Cold seeps in the Sea of Marmara: a refuge for “extremophile” foraminifera?

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In this study, we investigate living (stained) deep-sea foraminifera from the Sea of Marmara. We focus on faunal composition and geochemical signatures (trace elements and stable isotopes) in foraminiferal tests at two cold-seep sites, which are located at 329 and ~1240 m depth. Both study areas are bathed by dysoxic water mass ($O_2 < 20$ μmol/L). They present extreme conditions characterized by a remarkable spatial heterogeneity. This variability is expressed through (1) contrasted geochemical process (e.g., free methane gas seepages provoking sulfate reduction, authigenic carbonate precipitation), (2) various sedimentary facies (e.g., coarse facies related to gravity flow, Mn-carbonates-enriched sediments, sapropel layers) and (3) an obvious biozonation of benthic life (e.g. microbial mat observed at 329 m depth). Overall dysoxia prevailing at both study areas restricts foraminiferal diversity to very low values ($S < 9$, $H' < 0.97$). Stress-tolerant species *Bolivina vadescens* and *Globobulimina affinis* dominate living faunas in both environments, with the highest standing stock recorded in shallower site where bacterial mat spreads. We assume that filamentous bacterial mat consists in a refuge for “extremophile” foraminifera, which can thereby survive and proliferate in dysoxic and sulfidic ecosystems. Moreover, our biogeochemical results show that the interpretation of the foraminiferal Mn/Ca ratio as a reliable proxy for bottom water oxygenation is neither straightforward nor equivoque, and depends strongly on basin physiography, sedimentary process and water column structure in modern and past periods.

Keywords: Living (stained) benthic foraminifera, Sea of Marmara, Cold seeps, Extreme ecosystems, Trace elements, Stable isotopes

A more robust salinity proxy: towards a mechanistic understanding of sodium incorporation in foraminiferal calcite

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Seawater salinity and temperature gradients drive ocean thermohaline circulation and thereby play an essential role in regulating Earth’s climate. Salinity reconstructions largely rely on combined proxy approaches, which are inherently associated with relatively large uncertainties. Element incorporation in foraminiferal calcite might provide a more direct reconstruction tool for salinity (Na/Ca and potentially, K/Ca). However, element/Ca ratios in foraminiferal calcite, including these monovalent cations, generally show relatively large variability between species, between specimens and even across chamber walls. Origin and extent of intra- and inter- specimen variability in element/Ca ratios need to be understood and quantified, this way reducing uncertainties and adding to the robustness of the reconstructions.

We cultured two foraminiferal species under a range of salinities and analyzed the newly formed calcite for their average Na/Ca and its distribution across chamber walls using Electron Probe Micro Analysis and Nanoscale Secondary Ion Mass Spectrometry. Obtained maps show that Na and other incorporated elements (Mg, K, S, and P) occur in distinct bands adjacent to the primary organic sheet. The width and intensity of these bands differ between elements and between the two species investigated. We evaluated the intensity of the high-Na, -Mg, -K, bands as a function of salinity. Together, these results are the basis of a new calcification model that explains incorporation of these elements as a function of 1) seawater chemistry and 2) biological control during calcification by the foraminifer. This framework will be applied to test recently obtained calibrations for incorporation of Na (and other elements) as a function of salinity.

Keywords: Biomineralization, Foraminifera, Salinity proxy

Investigation of δ^{26}Mg in large benthic foraminifera as a temperature proxy

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In the last decade, stable magnesium (Mg) isotope fractionation in biogenic carbonates has been attracted for a new paleoenvironmental proxy, along with technological advance in mass spectrometry. Although δ^{26}Mg has been expected to serve as more robust temperature proxy from the dawn of their evaluation, considerable differences were observed between various biogenic carbonates having various Mg content. In this study, we investigated δ^{26}Mg in large benthic foraminifers producing high-magnesium calcite tests in order to evaluate them as a temperature proxy. *Amphisorus kudakajimensis* and *Calcarina gaudichaudii* were cultured in six temperature conditions (21℃-30℃), and measured δ^{26}Mg by MC-ICP-MS. In a previous study, both species showed clear relationships of linearity between Mg/Ca and temperature. Regardless of the previous studies reporting positive relationships between δ26Mg and temperaturethe, the δ^{26}Mg in both species showed negative temperature dependency. There was no significant correlation with the growth rate of foraminifers. Evaluation of Mg isotope fractionation process in large benthic foraminifera may give a profound insight into a foraminiferal biomineralization.

Keywords: Temperature proxy, Large Benthic Foraminifera, Mg isotope fractionation

What are constraint factores for foraminifers shape ? either Physics or Biology

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Benthic foraminifers show genera specific test shapes. Test morphologies are different from species to species. Thusfar, foraminiferal tests are used as taxonomic characters. Patellina corrugata (Williamson) is made from single crystal of calcite. Even though the species has multi-chambered form, test consits of single crystal. Test growth takes place when calcite crystal grows. Is foraminiferal growth constraint by crystal physics or constraint by genetic inoformation ? I would like to discuss this question during my presentation. To grow with crystal physics, or not to be constraint by crystal physics ? This is the question.

