

**Subsurface Source and Biological Fate of Hydrogen in Hot Spring Ecosystems.** M. R. Lindsay<sup>1</sup>, K. E. Fristad<sup>2</sup>, M. J. Amenabar<sup>1</sup>, M. R. Urschel<sup>1</sup>, K. M. Fecteau<sup>3</sup>, Randall V. Debes<sup>4</sup>, John R. Spear<sup>5</sup>, T. M. Hoehler<sup>2, 6</sup>, E. L. Shock<sup>3, 4, 6</sup>, and E. S. Boyd<sup>1, 6</sup>, <sup>1</sup>Montana State University - Department of Microbiology and Immunology, Bozeman, MT, <sup>2</sup>NASA Ames Research Center, Moffett Field, CA, Arizona State University – <sup>3</sup>School of Molecular Sciences, <sup>4</sup>School of Earth and Space Exploration, Tempe, AZ, <sup>5</sup>Department of Civil and Environmental Engineering Colorado School of Mines, Golden, CO, <sup>6</sup>NASA Astrobiology Institute, Mountain View, CA

Hydrogen (H<sub>2</sub>) is prevalent in many hot spring environments and serves as a key source of reductant for microbial inhabitants, including those in Yellowstone National Park (YNP) [1]. The concentration of dissolved H<sub>2</sub> varies in YNP thermal waters, which in turn could influence the extent to which this substrate can support microbial metabolism. However, little is known of the processes that influence H<sub>2</sub> concentrations in thermal waters and how this influences the distribution, abundance, and activities of hot spring populations.

Using previously compiled dissolved gas and aqueous geochemical data from 167 thermal features from a variety of YNP thermal areas [2], we investigated the influence of subsurface processes on the relative abundance of H<sub>2</sub> in hot spring environments. Specifically, we examined the source of H<sub>2</sub> using dissolved helium (He) concentrations as a proxy for deep gaseous input and methane (CH<sub>4</sub>) concentrations as a proxy for crustal gaseous input [2]. Additionally, as hydrothermal fluids undergo subsurface boiling and separation into a vapor (steam) and liquid phase, hydrogen sulfide (H<sub>2</sub>S) gas is thought to partition into the vapor-phase while chloride (Cl<sup>-</sup>) ions are thought to remain in the liquid-phase [3]. Condensation of H<sub>2</sub>S-rich steam in the near subsurface, followed by oxidation with oxygen can generate sulfate (SO<sub>4</sub><sup>2-</sup>) [4]. As such, SO<sub>4</sub><sup>2-</sup> is commonly used as a proxy for vapor phase influenced fluids while Cl<sup>-</sup> concentrations are used as a proxy for liquid-phase fluid input [3, 4].

H<sub>2</sub> concentrations (in proportion of total dissolved gas) were highest in springs with a combination of high mantle-derived gas input that had interacted with crustal rocks, as indicated by high He and CH<sub>4</sub> concentrations. Consistent with this observation, the proportion of H<sub>2</sub> in total dissolved gases was highest in fluids that were influenced by vapor-phase input, as indicated by low Cl<sup>-</sup> and high SO<sub>4</sub><sup>2-</sup> concentrations. It was found that springs with elevated H<sub>2</sub> concentrations were also located near caldera boundaries within the margin of the ring-fracture zone, or in highly faulted regions just outside of the caldera boundary [5]. We suggest that highly reactive steam released from hydrothermal fluids during subsurface phase separation can freely migrate to the surface along fault boundaries and underground channels [5], where it becomes enriched in H<sub>2</sub>

due to enhanced water-rock interactions with crustal minerals enriched in iron (e.g., basalts, rhyolites) [6, 7].

One hydrothermal area which is located in the fracture zone and which has elevated H<sub>2</sub> concentrations is Smokejumper Hot Springs located in the Summit Lake flow (rhyolite bedrock). We sampled three thermal features (SJ1, SJ2, and SJ3) in this area for dissolved gases (H<sub>2</sub>, CH<sub>4</sub>, and CO), aqueous geochemistry (i.e. Cl<sup>-</sup> and SO<sub>4</sub><sup>2-</sup>), and biomass for cultivation and molecular analysis. Among these three springs, SJ3 had the highest concentrations of H<sub>2</sub> (1.9 μM) and CH<sub>4</sub> (30 nM). Moreover, waters from this spring exhibited a Cl<sup>-</sup>/SO<sub>4</sub><sup>2-</sup> ratio among the lowest (0.01) measured in thermal waters from YNP, indicating that this spring has deep subsurface inputs of gas that interact with oxidized, near-surface, meteoric fluids. Cultivation-based approaches indicate that SJ3 also has the highest abundance (~10<sup>6</sup> cells per gram sediment) of H<sub>2</sub> utilizing autotrophic cells when compared to SJ1 and SJ2 (both ~10<sup>4</sup> cells gram sediment). These hydrogenotrophs are capable of utilizing the widest array of oxidants tested (O<sub>2</sub>, Fe<sup>3+</sup>, NO<sub>3</sub><sup>-</sup>, S<sub>2</sub>O<sub>3</sub><sup>2-</sup>, SO<sub>4</sub><sup>2-</sup>, and S<sup>0</sup>), with the sulfide oxidation intermediate product S<sub>2</sub>O<sub>3</sub><sup>2-</sup> yielding the highest abundance of cells. Hydrogenotrophs in the other two hot springs can only use a subset of the oxidants provided. This spring also has the highest abundance of 16S rRNA gene templates indicating that it is the most productive of the three springs sampled. Metagenomic sequencing and targeted transcript quantification reveals an abundance of diverse hydrogenases involved in H<sub>2</sub> cycling. The results suggest that processes that take place in the subsurface exert a strong influence on the abundance and function of microbial communities in surface hot spring environments at the level of H<sub>2</sub> cycling, and contribute necessary energetic substrates to chemolithotrophic communities.

[1] Spear J.R. et al. (2005) *PNAS*, 102.7, 2555-2560. [2] Bergfeld D. et al. (2014) *USGS*, 2011-5012. [3] Fournier R.O. (1989) *Ann. Rev. Earth Plan. Sci.*, 17, 13-53. [4] Nordstrom K. et al. (2009) *Appl. Geochem.*, 24, 191-207. [5] Christiansen, R.L. (2001) *USGS*, Prof. Pap. 729-G. [6] Stevens T. O., & McKinley J. P. (2000) *Environ. Sci. Technol.*, 34, 826-831. [7] McCollom T.M. (2015) *PNAS*, 113 (49), 13965-13970.