

Cruise Report

Atlantis/Alvin Voyage 11 Leg 20 (AT11-20)

San Diego, CA to Manzanillo, Mexico
November 5 to 26, 2004

WHOI Geomicrobiology Group

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1. Introduction

Cruise AT11-20 to the East Pacific Rise (EPR) between 9°28'N and 9°51'N had two primary scientific goals: (1) sampling and in situ chemical measurements of vent fluids, and (2) geomicrobiological investigation of basalt and sulfide weathering. The scientific party includes individuals from various universities and institutions (Table 1). The group from Woods Hole Oceanographic Institution (WHOI) (Edwards/Bach, PIs), for whom this report represents, is examining microbial controls on the weathering of seafloor materials using environmental samples as well as in situ incubation studies. Their program involved deployment and retrieval of baskets with polished chips of basalt glass, sulfides, oxides, olivine, plagioclase, and steel for in situ incubation studies at various locations on the EPR axis and upper flanks. In addition to microbiological studies, the WHOI group will conduct geochemical investigations of vent fluids and associated rocks and sulfides, including isotope geochemistry (Fe, Cu, Ge, S, Sr, O) and biomarker studies (McCollom, U Colorado).

1.1. East Pacific Rise 9°N – 10°N

The work area is a well-studied segment of the EPR between the Clipperton and Siqueiros Fracture Zones [e.g. Detrick et al. 1987; Macdonald et al., 1992; Haymon et al., 1991, 1993; Harding et al., 1993; Toomey et al., 1994; Perfit et al. 1994; Shank et al., 1998; Fornari et al. 1998a,b; Cochran et al., 1999; Von Damm, 2000; Engels et al. 2003; Von Damm and Lilley, 2004] (Figure 1). The focus of cruise AT11-20 was the ridge axis and uppermost shoulder of the EPR centered at 9°50'N; two dive took place in the 9°28'N to 9°30'N area, and one dive targeted hydrothermal vents in the 9°46'N to 9°49'N region. During a survey with ABE in 2001 [Tivey, Schouten, Fornari], high-resolution microbathymetry data were recorded in the 9°50'N and 9°29'N areas (Figure 2).

The neovolcanic zone in the 9°50'N and other areas of the EPR between 9°N and 10°N is marked by a pronounced axial summit trough (AST), a ridge-parallel, elongate collapse feature that can be up to 15 m deep (REF). Most of the volcanic eruptions along the EPR are sourced in the AST, but during large-volume eruptions lava flows can extend several km on either side of the axis [e.g., Hooft et al., 1996; Schouten et al., 1999]. Different flow morphologies, ranging from flat sheet flows in lava channels to pillowed flows with steep flow fronts can be distinguished. Some of the more pronounced flows can be picked in microbathymetry and sonar maps of the area (Figure 2). Faults are rare and show vertical throws of only 1 m or less. A ~10 graben on the western shoulder in the 9°28'N to 9°29'N region (the western graben) is the most pronounced extensional feature in the study area.

The distribution of known hydrothermal vents between 9°28'N and 9°51'N is shown in Figure 3. Hydrothermal activity ranges from focused discharge of phase-separated high-temperature (up to 390°C) fluids to low-temperature (<20°C) diffuse venting [Von Damm and Lilley, 2004], and the temporal evolution of vent systems in relation to magmatic events has been well documented [Von Damm et al., 1997; Von Damm, 2004].

1.2. Geomicrobiological studies

Chemosynthetic microorganisms around hydrothermal vents catalyze redox reactions to harness geochemical energy in environments where reduced and oxidized chemical species are brought together. Areas where hydrothermal fluids mix with seawater at the seafloor and in the subseafloor have been long recognized as important habitats for microbial activity [e.g., Jannasch and Mottl, 1985; McCollom and Shock, 1997; Huber et al., 2003; Butterfield et al., 2004]. Episodes of massive perturbation of magmatic-hydrothermal systems, for instance in relation to magmatic diking

events, have also been linked with increased microbial productivity [e.g., Delaney et al., 2000]. The largest pool of reduced components at and below the seafloor, however, is fixed in solids. Microbe-mineral interactions can support significant biomass production in the deep sea, if microorganisms are able to harness the geochemical energy released by oxidation of reduced species in rocks and sulfides (e.g., McCollom, 2000; Bach and Edwards, 2003]. Previous investigations by the WHOI geomicrobiology group revealed that the oxidation of sulfides is strongly controlled by Fe, Mn, and S oxidizing microorganisms that cover the mineral surface [Edwards et al., 2002; 2003; Rogers et al., 2003]. The microorganisms produce bio-films to regulate the oxygen level at the mineral-water interface such they can control the kinetic rates of oxidation. This process allows microorganisms to utilize the chemical energy associated with various weathering reactions to fuel their metabolic activities and fix inorganic carbon. However, many fundamental questions remain unanswered. Among the questions under investigation: which microorganisms are involved in weathering, how many of these organisms are present and/or active in the environment, what physiological traits might these organisms possess that enable them to exploit their deep-sea epi- and endolithic environments?

The principal goal of the WHOI group is investigating the role of microorganisms in seafloor weathering, their influence on redox cycling, specifically of Fe and S, and exploring theoretical and empirical approaches for quantifying biomass production related to weathering. Key to understanding mineral-microbe relationships is understanding how the morphological evolution of mineral surfaces and the development of alteration films on these surfaces relates to the physiology of microbial colonists. In situ incubation studies are well suited to study these processes and close the gap between laboratory culture experiments and field studies, because mineralogical properties of the substrate and exposure times are well known, while biases inherent to culture studies are avoided.

2. Cruise logistics, and operations

2.1. Atlantis operations

2.2. Alvin operations

2.3. Alvin Navigation

During all dives, Alvin navigation data were acquired using the bottom-lock Doppler navigation DVLNAV software [Whitcomb et al., 2003]. When within the network of transponders deployed near 9°50'N, the Doppler navigation was supplemented with long baseline (LBL) acoustic navigation. When LBL was not available (e.g., in the 9°29'N area or near transponder baselines off axis at 9°50'N), the raw Doppler navigation data (*.csv files saved by DVLNAV) were used as Alvin navigation. When only Doppler navigation was used, Alvin was surveyed in at the start of the dive. The navigation agreed very well with micro bathymetric terrain features and off-axis incubation sites in both the 9°29'N and the 9°50'N areas were generally located within minutes. The only exception is Dive 4049, when both LBL and Doppler navigation were poor, the former due to the proximity to a transponder baseline and the latter due to failure of the octans gyro and the forward starboard transducer on the Doppler. Dive tracks for all Edwards/Bach dives are depicted in Figures 4 and 5.

3. Dives summaries

This report covers the five Bach-Edwards dives (4049, 4053, 4055, 5057, 5059) and part of one half shared dive (dive 14: 4061) that were part of this program. The full transcripts can be found in Appendix A.

Dive #4049

Date: Nov. 12, 2004

Pilot: Bruce Strickrott

Port observer: Tom McCollom

Starboard observer: Olivier Rouxel

Our first objective was to recover baskets containing mineral samples for seafloor incubation deployed during cruise AT11-7 in April, 2004 by Wolfgang Bach/Cara Santelli. We spent ~1.5 hours looking for the baskets at target coordinates, but were unsuccessful and decided to abandon the effort. The navigation problems were due to poor LBL data resulting from the close proximity of the sub to the transponder baseline. We then moved to Tica to retrieve another set of baskets and deploy others. We recovered three baskets as well as a basalt plate deployed by Stace Beaulieu for biological studies. We then maneuvered to the main Tica chimney structure to obtain fluid & sulfide samples, but experienced several equipment failures. In a short period of time, the port manipulator arm malfunctioned and the high temperature probe failed. Shortly afterwards, the aft rudder of ALVIN grounded out and was no longer operating. Madness ensued. With a non-functioning port arm and limited maneuverability, no fluid or sulfide samples could be obtained. However, we were able to proceed to a patch of oxide-stained basalt to obtain a slurp sample and some oxide-coated basalt using the starboard arm. We then proceeded to the vicinity of Bio9, where we located a site where shimmering water was venting from a ~1 m oxide-stained crack in basalt. We obtained several oxide-coated rocks from this location. We spent the last few minutes unsuccessfully attempting to locate an extinct sulfide chimney in the area, when time forced us to end the dive.

Dive #4053

Date: Nov. 16, 2004

Pilot: Bruce Strickrott

Port observer: Wolfgang Bach

Starboard observer: Katrina Edwards

The objectives for Dive 4053 were (1) obtaining fluid and sulfide samples from K vent, (2) deploying short-term incubation experiments in the western graben near a fissure with tevidia colonies and presumed low-temperature venting, (3) further exploring the western graben for hydrothermal activity, and (4) retrieving incubation baskets at marker ESB#4 between the ASC and the western graben in the 9°28'N area.

We were able to locate K vent at x10137, y40046, 2562m and determined that the only form of venting was emanations of shimmering water from the beehive-like uppermost part of the structure. We broke off a spire from the beehive that was completely overgrown with Alvinella without destructing it during sampling. Removal of the spire opened a small orifice that vented

203°C hot clear to somewhat milky fluids. That fluid was sampled with a major sampler pair. Because the orifice was too small to get both nozzles in, the bottles were fired sequentially. Temperature after sampling was unchanged. A second spire, located about 10 cm left of the previously sampled one was picked for a biological sample. This spire is apparently not emanating fluid; it has some *Alvinella* near its tip, but is generally much less densely colonized than the previously sampled spire. We retrieved a sample of oxidized sulfide from a small field of chimney debris just north of K-vent, before leaving for the western graben.

Went down the western shoulder, bearing 205, over mostly lobate flow with numerous shallow collapse pits and reached the eastern wall of the western graben at x9521, y38610, 2571m. We proceeded to explore the graben, heading 350, and discovered hydrothermal staining on both graben walls and in the associated talus. We then headed 170 and found the fissure with tubeworm colonies at x9521, y38476, 2573 m. Explored graben wall further to the south, down to x9587, y38400, 2567m, but then decided to return to the fissure with Tevnia and deploy our incubation baskets there. All of the graben floor is sheet flows, which are strongly fissured in the center and faulted (offsets <1m) leading up to the graben walls. After the baskets were deployed at x9520, y38475, 2573m within a small patch with rusty staining, a slurp gun and rock sample were recovered. Marker ESB#8 was deployed at the incubation site. Finally, we took a Niskin water sample while hovering over the fissure. The fissure walls expose nothing but one single massive lava unit, implying the sheet flow we were on is the surface of a lava pond. The temperature remains at 1.8°C as we shoot the Niskin.

We took a heading of 075 for the ESB#4 site and cross numerous lobate flow ridges and sheet flows in shallow channels. Find site ESB#4, located x9909, y38289, 2563m on the top of a small lobate ridge between two lava channels and retrieved three incubation baskets and a Staudigel charge as well as a sample of a 5cm thick lobate crust.

Dive #4055

Date: Nov. 18, 2004

Pilot: Pat Hickey

Port observer: Wolfgang Bach

Starboard observer: Cara Santelli

The objectives of dive 4055 were (1) sampling sediments and off-axis lava for biological studies, (2) retrieving incubation baskets at ESB#7 and ESB#8 sites, and (3) further exploring the western graben for hydrothermal activity.

