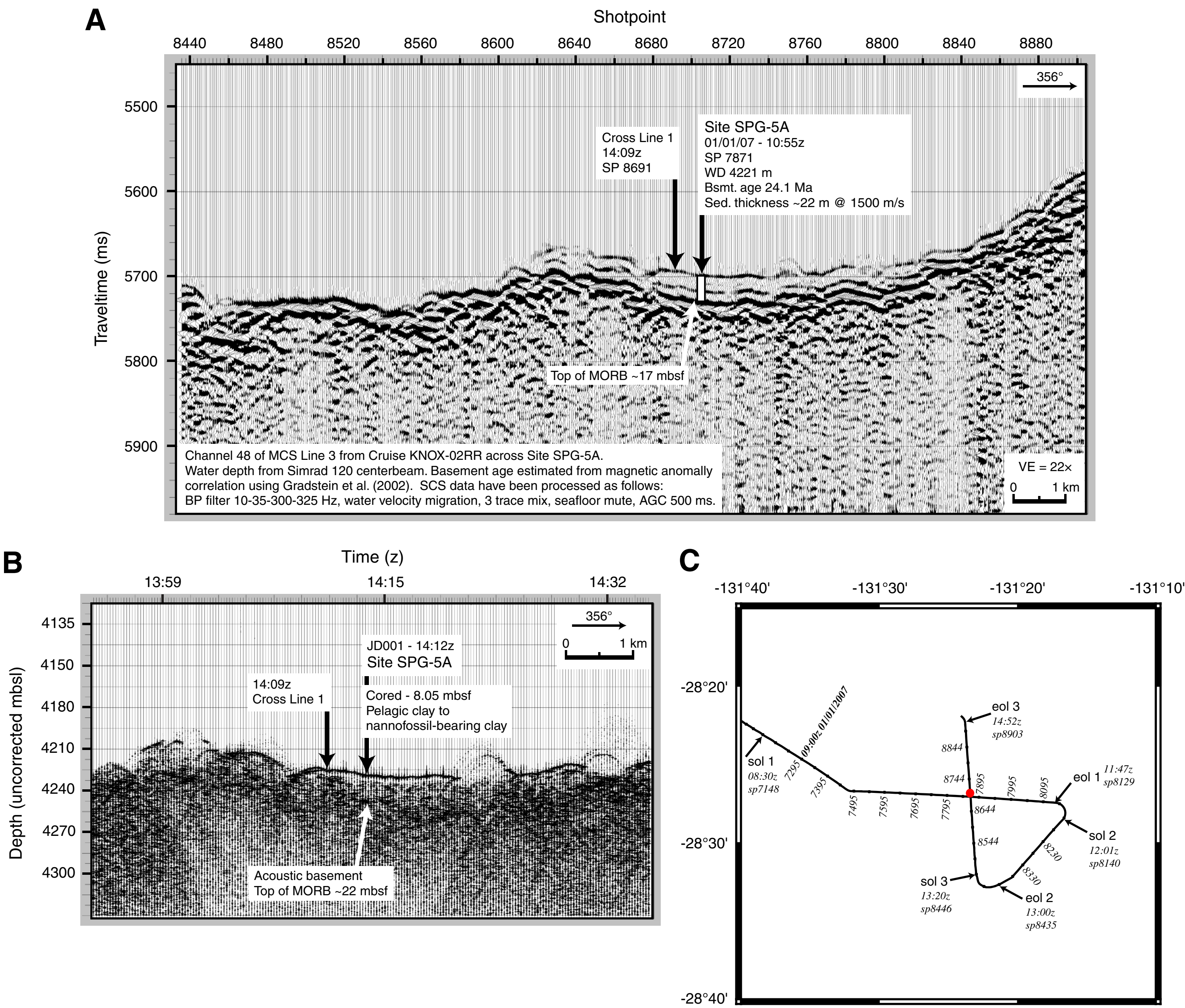


Figure AF14. Single-channel seismic (SCS) Line 1 from Cruise KNOX-02RR, proposed Site SPG-5A. C/C = course change, SP = shotpoint, WD = water depth, MORB = mid-ocean-ridge basalt, MCS = multichannel seismic, BP = band-pass, AGC = automatic gain control, VE = vertical exaggeration. **B.** Portion of 3.5 Hz seismic Line 1. **C.** Survey track chart, proposed Site SPG-5A (seismic Lines 1–3). Start of line (sol) and end of line (eol) indicated with time (z) and shotpoint (sp). Tick marks are every 10 min (50 shots); selected tick marks labeled with shotpoint. Red dot = proposed Site SPG-5A. (**Figure shown on next page.**)

Figure AF14 (continued). (Caption shown on previous page.)

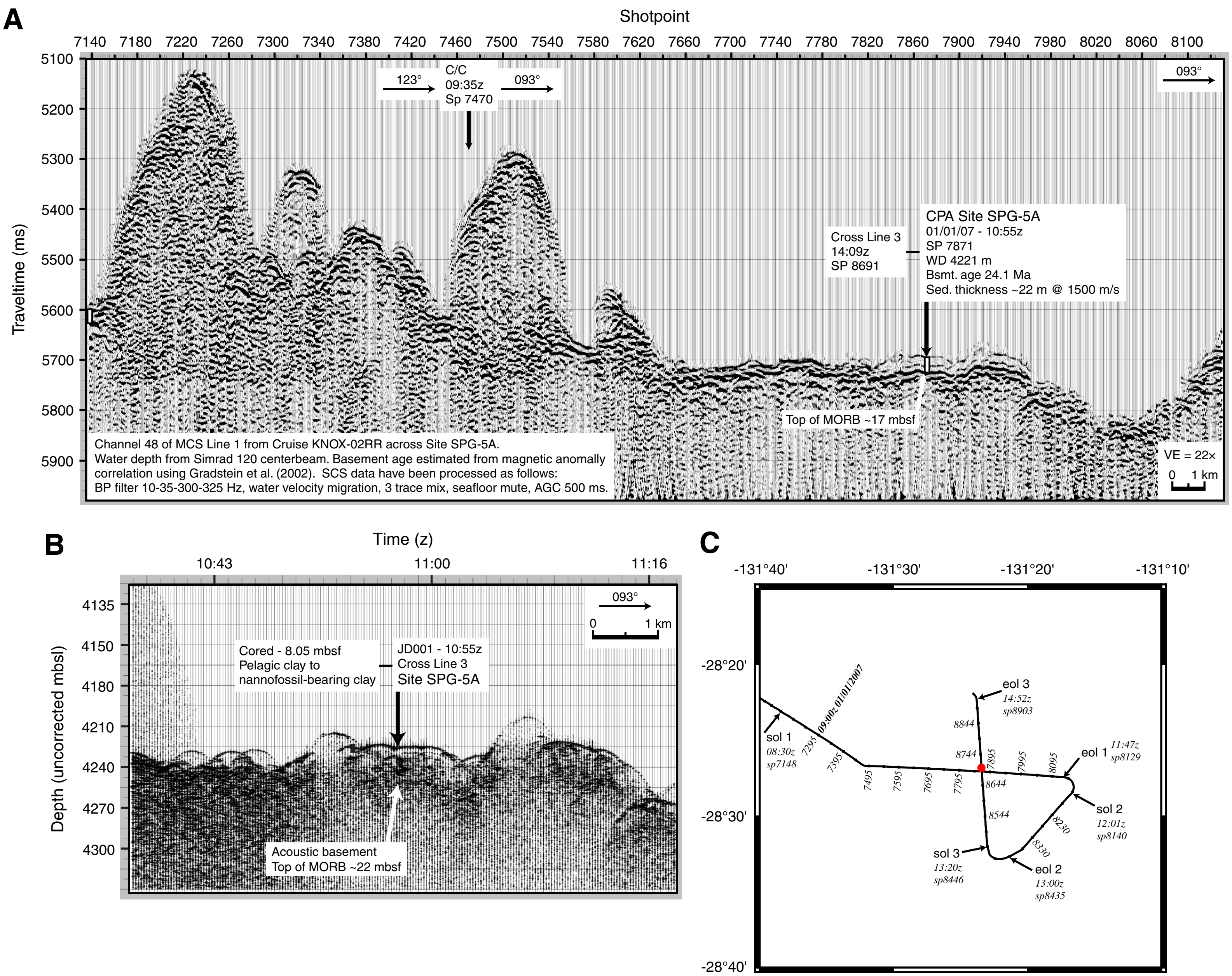


Figure AF15. Bathymetry and track chart, proposed Site SPG-5A. sol = start of seismic line, eol = end of seismic line, z = time, sp = shotpoint.

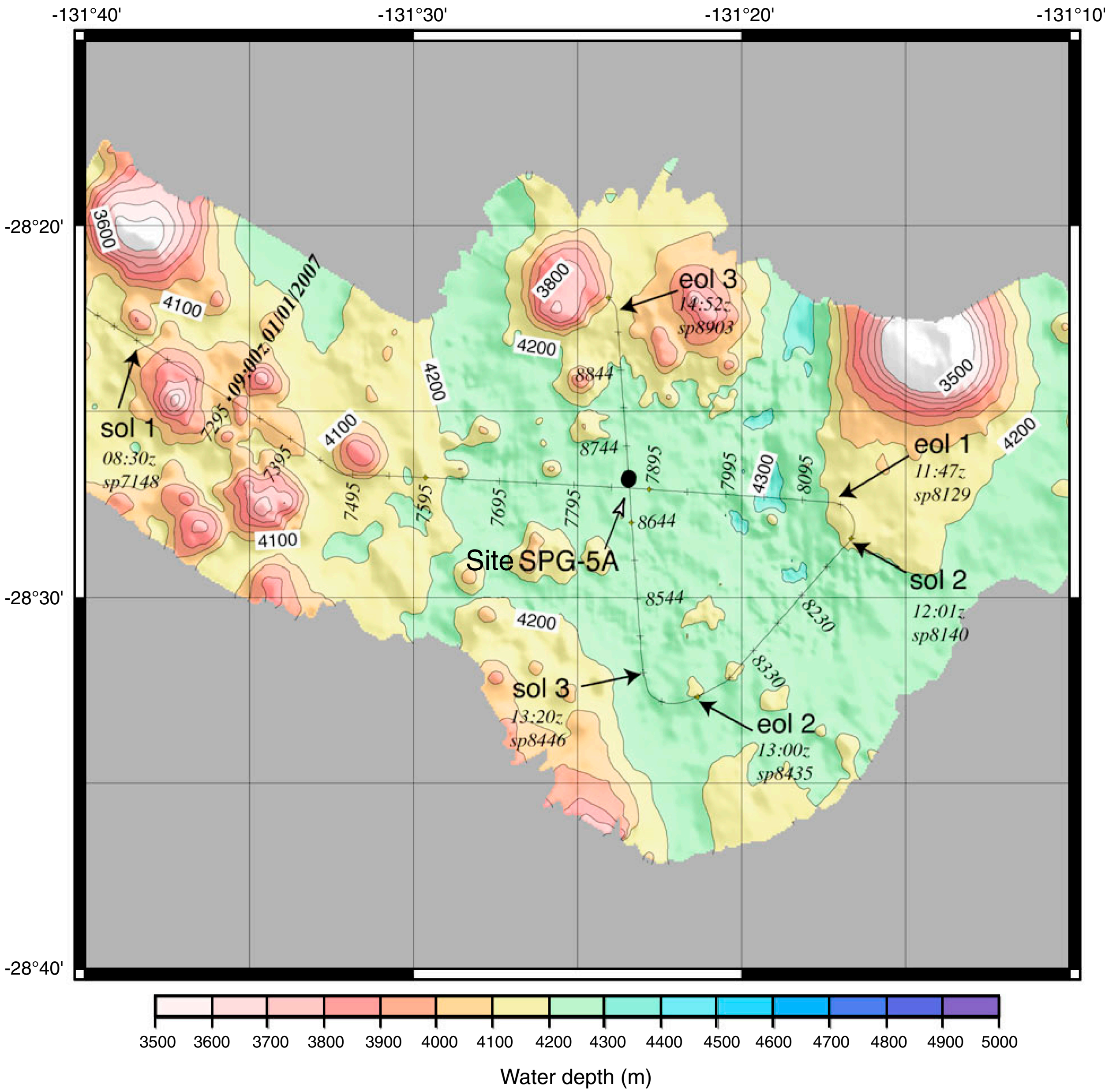


Figure AF16. Single-channel seismic (SCS) Line 2 from Cruise KNOX-02RR, proposed Site SPG-6A. SP = shotpoint, WD = water depth, MORB = mid-ocean-ridge basalt, MCS = multichannel seismic, BP = band-pass, AGC = automatic gain control, VE = vertical exaggeration. **B.** Portion of 3.5 Hz seismic Line 2. **C.** Survey track chart, proposed Site SPG-6A (seismic Lines 1, 2, 3, and 4). Start of line (sol) and end of line (eol) indicated with time (z) and shotpoint (sp). Tick marks are every 10 min (50 shots); selected tick marks labeled with shotpoint. Red dot = proposed Site SPG-6A. (**Figure shown on next page.**)

