AUV *Sentry* in Guaymas Basin 2018

Proposal by Andreas Teske (UNC-Chapel Hill), Carl Kaiser & S. Adam Soule (WHOI)

Background and scientific rationale. Hydrothermal fluids in Guaymas Basin are rich in microbial energy sources (sulfides, reduced metals, hydrocarbons) which migrate upwards through the sediment layers and ultimately reach the water column, at numerous on- and off-axis locations with hydrothermal seepage (Lonsdale and Becker 1985, Lizarralde et al. 2011, Teske et al. 2016). The chemical and biological oxidation, and microbial assimilation of hydrothermal compounds during migration has been termed "the microbial gauntlet" (Teske et al. 2014), where numerous functional classes of microorganisms participate. Essential players are the sulfate-dependent methane-oxidizing archaea in hydrothermal sediments (Teske et al. 2002; Biddle et al. 2012), diverse sulfate-reducing bacteria in sediments, often with hydrocarbon-oxidizing capabilities (Dhillon et al. 2003, Kniemeyer et al. 2007, Teske 2010), and nitrate-reducing filamentous sulfur-oxidizing bacteria at the sediment/seawater interface (Nelson et al. 1989; McKay et al. 2012: MacGregor et al. 2013). Metagenomic and -transcriptomic surveys of the water column and hydrothermal plumes in Guaymas Basin have identified aerobic metal-, hydrogen-, methane-, sulfur- and ammonia-oxidizing bacterial and archaeal populations that occur widely in the water column, and differ from their counterparts in the sediment (Dick and Tebo 2010; Lesniewski et al. 2012; Anantharaman et al. 2013, Dick et al. 2013).

Since these microbial surveys were based on CTD sampling of the water column, spatial resolution and depth resolution are limited. Samples fell essentially into generalized "hydrothermal plume" and "background" categories (Lesniewski et al. 2012); the plume samples were obtained from different localities at ca. 2000 to 1775 m depth, and were identified by CTD turbidity readings (Dick et al. 2009). Beyond these broad sample categories, little can be said about the relationship between microbial community structure and proximity to hydrothermal features and hydrothermal venting. Two possible working hypotheses about the spatial scale of the "microbial gauntlet" in the Guaymas Basin water column can be put forth: 1) Since hydrothermal fluids and plumes are rapidly diluted, their energy-rich compounds are quickly oxidized or consumed by microbes that are already present in the water column. 2) Alternatively, specific hydrothermal microorganisms occur in close proximity to venting hot spots and oxidize much of the hydrothermal energy sources; at some intermediate distance, commonly occurring water column microorganisms take over. Hard data to distinguish these scenarios are lacking, due to the difficulty of tracking and sampling hydrothermal plumes in close spatial resolution, near the seafloor and near hydrothermally active hot spots. In Guaymas Basin, hydrothermal activity is highly localized (Teske et al. 2016), and hydrothermal gradients are defined on a lateral and vertical scale of centimeters (McKay et al. 2012, 2016). Thus, hydrothermal activity in sediment and water column needs to be tracked as accurately as possible for closer study. In consequence, a finely resolved grid of in-situ geochemical and physical parameters is required to inform microbial sampling.