We took a sediment sample with push corer no. 5 at the landing site (x7215, y38427, 2673m). We then slowly moved ESE up the western shoulder. We stopped over a lobate lava flow and collected a sample of Mn-oxide coated lobate crust (x7302, y38371, 2665m), then took another basalt sample, a glassy knob on a large pillow, at x7327, y38345, 2664m – the base of a steep front of a pillowed flow unit. Using push corer no. 8, we took a second sediment sample at x7407, y38327, 2646m from a small sediment pond surrounded by lava pillows. A third basalt sample, a Mn-Ox coated piece from a ridge of hackly lava, is sampled at x7937, y38308, 2595m. After reaching ESB#7 site at x8064, y38371, 2588m, we picked up three baskets and deployed seven new baskets for long-term incubation experiments before sampling the lobate flow in the ESB#7 area and firing a Niskin bottle.

We reached the western graben wall at x9440, y38511, 2566m shortly before arriving at the ESB#8 site, where we reset the xy's to x9560, y385222 (determined during dive 3974 of cruise AT11-7). The short-term incubation experiments at ESB#8 were retrieved, a Niskin was fired at the incubation site, another one over the tubeworm fissure, and a close-up survey of the vent communities with the 3-chip camera indicated that the tubeworms are alive and vent crabs are abundant (Figure 6).

Further exploration of the graben, bearing 170, revealed another flat sheet flow with numerous tubeworm-colonized fissures at x9567, y38427, 2570 m. Hydrothermal fluid discharge could not be detected visually or by temperature measurements. Two more basalt samples were collected, one at x9567, y38427, 2570m, and one at x9570, y38494, 2570m.

Dive #4057

Date: Nov. 20, 2004

PIT: Anthony Berry

Starboard/Pilot: Anthony Tarantino

Port observer: Wolfgang Bach

Objectives of dive 4057 were (1) recovering and deploying incubation baskets at site ESB#2 (x6332, y76813, 2545m), and (2) sampling vent fluids and chimneys in the Bio9 area.

After locating the incubation baskets at site ESB#2 (x6330, 76812, 2545m), we retrieved three baskets plus a Staudigel charge and deployed two incubation baskets. A basalt sample of a hackly lava flow was also recovered and placed into a biobox. We then drove 2100m, heading 301, to the AST just south of the Bio9 area. The following flow morphology changes were observed during transit: hackly-pillow (x6303, y76825, 2545m), pillow-lobate (x6212, y76883, 2540m), lobate-hackly sheet flow (x6173, y76909, 2540m), pillow overlying hackly sheet flow (x6031, y77009, 2530m), pillow-lobate (x6031, y77009, 2530m). Lobate flow remain dominant from the last coordinates on all the way up to the ASC. Collapse pits increase in number and size as the ASC was approached. We changed our heading to 275 at x4860, y77843, 2506, after crossing a large, flat to ropey sheet flow. At x4713, y77867, 2504m, we noticed an area, several meters across that was littered with dead mussels and serpulid worms. We reached the ASC at x4665, y77880, 2501m and changed heading to 350.

We located Bio9'' at x4603, y77924, 2511m and measured the vent fluid temperature at 383°C using the high-T probe. A fluid sample was taken with a major sampler pair. The ICL probe recorded a temperature of 374°C during sampling. It was noted that the Bio9'' chimney has a wide trunk that bifurcates into two orifices, one that was venting vigorously, and a second one that was almost clogged but still emanated some black "smoke". When trying to break off the unobstructed orifice, the entire uppermost 40-50 cm of the chimney broke loose, including the part that had the HOBO probe (no. 4) cemented in. The dislodged chimney was collected and carefully broken up, first in the Alvin basket and later over an open biobox, in order to remove the HOBO probe. The probe was then re-deployed at the leftover chimney. The HOBO probe was not in the chimney between 19:16 and 19:44 EST. With only 15 minutes of bottom time left, we decided to shoot a Niskin bottle, while hovering over a patch of shimmering water immediately east of Bio9''. The temperature went up from 1.9 to 2.2°C, while the sample was taken. We then recovered a slurp gun sample of rusty, flocculent material associated with an

inactive sulfide structure, approximately 3 m north of Bio9''. A rock sample of the oxidized sulfide was also taken and placed in a biobox.

Dive #4059

Date: Nov. 22, 2004

Pilot: Bruce Strickrott

Port observer: Katrina Edwards

Starboard observer: Dan Rogers

The goals of this dive were; to retrieve 3 in situ incubation chambers at marker ESB#6, deploy 2 more chambers at ESB#6, sample high temperature fluids and sulfide from Tica, and sample extinct sulfides to the east of Bio9. Upon arrival at the bottom (2504m, 15:30) on a lobate flow we motored west toward the Tica vents and ESB#6. Our transit took us over a lightly sedimented lobate flow with some cave in pits and sparse macroscopic biology. Within 15min of landing on the bottom we had found ESB#6 (x3907, y78125, z=2517m, 15:45) and the in situ chambers sitting on top of the lobate flow. Bruce opened biobox #1 and deployed chamber #61 and #71 and retrieved #34 and #17. A large piece of basalt was placed in the milk crate and a second piece of glassy basalt was placed in biobox #1. A ship survey was executed and upon finishing we moved on to Tica (16:15). During our transit to Tica we crossed several contact margins indicating a later flow of pillow lava, lobate to pillow (z=2516, 16:20) and pillow to lobate (16:23). Much of the basalt seen during this transit and the entire dive was very glassy in composition. During this transit we found and recovered the lost major sampler (16:30) sitting on the lobate flow. As we approached the axial summit trough we crossed another flow contact, lobate to hackley (16:41) finally arriving at Tica at 16:45 (z=2512). Finding a black smoker, we used the HT probe to measure the temperature (344C, 16:53) and sampled the fluid using the black major sampler (17:03, 337C). The sulfide was sample and inboard Niskin bottle triggered (17:13-14). After changing the video tape we set off in search of UM1 and the Riftia patch and our next set of chambers. We arrived at the UM1 marker (x4585, y78208, z=2513m, 17:24) deployed chambers #58 and #72 and retrieved #52 and #54 (biobox 4). Around the slope (x4587, y78209, 17:35) we found the Riftia patch and deployed #66 and #68 and retrieved #49, #50 and one of Stace Beaulieu's 2 basalt panels and put them in biobox 2. After weeding out some of the Riftia we still could not find the second basalt panel and decided to move on to Bio9. We found a diffuse flow vent near Bio9 (x4634, y78003, 18:20) complete with anemones sitting in the flow path. We sample the 8C fluid using the red major samplers (18:27) and collect oxide particles off the basalt using the slurp gun (18:31). We also collected two glassy basalts in the milk crate and fired the second Niskin (18:40). To the southeast of Bio9 we found an extinct sulfide on top of a wall of basalt (x4658, y77995). We took one sample for biobox 5 and a larger piece for the milk crate. Our sample cracked the sulfide in two revealing a thick oxidized rind over a pyrite/chalcopyrite interior. Our final goal of the dive was to try and find an extinct sulfide previously identified by Pat Hickey, but first we spent some time over the lobate/pillow contact looking for a lost light, which was located near a sheet flow/hackley margin as imaged by a previous camera tow. We found the 9m tall extinct sulfide (311m from Bio9 on 085°, x5041, y77713, z=2506m, 19:51; Figure 8) but were low on power and unable to surface due to recovery of a buoy by the science crew still on the Atlantis, so we turned off the lights and sat in the dark. After waiting 20min we were informed the buoy was recovered and we again light up

the abyss and started to sample the magnificent sulfide. The small sulfides at the base of the tower proved too fragile for recovery so we moved to the top of the sulfide. There we were able to break a small piece of the top portion of the tower off and place it in biobox 6. At 20:06 we dropped our remaining weights and headed to the surface.

Dive 4061

Port Obs: Erin Banning

Stbd Obs: Marcel Vieira

Pilot: Pat Hickey

The major objectives of this dive were to obtain high- and low-temperature fluid and sulfide samples during a transect from Bio Vent to Choo Choo as well as check mooring positions, deploy a Hobo probe and basalt blocks and retrieve a basalt panel. Upon landing about 160 m east of Bio Vent, one major sampler pair missing an ICL was used successfully at 1523 GMT to collect an ambient seawater sample. We passed over lobate flows on our way to Bio Vent, where we observed at 1529 GMT a large beehive structure atop the vent chimney, surrounded by vent fauna (crabs, mussels, etc.) and large amounts of shimmering water venting both through the beehive and directly out of the chimney. The beehive virtually exploded on contact with Alvin's arm and was cleared out of the way at 1535 GMT to permit fluid sampling from a venting orifice. Two major sampler pairs and one gas-tight sampler were used to successfully sample Bio Vent high-temperature fluids. A fresh sulfide sample was collected from the top of the vent chimney and placed in the milk crate, after which we maneuvered lower on the chimney to collect a weathered sulfide sample from the side of the chimney, which was placed in a biobox. We left the Bio Vent area at about 1605 GMT and drove to Q Vent, passing over more lobate flows with extensive collapse features and bathtub ring features and observed many glassy patches on the lobate flows. As we approached Q Vent we observed a series of old Alvin weights before arriving at Q vent at about 1617 GMT. A gastight sampler was successfully used to collect a high-temperature fluid sample at 1623 GMT, and then a Hobo probe was deployed in the vent orifice before departing from Q Vent at 1626 GMT to drive towards the East Wall. Large amounts of rusty floc on lobate flows were observed enroute. The East Wall mooring was located at 1644 GMT. We departed and headed for Tica, passing over hackley flows with very irregular surfaces. We arrived at Tica at 1657 GMT and obtained a high-temperature probe reading on the venting fluid there. We maneuvered towards the Riftia patch and used a major sampler pair to obtain a diffuse flow sample at about 1713 GMT. A previously deployed incubation basket (#66) was temporarily moved (and then returned) to allow digging in the Riftia patch in an unsuccessful attempt to locate the basalt panel. At 1726 GMT we departed Tica and drove towards Choo. We passed by and obtained a good location on a Tolstoy OBS at 1735 GMT. As we continued towards Choo Choo, we passed over more predominantly sheet flows interspersed with pillows and hackley features. We arrived at Choo Choo at 1826 GMT and located the Choo Choo mooring at 1835 GMT. It was captured by Alvin's arm and moved closer to Choo Choo. After this, a dive flag and basalt blocks were deployed near Choo Choo starting at 1850 GMT and temperature readings were taken on the blocks after deployment. At 1910 GMT we returned to the Choo Choo mooring and began our ascent, following the mooring line. At 1922 GMT we passed the top of the mooring and continued our ascent.

5. Initial Science Results

Sampling and sample descriptions

A total of 15 basalts, 4 slurp gun samples, 10 sulfide samples, 2 sediment push cores, 12 Ti-bottle fluid samples, and 10 Niskin bottle water samples were collected. Recovery of solid samples for geomicrobiological and geochemical studies was achieved by breaking off piece of lava flows and chimney with one of Alvin's manipulator arms and placing the sample in a biobox (microbiological sample) or in a milk crate (geochemical sample) attached to the Alvin basket.