Figure AF16 (continued). (Caption shown on previous page.)

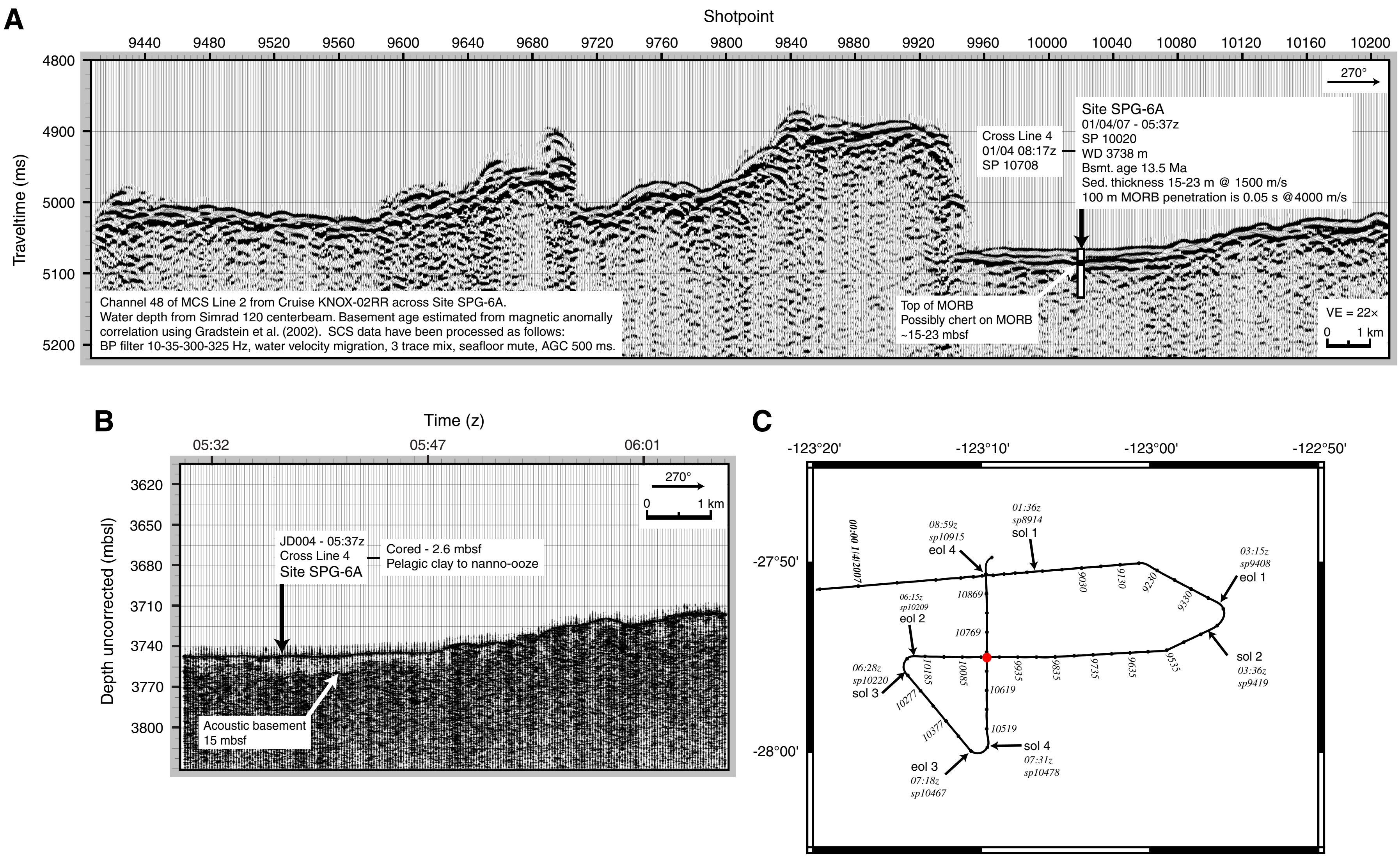


Figure AF17. Single-channel seismic (SCS) Line 4 from Cruise KNOX-02RR, proposed Site SPG-6A. SP = shotpoint, WD = water depth, MORB = mid-ocean-ridge basalt, MCS = multichannel seismic, BP = band-pass, AGC = automatic gain control, VE = vertical exaggeration. **B.** Portion of 3.5 Hz seismic Line 4. **C.** Survey track chart, proposed Site SPG-6A (seismic Lines 1, 2, 3, and 4). Start of line (sol) and end of line (eol) indicated with time (z) and shotpoint (sp). Tick marks are every 10 min (50 shots); selected tick marks labeled with shotpoint. Red dot = proposed Site SPG-6A. (**Figure shown on next page.**)

Figure AF17 (continued). (Caption shown on previous page.)

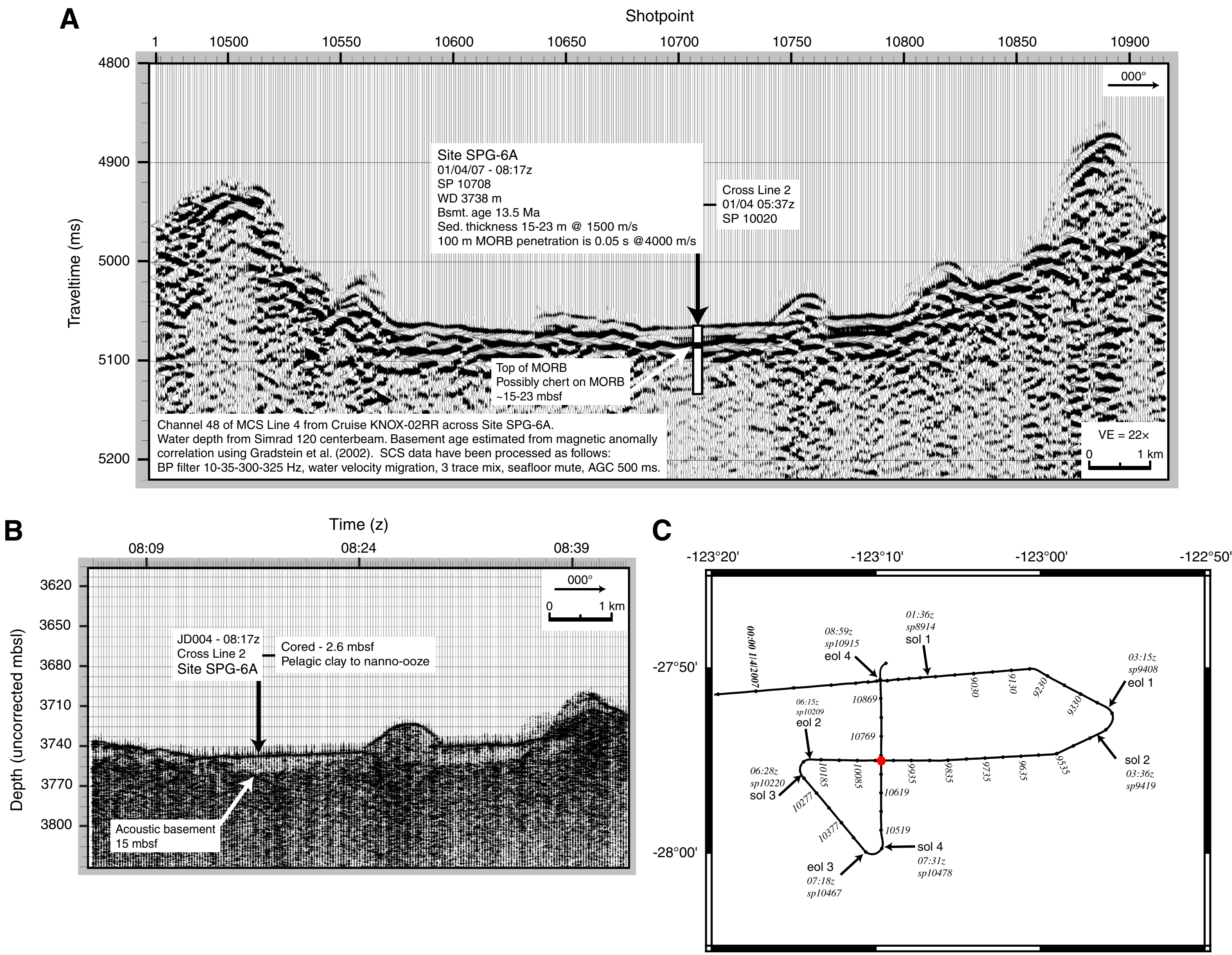


Figure AF18. Bathymetry and track chart, proposed Site SPG-6A. sol = start of seismic line, eol = end of seismic line, z = time, sp = shotpoint.

