



## **2022 Northeast Geobiology Symposium**

April 16<sup>th</sup>

Massachusetts Institute of Technology, Cambridge, MA

## Talks

### Session 1: Evolution, Phylogeny, and Omics

9:10 a.m.

#### **Distribution and phylogeny of denitrification genes within metagenomes and genomes from oxygen deficient zones**

*Irene H. Zhang, Xin Sun, Amal Jayakumar, Bess B. Ward, Gregory P. Fournier, and Andrew R. Babbin*

When oxygen is limiting, denitrifying microbes use nitrogen oxides as terminal electron acceptors, sequentially reducing nitrate ( $\text{NO}_3^-$ ) to nitrite ( $\text{NO}_2^-$ ), nitric oxide (NO), nitrous oxide ( $\text{N}_2\text{O}$ ), and dinitrogen ( $\text{N}_2$ ) through enzymes encoded by the genes *nar*, *nir*, *nor*, and *nos*, respectively. Complete denitrifiers possess the entire suite of genes necessary for reducing  $\text{NO}_3^-$  to  $\text{N}_2$ , while partial denitrifiers possess only one or several of these genes. The evolution of partial vs. complete denitrifiers, as well as their impacts on environmental nitrogen compound gradients, are not yet fully understood. Using large metagenomic datasets, we analyzed the vertical distribution of denitrification genes in two marine oxygen deficient zones (ODZs), hotspots of denitrification. We find *nirS*, *nor*, and *nos* correlate positively with each other but negatively with *nirK* throughout the water column. Additionally, we constructed the first large collection of high-quality metagenome-assembled genomes (MAGs) from ODZs. We demonstrate the prevalence of partial denitrifiers from these MAGs and perform phylogenetic analyses indicating horizontal gene transfer as a possible mechanism behind the fragmentation of the denitrification pathway within microbial genomes.

9:27 am

#### **Harnessing proteomics to explore the low zinc requirements of modern prokaryotes from an Archaean perspective**

*Annaliese C.S. Meyer, Matthew McIlvin, Dawn Moran and Mak A. Saito*

Introduction: Of the many distinguishing features between prokaryotes and eukaryotes, one of the most subtle and perplexing is the disparity in zinc usage. An estimated 30-40% of all proteins contain metals, and 40% of all enzymes with known structures require a metal for catalysis – of all proteins, those containing zinc make up 9% of all proteins in eukaryotes but only 5-6% in prokaryotes, and many of those are known to be cambialistic (i.e., can use more than one type of metal) (Del Prete et al. 2020). Until recently, euxinia was thought to dominate the Archaean Ocean, and the high quantities of sulfides would have led to a dearth of zinc through precipitation reactions (i.e. Canfield 1998). Low zinc content in the early Earth could explain the low zinc quota of prokaryotes (Saito 2003). However, recent studies suggest a ferruginous ocean with comparable zinc to today existed in the Archaean, adding complexity to the previous hypothesis (Scott et al. 2013). Additionally, changes in zinc isotope signals over geologic time (e.g. Isson et al. 2018) indicate that while total zinc abundance in the ocean may have remained relatively constant, the

bioavailability of zinc is likely to have varied over time (Robbins et al. 2013). As such, the reason for the minimal zinc requirement in prokaryotes remains enigmatic, and zinc use and transport in modern environmental prokaryotes has been insufficiently characterized to fully shed light on this question.

**Methods:** We used a model prokaryote, *Pseudomonas aeruginosa*, to investigate the proteomic response to zinc-depleted and zinc-replete media conditions. The parent strain, MPAO1, and several mutant strains with knockouts involved in zinc uptake and use (e.g. *zur*, *znu* ABC transporter genes, pseudopaline synthesis genes) formed the basis of our investigation. Each strain was cultured using trace metal clean methodology. We used liquid chromatography data dependent acquisition mass spectrometry for proteomic analysis (see McIlvin and Saito, 2021).

**Results:** Of the 1983 proteins identified, 142 were significantly differentially expressed between zinc limited and zinc replete conditions. Several proteins involved in copper resistance and efflux were upregulated in the zinc replete condition, corroborating previous observations that these proteins may also be implicated in zinc removal (Caille et al. 2020). Several metal-transporting P-type ATPases and zinc-containing proteases were also upregulated, with some variation between mutants. In the zinc depleted condition, a zinc-independent paralog of the zinc-requiring transcriptional regulator, *DksA*, was upregulated. Several hypothetical proteins that are closely associated in the genome were also upregulated and may form a novel zinc-transporting ABC-type transporter system. Multiple Ton-B dependent receptors also appeared primarily in the zinc-depleted condition. Ton-B dependent receptors have long been implicated in the uptake of zinc, so this finding is unsurprising (Wang et al. 2021, Pederick et al. 2015). Several genes involved in the synthesis of cobalamin, a cobalt-containing modified tetrapyrrole which is a cofactor for the conserved enzymes methylmalonyl-CoA and methionine synthase, appeared predominantly in the zinc-depleted condition. Despite these proteomic differences, there was little difference in growth rate between conditions and between mutants.

**Initial Conclusions:** Taken together, this suggests that modern prokaryotes have a surprising amount of flexibility in transition metal-protein interactions. Our data suggests that *P. aeruginosa* can respond to rapidly changing environmental conditions even with mutations to simulate a more streamlined genome. The prevalence of canonical copper resistance/toxicity genes (indicating an ability to regulate zinc as needed) and the upregulation of possibly non-specific metal transporters and permeases, and the presence of non-zinc paralogs to zinc proteins aids in this flexibility. The inferred lack of aerobic heterotrophy in an early anoxic ocean may also have contributed to a lower zinc requirement in early evolution. More data is needed to determine the evolutionary cause of this flexibility as it contrasts to the stricter zinc quota of eukaryotes.

9:44 am

## **Chimeric Inheritance and Crown-Group Acquisitions of Carbon Fixation Genes within Chlorobiales**

*Madeline Paoletti & Gregory Fournier*

The geological record of microbial metabolisms and ecologies primarily consists of stable isotope fractionations and the diagenetic products of biogenic lipids. Carotenoid lipid biomarkers are particularly useful proxies for reconstructing this record, providing information on microbial

phototroph primary productivity, redox couples, and oxygenation. The biomarkers okenane, hlorobactene, and isorenieratene are generally considered to be evidence of anoxygenic phototrophs, and provide a record that extends to ~1.64 Ga. The utility of the carotenoid biomarker record may be enhanced by examining the carbon isotopic ratios in these products, which are diagnostic for specific pathways of biological carbon fixation found today within different microbial groups. However, this joint inference assumes that microbes have conserved these pathways across the duration of the preserved biomarker record. Testing this hypothesis, we performed phylogenetic analyses of the enzymes constituting the reductive tricarboxylic acid (rTCA) cycle in Chlorobiales, the group of anoxygenic phototrophic bacteria usually implicated in the deposition of hlorobactene and isorenieretane. We find phylogenetically incongruent patterns of inheritance across all enzymes, indicative of horizontal gene transfers to both stem and crown Chlorobiales from multiple potential donor lineages. This indicates that a complete rTCA cycle was independently acquired at least twice within Chlorobiales and was not present in the last common ancestor. When combined with recent molecular clock analyses, these results predict that the Mesoproterozoic lipid biomarker record diagnostic for Chlorobiales should not preserve isotopic fractionations indicative of a full rTCA cycle. Furthermore, we conclude that coupling isotopic and biomarker records is insufficient for reliably reconstructing microbial paleoecologies in the absence of a complementary and consistent phylogenomic narrative.

10:01 am

### **Branching archaeocyathids as ecosystem engineers during the Cambrian radiation**

*Ryan A. Manzuk, Adam C. Maloof, Jaap A. Kaandorp, and Mark Webster*

The rapid origination and diversification of major animal body plans during the early Cambrian coincides with the rise of Earth's first animal-built framework reefs. Given the importance of scleractinian coral reefs as ecological facilitators in modern oceans, we investigate the impact of archaeocyathid reefs as engineered ecosystems during the Cambrian radiation. In this study, we present the first high-resolution, three-dimensional (3D) reconstructions of branching archaeocyathid individuals from three localities on the Laurentian paleocontinent. Because branched forms in sponges and corals display phenotypic plasticity that preserves characteristics of the surrounding growth environment, we compare morphological measurements from our fossil specimens to those of modern corals to infer the surface conditions of Earth's first reefs. These data demonstrate that archaeocyathid sponges could withstand and influence the flow of water, accommodate photosymbionts, and build topographically complex and stable structures much like corals today. We also recognize a stepwise increase in the roughness of reef environments in the lower Cambrian, which would have laid a foundation for more abundant and diverse coevolving fauna. We pair our models and measurements with high-resolution mapping of each reef in the field based upon drone-derived ortho-photographs and elevation models, as well as a dGPS-constrained observations and sample grids. In the lab, we have developed high-resolution multispectral imaging techniques to perform quantitative image analysis of thin sections and polished slabs from each sampled point, constraining lithology and biodiversity trends on each reef spanning both space and time. Analyzing these reef-scale patterns in concert with the 3D

morphology of Archaeocyathids, we identify the potential influence of reef construction on early Cambrian ecology and evolution.

## **Session 2: The Fossil Record & Diagenetic Processes**

10:50 a.m.

### **Investigating a Proposed Algal Origin for Precambrian Sponge Steranes**

*Juliana Drozd, Fatima Husain, Gareth Izon, Roger Summons*

Life leaves molecular traces in the fossil record, which can provide a lens to understand the history of life on Earth, even when body fossils are not present. One widely discussed molecular fossil, known as a biomarker, for unraveling the history of animal life on Earth is 24-isopropylcholestane (24-ipc), the C30 hydrocarbon derivative of 24-isopropylidene cholesterol, an uncommon marine sterol that is the major sterol in some species of demosponges. The hydrocarbon 24-ipc has been identified in many late Neoproterozoic rocks and oils just before first definitive appearances of metazoan fossils. The sponge biomarker hypothesis argues that this Neoproterozoic 24-ipc comes from ancient sponges, which would have implications for the timing of the evolution of animals. Despite the sponge biomarker hypothesis having considerable support in terms of the secular distribution patterns of 24-ipc and the limited biological distributions of its biological precursor, it has seen numerous challenges. In one example, Bobrovskiy et al. (2020) suggest that the sponge steranes found in the rock record have a diagenetic origin via methyl migrations from and to C29 algal sterols. Here, we examine the suggested diagenetic production of 24-ipc from environmental and purified algal sterols based on geologically-plausible diagenesis experiments in order to further understand the preservation pathways of algal sterols.

11:07 a.m.

### **Moldic preservation of microbial mats and animal tissues in silica-rich solutions: implications for the preservation of the Ediacara Biota**

*Silvina Slagter, Weiduo Hao, Noah J. Planavsky, Kurt O. Konhauser, Lidya G. Tarhan*

Fossils of the Ediacara Biota provide a critical window into the emergence of complex multicellular life. However, the mechanisms responsible for their exceptional "Ediacara-style" fossilization (moldic replication of soft tissues in sandstones) are still extensively debated. Fossils of these enigmatic organisms are often associated with "textured organic surfaces" (Gehling & Droser, 2009) recording the common presence of heterogeneous, mat-forming microbial communities on the Ediacaran seafloor, and which have been proposed to have fundamentally shaped the ecology of early animals. Here, we explore the role of microbial mats in fostering Ediacara-style fossilization, combining an experimental approach with a detailed, molecular-scale characterization of interactions between organic substrates and dissolved silica. We performed taphonomic experiments including cyanobacteria, green algae, and marine invertebrates embedded

in quartz sand under conditions representative of Ediacaran seawater (e.g., high dissolved silica levels and normal marine pH) to assess the role of organic substrates in mediating silica precipitation and macroorganism fossilization. Dissolved silica concentrations were observed to decrease with time, coincident with the precipitation of silica nano- and microspheres onto microbial mat and macroorganism surfaces. Amorphous silica precipitates preferentially nucleated onto microbial mats and surrounding pore spaces, cementing the sand grains into coherent molds. Fourier transform infrared spectroscopy and potentiometric acid-base titrations indicate that higher rates of silica precipitation were associated with the presence of particular functional groups present in the degrading organic matter of both micro- and macroorganisms. Our results provide evidence that soft tissues can silicify under conditions characteristic of Ediacara seawater and be preserved as coherent molds in sandy sediments on timescales of days to weeks. This observation indicates that microbial mats not only played an important role in the ecology and evolution of complex ecosystems but also in their exceptional preservation in the fossil record.

