

Rates of autotrophy peak in the anoxic interface of a deep hypersaline anoxic basin

EMILY R. PARIS¹, JEFF S. BOWMAN², ELLERY D. INGALL³, PETER T. DORAN⁴, MIGUEL DESMARAIS², CLAIRE ELBON³, JENNIFER B. GLASS³, STEFFEN BUESSECKER¹, CHAD POZARYCKI³, JORDAN MCKAIG³, VERONICA HEGELEIN⁵, MATTHEW MEISTER⁵, ANDREW D. MULLEN⁵, CATHRYN SEPHUS², ENRICA QUARTINI⁵, BRITNEY SCHMIDT⁵ AND ANNE E. DEKAS¹

¹Stanford University

²Scripps Institution of Oceanography

³Georgia Institute of Technology

⁴Louisiana State University

⁵Cornell University

Presenting Author: erparis@stanford.edu

Deep hypersaline anoxic basins (DHABs) are analogous to ocean worlds like Europa and Enceladus and contain a range of extremes that include high pressure, low water activity (a_w), and low oxygen availability. Here, we link microbial activity rates to taxonomic identity in Orca Basin, a 400 km², NaCl-dominated DHAB in the Gulf of Mexico. We employ two single-cell techniques: (1) stable isotope probing and nanoscale secondary ion mass spectrometry (nanoSIMS) and (2) fluorescence-activated cell sorting with subsequent metagenomics sequencing.

We quantified assimilation of ¹³C-bicarbonate and ¹⁵N-ammonium by 5,422 individual cells across 10 depths ranging from seawater (2085 m; $a_w = 0.983$) to hypersaline brine (2320 m; $a_w = 0.710$). Most autotrophic activity was found in the interface region, composing 48 to 53% of the total number of active cells at 2192 m and 2200 m, respectively. The average rate of inorganic carbon assimilation by autotrophs in the interface was 1.7X higher than those in seawater. No autotrophic activity was detected at depths below 2211 m.

Heterotrophs created 2-10% of their new biomass from inorganic carbon depending on the depth, which contributed ~15% of the total inorganic carbon assimilated by the active microbial community at 2200 m. Compared to several factors—including salinity, pH, and water activity—average growth rate had the strongest positive correlation with depletion of nitrate ($R^2 = 0.69$), which reached 0 μM by ~2217 m. The energy required by anaerobic microorganisms to maintain growth at high salt concentrations may be underlying this trend.

To link anabolic activity to taxonomic identity and potential catabolic pathways, we separated active from inactive cells using redox sensor green and fluorescence-activated cell sorting. Preliminary analysis of metagenomes generated from the active fraction of cells at 2202 m revealed that bacterial members of the *Ca. Marinimicrobia* phylum and *Scalindua* genus, as well as archaeal members of the Nitrosopumilaceae family (known autotrophic nitrifiers) were active in the interface. These results indicate a mixed population of active archaea and bacteria at the