Fluid samples were collected with "major" Ti-bottle samplers and Niskin bottles.

Sample details are listed in Appendix B. Maps with the locations of different sample types are shown in Figure 7.

5.1. Fluid sampling

Twelve fluid samples were obtained using major samplers (Ti-bottles) from six locations (Table 1 and Appendix B): three high temperature vents and two low temperature diffuse sites. For each fluid sample (~750mL), subsamples were saved for inorganic chemistry and archive (250mL), S and C isotopes (40mL), organic (formic) acids (20mL), Fe, Ge and Cu isotopes (250mL). In addition, an aliquot of each fluid (~100mL) was extracted for organic compounds using solid phase microextraction cartridges for later analysis. All samples were preserved (acidified with HCl, frozen, or filtered when appropriate) and stored for onshore analysis. Insoluble or precipitated particles remaining in the Ti-bottles are recovered (DREGS) by rinsing with Milli-Q water and acetone on 0.45 filters and saved for metal analysis.

Ten bottom seawater samples were obtained using the Niskin bottles on-board of the Alvin in the vicinity of diffuse flow area or basalts. 30mL of each were saved for microbial culture and 1L saved for geochemical analysis (Mn, Fe concentration, Ge/Si ratios and Fe and Ge isotopes when appropriate). Geochemical samples were filtered onboard using 0.45um vacuum filtration device and acidified with 1mL of concentrated suprapure HCl. Filters are saved for onshore geochemical analyses.

Geochemistry on board, cleaning, and sample preparation

A variety of samples were frozen for use in onshore analysis of organic compounds. These included: 8 basalt samples, 5 pieces of sulfide chimney, portions of 2 sediment cores, and 3 slurp-gun sediments. After return to the lab, these samples will be extracted and analyzed for organic compounds, with a focus on identification of biomarkers.

Geochemical fluids (from Majors and Niskins) are stored in 250mL and 1L HCl-cleaned HDPE bottles and acidified with suprapur HCl. DREGS, Niskins and low-temperature Major bottles are filtered on board using 0.45um Durapore filters and hand-pumping. Depending on suspended material quantity, slurp gun samples were either stored in HDPE 250mL bottles or filtered on 0.45um filtered and stored in centrifuge tubes.

Sulfide samples for geochemical analyses, petrographic description and archive have been dried under UV lamps and stored in bubble paper or wipes.

H₂S, S isotopes

Using guidelines and materials provided by Pat Shanks (USGS), we sampled fluid samples for determination of H₂S concentration and ³⁴S/³²S isotope ratios. Approximately 30 ml of fluid were transferred from the Ti-bottles directly into evacuated glass bottles that contain a pre-weighed amount of Zn-acetate [Zn(CH₃COO)₂ x 2H₂O]. All fluids were sampled in duplicate.

Organics

A 25 ml aliquot of all Ti-bottle fluid samples was collected in pre-washed 30 ml Nalgene bottles and immediately stored in the -18°C freezer for determination of formic acid contents by Jeff Seewald (WHOI).

pH

Immediately after retrieving the fluids from the sampler, the pH was measured with a handheld pH meter that was calibrated with pH 4 and pH 7 buffer solutions.

5.2. Sampling of rocks and sulfides

Seafloor samples were collected in individual bio-boxes attached to Alvin's basket to avoid cross contamination and minimize exposure to surface sea water. Once on board, the samples stored in the bio-boxes in the 4°C cooler until they were processed in the lab (typically within 30 to 180 minutes). The samples were placed on sterile Al-foil and sterile tools were used at all times to remove weathered surfaces from the rock samples. Sterile chisels and a mortar and pestle were used to break up large, coherent pieces of rock. Sterilization was achieved by spraying with ethanol and subsequent flaming, as well as autoclaving.

Samples were collected for DNA extractions in 2 ml sterile plastic vials and kept frozen at -70°C. Samples for shore-based fluorescent in situ hybridization microscopy (FISH) studies were fixed in 4% paraformaldehyde solution at room temperature for 4 hours, then rinse with 1:1 phosphate-buffer-solution:ethanol (PBS-ethanol) several times and stored in PBS-ethanol at -20°C.

The following enrichment cultures were started immediately following sampling: heterotrophic and autotrophic Fe-oxidizers in gradient tubes, heterotrophic and autotrophic microorganisms in the presence of crushed EPR basalt glass and pyrite in 15 ml centrifuge tubes, heterotrophic Fe and S reducing microorganisms in anaerobic serum bottles that were purged with N₂ gas after inoculation. The gradient tubes for culturing of Fe-oxidizing bacteria were designed to allow dissolved oxygen in solution to vary from atmosphere-saturated at the top to pyrrhotite-pyrite buffered at the interface with the FeS plug at the bottom. A list of the samples taken and cultures started during the cruise is reported in Appendix B.

5.3. Preliminary sample descriptions

5.3.1. Rocks:

A variety of lava flows, ranging from pillowed to lobate to ropey and hackly sheet flows were sampled. Pillow lavas commonly found at steep flow fronts (e.g., sample AT11-20_4055_B2). Lobates are generally common and particularly abundant near the AST where collapse pits are frequent. Sheet flows form the floors of lava channels and are also common in the floor of the western graben. Ridges of hackly lava can be found near the channel margins and interspersed with lobate and pillowed flows. Specific descriptions of the sampled basalt are provided in Appendix B.

All lavas are aphyric and a vesicular to sparsely vesicular. They typically have glassy rinds that grade into spherulitic to variolitic textures and have microcrystalline flow interiors. Lobate crusts typically reveal a non-glassy underside, often marked with lava drips and drip ridges. Samples of pillow basalts are usually in the form of glassy knobs broken off the flow surface. They have a concentric appearance with glass on the outside and a microcrystalline interior that can have several percent of vesicularity, sometimes as single tube-like vesicles with amygdoidal appearance. Cavities in lobate and sheet flow lavas can have smooth surfaces, in which case they likely represent lava stretching upon extrusion, or they have a rugged surface, which may indicate the presence of a vapor phase [e.g., Perfit et al., 2003].

Off-axis lavas are mostly fresh, except thin (<1 mm) coatings of red Fe-oxyhydroxide or whitish to yellowish or brownish staining, which may represent mixtures of clay and silica. Botryoidal Mn oxide coatings are common on rock samples from the EPR 9°29'N area, and can be as much as 2-3 mm thick on rock recovered 2.5 km off-axis. Hydrothermally altered basalt was recovered from two locations in the AST near 9°50'N, in the vicinities of Tica Vent and in the Bio9 area. In both examples, the rocks are covered with Fe-oxyhydroxide rich crusts with a rugged to botryoidal surface. Alteration halos extend up to 1 cm into the interior of hand specimen recovered from these locations. In the case of the Bio9 location, the rocks were exposed to 8°C warm diffuse fluid venting near the base of a large lava pillar. The locale near Tica Vent showed no active venting at the time of sampling.

5.3.2. Sulfides:

Summary:

Three main types of hydrothermal deposits have been recovered during AT11-20 cruise (*Figures 9, 10, and 11*) and include:

(1) Zn-rich chimneys and diffusers with variable enrichment in Fe (mainly pyrite).

Zn-rich chimneys are the most common hydrothermal deposits observed and are characterized by coarse-grained sphalerite lining a well-defined vent orifice. Zn-rich diffusers display multiple tortuous conduits lined with fine-grained sphalerite and probably wurtzite. Abundance of *Alvinella* tubes is a common feature of diffusers suggesting a possible role of biology for the evolution from active chimney to diffusers.

- Active chimneys: ALV-4059-M3

- Active diffusers: ALV-4053-M1(A3-5);

- Inactive chimneys: ALV-4053-M1(B); ALV-4053-M2; ALV-4059-M4

- Inactive diffusers: ALV-4053-M3

(2) Fe-rich hydrothermal deposits with variable enrichment of Zn likely reflect higher temperature venting resulting from less conductive and/or seawater mixing cooling of the high temperature fluid end-member. Fe-rich massive sulfides forming mounds in the vicinity of active chimneys are expected to result from the hydrothermal diagenesis of collapsed fragments of chimneys and diffusers.

- Active chimneys: ALV-4053-M1(A1)

- Inactive chimneys: ALV-4057-M2

- Inactive massive sulfides: ALV-4059-M3; ALV-4058-M1

(3) Cu-rich chimneys are only found at vents emitting fluids at temperature above ~350°C and are typical of black smokers. Only two representatives from Bio9''- and V-vent have been recovered and are characterized by a thick inner layer of well-crystallized chalcopyrite. Massive sulfides displaying significant enrichment in chalcopyrite are expected to result, in part, from the hydrothermal diagenesis of such chimneys.

Active chimney: ALV-4057-M1; ALV-4051-M1

Inactive massive sulfides: ALV-4059-M2

Description of samples:

ALV-4051-M1 (5553, 72380, z?): *K. Van Damm & M. Schulte sample. Sample from active chimney at V-Vent. 2 cm thick chimney wall lined with chalcopyrite. WHOI (O.R.) requested 2 subsamples corresponding to the top and bottom of the sample for geochemical analyses and archive.*

ALV-4053-M1 (x10135, y40041, z 2563): Geochemical sample from K-Vent. M1 has been recovered from a 3-4m high hydrothermal chimney characterized by a pronounced mushroom-shaped top (beehive). The trunk is 1-2 m wide and appears oxidized on outside with no significant animals and emanating fluid. The top is a beehive-like structure, approximately 3 m wide, with numerous little spires that emanate diffuse fluid, which is either clear or milky, light-gray to white in color. The beehive is heavily colonized by *Alvinella* and vent grabs. Sample M1 is from the upper part of the beehive and is a 73 cm long piece of chimney that is composed of two distinct vents (A) Zn-Fe-rich active chimney venting diffuse fluids and thickly colonized by *Alvinella* (and sea anemones). The removal of the chimney at the seafloor caused vigorous venting of fluid from a small, marcasite-lined orifice with a T between 198 and 200°C. The upper part of the chimney colonized by *Alvinella* is composed by fine-grained sphalerite and pyrite/marcasite. Small euhedral sphalerite and wurtzite crystals line the interior of the diffuser whereas the external wall is composed of more botryoidal-like sphalerite and increased amounts of pyrite. The active chimney has been divided into 5 sections (#A1 to #A5) from the bottom to the top (Figure 7). (B) Zn-rich inactive vent with internal conduit composed of coarse-grained euhedral sphalerite coated with soft, amorphous material (possibly clay) reflecting late-stage mineralization leading to extinction of hydrothermal activity. The external wall is composed of mixed assemblages of sphalerite,

pyrite, and silica and shows relicts of *Alvinella* tubes. The inactive chimney has been divided into 3 sections (#B1 to #B3) from bottom to top. Subsample C1 and D1 to D3 represent broken-off pieces of the active Zn-rich diffuser, the location of which with respect to the larger structure cannot be precisely located.