AUV Sentry is often used to conduct systematic chemical surveys of the water column; here we propose to use *Sentry* for a high-resolution survey of hydrothermal plume structure, based on our previous work in December 2016 when *Sentry* mapped the hydrothermal vent fields of the southern Guaymas Basin spreading center and some off-axis seep sites in high spatial and bathymetric resolution (Figure 1). Previous Sentry surveys have included temperature, oxidation/reduction potential (ORP), dissolved oxygen, turbidity, hydrocarbons from a fluorometer, and mass spectroscopy, and they have been carried out in a variety of patterns. The two most common are a 2D grid at a fixed altitude above bottom and a quasi 3D grid created by carrying out stacked 2D grids at a systematic set of altitudes above bottom. Additional patterns have included both linear "yo-yos" intended to simulate a CTD tow-yo but with a very close bottom approach, cylindrical patterns intended to help establish a control volume around a point source, zig-zag patterns intended to follow and characterize a plume, and fixed depth patterns intended to survey far up in the water column.. Our initial survey plan would include ORP, Dissolved Oxygen, Turbidity, and Methane sensors. We would run these surveys in a quasi 3D grid starting at 6 meters above bottom (mab) and then other altitudes based on observed data but likely repeating the grid at 10mab, 25mab, 50mab, and 100mab. We base these mab selections on Sentry observations in 2016 that show localized hydrothermal signatures close to the seafloor at 6-8 mab, which have dissipated at 100 mab (see Figure 2 for an example from the 2016 survey). Additionally, we believe that a "switch-back" approach up each spreading center wall of Guaymas Basin (approx. 100 m high, from 2000 to 1900 m depth, Figure 1) is possible provided that we are not too close to the bottom, for example at 20mab; such a survey would evaluate the transition from the ridge valley to the ridge flanks.

Southern Guaymas Survey. Hydrothermal plume mapping would be conducted at the previously surveyed southern Guaymas Basin vent area at a depth of 2000 m, located at approx. 27º01.00N/111º24.500W (Teske et al. 2016), and at the recently discovered [July 2015] off-axis “Smoker vent” site, at a water depth of ca. 1850 m, located approx. 25 miles further north at 27º24.700 N/111º23.250 W (Bernd et al. 2016). Physical and geochemical indicators such as temperature and redox potential, dissolved oxygen and methane concentrations will be used to map the vertical and lateral extent of hydrothermal plumes and methane seepage in Guaymas Basin, focused on these known locations of hydrothermal massifs. In December 2016 during *Atlantis* cruise AT37-06, *Sentry* mapped hydrothermal areas of the southern Guaymas Basin spreading center (Lonsdale and Becker 1985, Teske et al. 2016) in unprecedented bathymetric resolution Figure 1); five hydrothermal areas are particularly well-documented and suitable for plume mapping:

1) The “Mat Mound” massif, a large cluster [ca. 200 m diameter] of steep hydrothermal mounds, harbors abundant sulfur-oxidizing microbial mats and *Riftia* colonies, and is surrounded by aprons of hydrothermal sediments and *Beggiatoa* mats [27º00.45N/ 111º22.550W]. These mats are proxies for warm seepage where sulfide- and methane-rich fluids permeate the sediment (McKay et al. 2012).

2) A little to the northwest, the rocky hydrothermal edifices “Busted Mushroom” and “Rebecca’s Roost” [27º00.65N/111º24.40W] are rising within 100 m of each other from the seafloor sediments and emit abundant hydrothermal fluids into the water column; “Rebecca’s Roost” is approx. 20 m high and characterized by extensive flanges, lateral hydrothermal formations that resemble pagoda roofs and eaves (Teske et al. 2016).

3) Ca. 300 m to the east, the “Cathedral Hill” area [27º00.70N/111º24.250W] contains at least two [more likely three] adjacent mounds topped with a large hydrothermal edifice each, and surrounded by hydrothermal sediments and mats. Hydrothermal areas No. 1 to 3 are linked by smaller hot spots and mounds, and form a consecutive hydrothermal zone, extending ca. 1 km in southwest-northeast direction.

4) Ca. 500 m northwest of Rebecca’s Roost, a cluster of tall hydrothermal edifices includes the massive “Big Pagoda” Structure [27º00.91N/111º24.65W]; its trunk is overgrown with microbial mats and *Riftia*, indicative of hydrothermal seepage, and topped with extensive hydrothermal flanges (Teske et al. 2016).

5) Approx. 3 nautical miles north, two adjacent clusters of steep and rocky hydrothermal towers, ca. 20 m high and extending 200 m north-south [“Northern Towers” area, 27º02.75N/111º22.850W], are emitting hydrothermal fluids directly into the water column; mat-covered hydrothermal sediments are relatively rare (AT37-06 cruise report).