Keywords: Foraminifera, test morphology, single crystal, crystal physics, Biological constraints

Biomineralization as the basis for understanding proxie incorporation

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A mechanistic understanding of element transport and incorporation into calcifying organisms is the basis for translating empirical proxie relationships into robust tools for paleo-reconstructions. Not only will it allow us to better understand the functional link between a target parameter and its geochemical signal but it will also unveil potential interactions with other biotic or physicochemical processes.
There are currently two models proposed for the biomineralization in Foraminifera that are fundamentally different but maybe not mutually exclusive. One model, is based on vacuolarisation of seawater while the other model (Trans-Membrane Transport model) is based on active pumping of Ca^{2+} ions during chamber formation. I will introduce the TMT model and discuss it in the context of additional, mostly experimental, data that has been generated over the last 30 years.

Keywords: Biomineralisation, trans-membrane transport, Proxies, Foraminifera, geochemistry

Late Holocene and Present Tropical Atlantic Ocean sewater temperature comparison based on stable isotopic proxies

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The Atmosphere and the Ocean are shown to be warming, in average, in the last century. However, locally this trend might not be the rule. In the Tropical Western Atlantic, long temperature time series are lacking and temperature proxies, such as $\delta^{18}O$ obtained from coral skeletons are still on the process of being validated. Here we show results of an investigation on oxygen isotopes of 2 ky old coral skeletons from 13S in the Brazilian coastline. We investigated present and 2 ky old specimens of *Mussismilia braziliensis* and *Siderastrea spp.* (endemic) corals and show the effectiveness of recent *Mussismilia braziliensis* species as current environmental conditions archive of seawater temperature. Based on this relationship, we show that temperature seasonality in the Late Holocene was similar to what is experienced in the present, although the contrast between warm and cold months was smaller in the Late Holocene than in the present. Furthermore the temperature in the late Holocene may have been about 0.2˚C warmer, differing from the global trend.

Keywords: corals, stable isotopes, Tropical Western Atlantic Ocean

Influences of symbiotic algae on skeletal mineral phases of scleractinian coral cultured with different Mg/Ca mol ratios

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Modern scleractinian corals live with symbiotic algae and construct their skeleton by calcium carbonate in aragonite form. For revealing the effects of symbiotic algae to coral skeletal mineralogy and coral calcification, aposymbiotic scleractinian corals, *Acropora tenuis* and *Acropora digitifera*, were cultured in treatment seawater with different Mg/Ca molar ratio. Their mineralogical features were characterized by using micro X-ray diffraction analysis. The coral skeletons were consisted of only calcite at Mg/Ca less than 1.0, indicating that aposymbiotic corals can survive by forming calcific skeleton under very low Mg/Ca molar ratio. The deposition of whole calcific skeleton at low Mg/Ca molar ratio is similar to experimental abiotic deposition from treatment seawater rather than coral skeleton growing with symbiotic algae. It suggests that the calcification of scleractinian coral is strongly affected by symbiotic algae and Mg/Ca molar ratio of ambient seawater.

Keywords: scleractinian coral, symbiotic algae, calcification

Proteomic analysis of shell matrix proteins in the pond snail *Lymnaea stagnalis*: discrimination of potentially functional proteins from accidentally occluded proteins

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Matrix proteins have important roles in molluscan shell formation, and their amino acid sequences have been characterized for some species. However, the mechanisms of shell formation have barely been clarified. In order to setup a platform for a systematic functional analysis of shell matrix proteins, we performed a combined transcriptome and proteomic analysis of the shell matrix proteins for the pond snail *Lymnaea stagnalis*. We found a total of 207 shell matrix proteins from the shell matrix of *L. stagnalis*. A total of 165 amino acid sequences of them showed sequence similarities to known proteins, including four paralogs of dermatopontin, which was previously reported from the shell matrix of *L. stagnalis*, while the remaining 42 showed no similarity to the proteins in the current databases. In order to discriminate functional shell matrix proteins from those that were accidentally buried in the shells, we compared the levels of expression of these shell matrix proteins between the right side and the left side of the mantle tissue which makes the shell. Underlying assumption is that genuine functional shell matrix protein genes would be more strongly expressed in the right hand side of the mantle in the dextral shell, while there would be no such differential expression pattern for the proteins which were accidentally trapped within the shells. Our results suggest that Pif-like protein is a functional shell matrix protein, while actin is a protein trapped within the shell accidentally. Comparisons of the expression patterns between the mantle and the foot tissues indicated that a total of 29 genes are expressed specifically in the mantle tissue with 25 out of them being expressed stronger in the right hand side than in the left hand side of the mantle tissue. Principle component analysis of the gene expression data showed that, those supposed functional shell matrix proteins are distinguished from the other shell matrix proteins, which were possibly accidentally entombed within the shells.