ALV-4053-M2 (x10135, y40041, z 2563): Biological sample from K-Vent. M2 is located about 10 cm left of M1. The sample is an entire spire that is apparently not emanating fluid; it has some *Alvinella* near its tip, but is generally much less densely colonized than sample ALV-4053-M1. M2 is a Zn-rich chimney and is mineralogically and physically similar to the M1#B inactive chimney, being characterized by a well defined central conduit composed of coarse-grained euhedral sphalerite. Subsamples (#a) and (#b) represent the top to central part of the inactive chimney, respectively. The external and internal walls of the chimney are coated with clayey material of collomorph appearance.

ALV-4053-M3 (x10132, x40048, z2564): Biological sample from K-Vent. M3 is sampled from the numerous pieces that became dislodged from the K-vent structure and subsequently were exposed to sea floor weathering. M3 is a rubbly piece of rusty sulfide chimney composed mainly of sphalerite. The numerous traces of tube worm casings and the poorly defined internal conduit suggest that M3 is an ancient Zn-rich diffuser (beehive). Coarse to fine-grained sphalerite and possibly wurtzite compose the main internal part of the sample whereas pyrite becomes more abundant in the external part. The external crust is composed of Fe-Si minerals that could be Fe-rich opal CT (conchoidal, translucent surfaces).

ALV-4057-M1 (x4603, y77924, 2511m, heading 067): Geochemical and biological sample from Bio9''. The hydrothermal chimney has a wide trunk (12x12 inches) that bifurcates into two orifices, one that was venting vigorously, and a second one that was almost clogged but still emanated some black "smoke". The unobstructed orifice is lined with chalcopyrite and emits a vent fluid with temperature of 383°C. Sample M1 represents the entire uppermost 40-50 cm of a Cu-rich black smoker that has been broken apart during sampling. The entire sample M1 has been divided into 17 pieces (#A1 to #A6 and #B1 to #B11) that represent sections from bottom to top. M1 is characterized by a well-zoned mineralogy across the chimney wall that varies in thickness between 1 cm to up to 4 cm. The central conduit is composed of well developed chalcopyrite crystals with only minor sphalerite and pyrite. Anhydrite is ubiquitous associated with chalcopyrite within the chimney wall or as cm-wide patches inside the open conduit reflecting active incorporation of seawater and mixing with the high temperature hydrothermal fluid. Very fine-grained chalcopyrite, sphalerite and pyrite are intermixed with acicular anhydrite. The external cm-wide wall is remarkably poorly oxidized and is composed mainly of pyrite, sphalerite and minor chalcopyrite. Along the chimney wall, mm-wide layers of coarse to fine grained sphalerite can occur locally.

ALV-4057-M2 (x4603, y77927, 2511m): Biological sample of an extinct sulfide structure just north of Bio9'' vent. The sample appears to be related to Fe-rich type black smoker although sphalerite tends to be the major mineral in total abundance. Relics of the central vent conduit are lined with euhedral marcasite (needs verification) forming mm-thick

layers. The core of the ancient chimney wall is characterized by fine grained sphalerite with only minor marcasite and pyrite. The external wall is composed by fine grained pyrite/marcasite with smaller amounts of sphalerite. Red minerals that may correspond to Fe oxide-stained silica-rich minerals (need XRD for identification) make up the external crust. The overall texture of the sample (e.g. multiple former vent orifices) suggests that it is evolving from inactive chimney toward massive sulfide. The abundance of relict *Alvinella* tubes and the enrichment of Zn suggest also that this sample could be related to Zn-rich diffuser vent-type.

ALV-4058-M1 (x4628, y78035, 2511m) Marv Lilley & Brian Dable sample. Several pieces of a ~10cm large massive sulfide donated for geochemical analyses and archive at WHOI. This sample corresponds to a Fe-rich massive sulfide from the Bio9 area. Pyrite is the main mineral occurring as anhedral to euhedral fine to coarse grained crystals. Relict worm tubes are completely mineralized by pyrite and display locally some enrichment of euhedral sphalerite. Marcasite, chalcopyrite and sphalerite occur as accessory phases, disseminated in the pyrite-rich matrix. The external crust of the sample is composed of red translucent crystals (probably Fe-oxide bearing opal CT but need XRD confirmation). Overall, M1 features are very similar to ALV-4059-M2 & M3.

ALV-4059-M1 (x4590, y78205, z2512): Biological sample of small chimney from Tica Vent adjacent to the major high-temperature vent ($T^{\circ}=344^{\circ}\text{C}$). M1 is a Zn-rich active chimney characterized by a central conduit that appears to be clogged, suggesting possibly an abandoned vent orifice or wall. Euhedral fine-grained sphalerite constitutes the major mineral of the chimney whereas pyrite, possibly marcasite and chalcopyrite occur only as fine grained near the external wall. The entire sample is characterized by a remarkable white coating of possibly amorphous Si. There is no trace of *Alvinella* tubes.

ALV-4059-M2 (x4655, y77998, z2504): Geochemical sample from inactive sulfide mount SE Bio9. M2 corresponds to Fe-rich massive sulfide with local Cu enrichment. It is a large piece (8x8 inch) composed primarily of massive to euhedral pyrite. Numerous relict *Alvinella* tubes are replaced and lined by markedly euhedral pyrite. Locally, abundant euhedral chalcopyrite crystals occur along the former vent orifice. Most chalcopyrite grains display remarkable iridescence coloration due to minor alteration to secondary Cu-sulfides. Euhedral sphalerite developed locally in void spaces. The well-developed external crust of Fe-oxide reflects intensive seafloor oxidative alteration. Oxidation does not extent into the interior of the sample (although very porous) as suggested by the lack of chalcopyrite replacement.

ALV-4059-M3 (x4655, y77998, z2504): Biological sample recovered in the vicinity of M2 sample. M3 corresponds to Fe-rich massive sulfides with local Zn enrichment. Numerous relicts of *Alvinella* tubes and vent orifices appear randomly distributed in cross sections of the sample. Tube worm walls are mineralized with massive pyrite whereas void space inside the tube is filled with either euhedral pyrite or sphalerite. The external side of the massive sulfide was exposed to seawater and is heavily encrusted with and replaced by Fe-oxides. Overall, different generations of sulfides (from massive to euhedral pyrite)

reflect a protracted period of hydrothermal diagenesis of primary Fe-rich black smoker type.

ALV-4059-M4 (x5046, y78003, z2512): Biological sample recovered from the top 1m of a 9-m tall inactive chimney off-axis (300m from AST). Because the central vent orifice has not been sampled, M4 may not be representative of the entire edifice. M4 is a Zn-rich inactive chimney with significant Fe enrichment. The sample shows a complex succession of sulfide layers that do not permit the identification of a central orifice. The interior of the former active vent is apparently composed of euhedral sphalerite forming mm to cm wide layers, followed by fine grained assemblages of sphalerite, pyrite and possibly chalcopyrite. Botryoidal sphalerite and fine grained pyrite form the external walls that are coated with Fe-Si-rich red to ochreous material. Amorphous Si is locally enriched as large patches along surface exposed to seawater. The relatively fresh parts of sample interior contrast with the off-axis location of the chimney and may, together with some residual biological activity suggest relatively recent hydrothermal activity.

5.3.3. Fluids:

Table 1 gives an overview of the fluids sampled with the Ti-bottle “major” sampler. Further details on the sample location and relations between fluid and solid samples are provided in Appendix B.

Sample	Location	T (T probe)	T (ICL)	H ₂ S odor	precipitates	pH
4053_W1	K-vent	203	>76 ^a	y	n	4.53
4053_W2	K-vent	203	>150 ^a	y	n	5.30
4057_W1	Bio9"	383	374	y	y	3.21
4057_W2	Bio9"	383	374	y	y	3.30
4059_W1	Tica Vent	344	338	y	y	3.16
4059_W2	Tica Vent	344	338	y	y	3.13
4059_W3	Near Bio9	8	8	n	n	7.00
4059_W4	Near Bio9	8	8	n	n	7.00
4061_W1	Biovent	331	311	y	y	6.21
4061_W2	Biovent	331	311	y	y	6.07
4061_W3	Tica Riftia patch	NA	15	n	n	4.45
4061_W4	Tica Riftia patch	NA	15	n	n	3.79

a: ICL temperature probe was not fully inserted into the chimney orifice

5.4 Microbiological Studies

5.4.1. Incubation studies

During the AT11-7 cruise in February of 2004 we deployed chambers containing polished petrographic thick sections (4 thick sections per chamber) of basalt, basaltic glass, goethite, dunite, laboradorite, pyrite, marcasite, hematite, and synthetic glass. Two physical forms have been used in our thick section incubations. The first is a whole petrographic thick section, the second is a similar petrographic thick section that has been cut by a diamond blade into a grid, which enables the sample to be divided between various treatments. After 8 months

of reaction, we have retrieved selected chambers for culturing, fluorescent/electron/atomic force microscopy, and DNA libraries. We also deployed new chambers containing four different sulfide minerals (collected from the EPR 9°N vent sites by Dr. Meg Tivey), basalt and basaltic glass (also from the EPR 9°N area), pyrite, gelatin coated slides (to determine the in situ production of Fe precipitates), dunite, labradorite, and goethite. These incubation periods, coupled with incubations at the Juan de Fuca ridge (1 month) and Loihi seamount (years) will provide a systematic time series over a diverse range of environments. These experiments are likely to be important on ocean basin scales, which will help to elucidate the general weathering cycle of deep sea rocks and minerals. Our group was also able to perform two short-term incubations during this cruise. The first was a 48-hour deployment at the 9°28 area with chambers containing basalts, sulfides and gelatin. The second deployment was a week-long incubation at the 9°50 area with similar thick sections. Biologically sealed boxes (bioboxes) on the Alvin science basket were employed to transport the chambers to and from the seafloor, thereby limiting the chance of contamination by sea surface organisms. Upon retrieval of short- and long-term incubations, the chambers, like all of our samples were handled with aseptic techniques to prevent contamination. Each thick section was digitally imaged multiple times using 6.5X, 8X, 10X, 20X, 40X, and 50X objectives of the Zeiss 2000C microscope by Dr. Stace Beaulieu. When animals were present on the surfaces they were picked off by Dr. Beaulieu and the thick sections were then processed in the Bio-analytical lab. Sections cut into the grid pattern were divided by removing individual squares using sterile forceps. Generally, parts of the minerals were preserved for fluorescent in situ hybridization (FISH see below), microscopy (SEM see below), and culturing (see below). These analyses will be carried out at WHOI.