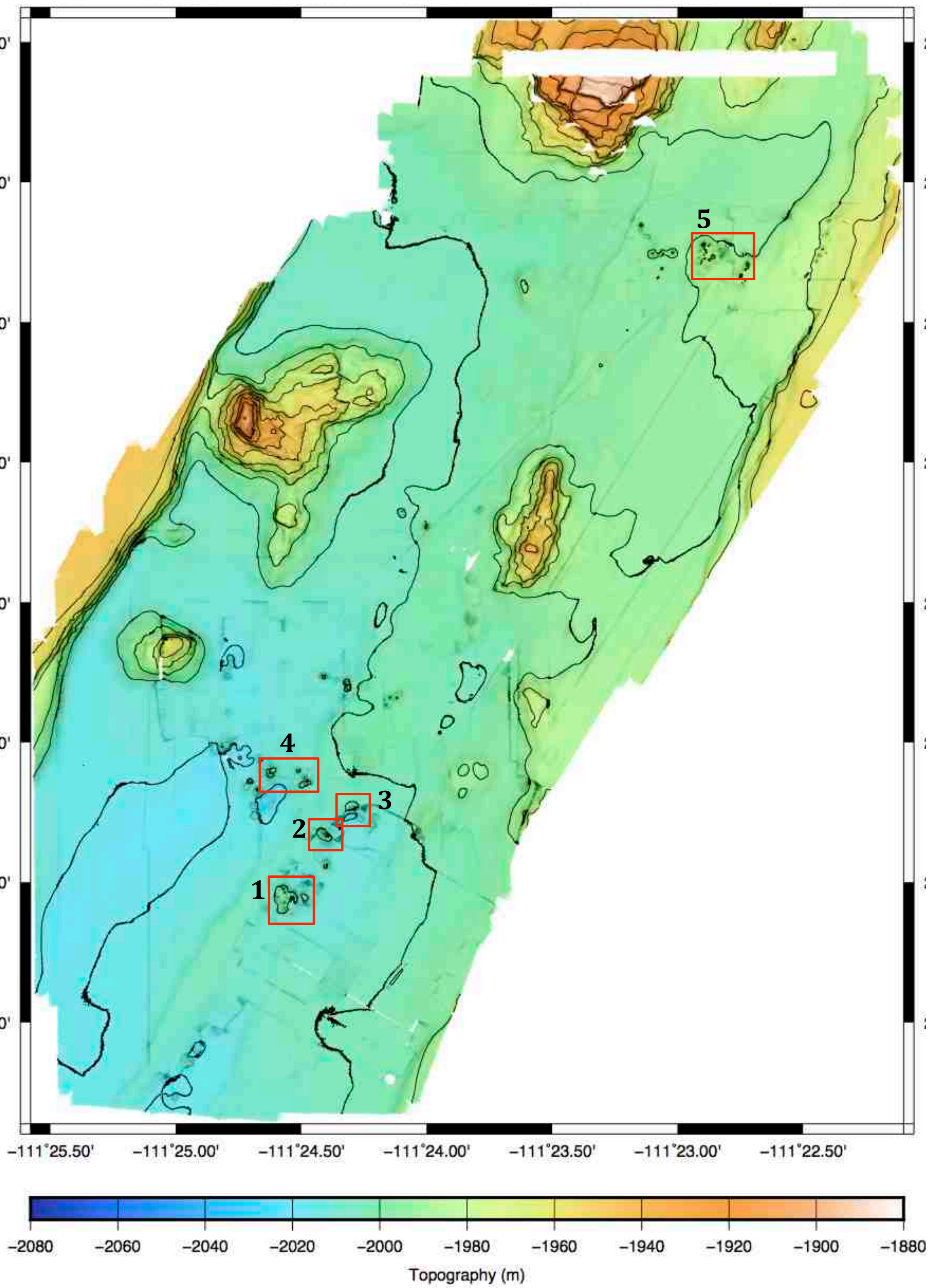

Figure 1. Sentry bathymetric map of the southern Guaymas Basin spreading center, with hydrothermal areas 1 to 5 marked in red boxes.