Keywords: Transcriptome, Proteomic analysis, Biomineralization, Shell formation, Matrix protein

Direct evidence for biogeochemical process in the formation of ferromanganese crust; Western Pacific Magellan Seamount

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Redox reaction is a ubiquitous process in the formation of ferromanganese crust that may reflect one of paleo-environments, particularly variations of Fe/Mn redox states and microbial diversity in the crust suggests the unique biogeochemical reactions when the ferromanganese crust layer forms. Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), Electron Energy Loss Spectroscopy (EELS), and Polymerase Chain Reaction (PCR) were utilized to determine the redox states of Fe/Mn and microbial diversity at each layer. A sample collected from Magellan Seamount (OSM11), western Pacific, was characterized in five well-defined crust layers, top to bottom (L1-5). Some microbial like structures of sheath-like with filaments (L1 –L3), capsule-shaped (L2), fossilized coccolith mounds with phosphatized globules (L4), and bean-shaped (L4) were detected in entire layers. The cross sectional observation of bean-shaped microbe like structures encrusted with Fe-vernadite (L3) by Scanning Transmission Electron Microscopy (STEM) and Focused Ion Beam (FIB) technique revealed ~ 1-μm diameter cavity in the center and porous structures of encrusting Fe-vernadite in periphery. Moreover, strong EELS profiles of organic carbon around the hole in the FIB-sectioned sample for microbe-like structure indicates that the microorganism used to occupy in the crusts and may play a role in the formation of Fe-Mn crusts. Indeed, presence of Fe- (*coxC*) and Mn-oxidizing gene (*cumA*), particularly displaying a strong PCR band of *coxC* in L2-3 indicate the dominant oxidizing conditions compared with L4 where CFA formed. The cloning and sequencing of DNA PCR fragments revealed the appearance of geobacter species in L3 (*G.* sulfurreducens and *G.* lovleyi). The present study collectively suggests that biogeochemical processes in the formation of Fe-Mn crust reveal unique paleo-environments of formation.

Keywords: Ferromanganese crust, EELS, TEM

Cellular Dissolution at Hypha- and Spore-Mineral Interface during Fungal Weathering

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Microbial weathering develops through intentional or unintended reactions between microbes/metabolites and minerals. Whereas the latter can be modeled by bulk dissolution, the former often involves complicated cell-mineral interfacial processes and hence is less understood. For fungus-mineral interaction, an additional but unique influence, i.e. the biomechanical forces, needs to be evaluated as surface-bound cells can apply physical pressure through hyphae to disrupt crystal structures. As high as 10-20 MPa12 appresssorial turgor pressure was reported during hyphal growth (approximately 100 times that of a typical car tire), strong enough for fungi to penetrate grain boundaries and break crystalline particles along the cleavage directions. What is more unique to fungi but yet largely unknown is the relative scales of cellular dissolution associated with different cell segments in light of the turgor pressure difference between hyphae and spores. Here we examine lizardite ($Mg_3Si_2O_5(OH)_4$) dissolution by single cells of a native fungal strain (*Talaromyces flavus*) from a serpentine mine using confocal laser scanning microscopy (CLSM), atomic force microscopy (AFM), and focused ion beam transmission electron microscopy-energy dispersive X-ray spectroscopy (FIB-TEM-EDX) to explore the mechanism, driving force, and magnitude of the interfacial reactions. Bulk experiments reveal that the fungi significantly enhanced dissolution. Moreover, dissolution was substantially stronger when cell-mineral contact was permitted in comparison to the cases where the cells were separated from the minerals grains via a semi-permeable membrane, suggesting the bioweathering results from combined active and passive microbial dissolution. In addition, the fungal effect appeared to steer the dissolution to a non-stoichiometric pathway. The molar ratios of Mg to Si during abiotic dissolutions varied between ~2 and ~1.3 but mostly stayed near the theoretical value of 1.5, signaling a congruent dissolution. In contrast, the ratio during bioweathering deviated progressively more strongly from the stoichiometric value as the dissolution continued and reached ~4 to ~7 at the end of experiments, indicating either a preferentially release of Mg or a re-precipitation of silica. Analyses of the cell-mineral interface show (i) significant pH reduction (~ 1 pH unit) in the vicinity of surface-bound cells upon mineral attachment, (ii) extensive occurrence of deep (~200 to ~2000 nm) channels and shallow (~50 nm) circular pits (features well resembling the size and shape of the hyphae and spores), (iii) exclusive Fe loss (by as much as 70%) from the mineral at the cell-mineral interfaces (i.e. in comparison to solution-mineral interfaces), and (iii) destruction of the mineral crystal structure below surface-colonized hyphae but not spores. Compared to the results from bulk experiments and at the mineral-water interface, these observations indicate (1) only attached cells release siderophores, and (2) biomechanical forces of hyphal growth are indispensable for fungal weathering and strong enough to breach the mineral lattice. Estimated mineral volume loss at the interface suggests that cellular dissolution can ultimately account for ~40-50% of the overall bioweathering, significantly larger than the previous estimate of ~1% contribution.

Keywords: fungal weathering, microbe-mineral interactions, lizardite, cell surface pH, interfacial reactions, siderophores