5.4.2. *Culturing*

To address what physiological groups of bacteria are present in and on deep sea rocks and minerals, an extensive suite of selective and non-selective (enrichment) media was inoculated with various rock, mineral and water samples from the hydrothermal vent fields and ocean crust regime of 9°N (see spreadsheet). Samples were brought onboard the Atlantis by the DSV Alvin in bioboxes (7 total) to limit contamination from microorganisms residing in the surface ocean. After Alvin was secured in its hanger, the bioboxes were removed from the science basket and placed in the cold room until processing. Water samples from each biobox were obtained for DNA extraction (see below) and analysis for sea surface microbe contamination. Inoculum for culturing was obtained from these retrieved samples either by scraping the material of interest (ex. Fe-oxides, Mn-oxides, microbial mats etc.) from the exterior and/or interior surface using sterile forceps and scoopulas or by grinding chunks of the rock's exterior in a mortar and pestle. The interior was accessed through physically breaking the rock or sulfide using a sterile chisel (sulfide and basalt equipment remained separated). Samples for inoculum were ground with a sterile mortar and pestle until a powder fine enough to pass through a 26G needle was obtained. This powder was suspended in filter-sterilized bottom water or artificial seawater and used as inoculum for the various culturing media and methods. The microorganisms of interest, selected for by our media composition, include those that conserve energy from the oxidation of iron, sulfide and manganese as well as from the reduction of iron and sulfate. Three culture media have been employed to select for iron-oxidizing bacteria (FeOB) from all available sample types: anaerobic liquid media containing 5 mM NO_3^- which selects for autotrophic FeOB; gradient tubes adapted from Kucera and Wolfe [1957] selecting for aerobic and microaerophilic autotrophic FeOB; and liquid media containing citrate and acetate as

carbon sources designed for anaerobic heterotrophic Fe-reducing bacteria. To select for sulfur-cycling microorganisms, microaerobic liquid cultures containing citrate and acetate were designed to select for chemoheterotrophic sulfide-oxidizers and chemoautotrophic sulfide-oxidizers are also selected for by the above-mentioned gradient tube method. In addition, acetate- and citrate-containing liquid culture media was designed to select for heterotrophic sulfate-reducing bacteria. Manganese (Mn) oxidizing organisms were enriched for using autotrophic liquid media (5 μ M and 100 μ M Mn(II)), autotrophic gradient tubes similar to those used for FeOB containing a heterogeneously distributed 10 μ M Mn(II) and agar plates containing 10 μ molal Mn(II). Non-selective enrichments include all the rock and mineral samples recovered from the abyss, placed in a 1:1 solution of filtered bottom water and an artificial seawater media. In total we have in excess of 800 cultures started from the sample obtained from 5 DSV Alvin dives in the 9°50 and 9°28 hydrothermal areas. These cultures will be brought back to WHOI for further cultivation and isolation of pure strains for various physiological studies providing a glimpse of the physiological diversity of organisms capable of exploiting the energy stored in the rocks and sulfides produced at mid-ocean ridges.

5.4.3. DNA libraries

To address the question of what organisms are present in and on the rock and sulfide minerals, we have constructed a large library of samples preserved for extraction of 16S rRNA genes and genomic DNA (referred to collectively as DNA) for the construction of large insert libraries. Preservation of samples for 16S rRNA libraries entails rapid freezing of small amounts of rock, mineral and water samples from the Niskin bottles and bioboxes for later processing ashore. The slurp gun samples of Fe-oxide microbial mat material provide an excellent substrate for our genomic DNA extraction techniques. This method requires the freezing of large volumes of Fe-oxide material and later extraction on shore using a CTAB extraction protocol followed by shearing and insertion of the DNA fragments into fosmid libraries. DNA from rock samples and incubation chamber materials will be extracted using a MoBio Soil DNA extraction kit or a phenol/chloroform freeze-thaw extraction method. Water samples from the bioboxes were treated by two techniques. One sample was treated in the normal manner (immediate freezing), while the second sample was filtered through a 0.02 μ m syringe filter and the DNA was extracted without freezing using a MoBio DNA extraction kit. PCR and phylogenetic analysis of all samples will be done at WHOI.

5.4.4. Fluorescent in situ Hybridization (FISH)

Often knowing which microorganisms are present in an environment is simply not enough information to constrain the ecological significance of these organisms in the environment. FISH is a method of staining the ribosomal RNA, which is only present at significant levels in active organisms (as opposed to DNA which is more refractory), for observation using fluorescence microscopy. The information garnered from FISH studies of the rock and sulfide minerals preserved from these Alvin dives can provide insight into which type of organism is dominant on specific minerals, or within specific zones of the mineral structure, as well as the association and potential interactions between the microbe and mineral. These samples have been preserved in a 1:1 paraformaldehyde:ethanol (vol:vol) solution and placed at -20°C for analysis upon return to Woods Hole.

5.4.5. Electron Microscopy

Portions of particularly interesting samples obtained by Alvin and petrographic thick sections have been preserved in 2%glutaraldehyde or 4% paraformaldehyde for SEM imaging at MBL and TEM imaging at the University of California, Berkeley. In particular we plan on performing SEM and TEM analyses of the filamentous microbial mats, weathered basalts, and sulfides obtained during this cruise, as well as on the prepared mineral sections from the short-term and eight-month incubation experiments. These analyses will provide us with insight into the various products and minerals formed from both chemical and biological alteration of seafloor rocks and minerals.

Figures and figure captions

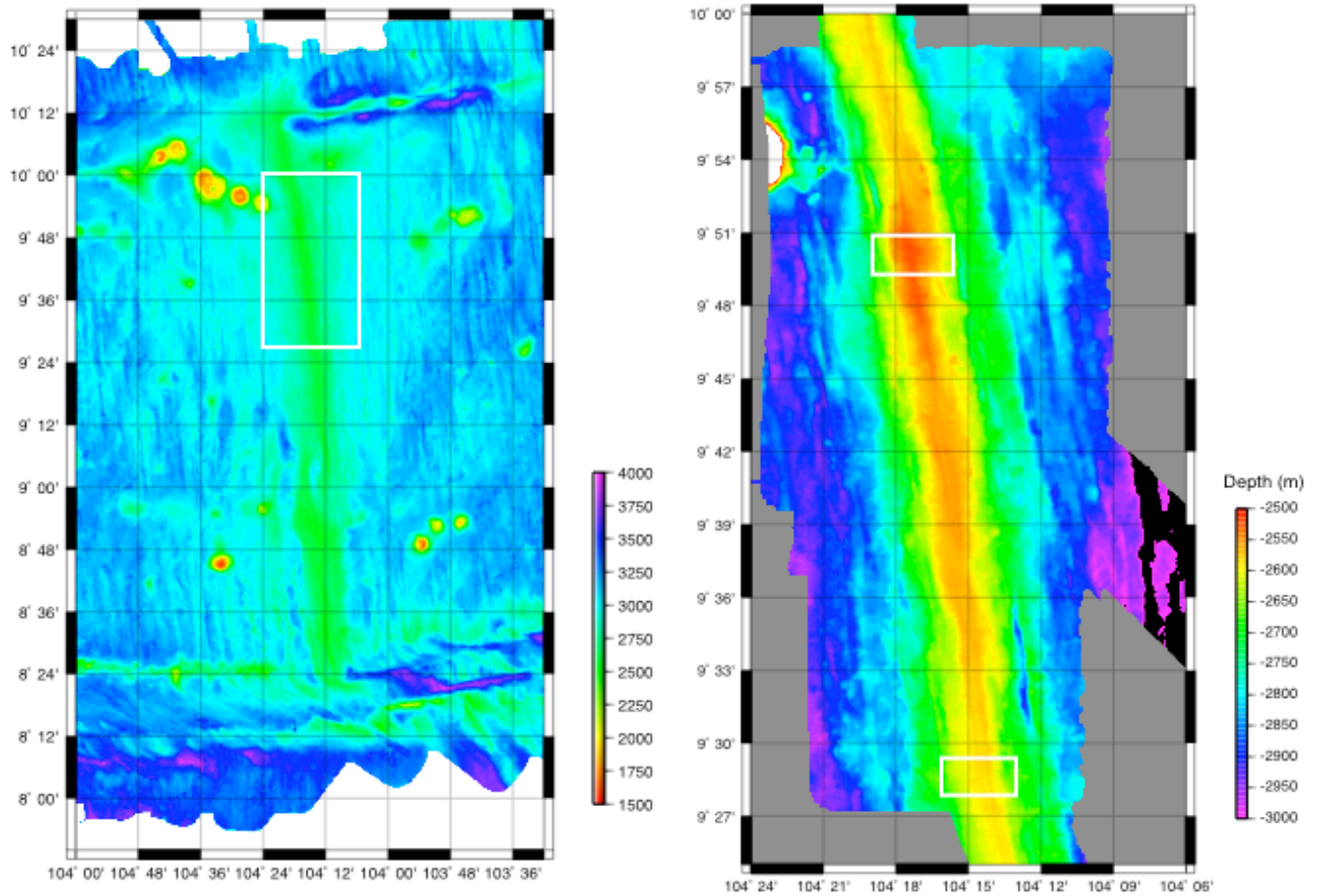


Figure 1: Bathymetric maps of the EPR. Left: regional map based on SeaMARC2 bathymetry [Macdonald *et al.*, 1992] of the EPR 9°N segment that is bordered by the Clipperton transform in the north and the Siqueiros transform in the south. Right: map of the area marked by a white box in the left panel that is based on multibeam sonar data [Cochran *et al.*, 1999]. Here white boxes represent areas for which microbathymetry data are available (see Figure 2).