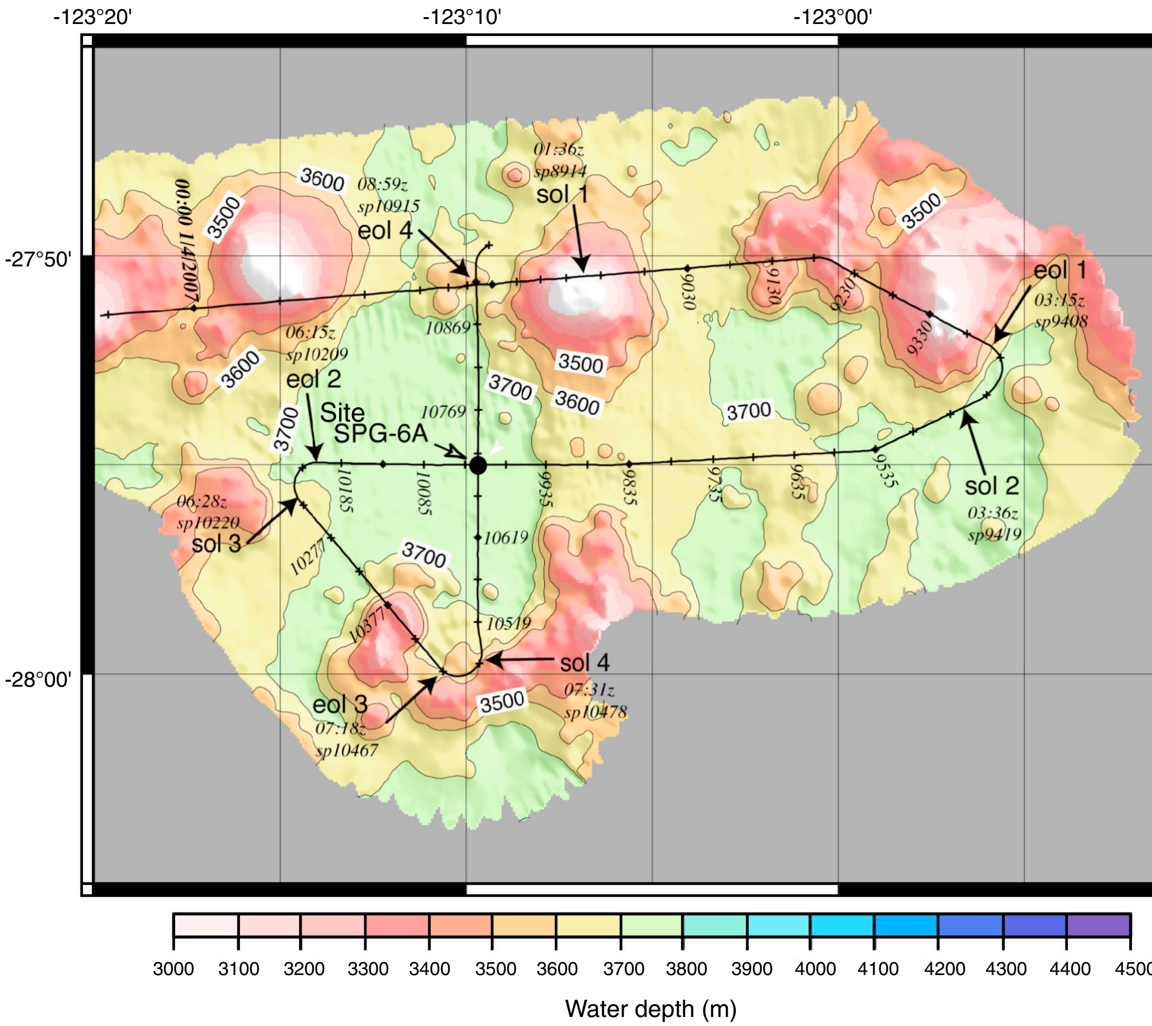


Figure AF19. Single-channel seismic (SCS) Line 3 from Cruise KNOX-02RR, proposed Site SPG-7A. SP = shotpoint, WD = water depth, MORB = mid-ocean-ridge basalt, MCS = multichannel seismic, BP = band-pass, AGC = automatic gain control, VE = vertical exaggeration. **B.** Portion of 3.5 Hz seismic Line 3. **C.** Survey track chart, proposed Site SPG-7A (seismic Lines 1–3). Start of line (sol) and end of line (eol) indicated with time (z) and shotpoint (sp). Tick marks are every 10 min (50 shots); selected tick marks labeled with shotpoint. Red dot = proposed Site SPG-7A. (**Figure shown on next page.**)

Figure AF19 (continued). (Caption shown on previous page.)

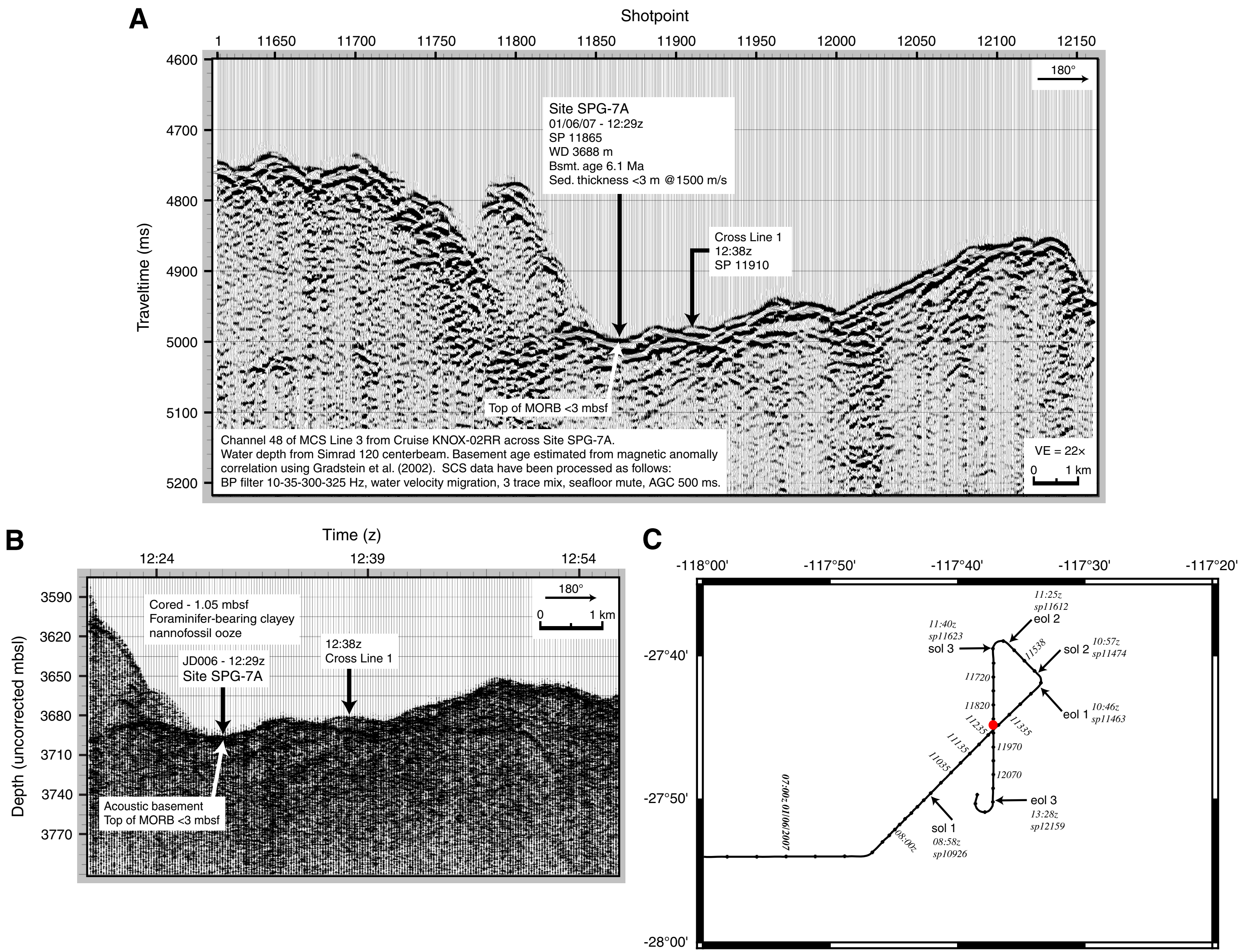


Figure AF20. Single-channel seismic (SCS) Line 1 from Cruise KNOX-02RR, proposed Site SPG-7A. SP = shotpoint, CPA = closest point of approach, WD = water depth, MORB = mid-ocean-ridge basalt, MCS = multichannel seismic, BP = band-pass, AGC = automatic gain control, VE = vertical exaggeration. **B.** Portion of 3.5 Hz seismic Line 1. **C.** Survey track chart, proposed Site SPG-7A (seismic Lines 1–3). Start of line (sol) and end of line (eol) indicated with time (z) and shotpoint (sp). Tick marks are every 10 min (50 shots); selected tick marks labeled with shotpoint. Red dot = proposed Site SPG-7A. (**Figure shown on next page.**)

Figure AF20 (continued). (Caption shown on previous page.)

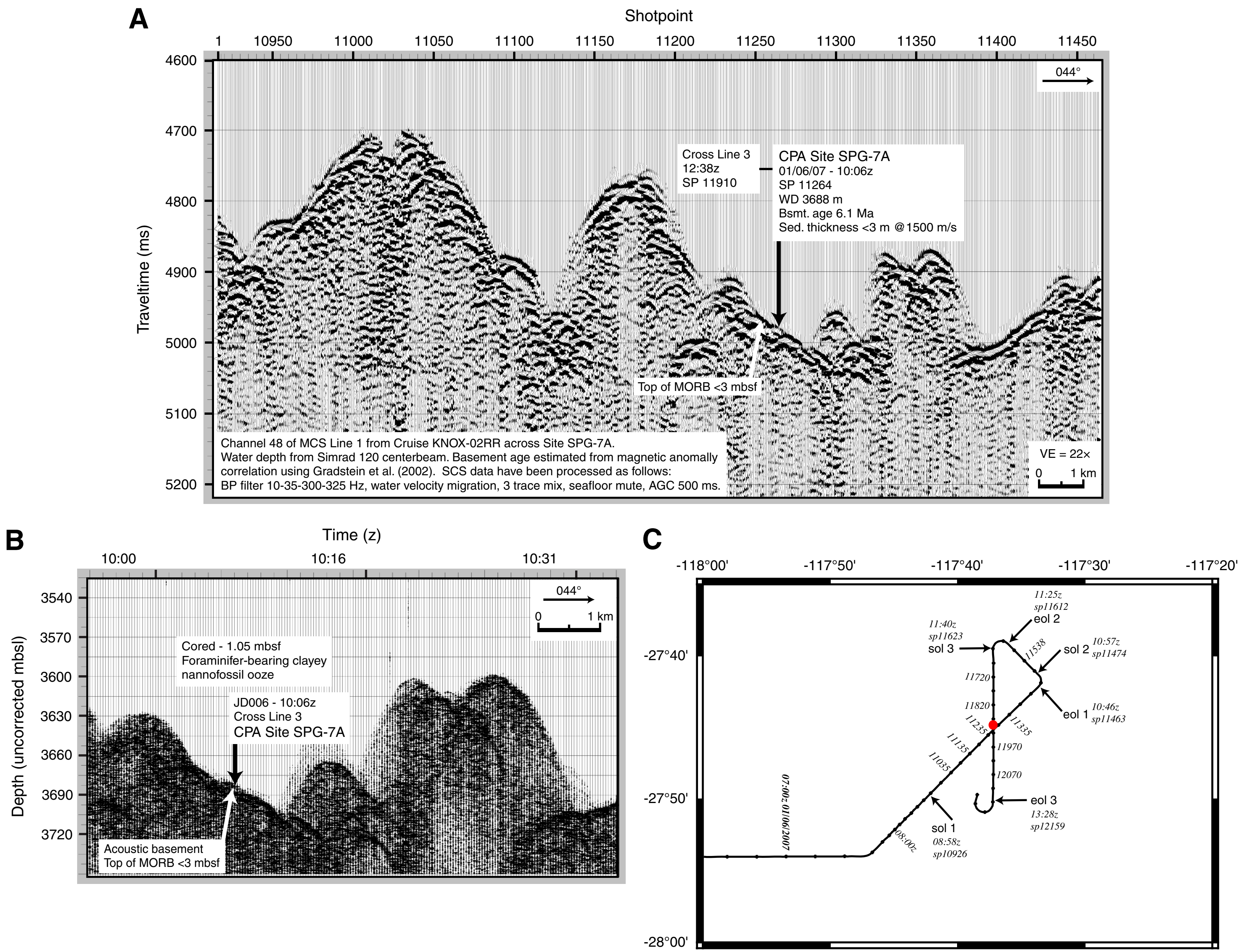


Figure AF21. Bathymetry and track chart, proposed Site SPG-7A. sol = start of seismic line, eol = end of seismic line, z = time, sp = shotpoint.