11:24 a.m.

### **The evolution of early Cambrian shallow marine environments: Refining the lower Cambrian stratigraphy of Esmeralda County, NV**

*Mary C. Lonsdale, Athena Eyster, Stacey H. Gerasimov, Nizhoni M. O'Connell, Sara B. Pruss, Emily F. Smith*

The early Cambrian (ca. 540 – 509 Ma) marks a turning point in evolutionary history, characterized by a rapid diversification of animal life and an associated dramatic reorganization of marine ecosystems. Lower Cambrian shallow marine strata capturing much of this biotic change are well preserved in the White-Inyo Ranges, CA, and Esmeralda County, NV, and form the most expanded lower Cambrian marine record deposited along the southwestern margin of Laurentia. Consequently, the lower Cambrian strata of this area have been the subject of a number of ecological and paleontological studies that, collectively, document the regional expression of early Cambrian ecosystems of southwestern Laurentia. The local environmental and depositional contexts of these ecosystems, however, have not been as thoroughly addressed. We aim to contribute to these contexts, addressing the question of how shallow marine environments changed during this evolutionary interval.

Here, we refine the early Cambrian stratigraphy and sedimentology of Esmeralda County, NV. We focus on a ~40 km<sup>2</sup> area NE of the ghost town Gold Point. We present a 1:24,000-scale geologic map of the site, as well as composite regional stratigraphy of the Poleta through lower Mule Springs formations, spanning parts of Stages 3 and 4 (Botomian and Toyonian stages). These units are deformed by high angle, conjugate fault sets associated with Basin and Range extension. We characterize and measure these strata across several fault blocks, capturing lateral variability within units along strike. In doing so, we contribute to the stratigraphic framework in which archaeocyathan reefs and reef-dwelling organisms (e.g. trilobites, hyoliths, and other small shelly fauna) are placed. Finally, we interpret the sequence stratigraphic and paleoenvironmental contexts of these units, characterizing the local evolution of the shallow marine environment at a turning point in evolutionary history.

11:41 a.m.

### **Calibrating the Neoproterozoic Hecla Hoek Succession, Svalbard, Norway**

*Alexie E.G. Millikin, Justin V. Strauss, Galen P. Halverson, Kristin D. Bergmann, Nicholas J. Tosca, Timothy M. Gibson, Ross P. Anderson, Alan D. Rooney*

The Tonian-Ediacaran Hecla Hoek succession of Svalbard, Norway is one of the most complete and well-preserved Neoproterozoic sedimentary archives worldwide. With diverse fossil assemblages, an extensive carbonate  $\delta^{13}\text{C}$  record, and sedimentological evidence for two distinct Cryogenian glaciations, this succession has and will continue to yield profound insights into the Neoproterozoic Earth system. Here we present the first direct radiometric age constraints from the Hecla Hoek succession. Two new Re-Os ages, initial Os values, and Os isotope chemostratigraphy tightly constrain the age of Neoproterozoic glaciation in Svalbard and provide insight into pre- and post-glacial weathering regimes. An age from the Russøya Member (Elbobreen Formation) facilitates correlation of the negative carbon isotope excursion recorded therein with the pre-glacial ca. 735 Ma “Islay” anomaly of the Callison Lake Formation (Windermere Supergroup) in Northwest Canada and the Didikama and Matheos formations (Tambien Group) in Ethiopia. Russøya Member vase-shaped microfossils can now also be allied to global records with pre-Cryogenian biostratigraphic significance. These results solidify global chemo- and biostratigraphic correlations for the late Tonian Period and lay the groundwork for assessing the synchronicity of perturbations to biogeochemical cycles during this interval. The data provide a unique opportunity to construct a temporally calibrated geochemical, biological, and geological framework to assess the timing and triggers of early eukaryotic evolution and the most extreme climatic changes in Earth history.

### **Session 3: Isotopes & Friends**

2:30 p.m.

#### **Combinatorial and rate effects on the multiply-substituted isotope signatures in methane during biological production and consumption**

*Jiawen Li, Beverly Chiu, Alec Cobban, Alison Piasecki, Vinitra Nathan, Jeemin H. Rhim, Edward D. Young, William D. Leavitt*

Methane is an important energy source, a potent greenhouse gas and a potential proxy for extraterrestrial life. Constraining the sources and sinks of methane is crucial for understanding methane cycling on Earth and other planets. The relative abundances of multiply-substituted (“clumped”) isotopologues  $^{13}\text{CH}_3\text{D}$  and  $^{12}\text{CH}_2\text{D}_2$  ( $\Delta^{13}\text{CH}_3\text{D}$  and  $\Delta^{12}\text{CH}_2\text{D}_2$ ) are potentially powerful tools to track processes of methane formation and destruction. However, substantial uncertainties exist in the interpretation of clumped isotope signatures of natural methane, in part due to a lack of understanding of fractionation mechanisms by microbial methanotrophy and anaerobic methanogenesis. Recent studies show that combinatorial effect is a key factor that

influences clumped isotope signatures. Other studies propose metabolic rates could affect clumped isotope signatures as well. However, more experimentation is needed to test these hypotheses.

We conduct two suites of experiments, one to test how the rate of aerobic methanotrophy affects methane clumped isotope signatures, and the other to investigate the role of combinatorial effects during different pathways of methanogenesis. In one suite of experiments, we monitor the change of  $\Delta^{13}\text{CH}_3\text{D}$  and  $\Delta^{12}\text{CH}_2\text{D}_2$  during aerobic oxidation by *Methylococcus capsulatus* Bath at different growth rates controlled by temperature. In the second suite of experiments, we test the combinatorial effects resulting from enzymatically-mediated kinetic steps within a cell (endogenous) and isotopically distinct external hydrogen pools such as growth medium (exogenous) on  $\Delta^{13}\text{CH}_3\text{D}$  and  $\Delta^{12}\text{CH}_2\text{D}_2$ . *Methanosarcina barkeri* Fusaro was cultivated on methanol, acetate or hydrogen/carbon dioxide. We see evidence for exogenous combinatorial effects during methylotrophic and acetotrophic methanogenesis, while hydrogenotrophic methanogenesis is mainly governed by endogenous combinatorial effects. More nuanced mixing of methane from different metabolic pathways is also identified during acetotrophic methane formation. The broader goal is to compare our results with microbial and environmental models, and to further develop  $\Delta^{13}\text{CH}_3\text{D}$  and  $\Delta^{12}\text{CH}_2\text{D}_2$  values as indicators of methane provenance.

2:47 p.m.

### **Carbon isotopic signatures of microbial trophic levels: insights from microbial mats**

*Ana C. Gonzalez-Nayeck, Wiebke Mohr, Tiantian Tang, Sarah Sattin, M. Nichole Parenteau, Linda Jahnke, Sharon L. Grim, Gregory J. Dick, Ann Pearson*

In modern ecosystems, the carbon stable isotope ( $\delta^{13}\text{C}$ ) ratios of consumers generally conform to the principle “you are what you eat, + 1 ‰.” However, this metric may not apply to microbial mat systems where diverse communities, utilizing a variety of carbon substrates via multiple assimilation pathways, live in close physical association and phagocytosis is minimal or absent. To interpret the  $\delta^{13}\text{C}$  record of the Proterozoic and early Paleozoic, when mat-based productivity likely was widespread, it is necessary to understand how a microbially driven producer-consumer structure affects the  $\delta^{13}\text{C}$  compositions of biomass and preservable lipids. Protein Stable Isotope Fingerprinting (P-SIF) is a recently developed method that allows measurement of the  $\delta^{13}\text{C}$  values of whole proteins, separated from environmental samples and identified taxonomically via proteomics. Here we used P-SIF to determine the trophic relationships in two modern microbial mat samples, one each from Yellowstone National Park and Lake Huron USA. In both cases, values of  $\delta^{13}\text{C}$  of producers and consumers were indistinguishable and indicative of primary production via the Calvin-Benson-Bassham cycle (CBB), indicating that “you are what you eat, + 1 ‰” is not applicable. To explain this finding, we hypothesize that sugar production and consumption dominate the net ecosystem metabolism, yielding a community in which producers and consumers share primary photosynthate as a common resource. Overall, the results confirm that the  $\delta^{13}\text{C}$  composition of microbial biomass and lipids is tied to specific metabolites, rather than to autotrophy versus heterotrophy or to individual trophic levels. Therefore, we suggest that aerobic microbial heterotrophy is simply a case of “you are what you eat.”



3:04 p.m.

### **Rise in the $^{15}\text{N}$ -to- $^{14}\text{N}$ Ratio of Otolith-bound Organic Matter Associated With Late Cretaceous Cooling**

*Zixuan C. Rao, Jessica A. Lueders-Dumont, Gary L. Stringer, Alfredo Martinez-Garcia, Gerald H. Haug, Daniel M. Sigman*

The nitrogen isotopes ( $\delta^{15}\text{N}$ ) of the organic matter preserved in fossil fish otoliths are a promising tool for reconstructing past changes in environmental conditions and trophic level. We analyzed the  $\delta^{15}\text{N}$  of fossil otolith-bound organic matter in Late Cretaceous *Eutawichthys maastrichtiensis*, *E. zideki* and *Pterothrissus* sp. otoliths from three different localities along the U.S. east coast. Despite morphological signs of alteration of the otolith biomineral material, there is no significant difference in otolith-bound organic matter  $\delta^{15}\text{N}$  between well-preserved and poorly preserved otoliths. Both genera showed a  $\sim 4\text{‰}$  increase in otolith-bound organic matter  $\delta^{15}\text{N}$  from Campanian ( $10.89 \pm 1.02\text{‰}$  in *Eutawichthys* spp. and  $11.08 \pm 0.79\text{‰}$  in *Pterothrissus* sp.) to Maastrichtian ( $14.87 \pm 1.25\text{‰}$  and  $14.78 \pm 0.80\text{‰}$ ). The similarity of the change in two separate genera argues against a trophic level change. Moreover, the proximity of the deposits and other geographic considerations argue against the locations of the host sedimentary deposits as the cause. Rather, the N isotopic change is best interpreted as an environmental (i.e., “baseline”) signal at the regional scale or greater. The lower  $\delta^{15}\text{N}$  in the Campanian may be a consequence of its warmer climate, consistent with warming-correlated declines in foraminifera-bound  $\delta^{15}\text{N}$  during the Cenozoic. More specifically, our otolith data are consistent with the foraminifera-based finding that warmer conditions are associated with a lower global rate of water column denitrification. This interpretation is speculative, and  $\delta^{15}\text{N}$  measurements from other fossil types and other regions are called for. Nevertheless, the large, coherent signal observed in this first study suggests that the nitrogen isotopes of fossil otoliths hold important information about the ocean and marine ecosystems over the Earth history.

3:21 p.m.

### **Experimental investigation of amino acid binding as a mechanism for fractionating metal stable isotopes**

*Corday Selden, Kathrin Schilling, Anirban Basu, Nathan Yee*

Background: Cellular metabolism depends on transition metals as protein co-factors. As cellular activity partitions metal isotopes, their ratios can be used to trace biological function. Ab initio calculations based on density functional theory suggest that the incorporation of transition metals into proteins can impart an isotopic fractionation dependent on the metal binding site and, particularly, on the identity of the coordinating amino acid ligands. To experimentally test this hypothesis, we investigated the isotopic fractionation of zinc and copper due to coordination by amino acids that commonly act as ligands at protein metal-binding sites (e.g., cysteine, histidine, glutamic acid, aspartic acid). The objective of this study was to constrain equilibrium isotope fractionation values for amino acid-bound metals.

**Materials and Methods:** Free and bound metal ions were separated via equilibrium Donnan dialysis using a cation-permeable membrane (Nafion™). Experimental solutions were prepared at least one day in advance of dialysis to allow for isotopic equilibration of bound and unbound metals. Initial metal donor solutions for dialysis contained metal ions and amino acids at a ratio of 100  $\mu$ M:200  $\mu$ M. All experimental solutions were prepared at pH = 6.0 using MES hydrate as a buffer (5 mM) and strontium nitrate (10 mM) as a background electrolyte. Donor solutions were dialyzed for one day against metal- and amino acid-free acceptor solutions. Isotope ratios of pre- and post-dialysis solutions were measured via MC-ICP-MS following column purification under clean laboratory conditions.