These hydrothermal features are located in the southern rift valley of Guaymas Basin, which is enclosed by steep walls that rise ca. 100 to 150 m towards the surrounding ridge flanks on both sides. This enclosed basin topography impedes circulation, as shown by consistently reduced oxygen concentrations throughout the deep-water column of Guaymas Basin (Calvert 1964) and in bottom water overlying hydrothermal sediments (Teske et al. 2016). Such conditions are suitable for hydrothermal plume formation and rise in the lower water column. Temperature and redox anomalies were actually observed as localized redox and temperature anomalies by *Sentry* dives 407, 408, 409, 413 and 414, when *Sentry* was used in photomosaic mode and cruised at an altitude of ca. 6-8 m above bottom while covering a hydrothermal area of interest in close spatial resolution (Figure 2).

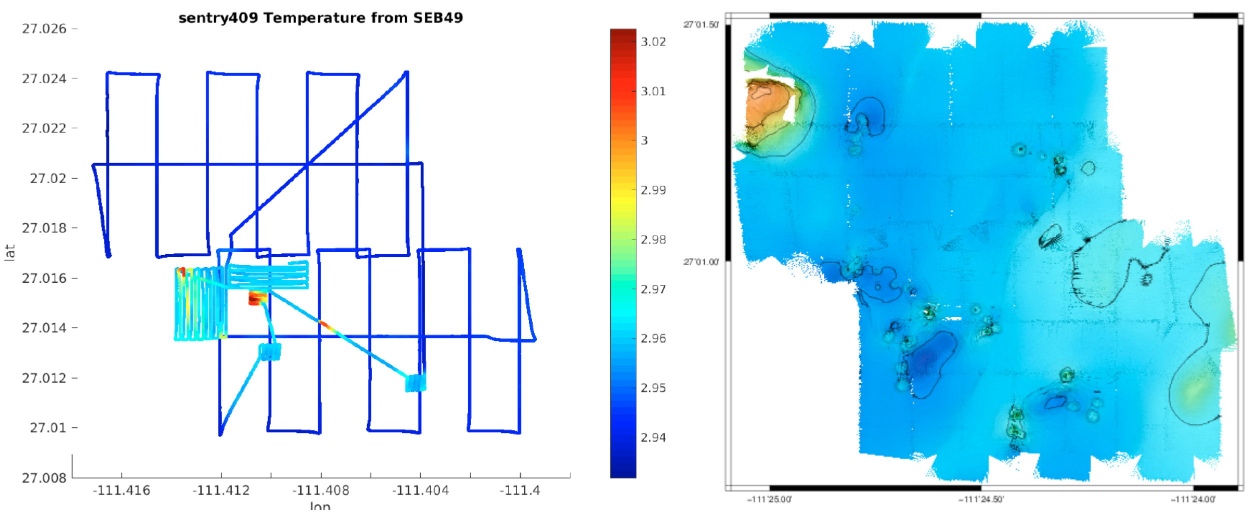

Figure 2. Comparison of temperature data from *Sentry* dive 409 [left] with seafloor bathymetry from the same dive [right], showing that thermal signals are associated with chimneys of the Big Pagoda area, located in the lower left survey area. Here, *Sentry* runs tightly spaced loops in photomosaic mode ca. 8 mab, and records highly localized thermal anomalies [marked in orange and red] indicative of hydrothermal plumes. The more widely spaced survey loops for bathymetry were run at ca. 100 mab [in deep blue] and do not record these thermal signals, which must have dissipated at this elevation.

Northern Guaymas survey. We also propose a survey of the off-axis hydrothermal mound discovered in 2015 just to the east of the northern spreading center (Bernd et al. 2016), approx. 25 miles north of the southern Guaymas spreading center. This steep, rocky hydrothermal mound, with a length of 1 km and a height of ca. 100 m, hosts several active hydrothermal vents on its ridge line, emitting hydrothermal plumes [marked by temperature, turbidity and methane anomalies] that extend ca. 200-300 m into the water column (Bernd et al. 2016, and SO241 cruise report). Thus, the large scale of focused venting at this location appears to be distinct from the widely dispersed, often diffuse and smaller-scale venting venting that characterizes the hydrothermal sediments and smaller mounds and chimneys of the southern Guaymas Basin. While the approximate extent of the plume has been mapped with shipboard CTD sampling and a ridgeline traverse by ROV (Bernd et al. 2016, and SO241 cruise report), finer-scale surveys on a lateral scale, a 3D reconstruction of the plume, and microbial studies of the plume are so far entirely missing.