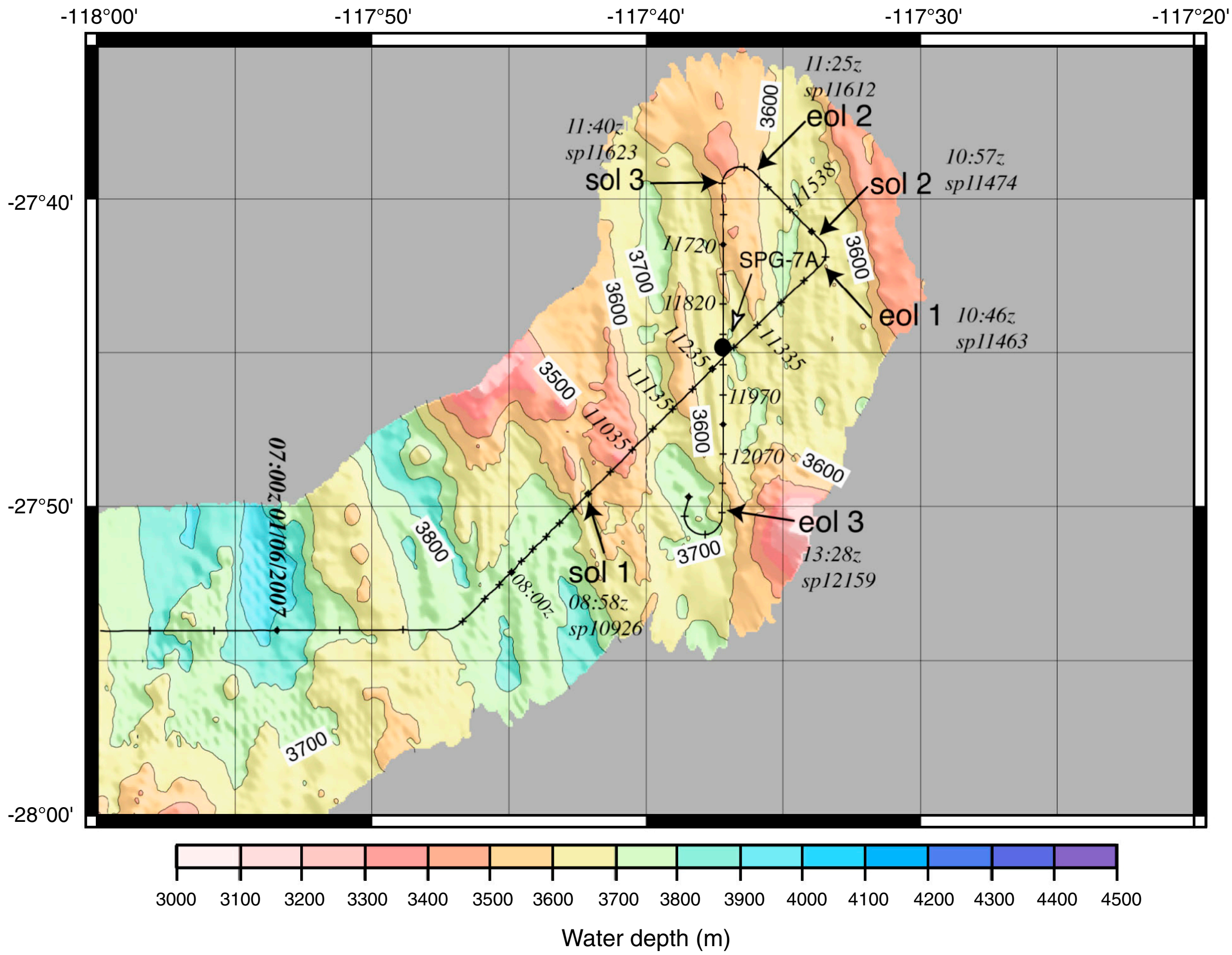


Figure AF22. Single-channel seismic (SCS) Line 4 from Cruise KNOX-02RR, proposed Site SPG-9A. SP = shotpoint, WD = water depth, MORB = mid-ocean-ridge basalt, MCS = multichannel seismic, BP = band-pass, AGC = automatic gain control, VE = vertical exaggeration. **B.** Portion of 3.5 Hz seismic Line 4. **C.** Survey track chart, proposed Site SPG-9A (seismic Lines 1–4). Start of line (sol) and end of line (eol) indicated with time (z) and shotpoint (sp). Tick marks are every 10 min (50 shots); selected tick marks labeled with shotpoint. Red dot = proposed Site SPG-9A. (**Figure shown on next page.**)

Figure AF22 (continued). (Caption shown on previous page.)

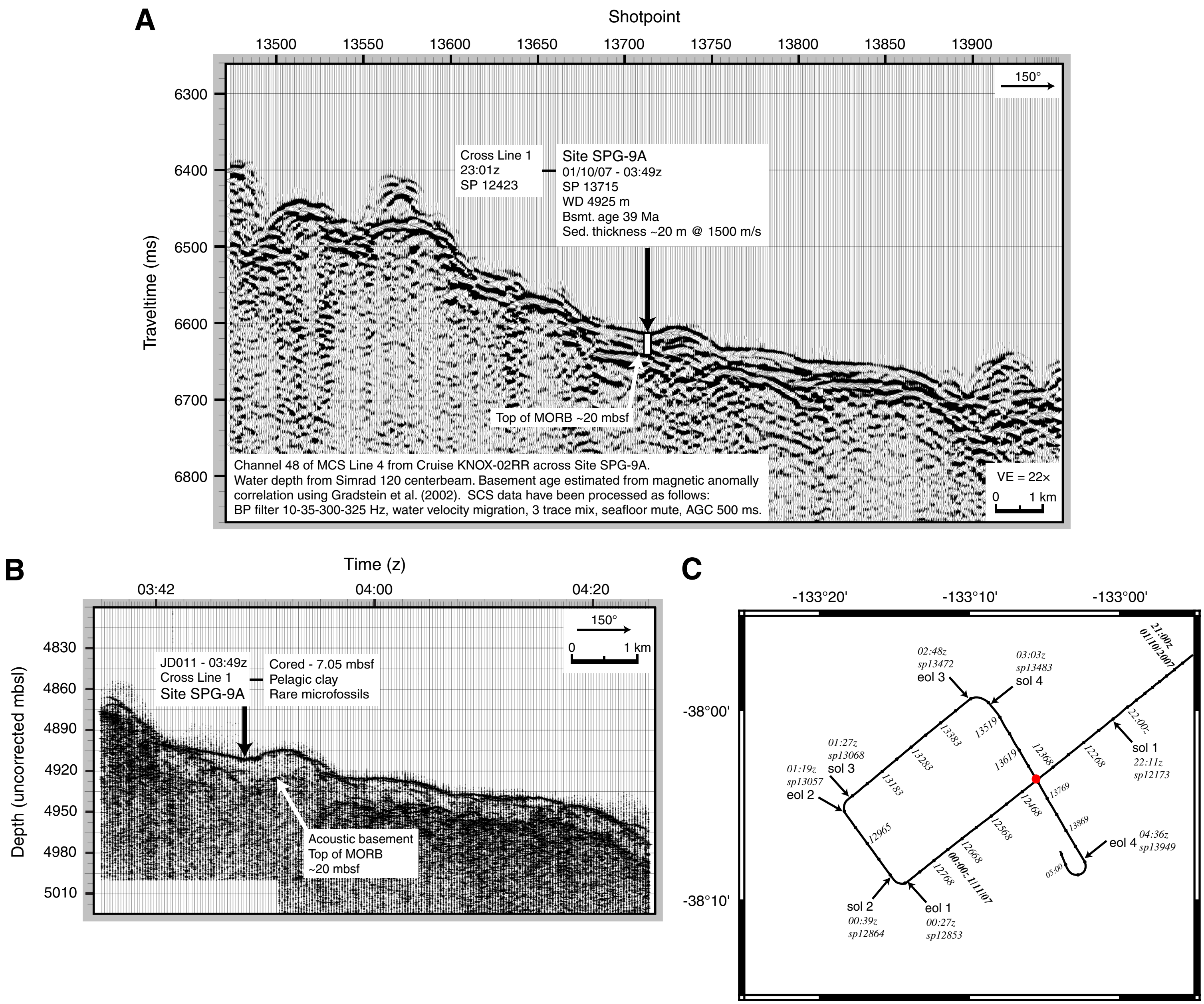


Figure AF23. Single-channel seismic (SCS) Line 1 from Cruise KNOX-02RR, proposed Site SPG-9A. SP = shotpoint, WD = water depth, MORB = mid-ocean-ridge basalt, MCS = multichannel seismic, BP = band-pass, AGC = automatic gain control, VE = vertical exaggeration. **B.** Portion of 3.5 Hz seismic Line 1. **C.** Survey track chart, proposed Site SPG-9A (seismic Lines 1–4). Start of line (sol) and end of line (eol) indicated with time (z) and shotpoint (sp). Tick marks are every 10 min (50 shots); selected tick marks labeled with shotpoint. Red dot = proposed Site SPG-9A. (**Figure shown on next page.**)

Figure AF23 (continued). (Caption shown on previous page.)