**Results and Discussion:** In the copper experiments, oxygen ligands (glutamic and aspartic acid) yielded small, negative  $\Delta^{65}\text{Cu}_{\text{complexed-free}}$  values ( $\sim 0.1\text{‰}$ ) while sulfur ligands (cysteine) yielded larger negative values ( $\sim 1.4\text{‰}$ ), suggesting that both preferentially bind the light isotope relative to water. Experimentally determined isotope separation values ( $\Delta x_{\text{Mcomplexed-free}}$ ) are consistent with published *ab initio* predictions. This work adds to the growing body of evidence that metalloprotein biosynthesis can affect the distribution of transition metal isotopes in biological systems.

## Posters

### Session #1

1 – 2:30 p.m.

#### **Shifting Antarctic Polar Front during the last deglaciation revealed by (micro)fossil-bound nitrogen isotopes**

*Xuyuan E. Ai, Anja S. Studer, Lena M. Thöle, Tony X. Wang, Julia Gottschalk, Alfredo Martínez-García, Elisabeth Michel, Xavier Crosta, Daniel M. Sigman, Gerald H. Haug*

Changes in deep water upwelling in the Southern Ocean have been proposed to explain a large portion of the changes in atmospheric CO<sub>2</sub> level during the last glacial-interglacial cycle. The mechanism encompassing different changes in productivity in different latitudinal zones of the Southern Ocean requires a northward shift of the polar front and wind-driven upwelling during the glacial times, of which direct evidence is lacking. Nitrogen isotopes of organic matter entrained in diatom frustules preserve signals of surface nutrient conditions, from which we can imply changes in upwelling intensity. We report a comparison of diatom-bound nitrogen isotope records since the Last Glacial Maximum (LGM) from two sites in the modern Antarctic Zone and the northern edge of the Polar Frontal Zone. At the Polar Frontal Zone site, distinct features of nutrient consumption level and productivity are observed during the deglacial transition. We interpret these features to be the result of two opposing effects on the Polar Frontal Zone surface layer imposed by meridional shifts in Southern Westerly Wind (SWW)-driven upwelling and associated hydrographic fronts during different deglacial events. Our interpretation is also supported by nitrogen isotopes of deep-sea coral residing at different depths within Drake Passage that reflect changes in the  $\delta^{15}\text{N}$  of the particulate nitrogen from different latitudes.

## **Identifying biological influences on hydrated magnesium carbonate mineral precipitation in photosynthetic benthic microbial mats**

*Matthew J. Baldes, Jian Gong, Tanja Bosak*

Magnesium carbonate deposits preserve some of the oldest evidence for life on Earth and have been identified as an important target in the search for potential biosignatures by the Perseverance Rover on Mars. Elucidating the microbial role in the formation of these minerals is essential in seeking to identify biosignatures in the rock record and to understand the carbonation of ultramafic rocks. This study explores the mechanisms by which oxygenic photosynthetic benthic microorganisms and their organic products influence the precipitation of high magnesium carbonates and the mineralogy of these precipitates, in terms of species and texture, relative to abiotically precipitated minerals. Coccoidal cyanobacteria *Chroococcidiopsis cubana* and filamentous cyanobacterial isolates from Lake Salda, Turkey were incubated in media whose composition replicates those of modern lakes where hydrated magnesium carbonate precipitation occurs. Analysis of mat and water samples from culture experiments with scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDS), and ion chromatography (IC) revealed the adsorption of magnesium onto cell surfaces. This initiated the precipitation of hydrated magnesium carbonate mineral spheroids identified as predominately dypingite with X-ray powder diffraction (XRPD) and Raman spectroscopy. Some hydromagnesite was also present along with minor components of calcium containing magnesium carbonates. No precipitation was observed in abiotic jars under identical conditions. pH titration experiments indicated that the precipitation of minerals requires photosynthesis to raise the pH to  $> 9.67$  ( $SI = 0$ ), but organic nucleation sites influence the mineral morphology and formation kinetics. Dypingite spheroids with radial internal structure rapidly precipitated in biological cultures, often encapsulating cells, and eventually mineralizing mats. Abiotic media titrated to  $pH = 10.3$  ( $SI > 1$ ) initially precipitated amorphous magnesium carbonate that evolved over several months to crystalline dypingite. Biologically precipitated spheroids were distinguishable in Raman spectra by the presence of organic peaks. We propose that metabolic changes in pH induce local environments of supersaturation and that organic compounds act as the nucleation sites and control the morphology of spheroidal dypingite. The biologically precipitated dypingite can be distinguished from abiotic precipitates because its clotted textures incorporate and preserve organic compounds. These clotted textures also closely resemble those found in samples of hydromagnesite microbialites from Lake Salda, Turkey. The comparison of natural and experimentally precipitated samples allows us to isolate biological influences on magnesium carbonate formation and better identify potential biosignatures.

## **Chemistry, Mineralogy, and Abundance of Meromictic Lakes in New Hampshire**

*Josephine Benson, Sarah Slotznick, William Leavitt*

Pyrite is ubiquitous in the rock record and may hold the key to understanding nutrient cycling in deep time; however, iron sulfide formation remains incompletely understood. Our study aims to characterize modern iron sulfide depositional environments, particularly examining whether iron

sulfides form in the water column. We hypothesize that greigite forms in the water columns of meromictic and euxinic lakes and acts as a pyrite precursor.

To evaluate this hypothesis, we began by identifying local settings where meromixis is likely. Prior to this study only three meromictic lakes had been identified in New Hampshire, a state with more than 650 lakes. These lakes shared several characteristics, including high depth to surface area ratios, low light penetration, and slightly acidic pHs. Using these parameters, we identified nine additional putative meromictic lakes.

We are characterizing putative and historic sites through seasonal water and sediment sampling, in addition to water column profiles. We have performed magnetic analyses on minerals filtered from water samples and on sediment samples. Geochemical characterization supports the classification of multiple sites as meromictic and as possible iron sulfide depositional environments.

New Hampshire's meromictic lakes may provide insight into the necessary conditions for iron sulfide formation and preservation. Increasing the inventory of these lakes is the first step to characterizing these enigmatic processes.

## **Distribution of GDGT Lipid Cyclization Genes Reflects pH in Terrestrial Hot Springs**

*Laura N. Blum, Daniel R. Colman, Emiley A. Eloie-Fadrosch, Matthew Kellom, Eric S. Boyd, Olga Zhaxybayeva, and William D. Leavitt*

Archaea produce unique membrane-spanning glycerol dibiphytanyl glycerol tetraether (GDGTs) lipids and these are key components of their ability to survive in hot and acidic environments. The addition of up to eight cyclopentane rings within the hydrophobic core of these isoprenoid-type lipids results in tighter packing and subsequently increases membrane stability, enabling cells to withstand thermal stress and acidity. Thus, the degree of GDGT cyclization has been considered a biomarker for microorganisms inhabiting high temperature and acidic conditions. An improved understanding of the environmental influences on cyclic GDGT production will help us interpret these structures in the rock record. Recently, the genes (*grsAB*) that encode enzymes responsible for GDGT cyclization were identified in the model thermoacidophile *Sulfolobus acidocaldarius* (Zeng et al. 2019). However, the diversity and distribution of this gene in natural settings and across archaea is not well-described. Numerous metagenomic samples from terrestrial hot springs spanning a wide gradient in pH and temperature are available and present an opportunity to study *grs* sequence diversity and abundance within well-characterized geochemical and ecological context. In this work, we surveyed global hot springs metagenomes and isolate genomes from preexisting datasets for *grs* homologs. In Yellowstone National Park hot springs, relative abundances of *grs* were significantly negatively associated with hot spring pH, with temperature playing a secondary role. Our results will help to inform interpretations of GDGT biomarkers in the rock record, expanding our view of the organisms that produce them and in what types of environments they are found. Moving forward, we continue to assess the degree to which pH and temperature have each shaped the evolutionary history of *grs*.

## **Carbon isotope signatures of marine GDGTs across the last deglaciation: implications for archaeal paleobarometry**

*Amanda Calhoun, Sam Phelps, Ann Pearson*

The paleoclimate record provides key constraints on the behavior of the climate system under different boundary conditions. To better predict how the current increase in atmospheric carbon dioxide will affect global climate, it is essential to understand how atmospheric CO<sub>2</sub> and climate have varied in the past, particularly under elevated CO<sub>2</sub> conditions. A recently proposed method for reconstructing CO<sub>2</sub> concentrations utilizes the biosynthetic isotope effect ( $\epsilon_{\text{Ar}}$ ) associated with glycerol dialkyl glycerol tetraether lipid (GDGT) production by marine archaea. In this proxy framework, values of  $\epsilon_{\text{Ar}}$  become smaller as ambient CO<sub>2</sub> increases, which makes GDGT carbon isotope ratios a potential CO<sub>2</sub> paleobarometer. However, whether this proxy records variations in CO<sub>2</sub> during time periods of known atmospheric CO<sub>2</sub> change has yet to be tested. Here we target the last deglaciation, during which atmospheric CO<sub>2</sub> levels rose from ~180 ppm to ~270 ppm. Using spooling-wire micro-combustion isotope ratio mass spectrometry, we measured the carbon isotope ratios of GDGTs in sediment samples from the Gulf of Tehuantepec in the Eastern Tropical North Pacific (ETNP) that cover the last ~25 kyr. We evaluate our data considering the known atmospheric CO<sub>2</sub> history and proposed controls on GDGT carbon isotope fractionation, testing the potential of the archaeal paleobarometer. Through comparison to existing paleoproductivity and nitrogen isotope records, we also explore how the archaeal community responded or participated in changes in nitrogen cycling through this period of dramatic global environmental change.

## **Exploring the relationships between total organic carbon and the preservation of Devonian organic-walled microfossils**

*Gwyneth Chilcoat and Phoebe Cohen*

Proterozoic paleontologists have long noticed that the presence and quality of preservation of organic-walled microfossils (OWMs) inversely correlate with total organic carbon (TOC). This observation was quantified for the first time by Woltz et al. (2021) in Proterozoic shales from the Chuar Group and Bylot Supergroup. These authors found that preservational quality of fossils decreases as TOC increases, and that high TOC shales do not contain OWMs. Contrary to this finding, high TOC shales (defined informally as greater than approximately 1.5% TOC) from the Devonian Appalachian Basin of New York do contain abundant OWMs. In order to interrogate the relationship between OWM preservation and TOC further, we adapted techniques from Woltz et al. 2021 to Devonian Kellwasser Event shales from 7 sites in the Appalachian Basin. We quantified the degree of pitting in individual microfossils from over 50 shale samples in horizons below, within, and above the Kellwasser Events and then compared those results against TOC. Preliminary results show that high-quality preservation correlates negatively with TOC, which supports Woltz et al.'s findings. Unlike Woltz et al., however, we also see well-preserved fossils in some high TOC settings. We also explore relationships between pyrite and fossil preservation, and find less conclusive results. These findings provide an enhanced understanding of the effect of TOC on preservation quality that contributes to studying overall patterns of organic walled microfossil abundance and environmental/taphonomic controls. Additionally, these data add a

dimension to existing research on Late Devonian OWMs that will allow us to better interpret our ongoing analyses of OWM abundance and diversity during the Late Devonian Biotic Crisis.

### **Tubular Microfossils from Cap Carbonates of Kyrgyzstan**

*Jane Dawson, Lyle L. Nelson, Kelsey R. Moore, Sara B. Pruss, Rustam Orozbaev, and Emily Smith*

In the Neoproterozoic Era, during the Cryogenian Period, two successive global Snowball Earth glaciations occurred, lasting c. 55 and 5 million years respectively. In the aftermath of each glacial epoch, layers of cap carbonates were deposited atop glacial deposits worldwide. Recent examination of cap carbonate samples has yielded the discovery of a microbiota including testate amoeba, pyritized cyanobacteria, and possible agglutinated foraminifera. This work focuses on recent analyses of Sturtian Cryogenian cap carbonate from the Naryn region of Kyrgyzstan, comprising ~5 meters of dark grey limestone and interbedded black shale that sharply overlie massive polymictic diamictite. A subset of samples were dissolved in dilute acetic acid, filtered, and examined, and one sample was found to be fossiliferous. The residues consist of tubular microfossils that consist of gently curved and straight tubes with a consistent diameter of 30  $\mu\text{m}$  and lengths that vary from approximately 100  $\mu\text{m}$  to 400  $\mu\text{m}$ . SEM images also show that the interiors of these fossils are hollow and smooth, while the exterior is rough. EDS analysis of the fossils revealed that they are composed of Si and O. Additionally, Raman spectroscopy showed distinct peaks at ~1360  $\text{cm}^{-1}$  and ~1600  $\text{cm}^{-1}$ , corresponding to disordered (D) and graphitic (G) carbon respectively. These peaks are characteristic of fossil organic matter. This work adds to the existing fossil record of a thriving post-Snowball biota and further documents the diversity of organisms that lived in the aftermath of global glaciation.