Sampling plans. Since *Sentry* in itself is not designed for water sample collection or microbial sampling, we will use the *Sentry*-generated data grids on hydrothermal anomalies as a guideline for *Alvin* and CTD sampling. The smaller-scale hydrothermal plumes in the southern Guaymas area require up-close localization and navigation, and are therefore best sampled using the five 10-liter Niskin bottles carried by *Alvin*, sufficient for a vertical or lateral sample series during one dive. From previous dives in 2016 we have good experiences with the *Alvin* Niskin bottle system, although it was used mainly for occasional spot sampling, without mapping gradients systematically. At the hydrothermal mound near the northern Guaymas spreading center, the plumes are large enough to be accessible by CTD profiling and sampling, to supplement *Alvin* sampling. For overall water column context, for example profiling the extensive oxygen minimum zone of Guaymas Basin that runs from ca. 400 m depth to the bottom, we will use CTD sampling. Water samples will be processed for geochemical sampling analogous to sediment porewater samples (Dowell et al. 2016, McKay et al. 216); filter samples will be prepared and frozen for microbial cell counts, DNA/RNA sequencing (itag screening; metagenomics and transcriptomics; see Dombrowski et al. 2017) and geochemical analyses of particulate matter.

Imaging improvements. As a secondary objective for *Sentry*, we will improve photomosaic imaging of Guaymas Basin targets. During the 2016 trip to Guaymas Basin, *Sentry* images of the seafloor, of microbial mats and of hydrothermal structures were relatively dark, due to insufficient lighting that was attributed to very high turbidity as measured by on-board instrumentation and confirmed visually. The resulting images were sufficient and indeed indispensable for *Alvin* dive planning purposes, but not usable for publications [at least not without extensive digital editing and manipulation]. If *Sentry* returns to Guaymas Basin with an imaging mission, the *Sentry* team will attempt to add additional light through the inclusion of up to four Arctic Rays fill strobes; the camera in one of the housings could also be exchanged for a lower resolution, higher sensitivity model. Since AUV multibeam maps of the vent fields are now available, photo surveys can be planned at a lower altitude than previously (4mab vs 6mab) to cover the most promising portions of the terrain.

Operational considerations and synergy. A *Sentry* deployment in Guaymas Basin in November 2018 is easily arranged, since *Sentry* and its crew will be available on R/V *Atlantis* during scheduled cruises before and after Guaymas Basin. No additional loading, offloading, or shipping [a risky proposition in Mexican harbors] is required, as *Sentry* remains on R/V *Atlantis* and is used to maximum advantage in Guaymas Basin. Finally, no additional costs are incurred for NSF supplements to the Teske/Joye/Peterson cruise party; instead, the *Sentry* deployments for plume mapping add considerable scientific value to the Guaymas Basin cruise and will enable an unprecedented endeavor: Coordinated mapping, geochemical and microbiological investigations of hydrothermal plumes in Guaymas Basin will become possible at vertically and laterally finely resolved spatial scales. Last but not least, the availability of plume samples with good pedigree will be a boon for our microbial collaborators who will take their cutting-edge approaches to Guaymas Basin. For example, the BONCAT labeling approach visualizes active microbial cells as they react to different chemical and physical stimuli, and reveal their physiological responses to Guaymas Basin environmental controls (Hatzenpichler et al. 2016). Stable carbon isotope probing experiments for hydrocarbon utilization, combined with metagenomic reconstruction of the active microbial fraction was very successful in oil-contaminated Gulf of Mexico water samples after the Deepwater Horizon oil spill (Dombrowski et al. 2016), and will open up new perspectives in Guaymas Basin as well. As always, fortune favors the prepared.