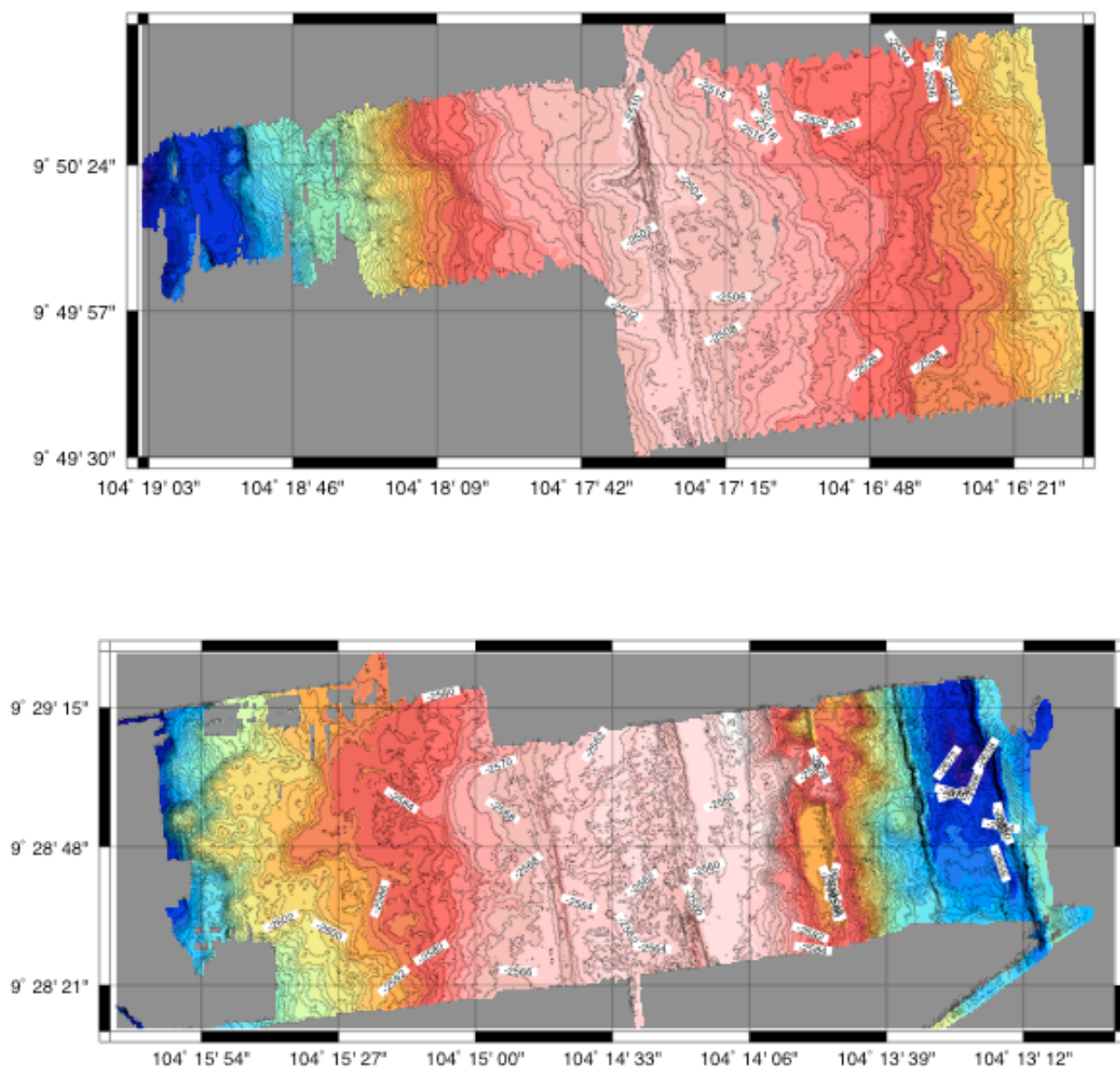


Figure 2: ABE microbathymetric maps of the EPR in the 9°50'N area (upper panel) and the 9°28'N area (lower panel).

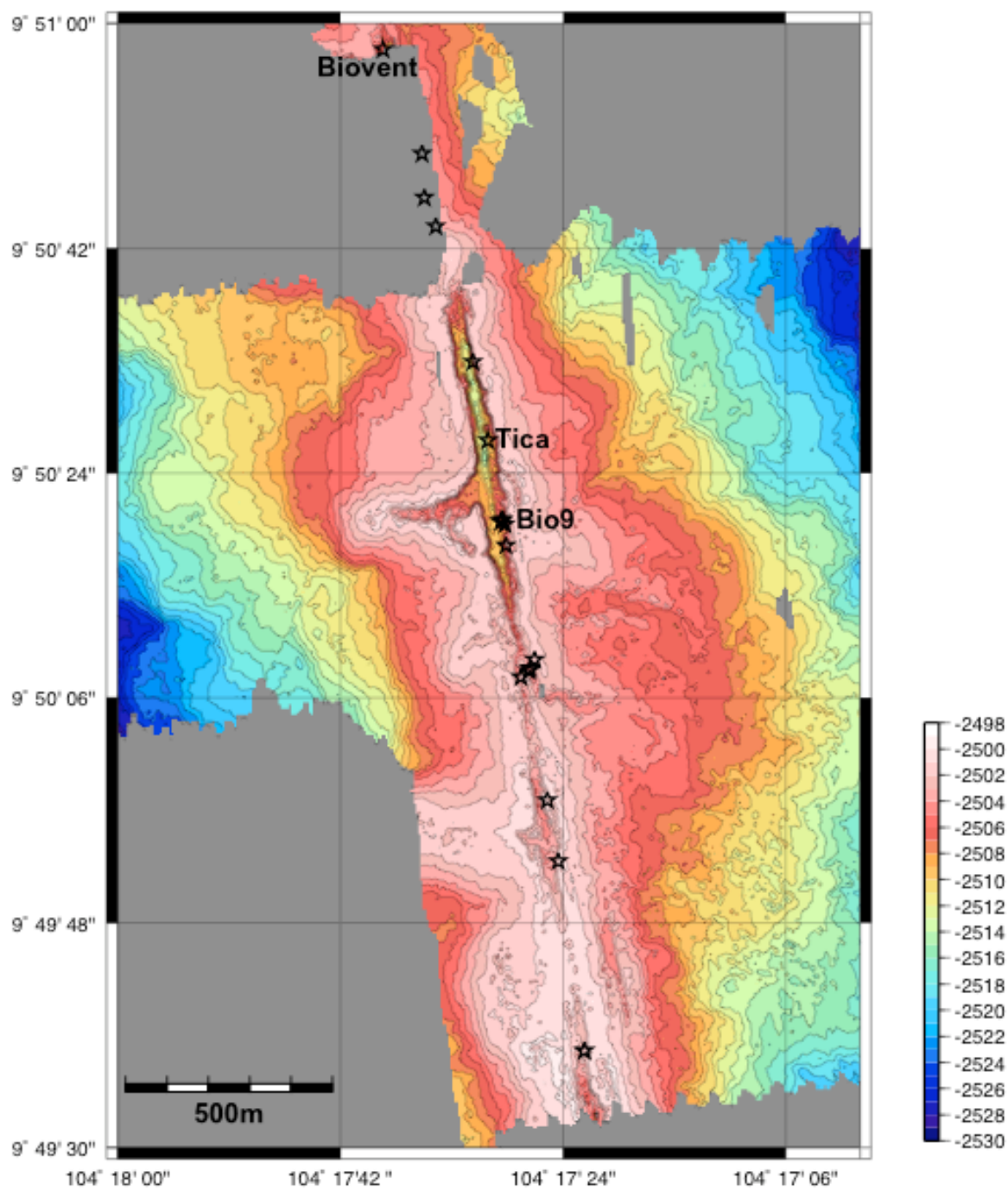


Figure 3: Location map of known hydrothermal vents in the 9°50'N area superimposed onto the ABE microbathymetry map. Only sites samples by our group during cruise AT11-20 are labeled.

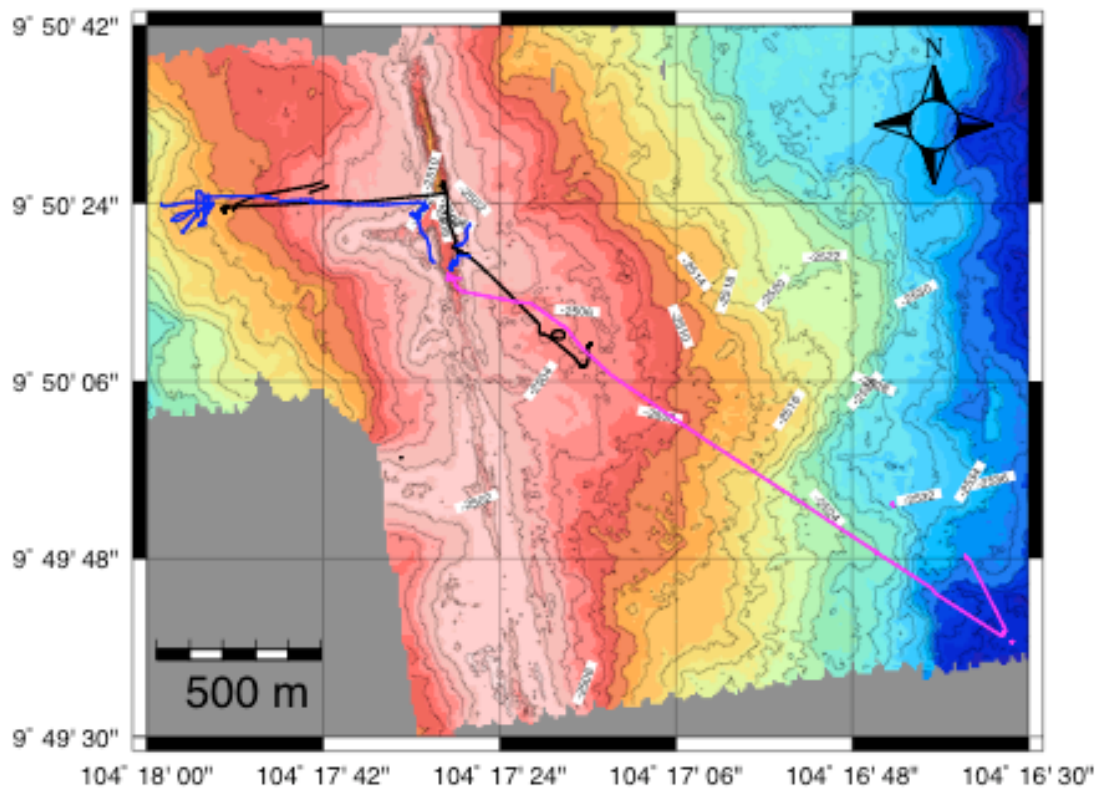


Figure 4: Dive tracks in the 9°50'N area superimposed onto an ABE microbathymetry map. Blue: Dive 4049, magenta: Dive 4057, black: dive 4059.

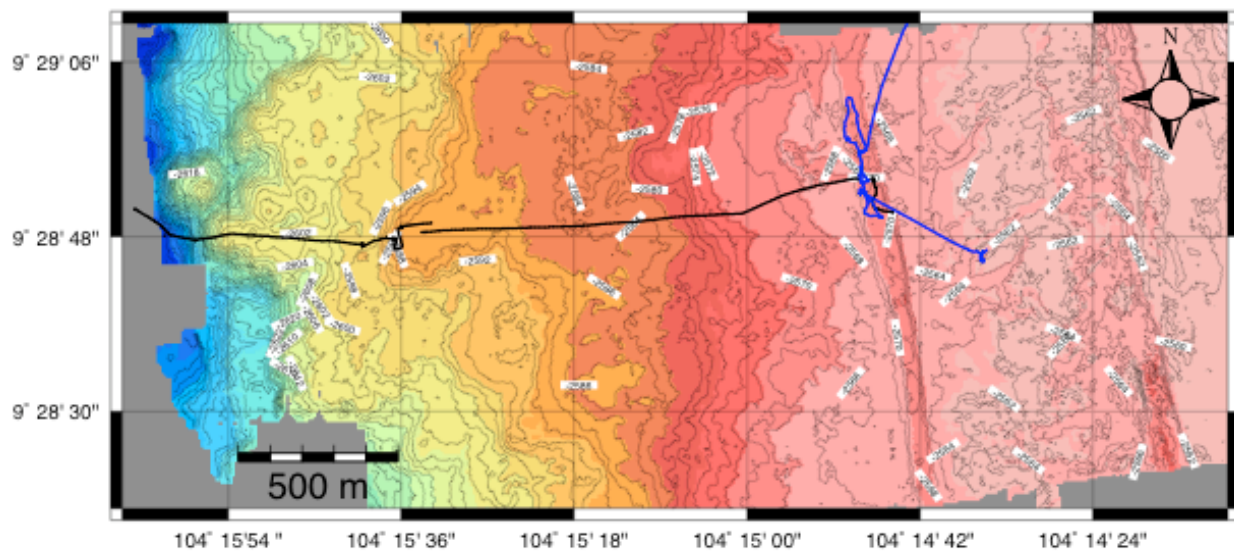


Figure 5: Dive tracks in the 9°29'N area superimposed onto an ABE microbathymetry map. Blue: Dive 4053, black: dive 4055.