### **Magnetic exploration of 3.2 Ga microbial mats from the Moodies Group of South Africa**

*Roger R. Fu, Sarah P. Slotznick*

An ongoing International Continental Scientific Drilling Program (ICDP) project in South Africa aims to provide the first unweathered samples of microbial mat and sub-aerial deposits from the 3.2 Ga Moodies Group. We will discuss our plans to constrain the redox environment of the microbial mats using rock magnetic tools, potentially testing for the presence of oxygenic photosynthesis. We also plan to use paleomagnetic techniques to identify the occurrence of lightning on the sub-aerial surface and therefore estimate the frequency of Archean lightning, which holds implications for the nitrogen cycle.

## **Pyrite sulfur isotope ratios in heterogeneous estuarine sediments reveal apparent constraints imposed by pore water sulfate concentration**

*Kalev Hantsoo; Maya Gomes; Sairah Malkin; Jeff Cornwell*

The stable isotope ratio of sulfur in sedimentary pyrite ( $\delta^{34}\text{S}_{\text{pyr}}$ , ‰-VCDT) is a geochemical signature commonly used to evaluate geochemical and environmental conditions in Earth's past. However, recent work has highlighted the potential for localized diagenetic factors to affect  $\delta^{34}\text{S}_{\text{pyr}}$  values, thereby complicating interpretations of the sedimentary record. In order to determine primary controls on  $\delta^{34}\text{S}_{\text{pyr}}$  values in a heterogeneous environment, we collected sediment cores of 30-60 cm length from nine sites in the Chesapeake Bay. This estuary features strong spatial and temporal variability in sedimentation rate, sulfate and oxygen concentration, infaunal sediment mixing, and organic carbon abundance and lability – all of which can affect  $\delta^{34}\text{S}_{\text{pyr}}$ . This variability makes the Chesapeake Bay a useful setting to investigate what kinds of environmental information can be captured in  $\delta^{34}\text{S}_{\text{pyr}}$  values. Results show that depth profiles of  $\delta^{34}\text{S}_{\text{pyr}}$  differ markedly between the nine sites, likely reflecting localized variation in the estuary's environmental conditions. However, the aggregate of  $\delta^{34}\text{S}_{\text{pyr}}$  values ( $n=156$ ) across all sites and sampling depths reveals a distinctive correlation between  $\delta^{34}\text{S}_{\text{pyr}}$  and pore water sulfate concentration. The envelope defined by the set of  $\delta^{34}\text{S}_{\text{pyr}}$  vs.  $\log[\text{sulfate}]$  has sharp upper and lower boundaries but generally uniform distribution of  $\delta^{34}\text{S}_{\text{pyr}}$  within those boundaries, meaning that for a given sulfate concentration in the measured range (0.3–13.1 mM),  $\delta^{34}\text{S}_{\text{pyr}}$  varies uniformly within 16‰ above or below the value predicted by the line of best fit. Furthermore, positive deviation from the line of best fit is correlated with (a) higher  $\delta^{34}\text{S}$  values of coexisting zero-valent sulfur and (b) higher organic carbon content. Thus, in the Chesapeake Bay and potentially in other estuarine settings, the  $\delta^{34}\text{S}_{\text{pyr}}$  of a given sample appears to be confined to a ~32‰ range that is determined by the sulfate concentration under which the bulk of the pyrite formed.

## **Using a Data Driven Approach to Predict Chlorophyll Content in the Northern Gulf of Mexico Through River Nutrient Data**

*Ellie Gellerson, Shuang Zhang*

The complex and dynamic coastal zone is one of the most susceptible ecosystems to climate change due to its proximity to anthropogenic activities. These activities can carry excess nutrients and pollutants into coastal zones which can fuel intense phytoplankton growth, cause eutrophication, alter trophic dynamics and effect water quality. Large loads of nitrogen and phosphorus derived from the agricultural lands in the Mississippi watersheds are delivered to the Gulf of Mexico through the Mississippi River each year. The objective of this study is to build a machine learning framework that integrates river nutrient data with coastal ocean conditions to predict chlorophyll content in the Gulf of Mexico. River nitrogen and phosphorus fluxes were collected from the USGS National Water Quality Network, and marine data (i.e. temperature, chlorophyll, etc.) were collected from the GCOOS data portal. Two ocean stations, one at the mouth of the Mississippi and one off Tampa, FL, were used to train the machine learning framework. Our model showed that river nutrients and water temperature are closely linked to the

variation of the coastal chlorophyll concentrations. Analysis of variable importance using either impurity or permutation method for both sites showed that water temperature, phosphorus flux, and nitrogen flux were the strongest predictor. We suggest that further investigations on additional ocean sites (coupled with the local river fluxes) are needed to draw a more complete picture of the relative importance of different parameters in controlling the coastal ecosystem.

### **Mechanistic Insights on Cellular Nitrogen Isotope Fractionation by N<sub>2</sub>-reducing Mo-nitrogenase**

*Eunah Han, Ashley E. Maloney, Xuyuan Ellen Ai, Daniel M. Sigman, Sebastian Kopf, Xinning Zhang*

The nitrogen stable isotope (<sup>15</sup>N/<sup>14</sup>N) composition of biomass is a powerful tool for reconstructing N cycling, but its interpretation depends on understanding the <sup>15</sup>N-fractionation of biological nitrogen fixation (BNF;  $15\epsilon_{fix} = \delta^{15}N_{dissolved\ N_2} - \delta^{15}N_{N_2\ fixer\ biomass}$  where  $\delta^{15}N$  reflects <sup>15</sup>N/<sup>14</sup>N). It remains unknown specific causes why molybdenum nitrogenase BNF leads to low  $15\epsilon_{fix}$  values (~1-2 ‰) while BNF by less abundant alternative vanadium and iron-only isoforms result in larger  $15\epsilon_{fix}$  values (~5-7 ‰). Potential reasons include (1) variations in nitrogenase isoform KIE and (2) variations in cellular scale expression of the kinetic isotope effect (KIE) of nitrogenase due to changes in reaction reversibility. To better understand how nitrogenase structure-function constrains  $15\epsilon_{fix}$ , we examine  $15\epsilon_{fix}$  sensitivity to variations in key functional residues for N<sub>2</sub>, proton, or electron delivery to the Mo-nitrogenase active site in the model nitrogen fixer *Azotobacter vinelandii* using batch and chemostat cultures of wild type and nitrogenase mutant strains. Values of  $15\epsilon_{fix}$  varied from 2 to 7 ‰, with largest  $15\epsilon_{fix}$  variations observed for N<sub>2</sub> substrate channel mutants. Using an isotope fractionation model constrained by nitrogenase reaction mechanism, the data suggest that low  $15\epsilon_{fix}$  values reflect low reversibility of net N<sub>2</sub> diffusion into the Mo-nitrogenase active site ( $\phi_{out}/\phi_{in} = f_{diff} < 20\%$ ), leading to limited cell-scale expression of a large intrinsic KIE for N<sub>2</sub> reduction (>10‰). This interpretation is consistent with the lack of a global correlation between  $15\epsilon_{fix}$ , growth rate, and specific activity across culture conditions and strains, suggesting that the rate limiting step of BNF shifts between isotopically sensitive and insensitive steps of the nitrogenase reaction mechanism. Highly conserved amino acid residues for the N<sub>2</sub> channel of Mo-nitrogenase among all natural extant variants supports the preponderance of low  $15\epsilon_{fix}$  for Mo-nitrogenase BNF.

### **Remote and In-Situ Characterization of Mars Analogs: Coupling Scales to Improve the Search for Microbial Signatures on Mars**

*Carolynn M. Harris\*, Matthew T. Maclay\*, Katherine A. Lutz, Vinitra Nathan, Noemi A. Ortega Dominguez, William D. Leavitt, Marisa C. Palucis (\*Co-first authors)*

Abundant water has been demonstrated for past environments on Mars and with it, the potential for microbial inhabitants. The possibility of habitable environments on Mars necessitates detailed characterization of analogous environments on Earth, where life detection strategies can be developed and validated. To this end, is critical to understand (1) how terrestrial microorganisms



generate remotely detectable biosignatures in response to extreme stressors, and (2) how different environments and mineralogies can preserve microbial biomarkers, and at what scales these are detectable. We surveyed five environments on Earth that are analogs for proposed climates during the Late Noachian to Early Hesperian, when crater lakes were active on Mars. This effort is timely given the recent landings of the Perseverance and Curiosity rovers in Jezero and Gale craters, respectively. Both craters hosted ancient lakes that experienced periods of active hydrologic cycling followed by prolonged intervals of drying. The analog sites range from active to desiccated lake systems, temperate to hyper-arid climates, and acidic to neutral-pH and hypo- to hyper-saline waters. All analogs host microorganisms that are adapted to multiple extremes (polyextremophiles), including aspects of water availability (i.e., surface waters versus shallow subsurface water versus groundwater) and physiochemistry (e.g., water activity, salinity, alkalinity, pH, redox potential, and temperature). Unlike on Mars, where orbital-scale and rover-scale data are coupled to determine potential habitability (modern or paleo), we found that most of these analogs lacked studies that explicitly connect local analysis with remote sensing data. This dearth makes it difficult to map how spatial patterns in mineralogy or physical characteristics of environments correlate to modern-day microbial communities or preserved biomarkers, which are important data for guiding sample collection locations within craters like Jezero. We conclude that generating these multi-scale datasets is critical to aid the search for life in evaporative martian basins (and on other terrestrial bodies) because they can provide vital information on whether “topographic signatures of life” can be preserved, and at what scale those biosignatures can be remotely detected.

### **Vanadium and thallium isotope constraints on the global ocean redox state approximately 1.4 billion years ago**

*Andy Heard, Yi Wang, Chadlin Ostrander, Maureen Auro, Donald Canfield, Shuichang Zhang, Huaijian Wang, Xiaomei Wang, Sune Nielsen*

The oxygenation state of the Mid-Proterozoic (1.8 – 0.8 Ga) ocean and atmosphere is heavily debated and has implications for the relationship between environmental O<sub>2</sub> and the emergence of complex life. While recent geochemical proxy studies of Mesoproterozoic marine sedimentary rocks inform a picture of ocean redox dominated by widespread ferruginous (iron-rich and anoxic) conditions, a growing number of datasets indicate that short-lived ocean oxygenation events were recurrent features in this time interval. The spatial extent of previously reported Mesoproterozoic oxygen pulses is unclear as they are observed via fundamentally localized environmental proxies. We report a novel combined application of the vanadium (V) and thallium (Tl) isotope paleoredox proxies to provide a global perspective to this time interval. We reconstructed seawater V ( $\delta^{51}\text{V}$ ) and Tl ( $\epsilon^{205}\text{Tl}$ ) isotopic compositions for shales from Unit 2 of the 1.38-1.39 Ga Xiamaling formation (North China craton) and observed an up-section shift to higher average  $\delta^{51}\text{V}$  values and a short-lived negative perturbation in  $\epsilon^{205}\text{Tl}$  that are stratigraphically near-coincident. Mass balance models for both isotopic systems are consistent with these isotopic shifts representing an expansion of oxic sedimentary environments from an anoxic (ferruginous) baseline ocean state, with the delayed shift in  $\epsilon^{205}\text{Tl}$  marking the onset of substantial Mn oxide burial. The long ocean residence time of V and Tl requires that this oxygenation event was regional-to-global in its spatial

extent, and may reflect a deepening of the oxycline; either as a purely oceanic phenomenon, or driven by ocean equilibration with a short-lived pulse of atmospheric oxygenation.