References.

Anantharaman, K., J.A. Breier, C.S Sheik, and G.J. Dick. 2013. Evidence for hydrogen oxidation and metabolic plasticity in widespread deep-sea sulfur-oxidizing bacteria. Proc. Natl. Acad. Sci. USA 110:330-335.

Berndt, C., C. Hensen, C. Mortera-Gutierrez, S. Sakar, G. Geilert, M. Schmidt, V. Liebertrau, R. Kipfer, F. Scholz, M. Doll, S. Muff, J. Karstens, S. Planke, S. Petersen, C. Bottner, W.-C. Chi, M. Moser, R. Behrendt, A. Fiskal, M.A. Lever, C.-C. Su, L. Deng, M.S. Brennwald, and D. Lizarralde. 2016. Rifting under steam - how rift magmatism triggers methane venting from sedimentary basins. Geology G38049.1; doi: 10.1130/G38049.1

Biddle, J.F., Z. Cardman, H. Mendlovitz, D.B. Albert, K.G. Lloyd, A. Boetius, and A. Teske. 2012. Anaerobic oxidation of methane at different temperature regimes in Guaymas Basin hydrothermal sediments. The ISME Journal 6:1018-1031.

Calvert, S.E. 1964. Factors affecting distribution of laminated diatomaceous sediments in the Gulf of California. pp. 311-330. In: Marine Geology in the Gulf of California, Vol. 3, T.H. Van Andel and G.G. Jr Shor. American Association of Petroleum Geologists Memoir, Tulsa, OK.

Dhillon, A., A. Teske. J. Dillon, D.A. Stahl, M.L. Sogin. 2003. Molecular characterization of sulfate-reducing bacteria in the Guaymas Basin. Appl. Environ. Microbiol. 69:2765-2772.

Dick, G.J., B.G. Clement, F.J. Fodrie, S.M. Webb, J.R. Bargar, and B.M. Tebo. 2009. Enzymatic microbial Mn(II) oxidation and Mn biooxide production in the Guaymas Basin hydrothermal plume. Geochim. Cosmochim. Acta 73:6517–6530.

Dick. G.J. and B.M. Tebo. 2010. Microbial diversity and biogeochemistry of the Guaymas Basin deep-sea hydrothermal plume. Environmental Microbiology 12:1334-1347

Dick, G.J. K. Anantharaman, B.J. Baker, M. Li, D.C. Reed, and C.S. Sheik. 2013. The microbiology of deep-sea hydrothermal vent plumes: ecological and biogeographical linkages to seafloor and water column habitats. Frontiers in Microbiology 4:124.

Dombrowski, N., J. Donaho, T. Gutierrez, A.P. Teske, and B.J. Baker. 2016. Metabolic pathways of hydrocarbon-degrading bacteria from the Deepwater Horizon oil spill. Nature Microbiology 1:16057

Dombrowski, N., K. Seitz, A. Teske, and B. Baker. 2017. Genomic insights into potential interdependencies in microbial hydrocarbon and nutrient cycling in hydrothermal sediment communities. Microbiome 5:106, doi: 10.1186/s40168-017-0322-2.

Dowell, F., Z. Cardman, S. Dasarathy, M.Y. Kellermann, L.J. McKay, B.J. MacGregor, S.E. Ruff, J.F. Biddle, K.G. Lloyd, J.S. Lipp, K-U. Hinrichs, D.B. Albert, H. Mendlovitz, and A. Teske. 2016. Microbial communities in methane and short alkane-rich hydrothermal sediments of Guaymas Basin. Frontiers in Microbiology 7:17, doi: 10.3389/fmicb.2016.00017.