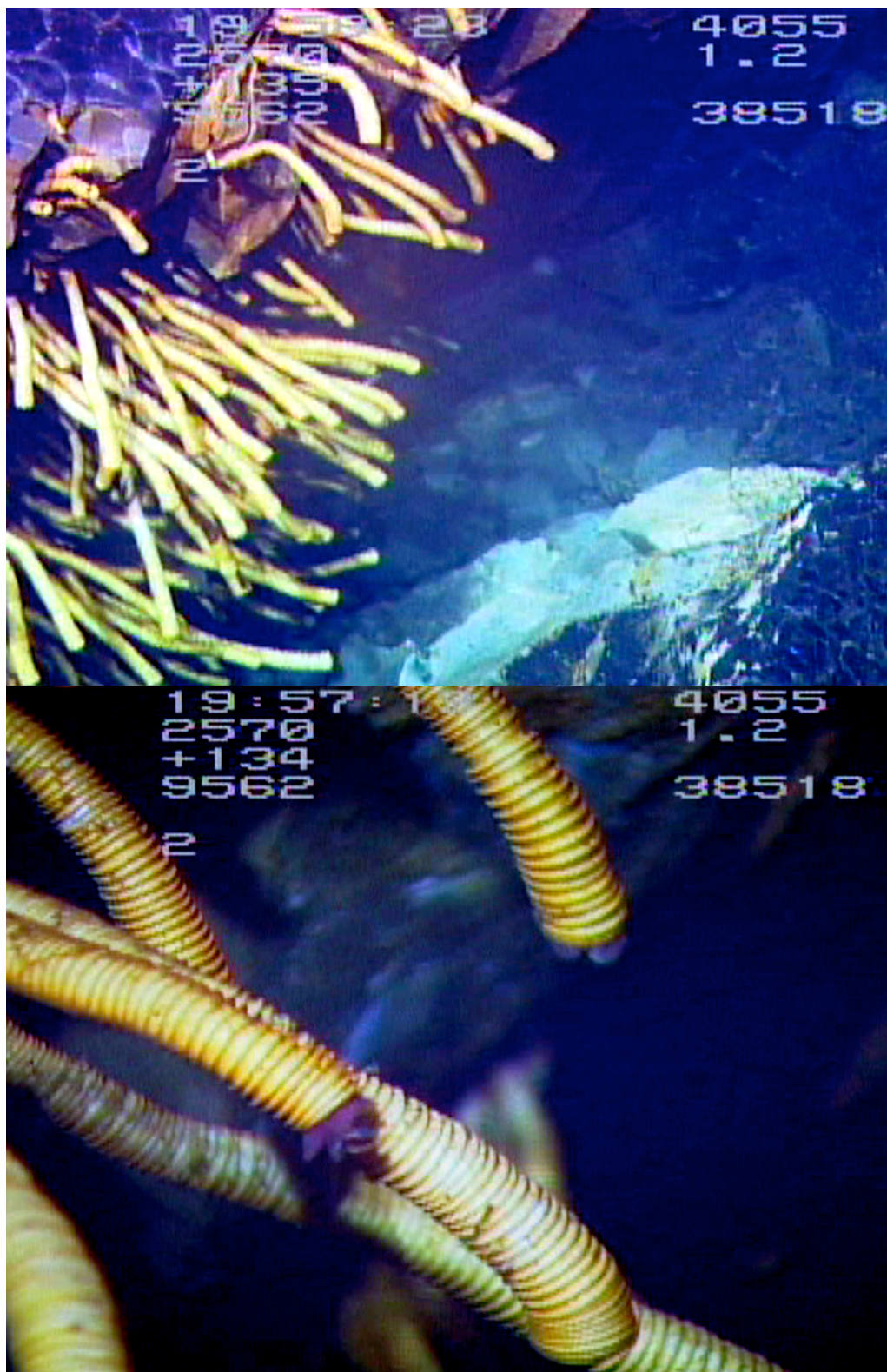


Figure 6. Images of the *Tevnia* colony observed in the Western Graben on dive 4055 (near Marker ESB#8).

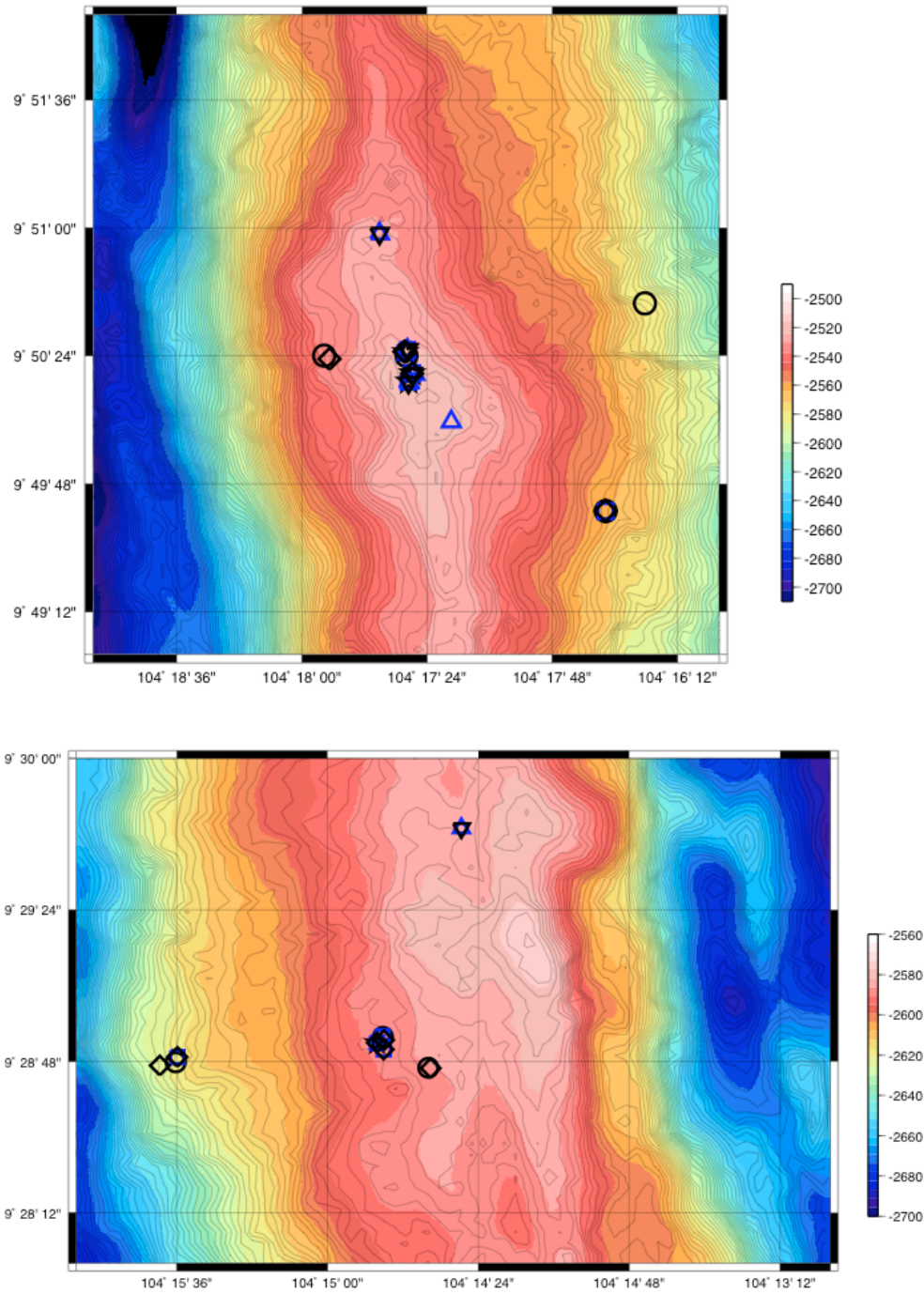
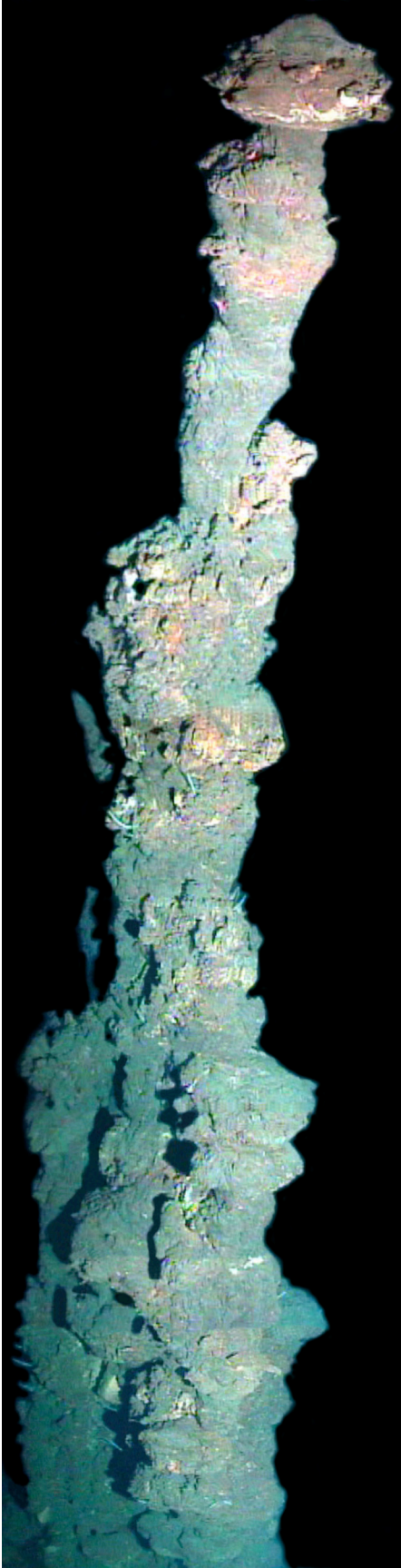


Figure 7: Sample and incubation basket locations in the 9°50'N area (upper panel) and the 9°28'N area (lower panel). Circles: incubation baskets, diamonds: basalts, triangles: sulfides, stars: slurp gun sample, inverted triangle: major fluid sample, squares: Niskin samples

Figure 8. Large, extinct, off-axis sulfide chimney sampled on Dive 4059. This sulfide is 9 meter high and is 1-2 meters wide. A small sample was taken from the top of the structure. Worms can be seen in this image and in the video at various places along the structure, and the sample retrieved exhibited only minor amounts of weathering. These observations suggest that hydrothermal activity was fairly recent. The type of worms on this chimney were not known to shipboard vent biologist Stace Beaulieu. Expedition leader Pat Hickey originally discovered this chimney on an off-axis dive, and indicates that there are several other similar chimneys in close proximity. The divers on 4059 did not observe any other chimneys in this area but the use of lights at this site was extremely limited due to battery power issues.