## **An Experimental Investigation of Clumped Isotope Budgets in Upper Atmospheric O<sub>2</sub> and CO<sub>2</sub>**

*Eleanor R. Hughes, Teresa L. Jackson, Gregory A. Henkes, Kevin M. Sutherland, Mark H. Thiemens, David T. Johnston*

The dynamics of atmospheric CO<sub>2</sub> and O<sub>2</sub> can be traced using their stable isotope compositions. While most atmospheric studies have so far been restricted to bulk isotope abundances, high-precision measurements are emerging that instead determine how rare isotopes are distributed between molecules. These measurements target the abundance of the multiply-substituted ('clumped') O<sub>2</sub> isotopologues at masses 35 and 36 (<sup>17</sup>O<sup>18</sup>O and <sup>18</sup>O<sup>18</sup>O; represented by D35 and D36), and the abundance of the multiply-substituted CO<sub>2</sub> isotopologue at mass 47 (<sup>16</sup>O<sup>13</sup>C<sup>18</sup>O; D47). Recent analyses indicate that variations in D35, D36 & D47 correspond closely to changes in the physical and chemical structure of the atmosphere, suggesting that clumped isotopes could be used as novel tracers of atmospheric processes. We investigate the mechanistic basis for these variations by using continuous irradiation experiments to simulate CO<sub>2</sub>-O<sub>2</sub>-O<sub>3</sub> photochemistry under stratospheric conditions. These experiments reveal that CO<sub>2</sub>-O<sub>2</sub>-O<sub>3</sub> interactions can produce non-equilibrium and non-stochastic D35, D36 & D47 compositions at steady-state. These D35, D36 & D47 are sensitive to the total pressure and pCO<sub>2</sub>/pO<sub>2</sub> of each experiment. From these results we suggest that – as previously shown for bulk stable isotopes – photochemical oxygen cycling could produce distinctive clumped isotope signatures in CO<sub>2</sub> and O<sub>2</sub>. Future work will use photochemical experiments at shorter (Ly- $\alpha$ ) wavelengths to target the mesospheric contribution to atmospheric D35, D36 & D47.

## **Characterizing sterol biomarkers from transient Antarctic microbial oases**

*Fatima Husain, Thomas W. Evans, Tyler J. Mackey, Ian Hawes, and Roger E. Summons*

Cold environments on Earth are under threat due to climate change, but they may hold secrets about the expansion of life following planet-scale glaciation events. Today, transient microbial oases in meltwater ponds thrive upon debris-covered ice shelf ecosystems during warm seasons on the McMurdo Ice Shelf (MIS) in Antarctica (Hawes et al., 2018). Previous studies of the meltwater ponds suggest that long-term stable microbial communities inhabit the transient environments, dwelling in both the water column and in microbial mat ecosystems beneath ice-covered lakes (Hawes et al., 2018; Jungblut et al., 2008). Sterol biomarkers are relatively resistant to degradation over short geological timescales and their presence in Antarctic meltwater ponds may help to characterize how life adapts to cold environments subject to perturbations from climate change (Volkman et al., 2003).

Here, we conduct an in-depth sterol biomarker analysis of twelve modern microbial mats from McMurdo Ice Shelf meltwater ponds that harbor stable populations of diverse microbial life. We detect and characterize a range of C<sub>26</sub> to C<sub>29</sub> sterols associated with corals, plants, and other

eukaryotes in different proportions across all ponds which are likely linked to specific pond physiochemistries. If distinct physiochemical environments can exist in such proximity annually and support distinct assemblages of eukaryotic microbial life, this suggests that these environments may have sheltered, supported, and contributed to the radiation of eukaryotic life during large-scale glaciation events.

### **Quantitative Modeling of Calcium and Strontium isotope cycles to the Ocean to predict responses to flux perturbations**

*Karas, J and Lau, K.V.*

The calcium isotope ( $^{44}\text{Ca}/^{40}\text{Ca}$ ), radiogenic strontium isotope ( $^{87}\text{Sr}/^{86}\text{Sr}$ ), and stable strontium isotope ( $^{88}\text{Sr}/^{86}\text{Sr}$ ) systems have been utilized to evaluate changes in mass fluxes during environmental perturbations, such as following massive volcanism. Environmental conditions for events throughout Earth's history, such as Oceanic Anoxic Events that took place during the Cretaceous, have been reconstructed using these isotope systems. However, these systems can react in unique ways depending on the driver (e.g., hydrothermal/volcanism, weathering, precipitation rate changes), and a quantitative model showing these changes has yet to be produced. To better understand these interactions, we have created a box model using modern and Cretaceous values for input and output fluxes for  $^{44}\text{Ca}/^{40}\text{Ca}$  ( $\delta^{44}\text{Ca}$ ),  $^{87}\text{Sr}/^{86}\text{Sr}$  and  $^{88}\text{Sr}/^{86}\text{Sr}$  ( $\delta^{88}\text{Sr}$ ). Calcium isotopes have been interpreted to record changes in hydrothermal, weathering, and precipitation rates and can also be an indicator of diagenetic alteration.  $^{87}\text{Sr}/^{86}\text{Sr}$  are not impacted by diagenetic alteration in the same way that calcium isotopes are, and are commonly interpreted as a proxy for weathering. Therefore,  $\delta^{44}\text{Ca}$  and  $^{87}\text{Sr}/^{86}\text{Sr}$  can work in tandem in response to weathering and hydrothermal changes but differ when it comes to changes in carbonate mineral precipitation and diagenesis.  $\delta^{88}\text{Sr}$  is a relatively new isotope proxy whose application is still under development but has been shown to be a constraint on the other proxy systems. Because  $\delta^{88}\text{Sr}$  varies with precipitation rate, similar to  $\delta^{44}\text{Ca}$ , it can be a line of evidence for changes in precipitation rate when compared to  $\delta^{44}\text{Ca}$ . The mass-balance model predicts the direction and magnitude of change for each isotope proxy in the oceans for each flux perturbation and improves on previous models that do not account for changes in alkalinity, a key driving factor in precipitation rates and fluxes. Our model predicts that increases in volcanism lead to an initial decrease in  $\delta^{88}\text{Sr}$  and  $\delta^{44}\text{Ca}$  before increasing back to steady state values, while  $^{87}\text{Sr}/^{86}\text{Sr}$  decreases quickly before slowly returning to steady state. With increasing weathering,  $\delta^{44}\text{Ca}$  and  $\delta^{88}\text{Sr}$  decrease initially before returning to steady state, while  $^{87}\text{Sr}/^{86}\text{Sr}$  increases before decreasing back to steady state. This model can provide further insight on the driving factors of volcanic events on geochemical cycles, including but not necessarily limited to OAE's, by identifying the relative importance of these driving factors. This can shed light on the debate between the importance of continental weathering and increased volcanic or hydrothermal activity as well as which are more likely to be the root cause of many other geochemically significant events throughout Earth's history.

## **Genomic and Taxonomic Characterization of a Novel Chloroflexi Genus**

*Elm Markert, Elizabeth Trembath-Reichert, L. M. Ward*

Chloroflexi are commonly anoxygenic phototrophs that may have played important roles in the evolution of photosynthesis and the productivity of the biosphere through time. We present the analysis of a draft metagenome-assembled genome of a novel genus of candidate Chloroflexi. The characterization of the genome of this organism will expand understanding of extant bacterial diversity by characterizing a novel genus and provide useful data to construct insights into the evolution of photosynthesis in bacteria in deep time.

## **Dissolved distribution of barium in seawater and its relationship to silicon**

*Öykü Z Mete, Heather H Kim, Ann G Dunlea, and Tristan J Horner*

Barium (Ba) is a trace metal that exhibits a similar marine distribution to the algal nutrient silicon (Si, as silicic acid). This similarity underpins a number of proxies whereby measurement of Ba stands in for Si. However, the global extent of—and spatial variability within—the Ba–Si relationship is unknown owing to the relative sparsity of dissolved Ba concentration ( $d[Ba]$ ) measurements in large parts of the ocean. A global assessment of the Ba–Si relationship thus requires a model that can simulate  $d[Ba]$  in regions of the ocean with few direct measurements. Accordingly, we developed an accurate simulation of global average annual  $d[Ba]$  using a Gaussian regression machine learning model. Model development and testing comprised three stages. First, we ingested datasets from GEOTRACES that were complete for 12 core features that would serve as predictors for simulating  $d[Ba]$  (geospatial information, nutrients, surface chlorophyll, etc.). Second, the optimal feature set for predicting  $d[Ba]$  was identified by creating, training, and validating models based on every possible permutation of feature combinations. Third, global simulations of  $d[Ba]$  were generated by feeding data from the World Ocean Atlas into various predictor models whose accuracy was assessed via cross-validating against withheld  $d[Ba]$  data from the Indian Ocean. This assessment reveals that several of the machine learning models can accurately simulate global  $d[Ba]$ , which we use to explore regional variations in the Ba–Si relationship and to constrain the total Ba budget of the ocean.

## **Suspicious minds: a molecular taphonomic approach to preservation of the central nervous system in the fossil record**

*Alexandra L. Morton-Hayward*

To-date, >2100 brains have been unearthed globally from the fossil record. By stark contrast, laboratory decay experiments suggest that the central nervous system (CNS) is amongst the first organs to decompose post-mortem. It has been suggested that decay resistance is an imperfect indicator of fossilization potential, given exceptionally preserved soft tissues arise through a complex system of interrelating bio- and geochemical processes (including early autolysis and putrefaction, microbially-mediated mineralization, and eventual diagenesis). Analysis of the

resulting organic chemistry of fossil soft tissues is the keystone for understanding their unexpected preservation; yet <1% of ancient brains excavated have been investigated biochemically, and their molecular composition remains unknown. This project, the first of its kind, adopts a multi-omics approach to unravel how and why the brain persists when other organs perish.

Whereas previous taphonomic experimentation with non-neural soft tissues has suggested a bacterial mineralization pathway to preservation, previous palaeontological work has hinted at the role of protein-lipid cross-linking in stabilising otherwise labile biomolecules in deep-time. To explore these hypotheses in parallel, we aim to unite experimental decay of modern brain tissues with molecular characterisation of ancient samples: the former marries metagenomics and environmental metabolomics to investigate the microbial communities colonising the decomposing CNS, while the latter exploits proteomics and lipidomics to probe the nature and degree of alteration of brains preserved in the fossil record.

Where preserved, the CNS might provide unique insights into palaeobiological and evolutionary investigations of extinct taxa across the animal kingdom. Constraining (a)biotic controls on the taphonomic processes acting to preserve the CNS through deep time, however, is an essential first step to appreciating the molecular and morphological information we can glean from these fossil remains. Further, an improved understanding of soft tissue chemical taphonomy is fundamental not only to deepening our knowledge of known fossils, but to targeting our search for new fossil sites worldwide: understanding the preservational pathway at play will aid in identifying lithologies where we might find soft-bodied organisms, like the earliest eukaryotes, either little-known or as-yet undiscovered."

## **Toward rate control of methanogen isotopologue signatures**

*Vinitra Nathan<sup>1</sup>, Beverly Chiu<sup>1</sup>, Jiawen Li<sup>1</sup>, Jeemin H. Rhim<sup>1</sup>, Edward D. Young<sup>2</sup>, William D. Leavitt<sup>1,3</sup>*

<sup>1</sup>*Department of Earth Sciences, Dartmouth College, Hanover, NH, USA*

<sup>2</sup>*Department of Earth, Planetary, and Space Sciences, UCLA, Los Angeles, CA, USA*

<sup>3</sup>*Department of Chemistry, Dartmouth College, Hanover, NH, USA*

Methane constitutes an important part of Earth's carbon cycle. Of the major greenhouse gases, its residence time in the atmosphere is second only to water vapor, trapping 28 times as much carbon dioxide over a century-long cycles. Historically, methane has been a vehicle for reduced carbon through geological systems as well as biological systems. Due to its ubiquity in the Earth system, contribution to climate change, importance in biological systems, as well as its intriguing presence in extraterrestrial settings, there is great interest in developing robust analytical methods to trace methane provenance with high fidelity. Methane 'bulk' carbon ( $^{13}\text{C}/^{12}\text{C} = \delta^{13}\text{C}$ ) and hydrogen ( $\text{D}/\text{H} = \delta\text{D}$ ) and multiply substituted ('clumped') isotopologues ( $^{12}\text{CH}_2\text{D}_2$  and  $^{13}\text{CH}_3\text{D}$ ) have been employed to characterize both sources and processes. While bulk isotopes integrate information about methane sources/sinks and formation/loss processes,  $\delta^{13}\text{C}$  and  $\delta\text{D}$  ratios do not clearly distinguish source from process. As such, the analysis of the multiply substituted isotopologues of nominal mass-18 ( $^{12}\text{CH}_2\text{D}_2$  and  $^{13}\text{CH}_3\text{D}$ ) may allow us to more uniquely differentiate source and process. The major process of low-temperature methane formation, microbialgenic methanogenesis, is a rich target to deploy these high resolution isotope tools.