Hatzenpichler, R., S. Connon, D. Goudeau, R.R. Malmstrom, T. Woyke and V.J. Orphan. 2016. Visualizing in situ translational activity for identifying and sorting slow-growing archaeal-bacterial consortia. Proc. Natl. Acad. Sci. USA 113:E4069-E4078

Kniemeyer, O., F. Musat, S. Sievert, K. Knittel, H. Wilkes, M. Blumenberg, W. Michaelis, A. Classen, C. Bolm, S.B. Joye, and F. Widdel. 2007. Anaerobic oxidation of short-chain hydrocarbons by marine sulphate-reducing bacteria. Nature 449:898-901.

Lesnieswki, S. Jain, K. Anantharaman, P.D. Schloss, and G.J. Dick. 2012. The metatranscriptome of a deep-sea hydrothermal plume is dominated by water column methanotrophs and lithotrophs. The ISME Journal 6:2257-2268.

Lizarralde, D., A. Soule, J. Seewald, and G. Proskurowski. 2011. Carbon release by off-axis magmatism in a young sedimented spreading centre. Nature Geoscience 4, 50–54.

Lonsdale, P. and K. Becker. 1985. Hydrothermal plumes, hot springs, and conductive heat flow in the Southern Trough of Guaymas Basin. Earth Planet. Sci. Lett. 73, 211–225.

MacGregor, B.J., J.F. Biddle, J.R. Siebert, E. Staunton, E. Hegg, A.G. Matthysse, and A. Teske. 2013. Why orange Guaymas Basin *Beggiatoa* spp. are orange: Single-filament genome-enabled identification of an abundant octaheme cytochrome with hydroxylamine oxidase, hydrazine oxidase and nitrite reductase activities. Appl. Environ. Microbiol. 79:1183-1190.

McKay, L.J., B.J. MacGregor, J.F. Biddle, H.P. Mendlovitz, D. Hoer, J.S. Lipp, K.G. Lloyd, and A.P. Teske. 2012. Spatial heterogeneity and underlying geochemistry of phylogenetically diverse orange and white *Beggiatoa* mats in Guaymas Basin hydrothermal sediments. Deep-Sea Research I, 67:21-31.

McKay, L., V. Klokman, H. Mendlovitz, D. LaRowe, M. Zabel, D. Hoer, D. Albert, D. de Beer, J. Amend, A. Teske. 2016. Thermal and geochemical influences on microbial biogeography in the hydrothermal sediments of Guaymas Basin. Environ. Microbiol. Reports 8:150-161.

Nelson, D.C., C.O. Wirsen, and H.W. Jannasch. 1989. Characterization of large, autotrophic *Beggiatoa* spp. abundant at hydrothermal vents of the Guaymas Basin. Appl. Environ. Microbiol. 55:2909-2917.

Teske, A., K.-U. Hinrichs, V. Edgcomb, A. de Vera Gomez, D. Kysela, S. P. Sylva, M. L. Sogin, and H. W. Jannasch. 2002. Microbial diversity in hydrothermal sediments in the Guaymas Basin: Evidence for anaerobic methanotrophic communities. Appl. Environ. Microbiol. 68:1994-2007

Teske, A. 2010. Sulfate-reducing and methanogenic hydrocarbon-oxidizing microbial communities in the marine environment. Part 21: Microbial Communities based on hydrocarbons, oils and fats: Natural habitats. Pp. 2203-2223. Handbook of Hydrocarbon and Lipid Microbiology, editor K. Timmis. Springer, DOI 10.1007/978-3-540-77587-4_160

Teske, A., A.V. Callaghan, and D.E LaRowe. 2014. Biosphere frontiers of subsurface life in the sedimented hydrothermal system of Guaymas Basin. Frontiers in Microbiology 5:362; doi:10.3389/fmicb.2014.00362

Teske, A., D. de Beer, L. McKay, M.K. Tivey, J.F. Biddle, D. Hoer, K.G. Lloyd, M.A. Lever, H.Røy, D.B. Albert, H. Mendlovitz, B. J. MacGregor. 2016. The Guaymas Basin hiking guide to hydrothermal mounds, chimneys and microbial mats: complex seafloor expressions of subsurface hydrothermal circulation. Frontiers in Microbiology 7:75, doi: 10.3389/fmicb.2016.00075