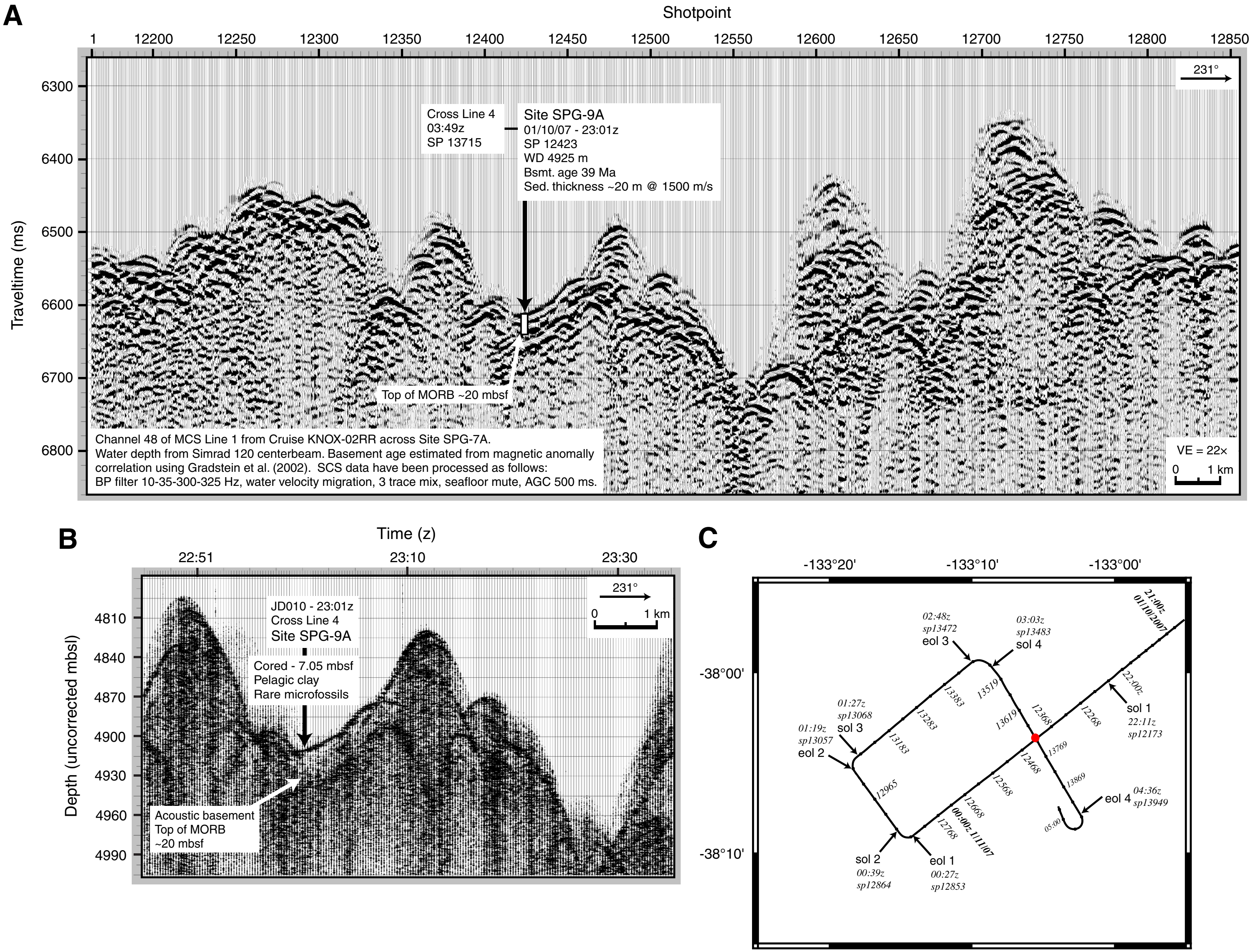


Figure AF24. Bathymetry and track chart, proposed Site SPG-9A. sol = start of seismic line, eol = end of seismic line, z = time, sp = shotpoint.

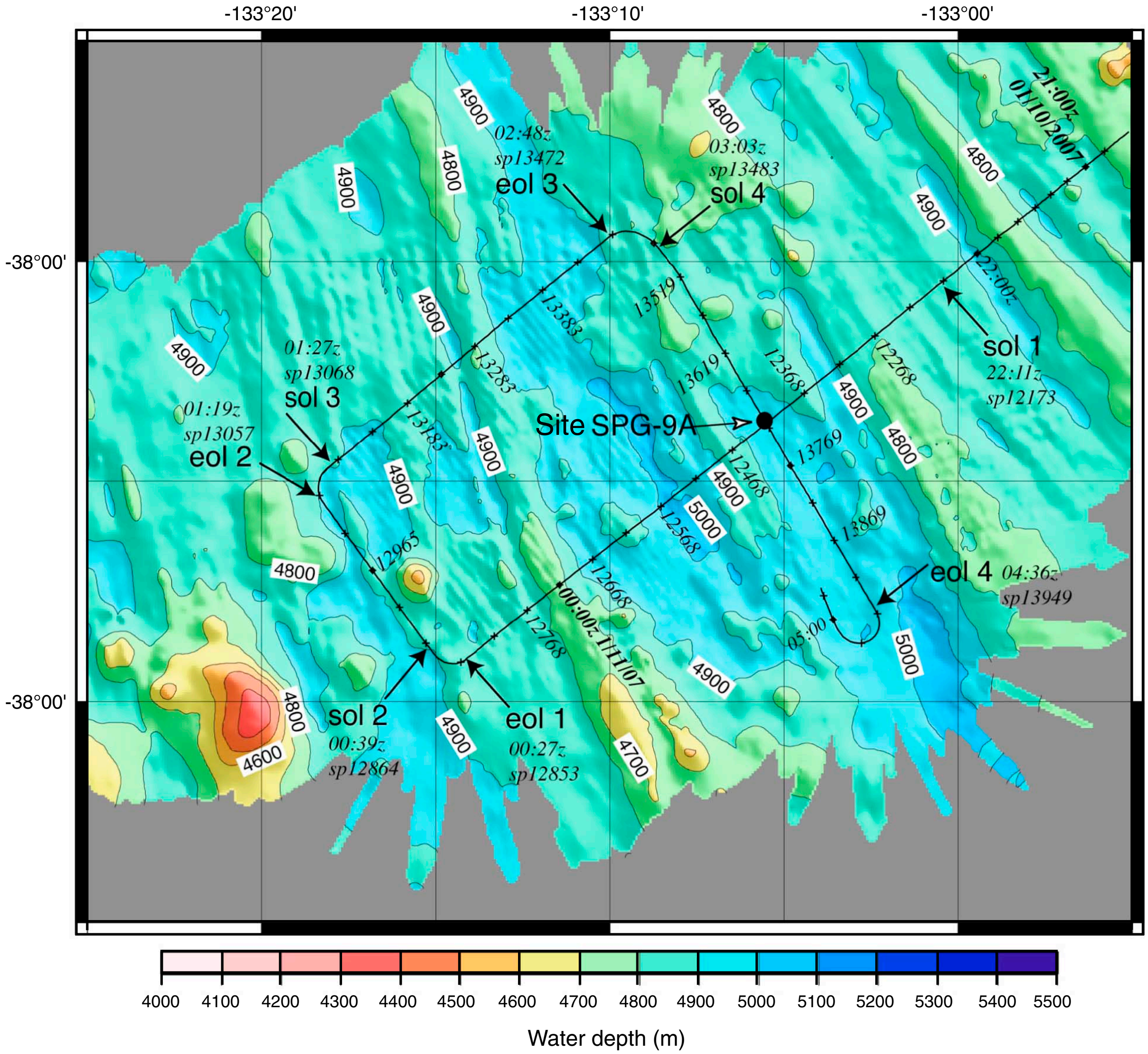


Figure AF25. Single-channel seismic (SCS) Line 6 from Cruise KNOX-02RR, proposed Site SPG-10A. SP = shotpoint, WD = water depth, MORB = mid-ocean-ridge basalt, MCS = multichannel seismic, BP = band-pass, AGC = automatic gain control, VE = vertical exaggeration. **B.** Portion of 3.5 Hz seismic Line 6. **C.** Survey track chart, proposed Site SPG-10A (seismic Lines 1–6). Start of line (sol) and end of line (eol) indicated with time (z) and shotpoint (sp). Tick marks are every 10 min (50 shots); selected tick marks labeled with shotpoint. Red dot = proposed Site SPG-10A. (**Figure shown on next page.**)

Figure AF25 (continued). (Caption shown on previous page.)

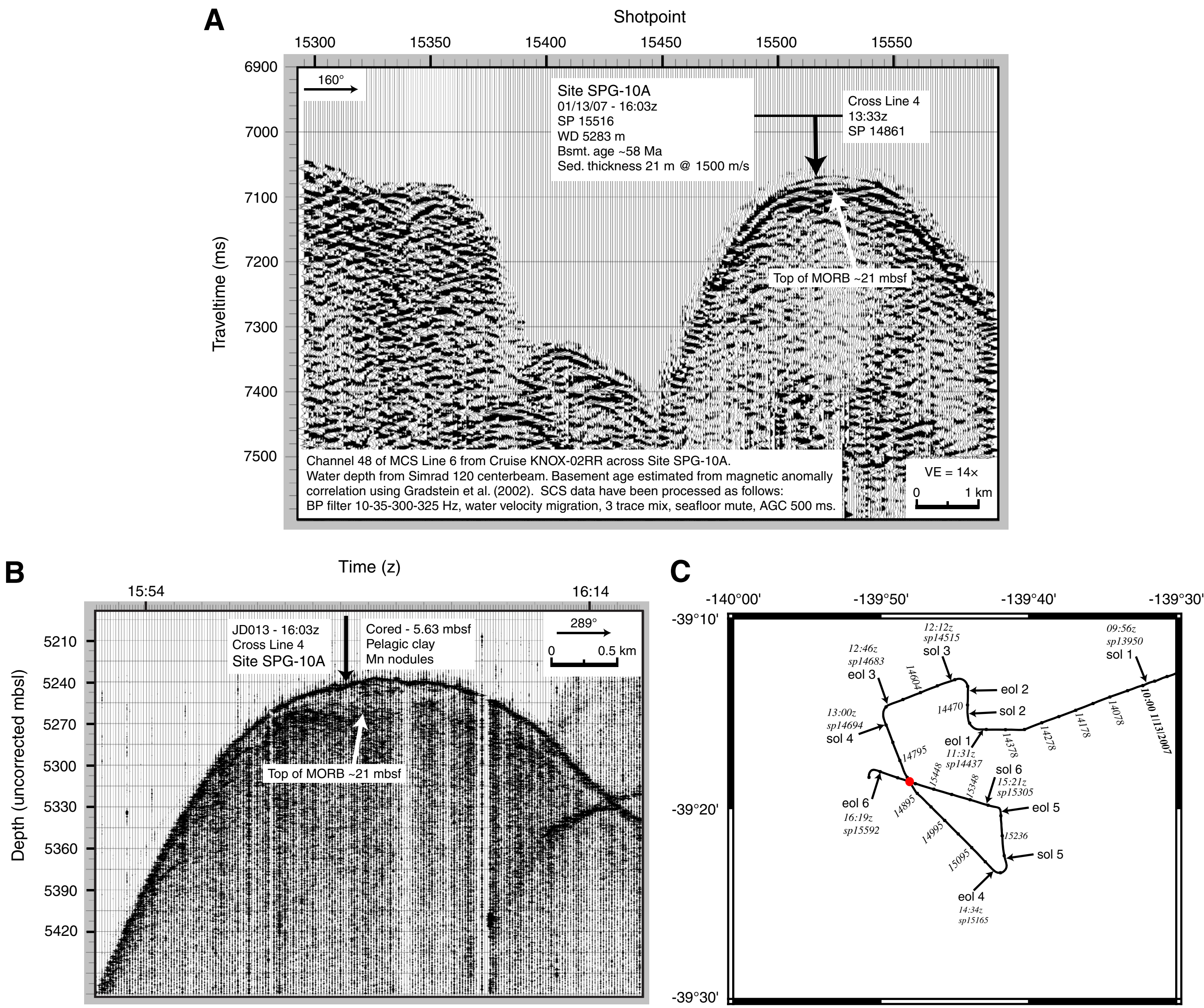


Figure AF26. Single-channel seismic (SCS) Line 4 from Cruise KNOX-02RR, proposed Site SPG-10A. SP = shotpoint, WD = water depth, MORB = mid-ocean-ridge basalt, MCS = multichannel seismic, BP = band-pass, AGC = automatic gain control, VE = vertical exaggeration. **B.** Portion of 3.5 Hz seismic Line 4. **C.** Survey track chart, proposed Site SPG-10A (seismic Lines 1–6). Start of line (sol) and end of line (eol) indicated with time (z) and shotpoint (sp). Tick marks are every 10 min (50 shots); selected tick marks labeled with shotpoint. Red dot = proposed Site SPG-10A. (**Figure shown on next page.**)

Figure AF26 (continued). (Caption shown on previous page.)