In this study, we aim to understand how differences in microbial growth rate when controlled by steady-state nutrient limitation manifest in methane clumped isotope compositions  $\Delta^{12}\text{CH}_2\text{D}_2 / \Delta^{13}\text{CH}_3\text{D}$  space. We predict that the clumped isotope fractionation factor(s) will reflect process (microbial methanogenesis), even when cellular growth and metabolic rates change, whereas bulk isotope values will reflect shifts in rate. To test this hypothesis we will manipulate growth rate by phosphate limitation in chemostat cultures of the model methanogen, *Methanosarcina barkeri* Fusaro, cultivated on either methanol or acetate. Bulk and clumped isotopologue measurements from these experiments will help test predictive models for methanogen isotope fractionation, and subsequently expand on the knowledge base of the field.

### **Animal abundance and changing redox conditions during the Furongian Cambrian SPICE event, western Utah**

*Amelia E. Olsen, David S. Jones, David A. Fike, Sara B. Pruss*

The Steptoean Positive Isotopic Carbon Excursion (SPICE) is one of the best-known, and globally reported, carbon isotope excursions from the Cambrian. It co-occurs with an extinction of trilobites and a major sea level change at the Sauk II/III boundary. Whereas much work has focused on the cause of the excursion and the extinction, little work to date has focused on the other biological change(s) that occurred throughout this interval. Two of the best-studied and first-reported sections hosting the SPICE are exposed in the House Range and at Lawson Cove in western Utah. These sections are thick ( $> 100$  m), fossiliferous, and well preserved. Here, we report new geochemical and fossil data from strata that span the SPICE at both localities. We use geochemical redox proxies to better understand the timing of local environmental change during the SPICE and we report skeletal abundance and diversity of fossils from both sections in order to recognize shifts in marine organism assemblages throughout the event. The goal of this research is to ascertain how the SPICE event and concurrent environmental changes influenced the marine organisms of the Cambrian in the shallow marine realm.

### **New constraints on the oxygen isotope composition of the Archean ocean**

*Haley Olson, Nadja Drabon, and Dave Johnston*

The oxygen isotope composition of chemical sediments throughout the geologic record have often been used to reconstruct sea-surface temperatures (SSTs) through time. Attempts to build these reconstructions using oxygen isotope records have led to three end-member interpretations, all of which carry implications for the conditions in which early life first proliferated. It has been proposed that the observed enrichment of  $^{18}\text{O}$  relative to  $^{16}\text{O}$  in mineral records reflects: 1.) a change in sea-surface temperatures (SSTs), 2.) a change in the  $^{18}\text{O}$  composition of the contemporaneous water, or 3.) that the primary composition has been overprinted by alteration fluids. These hypotheses become most salient in the Archean, where estimates of the isotopic composition of the ocean span  $\sim 20\%$ , with a correspondingly wide range in estimated SSTs derived from these compositions. Here, we use the triple oxygen isotope composition of barite ( $\text{BaSO}_4$ ) as a robust new proxy for the oxygen isotope composition of the Archean ocean. Using

a Monte Carlo approach, we identify the sources of sulfate to the barite deposits and constrain the possible isotopic composition of contemporaneous seawater. Our results show that abiotic oxidation of more reduced sulfur species was the primary source of sulfate to the Archean marine reservoir, with smaller contributions from sulfate derived from the atmosphere and microbial sulfur cycling. Our results support an Archean ocean with a somewhat enriched oxygen isotope composition ( $\sim 0\text{--}5\text{‰}$ ), with the exact composition dependent on the relative contribution from each sulfate production pathway. This result is consistent with a decrease in low-temperature weathering as compared to today and, to explain the much debated chemical sediment record, with elevated Archean SSTs.

### **Rise of animals in a low-O<sub>2</sub> Ediacaran ocean**

*Chadlin M. Ostrander, Christian J. Bjerrum, Anne-Sofie C. Ahm, Simon R. Stenger, Nicholas J. Butterfield, John P. Grotzinger, Mohamed El-Ghali, Sune G. Nielsen*

The environmental conditions that played host to the rise of animals in the late-Ediacaran remain debated. One particularly polarizing question is whether this evolutionary milestone took place in an ocean that was overwhelmingly anoxic as for most of the preceding Proterozoic or in an ocean that was much better oxygenated as for most of the ensuing Phanerozoic. From two siliciclastic-dominated successions from Oman deposited in widely separated basins coeval with the Avalon assemblage of the Ediacara biota in Newfoundland, we uncover near-crustal thallium isotope ratios and muted redox-sensitive element enrichments. These trends require limited Mn oxide burial and widespread marine anoxia over large areas of the global seafloor, placing the rise of animals in a largely reducing Ediacaran ocean typical of the Proterozoic.

### **Total constraint on the marine carbonate system from B, Ca, and stable Sr isotopes during Ocean Anoxic Event 1a**

*Brian J. Beaty[1], Jiuyuan Wang[1], Noah J. Planavsky[1], Andrew D. Jacobson[2]*  
[1] Department of Earth and Planetary Sciences, Yale University, New Haven, CT 06511  
[2] Department of Earth and Planetary Sciences, Northwestern University, Evanston, IL 60208

For the first time, we directly constrain all carbonate system parameters across an ancient carbon cycle perturbation using a novel combination of the B, Ca, and stable Sr isotope systems. We present new  $\delta^{11}\text{B}$  measurements that constrain pH changes across Ocean Anoxic Event 1a (OAE1a, ca. 120 Myr) and pair them with previously-published  $\delta^{44}/^{40}\text{Ca}$  and  $\delta^{88}/^{86}\text{Sr}$  measurements from the same samples that constrain carbonate precipitation rates [Wang et al. (2021) *Geology*]. Negative excursions in  $\delta^{11}\text{B}$  prior to OAE1a parallel positive excursions in  $\delta^{44}/^{40}\text{Ca}$  and  $\delta^{88}/^{86}\text{Sr}$ , indicating ocean acidification and a reduction in carbonate precipitation rates, likely as a result of rapid CO<sub>2</sub> emission from the Ontong Java Large Igneous Province (LIP). Likewise, a large positive  $\delta^{11}\text{B}$  excursion across OAE1a parallels negative excursions in  $\delta^{44}/^{40}\text{Ca}$  and  $\delta^{88}/^{86}\text{Sr}$ , recording a return to more alkaline conditions and restoration of biogenic carbonate production after LIP emplacement. Interestingly, the magnitude of the positive  $\delta^{11}\text{B}$  excursion requires a rate-driven change in B isotope fractionation from seawater during precipitation, which

has been demonstrated experimentally but never within the geochemical record, nor has it been incorporated into previous  $\delta^{11}\text{B}$ -based pH reconstructions. Our results demonstrate that shifting carbonate precipitation rates amplify the  $\delta^{11}\text{B}$  response to changing seawater pH as recorded in bulk carbonates, and suggest that existing  $\delta^{11}\text{B}$  records for other ancient carbon cycle perturbations may overestimate pH changes. Moreover, our results demonstrate that combined analysis of B, Ca, and stable Sr isotopes in shallow marine carbonates can directly constrain changes in atmospheric  $\text{CO}_2$  by independently constraining pH and alkalinity.

## **Session #2**

4 – 5:30 p.m.

### **Mg-calcite and iron sulfide minerals record microbial influence on the formation of ooids in Shark Bay, Western Australia**

*Juliet Ramey-Lariviere, Tanja Bosak, Jian Gong, Matthew Baldes, and Sara B. Pruss*

Ooids are concentrically laminated calcium carbonate grains that form in warm and agitated waters. Many details of ooid formation are still debated, including whether or not microorganisms play a role in the accretion of ooid nuclei or some layers. Here we investigated the mechanisms of ooid formation in Shark Bay, Western Australia. The nuclei of dark grey ooids contain more organics and iron sulfide minerals relative to the outer layers. The 10-15 micrometer thin carbonate layers directly surrounding the unlaminated nuclei are enriched in Mg and surrounded by Mg-poor cortical layers. SEM-EDS, microXRD, microXRF, XANES and Raman analyses detect high-Mg calcite and organics in the Mg-rich layers and organic-poor aragonite in the Mg-poor layers. The relationship between the Mg-calcite layers, organics, trace metals and iron sulfide-rich nuclei point to the role for microbial sulfate reduction in the formation of iron sulfide phases and the precipitation of aragonite in the nuclei. The lack of sulfide phases and the presence of organics support the stabilization of Mg-calcite layers in sulfide-poor and organic rich diffusive layers around the nuclei, followed by the precipitation of aragonite layers in suspension or during occasional burial. Organics in the nuclei and Mg-calcite layers as well as the microscopic iron sulfide phases and Mg-calcite rings in ooids reflect microbial influences on ooid formation in Shark Bay.

### **Archaeal Lipid $\delta^2\text{H}$ as a Potential Paleoenvironmental Proxy: Experiments with a Metabolically Flexible Thermophilic Archaeon**

*Jeemin H. Rhim,Carolynn Harris, Harp Batther, Jamie McFarlin, Sebastian Kopf and William D. Leavitt*

The hydrogen isotope compositions ( $\delta^2\text{H}$ ) in lipid biomarkers track metabolic processes and can remain stable over geologically relevant time scales, with hydrogen isotope exchange rates ranging from 104 to 108 years (Sessions et al., 2004). The lipid  $\delta^2\text{H}$  in photosynthesizing eukaryotes (e.g., leaf wax  $\delta^2\text{H}$ ) has therefore been widely used for paleoclimate reconstructions (Sachse et al.,



2012). Microbial lipid  $\delta^2\text{H}$  may reveal additional information about geobiological processes, as microbes live in a broad range of habitats and have wider metabolic capabilities. While bacterial lipid  $\delta^2\text{H}$  has been extensively studied and shown to be quantitatively related to central metabolism (Wijker et al., 2019), archaeal lipid  $\delta^2\text{H}$  remains largely understudied. In this study, we conducted experiments with pure cultures of *Archaeoglobus fulgidus*, an anaerobic hyperthermophilic sulfate-reducing archaeon, to evaluate the potential of archaeal lipid  $\delta^2\text{H}$  as a paleoenvironmental proxy. The metabolic flexibility of *A. fulgidus* allowed us to investigate the effects of carbon metabolism (autotrophy vs. heterotrophy) and redox potentials (lactate vs.  $\text{H}_2$  as electron donors) on their lipid  $\delta^2\text{H}$ . All experiments were conducted with different  $\delta^2\text{H}$  of growth medium to apportion the relative contributions of H from different sources—protons in water, hydride carrier molecules, and organic substrates—to the final lipid products. Preliminary  $\delta^2\text{H}$  data from these experiments indicate that more lipid-bound hydrogens derive from water during autotrophy compared to heterotrophy. In light of the experimental results, we discuss how this approach can include other metabolisms to enable a generalized framework for interpreting archaeal lipid  $\delta^2\text{H}$ . The empirical framework can be validated by analyzing the  $\delta^2\text{H}$  of relevant environmental samples, allowing us to assess the potential application of the H isotope composition of archaeal lipids as paleoenvironmental proxies.

### **Using single-fossil organic carbon isotopes to illustrate the paleoecology and depositional conditions of the Mesoproterozoic Roper Group Velkerri Formation of Australia**

*Roman Ruiz, Christopher K. Junium, Quinlan Byrne, Phoebe Cohen*

The Mesoproterozoic Roper Group of Australia contains some of the oldest definitive eukaryotic fossils and thus is an important window into the early evolution of eukaryotes. Considerable work has been done on the Roper Group on both this fossil record as well as geochemical proxies, especially those relating to redox. Analyses of carbon isotopes within these strata have primarily focused on measuring the organic or inorganic carbon from bulk samples. While this has provided us with new information concerning broad biogeochemical trends, the results are also time- and community-averaged.

In this study, we look instead at carbon isotope measurements of individual microfossils and bulk organic carbon from the Roper Group Velkerri Formation to obtain a more short-term view of the conditions of the water column during the deposition of sediments as well as information on fossil paleoecology. We obtained carbon isotopic data from individual leiosphere microfossils via nano-EA mass spectrometry, and found a range of average  $\delta^{13}\text{C}_{\text{fossil}}$  per horizon from -31.7‰ to -24.03‰ compared to the bulk rock  $\delta^{13}\text{C}_{\text{Org}}$ , which ranges between -35.22‰ and -27.74‰. In the standard model of eukaryotic evolution, eukaryotes were limited to oxygenated surface waters in a stratified ocean with strong  $\delta^{13}\text{C}$  and oxygen gradients. Such conditions would leave the microfossils'  $\delta^{13}\text{C}_{\text{Org}}$  values partially determined by their location in the water column, and if living at the surface, the  $\delta^{13}\text{C}$  of organisms would be greater than the  $\delta^{13}\text{C}$  of the bulk organic matter because the biological pump would leave the surface  $\delta^{13}\text{C}$ -enriched. Our results show that fossil  $\delta^{13}\text{C}_{\text{Org}}$  values are consistently equivalent to or heavier than the bulk rock, with some samples enriched from the bulk by up to 10‰, with the exception of two horizons at which some fossil  $\delta^{13}\text{C}_{\text{Org}}$  values are less than the bulk rock values. Results from these two horizons potentially indicate that the fossils' habitats were located out of the surface ocean, perhaps in

anoxic portions of the water column. However, the bulk of our data supports the hypothesis that eukaryotic organisms were mainly living in surficial and partially oxygenated waters. Critically, our interpretation of these data relies heavily on the veracity of bulk redox proxies, which have recently been called into question. If the Velkerri Formation was not a consistently stratified, anoxic-at-depth basin, then our data may instead be tracking changes in the water column  $\delta^{13}\text{C}$  gradient recorded by organisms living in the surface ocean.