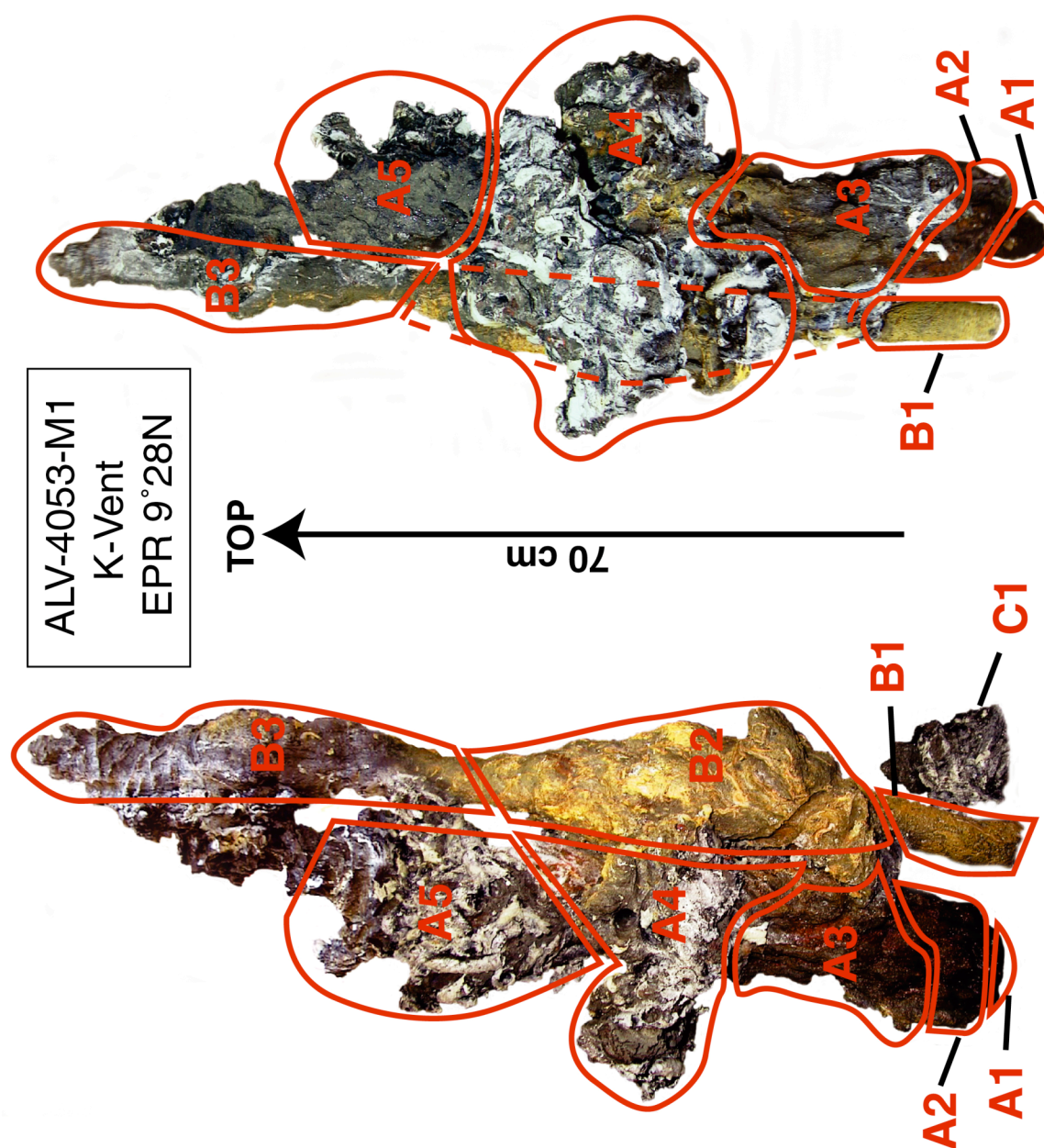


Figure 9: Photomosaics of sample AT11-20_4053_M1. Red lines and labels mark subsamples for onshore geochemical studies. See text for a detailed description.

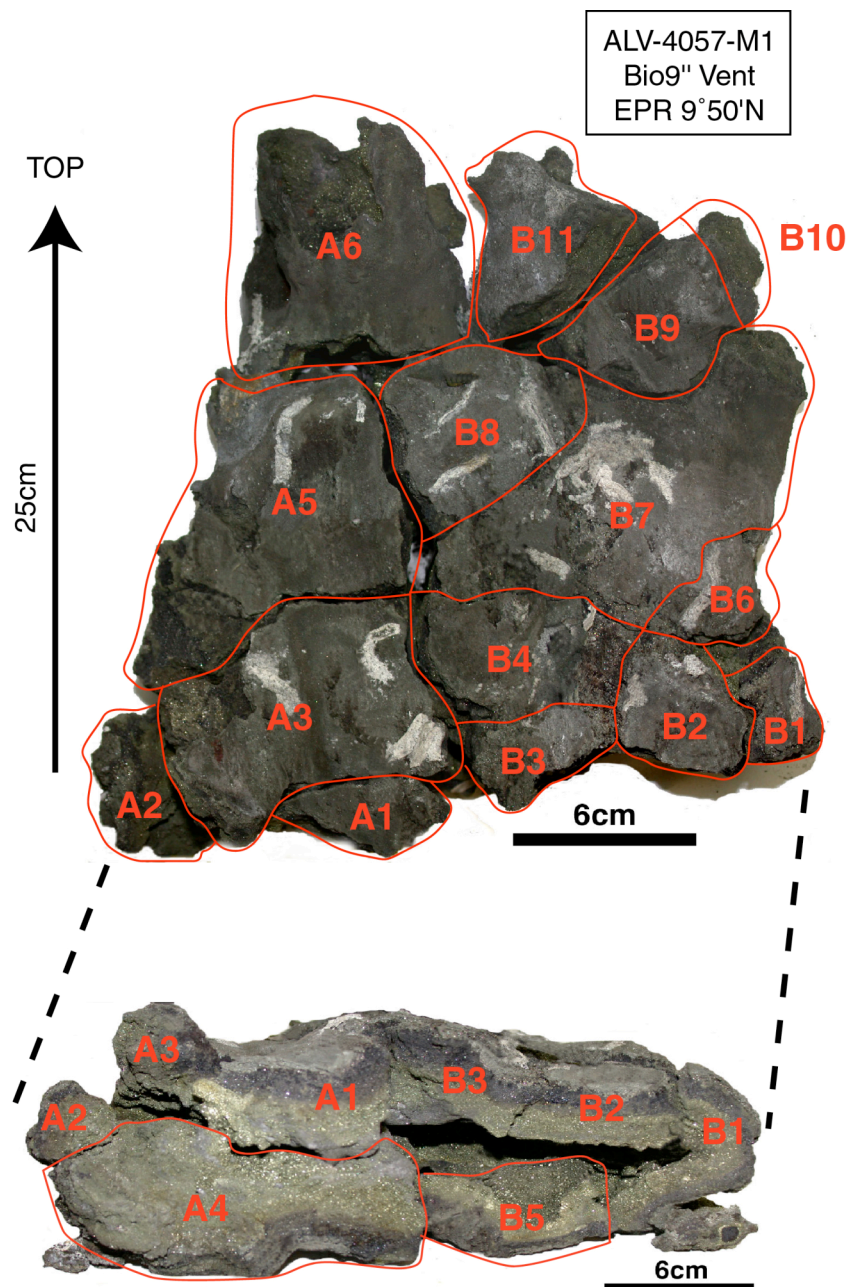


Figure 10: Photomosaics of sample AT11-20_4057_M1. Red lines and labels mark subsamples for onshore geochemical studies. See text for a detailed description.

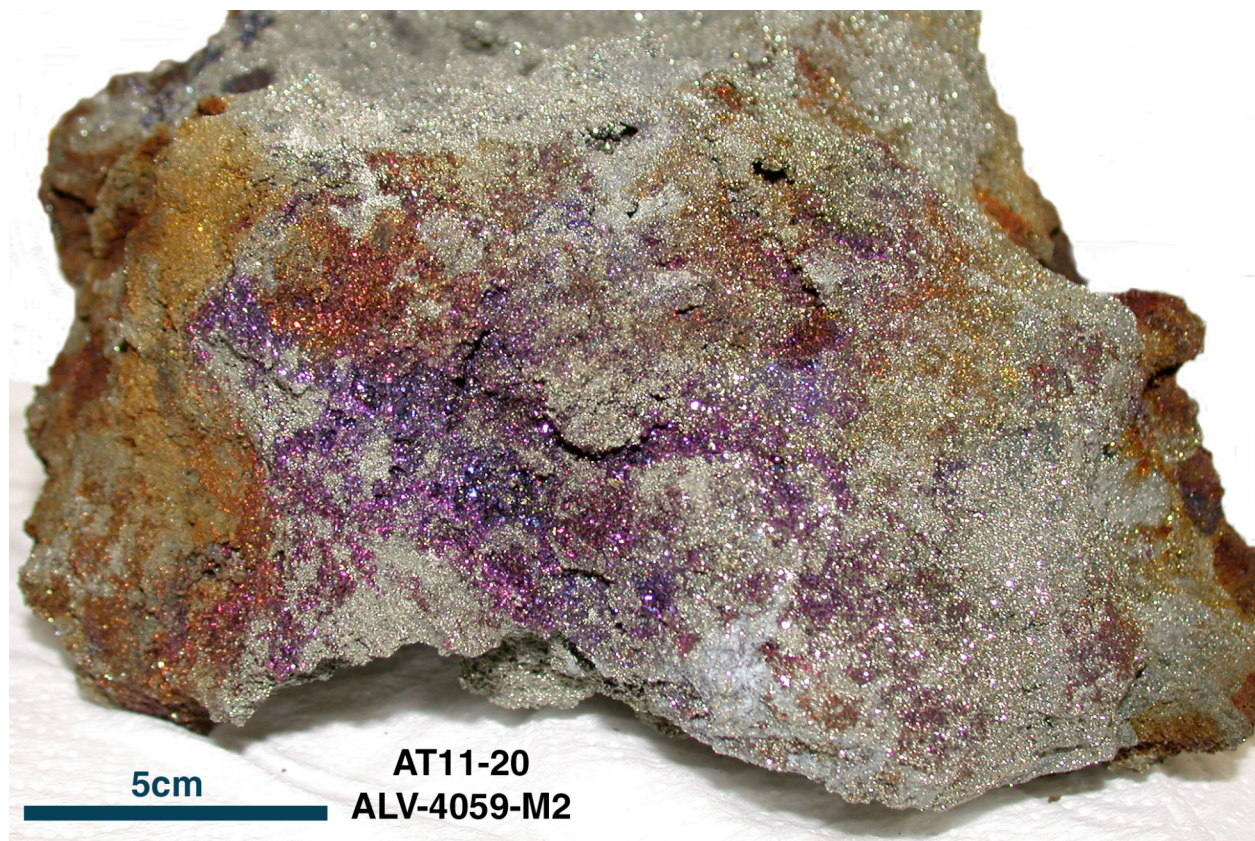


Figure 11: Photo of sample AT11-20_4059_M2.. See text for a detailed description.