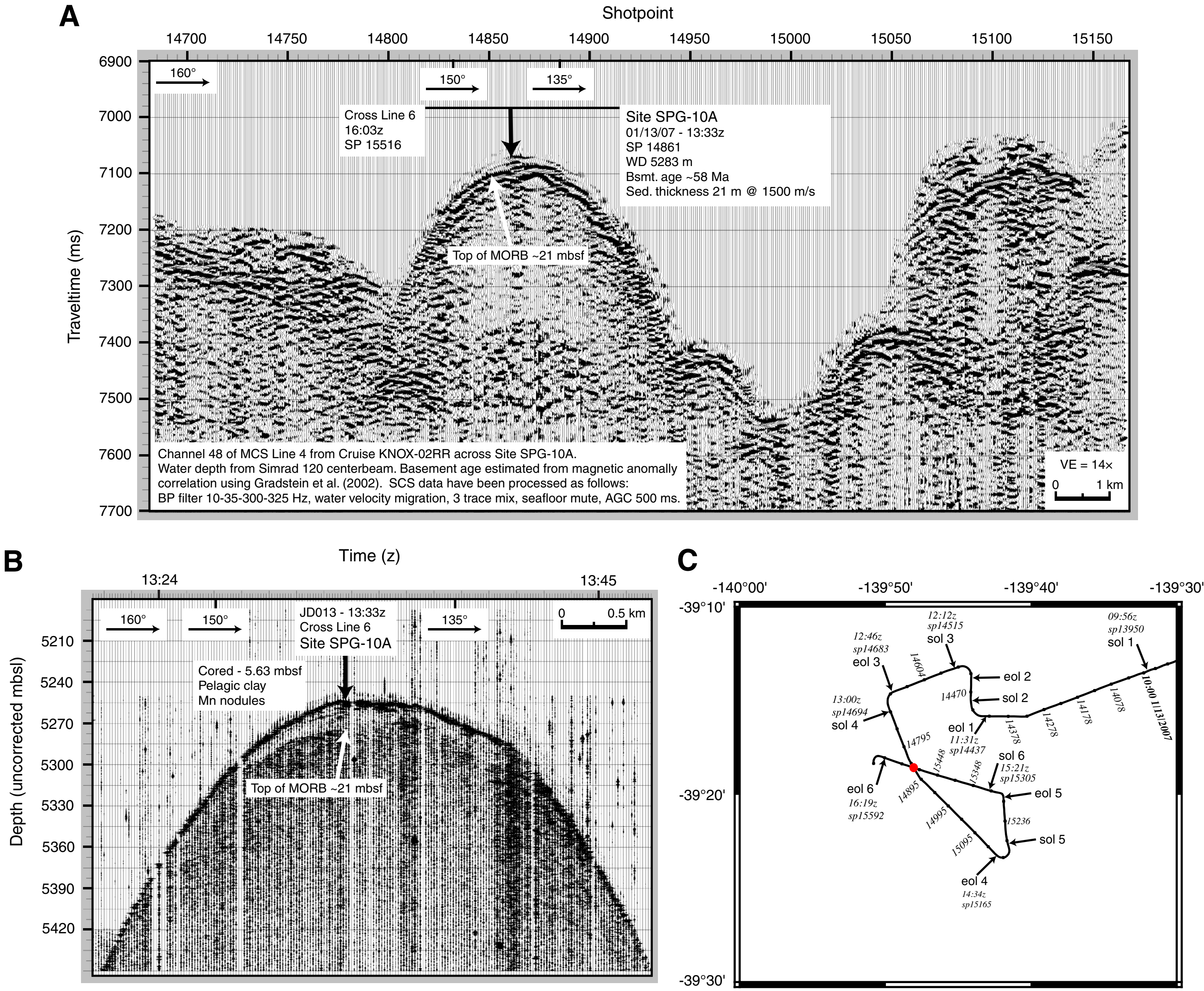


Figure AF27. Bathymetry and track chart, proposed Site SPG-10A. sol = start of seismic line, eol = end of seismic line, z = time, sp = shotpoint.

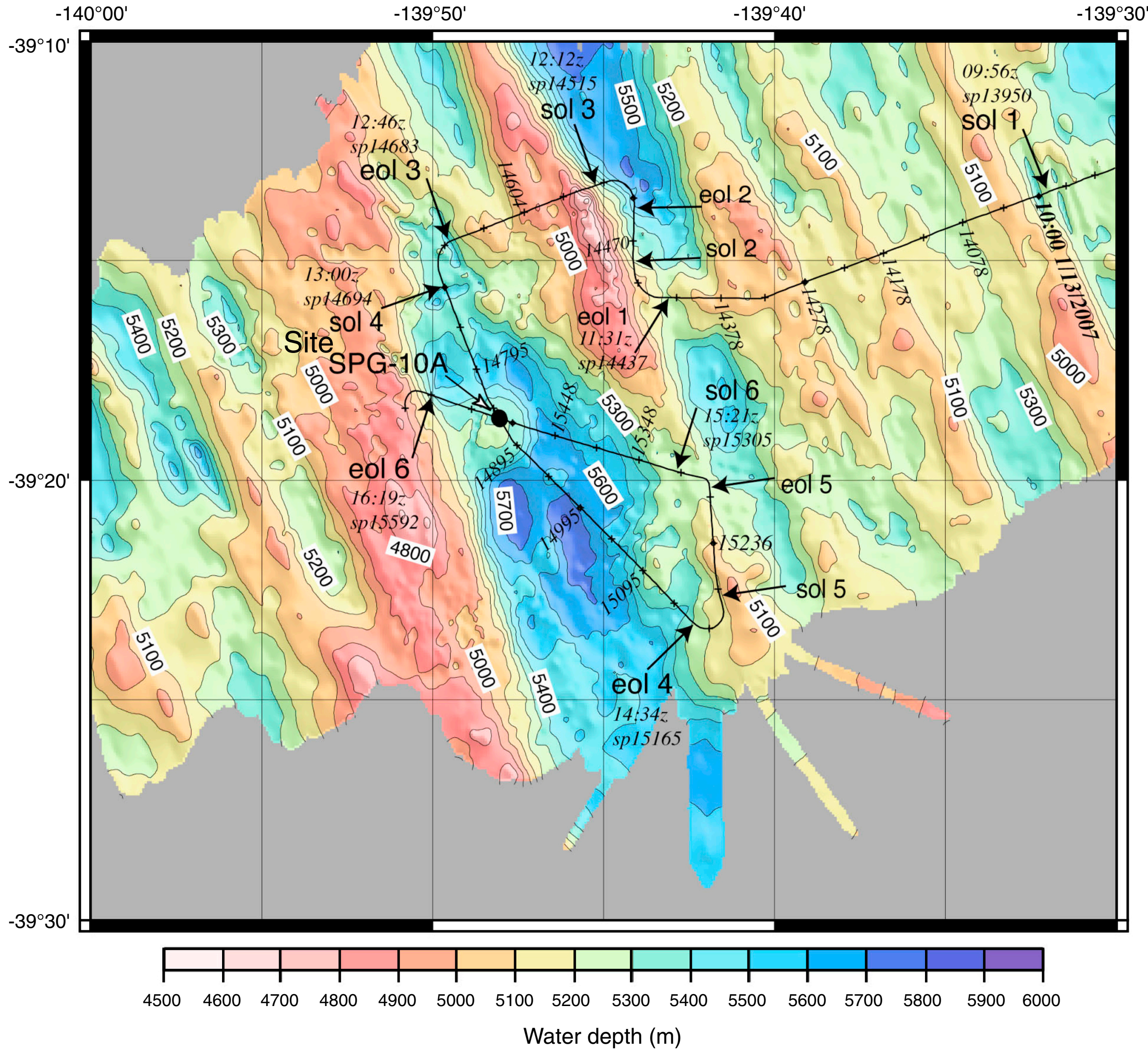


Figure AF28. Single-channel seismic (SCS) Line 3 from Cruise KNOX-02RR, proposed Site SPG-11B. SP = shotpoint, WD = water depth, MORB = mid-ocean-ridge basalt, MCS = multichannel seismic, BP = band-pass, AGC = automatic gain control, VE = vertical exaggeration. **B.** Portion of 3.5 Hz seismic Line 3. **C.** Survey track chart, proposed Site SPG-11B (seismic Lines 1–3). Start of line (sol) and end of line (eol) indicated with time (z) and shotpoint (sp). Tick marks are every 10 min (50 shots); selected tick marks labeled with shotpoint. Red dot = proposed Site SPG-11B. (**Figure shown on next page.**)

Figure AF28 (continued). (Caption shown on previous page.)

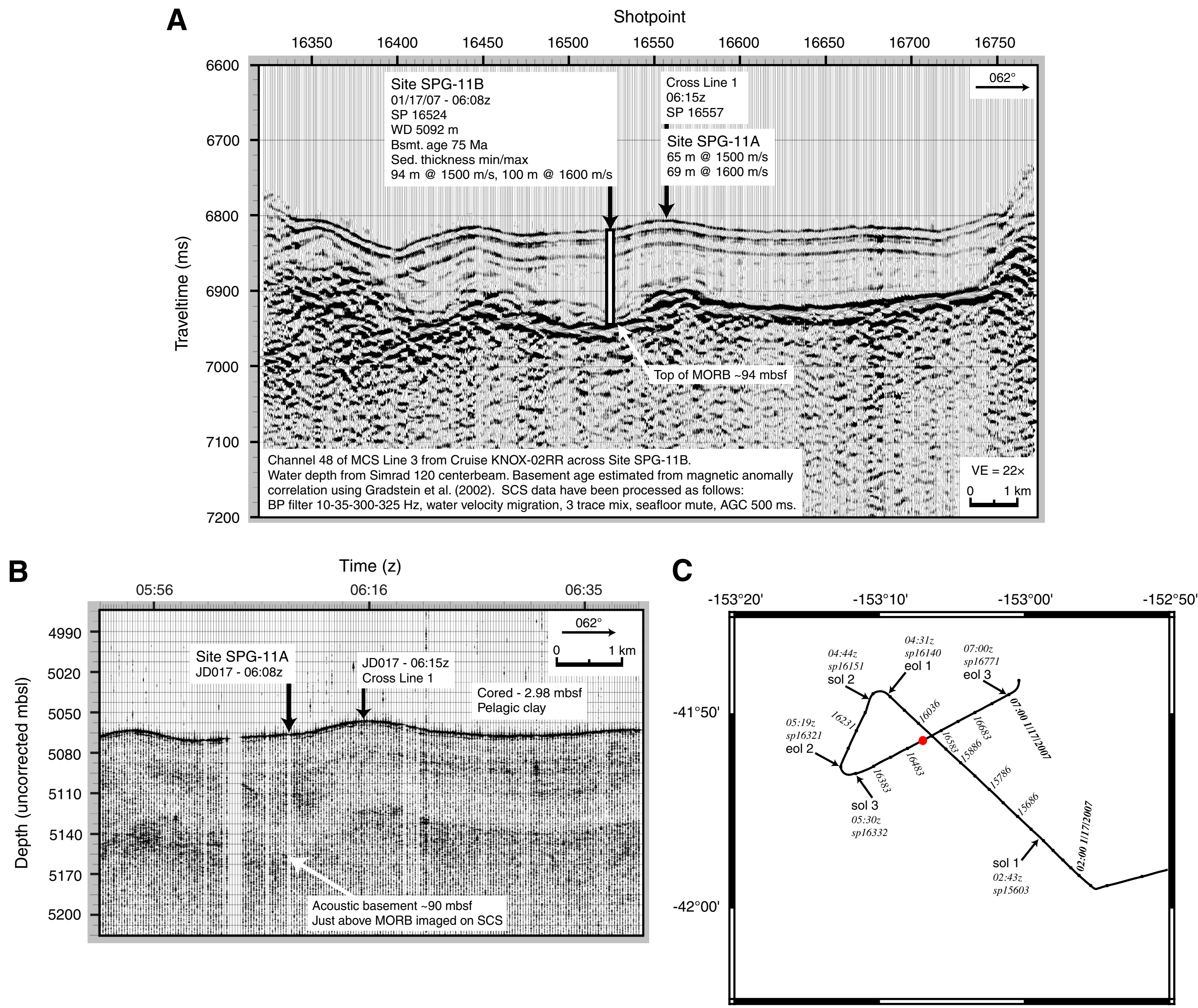


Figure AF29. Single-channel seismic (SCS) Line 1 from Cruise KNOX-02RR, proposed Site SPG-11B. CPA = closest point of approach, SP = shotpoint, WD = water depth, MORB = mid-ocean-ridge basalt, MCS = multichannel seismic, BP = band-pass, AGC = automatic gain control, VE = vertical exaggeration. **B.** Portion of 3.5 Hz seismic Line 1. **C.** Survey track chart, proposed Site SPG-11B (seismic Lines 1–3). Start of line (sol) and end of line (eol) indicated with time (z) and shotpoint (sp). Tick marks are every 10 min (50 shots); selected tick marks labeled with shotpoint. Red dot = proposed Site SPG-11B. (**Figure shown on next page.**)

Figure AF29 (continued). (Caption shown on previous page.)