### **Nitrogen isotopic composition of chloropigments and bulk suspended particles in North Atlantic and North Pacific surface waters**

*Yeongjun Ryu, Ashley Maloney, Victoria Luu, Sarah Fawcett, Meytal Higgins, Nicolas van Oostende, Bess Ward, and Daniel Sigman*

The  $\delta^{15}\text{N}$  of chloropigments ( $\delta^{15}\text{NChl}$ ) provides insight into the nature of primary production in the modern and past ocean. Culture studies have reported that the nitrogen isotope fractionation during chlorophyll synthesis varies taxonomically, suggesting that the  $\delta^{15}\text{N}$  difference between chloropigments and bulk organic nitrogen can be used to constrain the relative importance of different taxonomic groups, in particular, prokaryotic and eukaryotic phytoplankton, to marine primary production. In this study, we measured bulk  $\delta^{15}\text{N}$  and  $\delta^{15}\text{NChl}$  from suspended particles in the North Atlantic and North Pacific surface waters. Overall,  $\delta^{15}\text{NChl}$  was positively correlated with  $\delta^{15}\text{N}$  of particulate N ( $\delta^{15}\text{NPN}$ ), indicating a shared control by N sources. However, the  $\delta^{15}\text{NChl}$  varied less among the regions than  $\delta^{15}\text{NPN}$ , such that a higher  $\Delta\text{Chl-PN}$  ( $\Delta\text{Chl-PN} = \delta^{15}\text{NChl} - \delta^{15}\text{NPN}$ ) of  $-1.7\text{‰}$  was observed in the cyanobacteria-rich subtropical gyres and a lower  $\Delta\text{Chl-PN}$  ( $-5.2\text{‰}$ ) in the eukaryote-rich subpolar North Atlantic and eastern tropical North Pacific. In stable ecosystems, the relative fraction of cyanobacteria to eukaryotic algae estimated from  $\Delta\text{Chl-PN}$  generally agreed with flow cytometric observations of live cells. At specific North Atlantic stations in the midst of the spring bloom, however,  $\Delta\text{Chl-PN}$  disagreed with the observed ratio of cyanobacteria to eukaryotic algae; instead,  $\Delta\text{Chl-PN}$  was consistent with the prokaryote-to-eukaryote ratio of phytoplankton from the previous season. These data suggest that particulate organic matter and chloropigments persist in surface waters for at least several weeks.

### **A multi-isotope approach to reconstructing dinosaur diets: trophic structure and niche partitioning**

*Mason A. Scher, Tyler R. Lyson, Henry C. Fricke, John A. Higgins*

Paleodietary information for dinosaurs has been inferred using a variety of methods including morphological studies of teeth, jaw mechanics, rare cases of fossilized stomach contents, and coprolites. Though these methods glean valuable information, coprolites and stomach content analyses are only snapshots of paleodiets, and while morphological studies of jaws and teeth can infer the general class of food that was eaten, they lack specificity. The enamel matrix of teeth is an excellent archive for geochemical reconstructions of diets; traditional carbon and oxygen isotope analyses (of carbonate substituted into the hydroxyapatite matrix) have been used to infer feeding locations, the possibility of migratory behavior, and niche partitioning (Fricke & Pearson,

2008; Fricke et al., 2008, 2011). Relatively recent developments in instrumentation have enabled measurements of non-traditional stable isotopes like calcium and magnesium ( $\delta^{44}/^{40}\text{Ca}$  and  $\delta^{26}\text{Mg}$ ) in fossil enamel. Specifically, within dinosaur ecosystems, paleodietary reconstructions can help answer big questions: How were Cretaceous systems able to support such a large quantity of physically large animals? How were resources partitioned among animals in the same trophic level? The utility of  $\delta^{44}/^{40}\text{Ca}$  has been demonstrated in dinosaur ecosystems to look at trophic relationships like predator diet ‘fishiness’ (Hassler et al., 2018; Martin et al., 2022) and herbivore niche partitioning (Martin et al., 2022).  $\delta^{26}\text{Mg}$  has been shown to be a useful trophic-level indicator in modern animals (Martin et al., 2015). In this work, we present the first known coupled measurements of  $\delta^{44}/^{40}\text{Ca}$  and  $\delta^{26}\text{Mg}$  in dinosaur tooth enamel and gar scale ganoine to investigate trophic structure and herbivore niche partitioning in Cretaceous ecosystems. We construct trophic relationships for theropods, gar, and herbivorous dinosaurs in three late Cretaceous localities. We propose that a negative correlation between  $\delta^{44}/^{40}\text{Ca}$  and  $\delta^{26}\text{Mg}$  in herbivorous dinosaurs reflect vertical niche partitioning between hadrosaur and ceratopsian dinosaurs. The multi-isotope approach presented in this work allows for more nuanced conclusions to be drawn about the diets of extinct animals and will be a useful tool for a variety of settings.

### **Fish and Shark diversity inferred from marine microfossils: Lessons from the Paleogene Greenhouse**

*Elizabeth C. Sibert, Leah D. Rubin, Douglas W. Tomczik, Daniel E. Gaskell, Gregory L. Britten and Richard D. Norris*

Isolated microfossil fish teeth and shark dermal scales (denticles) preserved in sediments (together called ichthyoliths) provide one of the best records of vertebrate evolution in the paleontological record. However, these isolated microfossils are preserved independently from any skeletal material that could provide taxonomic or ecological context about the fish and sharks that produced them. This makes ichthyoliths a challenge to interpret in an ecological or taxonomic context. Despite this limitation, both teeth and scales play significant functional roles in the ecology and life history of fish and sharks. For example, teeth are used in prey capture and handling, and thus the size, shape, and placement of teeth in the fish’s jaw are all impacted by the type of diet and lifestyle that the fish leads. Similarly, denticles form a dense coating along the body of most shark species, providing an interface between the shark and its environment, such as protection from physical abrasion, drag reduction while swimming. Here we present results from a series of ongoing morphological studies of modern bony fish teeth and shark dermal scales, as well as present results from a series of case-studies looking at ichthyolith assemblages throughout the Paleogene Greenhouse, and across intervals of both rapid and long-term global warming. We find that while there are major changes in fish abundance and sometimes in short-term community composition during rapid global warming events, these excursions do not have a long-term evolutionary impact on open-ocean fish or sharks.

## **A multispectral imager for large-format thin sections**

*Devdigvijay Singh, Ryan Manzuk, Akshay Mehra, Adam Maloof*

Accurately assessing the shape, size, and modality of features in rock samples is a longstanding problem in geology. Recent advances in machine learning have made it possible to improve upon point counts with automated image classification; however, these techniques perform best with enhanced contrast to help differentiate classes. To leverage these methods for geological applications, we need a way to acquire high-resolution images of thin sections with a field of view large enough to resolve entire crystals, fossils, bedforms, etc. We present a novel multispectral light table equipped with 5-band (470-940 nm) spectral resolution and computer controlled broadband polarizers that can rotate in 0.1 degree increments in both a plane-polarized and cross-polarized transmitted light configuration. Paired with a 150 MP panchromatic camera that can acquire images at  $\sim 3.76 \mu\text{m}$  per pixel spatial resolution over a 4 cm by 4 cm field of view, the new system is a high-throughput thin section imager. The additional spectral bands outside the visible range, combined with cross-polarized rotations, encode rock properties that heighten image contrast through wavelength-dependent birefringence and differential extinction. Our setup provides an efficient way to (1) build reproducible image archives of petrographic thin sections that complement field observations, (2) classify and segment those images, and (3) quantitatively compare lithofacies and fossil assemblages with complementary geochemical measurements.

## **Metagenomic, biochemical, and microscopic analyses reveal the potential for the cycling of sulfated EPS in Shark Bay pustular mats**

*Emilie J. Skoog, Kelsey R. Moore, Jian Gong, Davide Ciccarese, Lily Momper, Elise Cutts, Tanja Bosak*

Cyanobacteria and extracellular polymeric substances (EPS) in peritidal pustular microbial mats have a two-billion-year-old fossil record. To understand the composition, production, degradation, and potential role of EPS in modern analogous communities, we sampled pustular mats from Shark Bay, Australia and analyzed their EPS matrix. Biochemical and microscopic analyses identified sulfated organic compounds as major components of mat EPS. Sulfur was more abundant in the unmineralized regions with cyanobacteria and less prevalent in areas that contained fewer cyanobacteria and more carbonate precipitates. Sequencing and assembly of the pustular mat sample resulted in 83 high-quality metagenome-assembled genomes (MAGs). Metagenomic analyses confirmed cyanobacteria as the primary sources of these sulfated polysaccharides. Genes encoding for sulfatases, glycosyl hydrolases, and other enzymes with predicted roles in the degradation of sulfated polysaccharides were detected in the MAGs of numerous clades including Bacteroidetes, Chloroflexi, Hydrogenedentes, Myxococcota, Verrucomicrobia, and Planctomycetes. Measurable sulfatase activity in pustular mats and fresh cyanobacterial EPS confirmed the role of sulfatases in the degradation of sulfated EPS. These findings suggest that the synthesis, modification, and degradation of sulfated polysaccharides influence microbial interactions, carbon cycling, and biomineralization processes within peritidal pustular microbial mats.

## **Banded Chert Depositional Environments and Ocean Stratification at 3.4 and 3.2 Ga, Evidenced from the Barberton Greenstone Belt, South Africa**

*Emily Stoll, Nadja Drabon, Donald R. Lowe*

Chemically precipitated banded cherts, including banded iron formation (BIF), banded ferruginous chert (BFC), and black and white banded chert (BWC), provide insight into the ocean composition at their time of deposition. This study uses lithologic associations, petrographic observations, and geochemical analyses to determine mineral formation locations and depositional environments of banded cherts from the 3.42 Ga Buck Reef Chert and the 3.28-3.23 Ga Fig Tree Group of the Barberton Greenstone Belt to constrain Paleoproterozoic ocean stratification. A stratified ocean would have significant impacts on the development of life and life-sustaining conditions on the early Earth. For example, stratification can establish a barrier that bottom waters cannot surpass, leaving surface waters depleted in nutrients, affect the carbon cycle, and impact the ability of oxygen to migrate throughout the water column.

BWC is composed of alternating carbonaceous and pure chert bands. It has ubiquitous current-and/or wave-deposited carbonaceous grains and widespread storm-deposited chert plate breccias, indicating it formed above storm wave base (SWB). BFC has alternating siderite and pure chert bands. It has abundant detrital carbonaceous grains, isolated current structures, and some chert plate breccias but lacks wave structures. This suggests formation below SWB yet close enough to shore to have prevalent organic matter introduced and experience some current activity. BIF has alternating hematite and jasper bands +/- siderite bands bearing volcanoclastic debris. It was mostly deposited via suspension settling and lacks organic matter, indicating deposition below SWB and in the deepest water of the three banded cherts.

All bands—except BIF siderite bands—have low concentrations of  $\text{Al}_2\text{O}_3$ ,  $\text{TiO}_2$ , Sc, Th, and Zr, indicating negligible detrital influx. This suggests the bands' precursor minerals precipitated out of seawater. Chert and jasper bands are enriched in heavy rare earth elements (REE), consistent with a marine surface water signature. They also have relict silica granules of a similar size across depositional depths, which suggests silica precipitated only in the upper part of the water column. Iron-rich minerals are absent in banded cherts deposited above SWB. Additionally, BFC siderite and BIF hematite bands are enriched in light and medium REE, indicating a hydrothermal influence. Altogether, this suggests iron precipitated below silica at a chemocline near SWB that separated iron-oxidizing surface waters from bottom waters enriched in hydrothermal fluids. Thus, we conclude the ocean was stratified at 3.2 and 3.4 Ga.