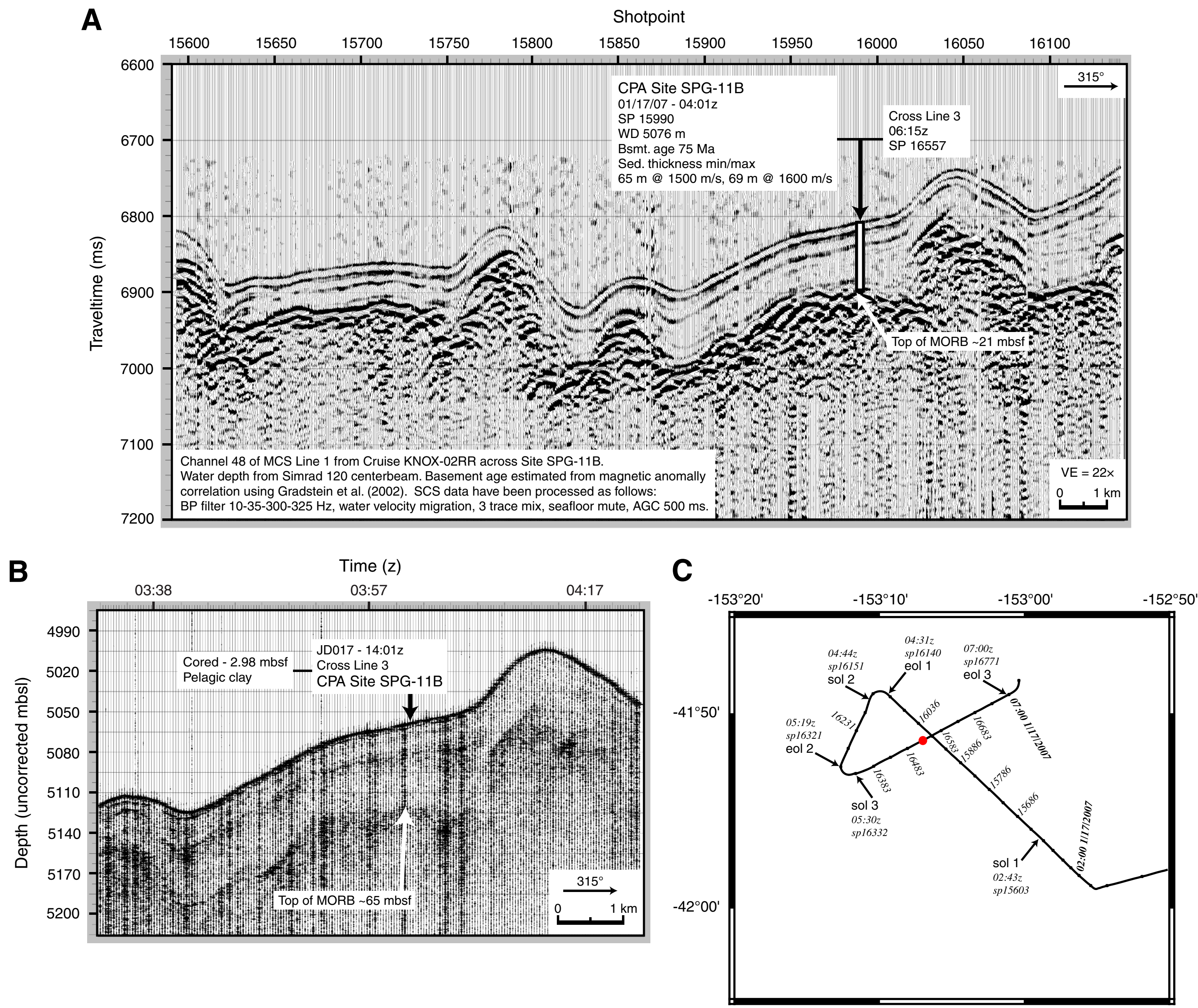


Figure AF30. Bathymetry and track chart, proposed Site SPG-11B. sol = start of seismic line, eol = end of seismic line, z = time, sp = shotpoint.

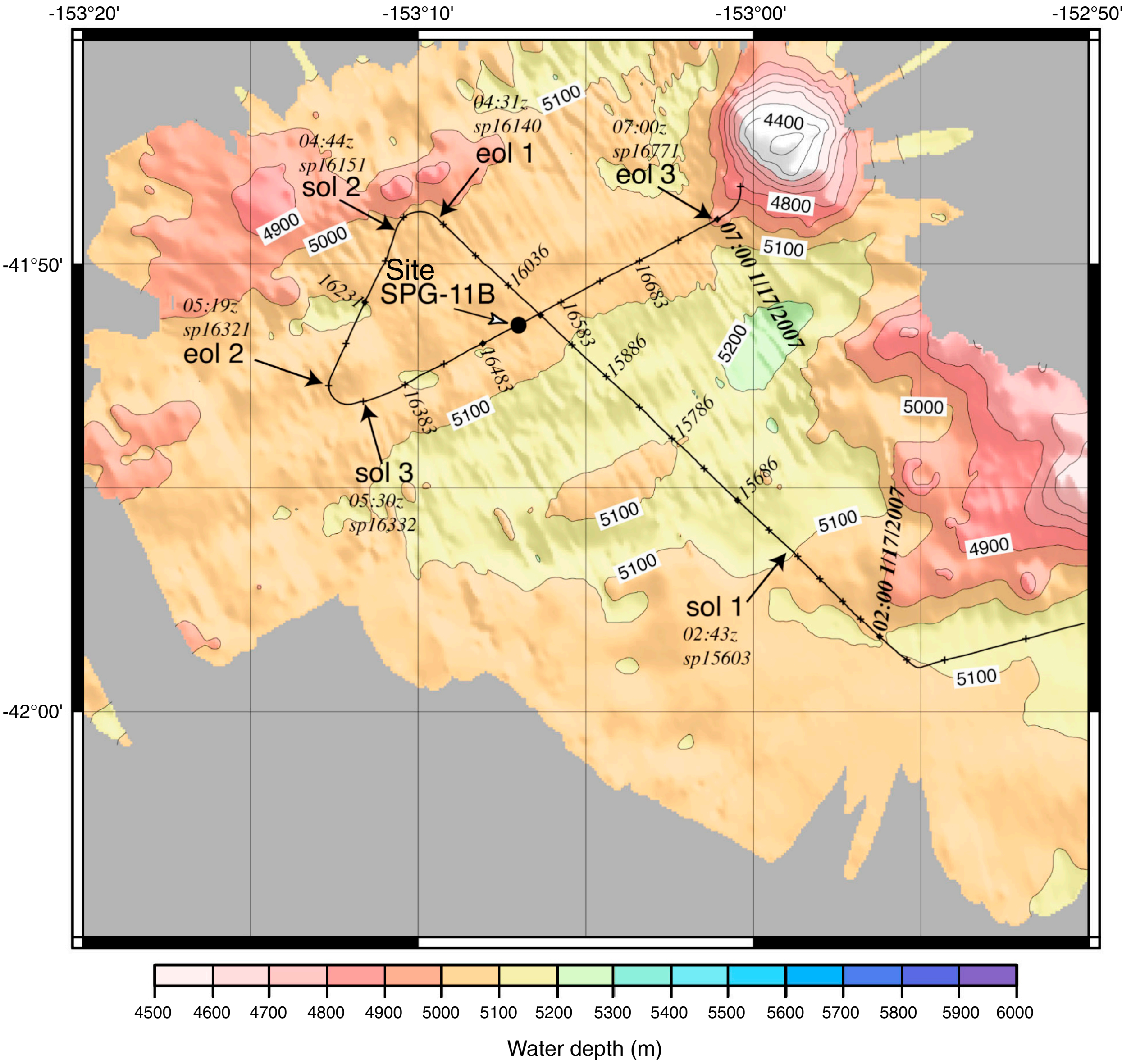


Figure AF31. Single-channel seismic (SCS) Line 3 from Cruise KNOX-02RR, proposed Site SPG-12A. SP = shotpoint, WD = water depth, MORB = mid-ocean-ridge basalt, MCS = multichannel seismic, BP = band-pass, AGC = automatic gain control, VE = vertical exaggeration. **B.** Portion of 3.5 Hz seismic Line 3. **C.** Survey track chart, proposed Site SPG-12A (seismic Lines 1–3). Start of line (sol) and end of line (eol) indicated with time (z) and shotpoint (sp). Tick marks are every 10 min (50 shots); selected tick marks labeled with shotpoint. Red dot = proposed Site SPG-12A. (**Figure shown on next page.**)

Figure AF31 (continued). (Caption shown on previous page.)

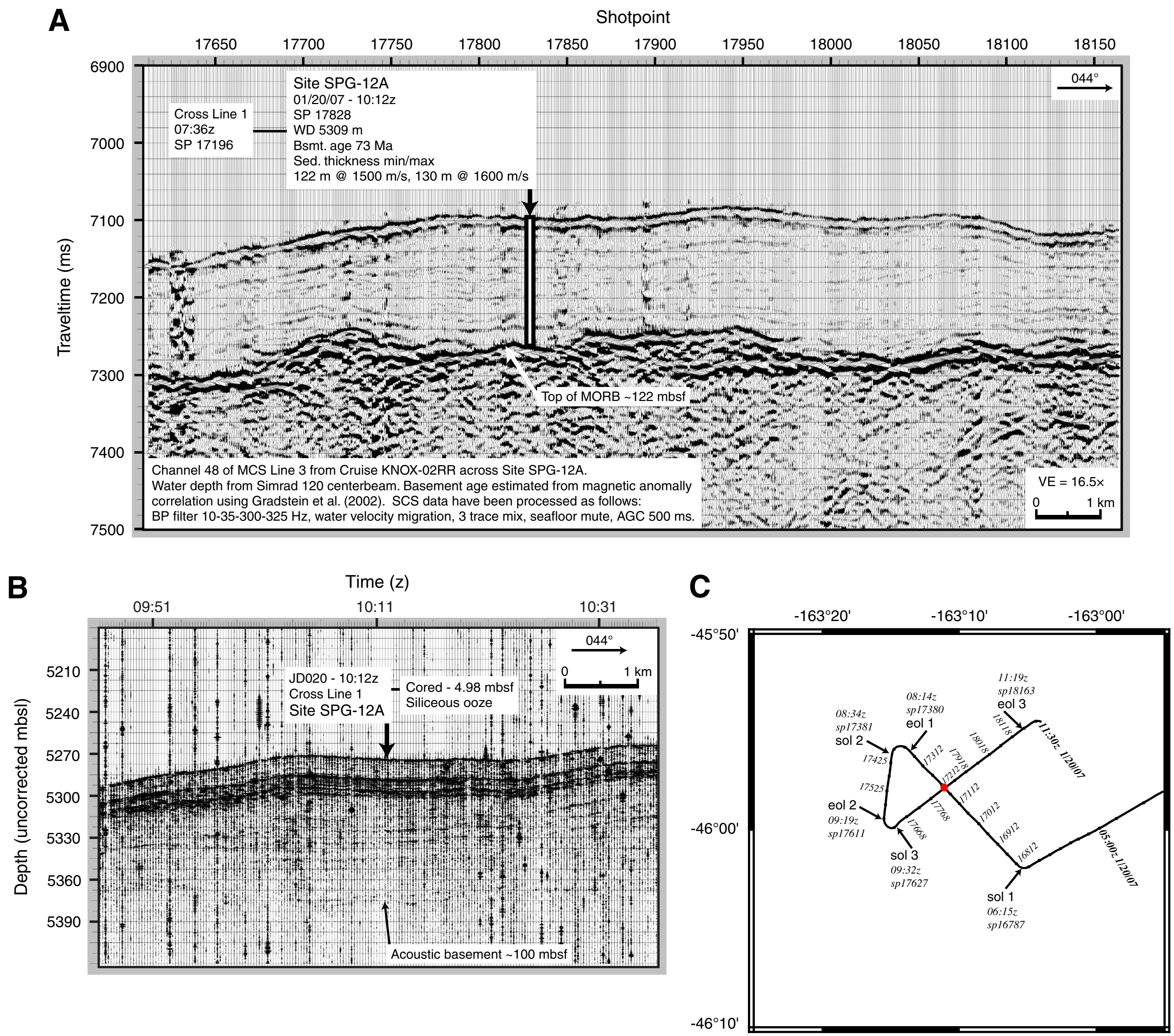


Figure AF32. Single-channel seismic (SCS) Line 1 from Cruise KNOX-02RR, proposed Site SPG-12A. CPA = closest point of approach, SP = shotpoint, WD = water depth, MORB = mid-ocean-ridge basalt, MCS = multichannel seismic, BP = band-pass, AGC = automatic gain control, VE = vertical exaggeration. **B.** Portion of 3.5 Hz seismic Line 1. **C.** Survey track chart, proposed Site SPG-11B (seismic Lines 1–3). Start of line (sol) and end of line (eol) indicated with time (z) and shotpoint (sp). Tick marks are every 10 min (50 shots); selected tick marks labeled with shotpoint. Red dot = proposed Site SPG-11B. (**Figure shown on next page.**)

Figure AF32 (continued). (Caption shown on previous page.)

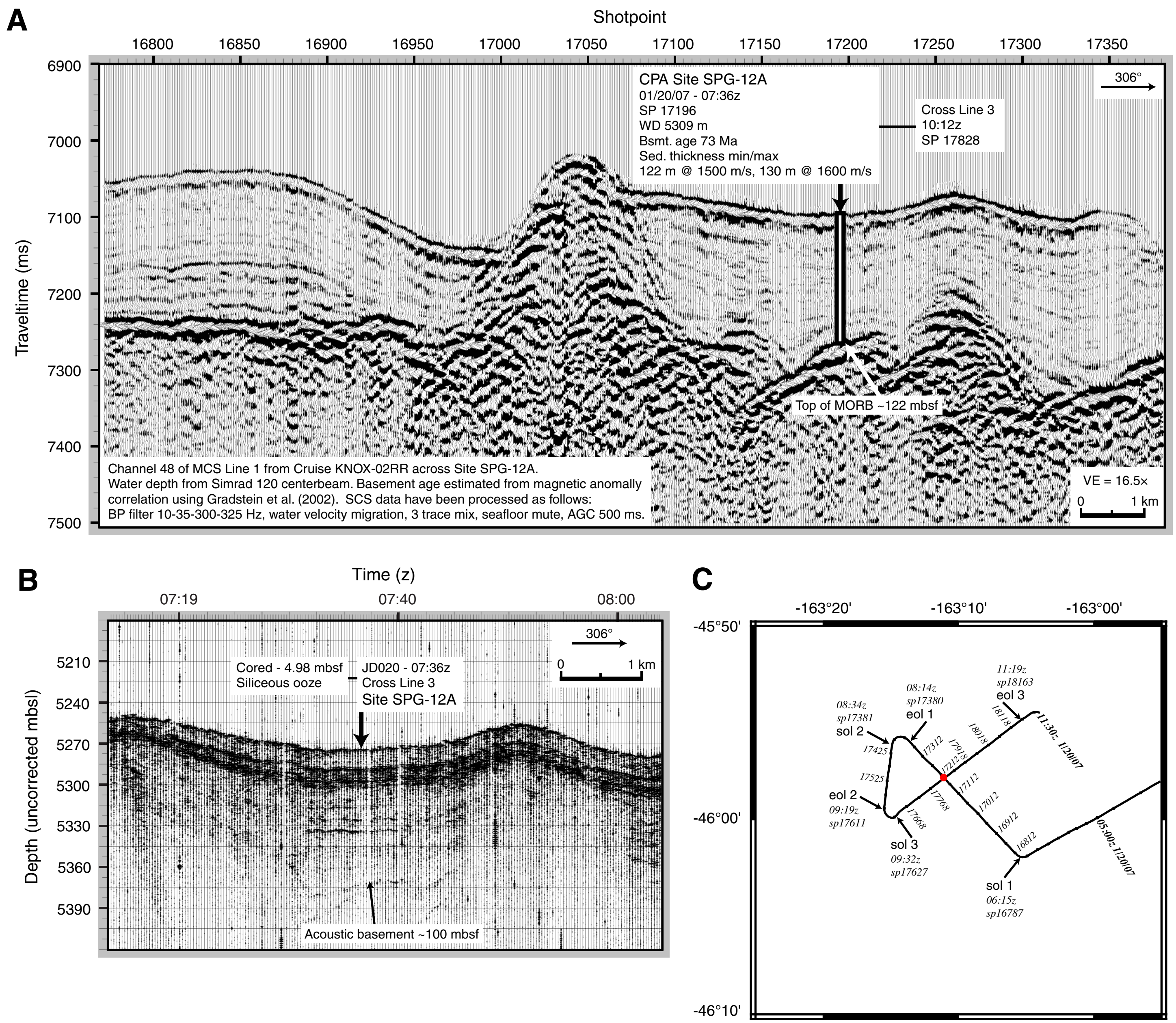
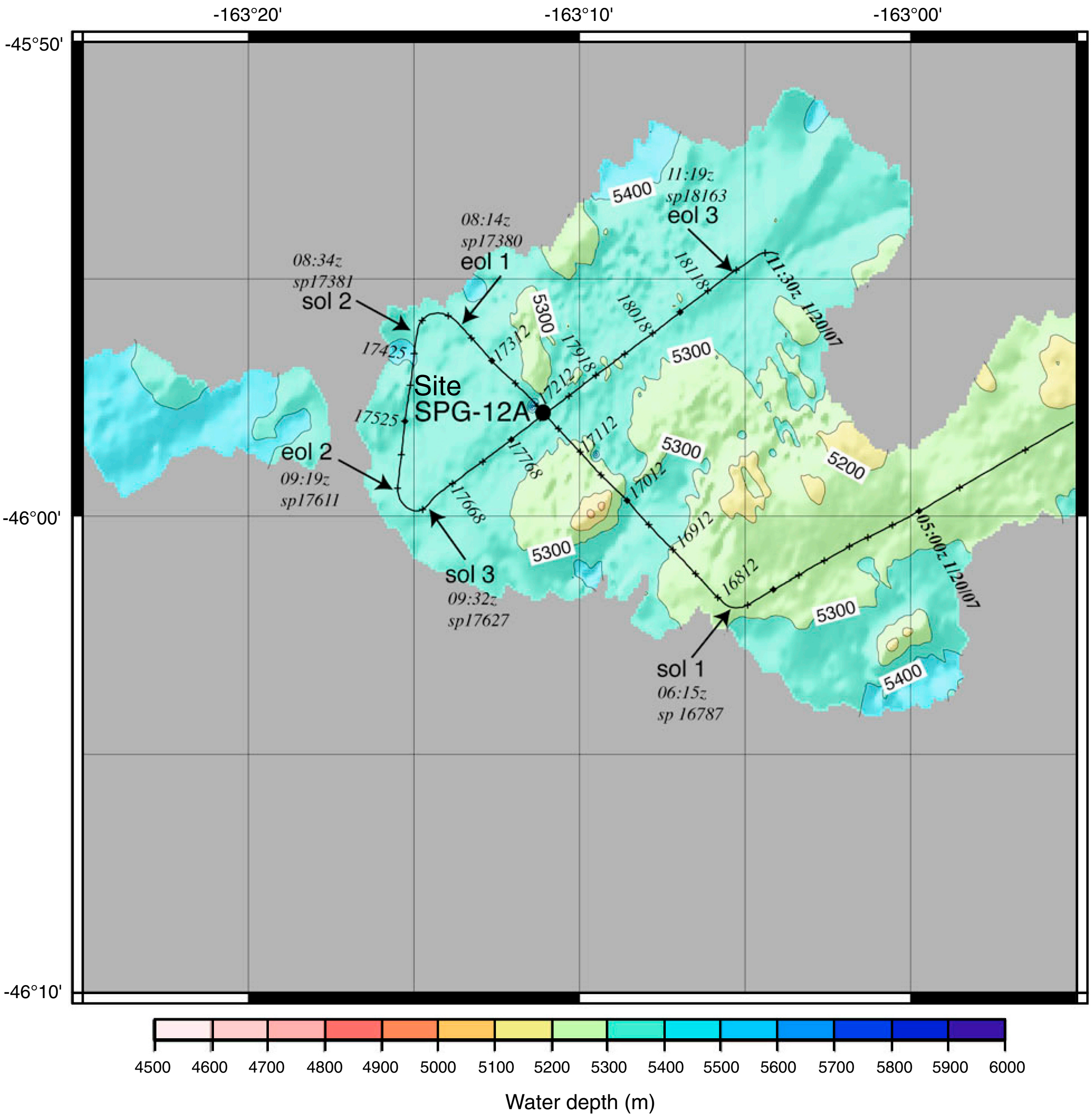


Figure AF33. Bathymetry and track chart, proposed Site SPG-12A. sol = start of seismic line, eol = end of seismic line, z = time, sp = shotpoint.



Expedition scientists and scientific participants

The current list of participants for Expedition 329 can be found at **iodp.tamu.edu/scienceops/precruise/spacificgyre/participants.html.**