## **Investigating the Complexity in the Marine Oxygen Cycle with Oxygen Isotopologues**

*Kevin M Sutherland, David T Johnston*

The biogeochemical fluxes that produce and consume  $\text{O}_2$  play a critical role in regulating Earth's climate and habitability. The triple oxygen isotope method is one of the most robust tools for directly tracing oxygen cycling and measuring gross primary productivity in the marine environment. This method assumes that photosynthesis, microbial oxygen consumption (in the form of respiration), and gas exchange are the primary influences on dissolved oxygen, and that they have predictable, consistent isotopic end-members. Recent work, however, has highlighted

the ubiquitous production of extracellular reactive oxygen species (e.g., superoxide and hydrogen peroxide) by microorganisms as a major sink of dissolved O<sub>2</sub>. Additionally, decades of research have demonstrated that abiotic photooxidation of dissolved organic carbon is also a significant sink of O<sub>2</sub> in the surface ocean. Quantifying the impact of these non-respiratory sinks of dissolved O<sub>2</sub> is essential to a complete understanding of the modern oxygen cycle and the trajectory of future ocean deoxygenation. We investigate the triple oxygen isotope fractionation of these seldom considered, yet environmentally widespread reactions. We demonstrate that the triple oxygen isotope mass laws associated with these reactions span virtually the entire range of the mass-dependent domain. These results highlight the tremendous spatio-temporal uncertainty in triple-oxygen derived productivity estimates, a method which is often considered the gold-standard for gross-productivity estimates. Lastly, we provide a first look at the clumped oxygen isotope composition of dissolved O<sub>2</sub> in the water column, and explore the ways in which clumped measurements may address outstanding uncertainty in marine oxygen cycling.

### **Recent origin of modern iron oxidation and some other iron metabolisms**

*Erik Tamre, Gregory P. Fournier*

Cytochrome-porin Cyc2 has recently been identified as the initial electron acceptor in iron oxidation pathways used by chemolithotrophic Gallionellales and Zetaproteobacteria as well as photoferrotrophic Chlorobi. Our phylogenetic analysis identified copies of Cyc2 in gammaproteobacterial endosymbionts of lucinid bivalves whose radiation is constrained in time by the availability of suitable sea-grass meadows starting in the Cretaceous. Thus, we were able to use the well-dated fossil record of lucinid bivalves to time-calibrate the Cyc2 phylogeny. The resulting molecular clock shows that iron oxidation with Cyc2 in modern Gallionellales, Zetaproteobacteria, and photoferrotrophic Chlorobi is a relatively young trait, originating in the late Neoproterozoic or the Phanerozoic.

Even though fossils morphologically similar to modern chemolithotrophic iron oxidisers date back to earlier periods, the shallow origin of the modern clades suggests that these similarities do not permit phylogenetic assignment of the fossils. While chemolithotrophic iron oxidation and photoferrotrophy as metabolisms are very ancient, the taxonomy of responsible organisms has changed profoundly through time – perhaps reflecting that niches with sufficient reduced iron do not persist over long stretches of Earth history. This finding is reinforced by exploratory analysis of proteins involved in other aspects of iron metabolism, such as siderophore processing: they show but shallow histories, suggesting that iron metabolism in general is characterised by little taxonomic stability through Earth history.

### **Event, Episodes, or Endurance? Reframing the Geochemical Record of Atmospheric Oxygenation**

*Benjamin Uveges, Gareth Izon, Shuhei Ono, Nic Beukes, Roger Summons*

Understanding the timing and trajectory of atmospheric oxygenation is fundamental to deciphering its causes and consequences. The geological record of mass-independent sulfur isotope

fractionation (S-MIF) has been used to argue that oxygenation occurred either as a single event or via multiple protracted oscillations between 2.45–2.2-billion-years-ago (Ga). However, the confounding issue of sulfur sourced from the oxidative recycling of continental sulfides, commonly termed the crustal memory effect (CME), potentially disconnects the sedimentary record from syndepositional atmospheric chemistry. Here we examine the magnitude and duration of a hypothetical CME using an updated accounting of the geographical and temporal distribution of S-MIF records. Our analysis conclusively demonstrates that the CME is smaller than previously thought, but also that the discrepancies between the South African and Australian S-MIF records can be reconciled by geographical heterogeneity of the CME. Furthermore, we provide statistical, and data supported estimates for the longevity of recently described post-2.3 Ga oxygen fluctuations. Our results provide a robust framework for the interpretation of the S-MIF record and directions towards developing a unified model for the rise of oxygen.

### **Temporal and spatial variability in $\Delta^{13}\text{CH}_3\text{D}$ values and methane flux from ebullition in a mid-latitude lake**

*Amber Velez, Ellen Lalk, and Shuhei Ono*

Quantification of global methane emissions is important for climate change policy and environmental remediation efforts, but natural sources of methane, particularly from bodies of freshwater, are poorly constrained. Methane is produced either thermally or microbially, and the isotopes of methane (most often  $\delta^{13}\text{C}-\text{CH}_4$ , but also  $\delta\text{D}-\text{CH}_4$ ) are used as atmospheric tracers to identify sources of methane in the environment. However, these tracers can be ambiguous when methane sources have overlapping isotope values. Recently, measurement of clumped isotopologues of methane ( $^{13}\text{CH}_3\text{D}$  and  $^{12}\text{CH}_2\text{D}_2$ ) has become analytically feasible, by tunable infrared laser direct absorption spectroscopy (TILDAS) (Ono et al., 2014). In order to leverage clumped isotopologues of methane to fingerprint local contributions to the atmospheric methane pool, accurate characterizations of the isotopologue signatures and fluxes of major sources and sinks are required. However, the dataset of isotopologues is currently limited. Some clumped isotopologue values of methane have been previously measured from lakes in Sweden, MA, and Alaska, with one timepoint per location. Here, we characterize the isotopologue signatures and flux of methane released via ebullition from Upper Mystic Lake, MA between May and November, 2021. Our research quantifies  $\delta^{13}\text{CH}_3\text{D}$  content of methane samples that vary both spatially and temporally, providing a dataset that spans various depths of freshwater and several months. Our dataset also shows that methane production decreased slightly over time as ambient temperature decreased. The relative abundance of  $\delta\text{D}$ ,  $\delta^{13}\text{C}$ , and  $\delta^{13}\text{CH}_3\text{D}$  also decreased slightly over time, although these values did not vary according to change in water depth. Our values for  $\delta^{13}\text{CH}_3\text{D}$  range from 1.9 to 4.2,  $\delta\text{D}$  values from -280.8 to -228.3, and  $\delta^{13}\text{C}$  values from -72.7 to -58.4. This is the largest collection of  $\delta^{13}\text{CH}_3\text{D}$  values measured from lakes, which allows us to characterize the spatial and temporal variability of this tracer for methane produced in a mid-latitude lake environment. These data can be incorporated into atmospheric methane models to more accurately predict natural methane emissions.

## **The evolution of the marine carbonate factory**

*Jiuyuan Wang, Lidya G. Tarhan, Noah J. Planavsky, Andrew D. Jacobson*

Formation of calcium carbonate ( $\text{CaCO}_3$ ) is the primary pathway by which carbon is returned from the ocean and atmosphere system to the solid Earth. On long timescales, the wax and wane of the carbonate reservoir reflects changes in carbon inputs to Earth's surface environments from  $\text{CO}_2$  degassing and carbon removal tied to weathering. The removal of carbon dissolved in the oceans by precipitation of carbonate minerals—the marine carbonate factory—plays a critical role in shaping marine biogeochemical cycling and contributes to modulation of atmospheric  $\text{CO}_2$  concentrations. There are currently widely varying views on how the marine carbonate factory has changed through time and a paucity of empirical constraints that are widely accepted to track this process. Here, we report the stable strontium isotope ratios ( $\delta^{88}/^{86}\text{Sr}$ ) for Precambrian calcites spanning from 3.0 to 0.65 Ga and present a new view of the evolution of the marine carbonate factory and seawater carbonate saturation state. We provide evidence demonstrating that the rise of the skeletal carbonate factory significantly decreased marine carbonate saturation states, and suggest that carbonate formed in non-platformal settings, including authigenic carbonate, may have been a major carbonate burial sink for the majority of Earth's history.

## **An exploration of pyritization through experimental decay and computed tomography**

*Madeleine Waskom, Sarah R. Losso, Javier Ortega-Hernández*

Pyritization represents one of the most common modes of exceptional preservation in the fossil record and has the potential to capture highly detailed morphological information of both animal and plant macrofossils. The preservation of delicate morphological detail in pyritized Paleozoic animal macrofossils suggest that this process likely took place during early diagenesis before the loss of information by extensive decay. Despite its palaeobiological significance and a working understanding of the pyritization process, there is much that remains unresolved. Although the fossilization of soft-tissues indicates that pyritization must happen rapidly, pyritization is microbial-mediated and mineralization is typically a longer process. Experimental taphonomy can be employed to better understand the complex dynamics between the short- and long-term diagenetic process and establish a timeline for the formation of pyritized remains in the fossil record. By optimizing experimental conditions through biogeochemical methods, we follow pyritization in *Triops* using organic-poor sediment with sulfur reducing bacteria, which recreate realistic conditions. We employ micro-computed tomography to track differences in density in the experimental vials where we can observe the precipitation of minerals and density decrease in decaying specimens providing a noninvasive and non-destructive method to investigate the timeline of pyritization and fossil formation.



## **Paleoredox context of Proterozoic microfossils from the McArthur Basin (NT, Australia): insights from Fe speciation analyses**

*Maggie Whelan (McGill University), Maxwell Lechte (McGill University), Leigh Anne Reidman (UC Santa Barbara), Susannah Porter (UC Santa Barbara), Angelo dos Santos Jr (McGill University), Galen Halverson (McGill University)*

The c.a. 1.75 – 1.3 Ga Greater McArthur Basin of the Northern Australian Craton comprises multiple Proterozoic sedimentary basins in Northern Territory, Australia. The well-preserved, mixed carbonate–siliciclastic successions record deposition in a range of marine depositional environments from supratidal to offshore settings and host some of the oldest putative eukaryotic microfossils. In order to investigate the possible relationship between the interpreted paleoredox conditions of the depositional environment and the preserved microfossil assemblage, we present iron speciation data, total organic carbon content, and major element geochemistry coupled with microfossil data for 428 samples across 8 cored drill holes and 19 sedimentary formations from the McArthur and Birrindudu Basins. Paleoredox conditions can be inferred using iron speciation analysis, which indicates deposition in a range of anoxic (ferruginous or euxinic) to oxic marine paleoenvironments, consistent with the emerging model for redox stratified basins during the middle Proterozoic. We further analyze the relationship between these paleoredox data and fossil abundance and diversity. In this study, we analyze the relationship between these paleoredox data and fossil abundance and diversity. This study provides an example of how performing high resolution geochemical analyses on the same rock samples known to contain microfossils can elucidate our understanding of the habitats of early eukaryotic life.

## **Where do top ocean predators source their carbon? Evaluating the contributions of epi- vs mesopelagic food webs to tuna & swordfish**

*Ciara Willis, Kayla Gardner, Walt Golet, Leah Houghton, Simon Thorrold*

Bigeye tuna (*Thunnus obesus*), broadbill swordfish (*Xiphias gladius*), and yellowfin tuna (*Thunnus albacares*) are top ocean predators of substantial value to commercial fisheries, including those in the North Atlantic. Globally distributed between c. 50°N and 50°S, these species routinely dive to mesopelagic depths during the day, presumably to forage. While their behaviours and diets have been studied, most diet analyses have used gut content analysis. Gut content analysis has several shortcomings, including lack of recognition or identification of species without distinctive hard structures (e.g. bones), the possibility for contents to be regurgitated upon capture, and representation of only one time point of the animal's diet. These gaps can be filled in by stable isotope analysis (SIA). In particular, compound-specific SIA, usually focusing on individual amino acids, has been shown to have significantly more resolution than traditional bulk C and N isotope analysis. Essential amino acids are transferred between trophic levels with negligible fractionation of carbon, meaning that they can be directly linked to their source at the base of the food web. Here we use stable C isotopes of essential amino acids to determine the reliance of the three study species on surface and mesopelagic productivity, and to consider 1) how this influences the structure and function of pelagic ecosystems and 2) how emerging fisheries on mesopelagic forage fauna may impact top predator populations.