

Aubri JENSON
June 2016

Lewis and Clark Fund for Exploration and Field Research
Evidence of Microbially Mediated Dissolution in a Submerged Cave

Previous Work

In January 2016, I began a series of standard tablet experiments to observe mass loss in Cenote San Carlos (Figure 1A; 20° 47' 28.65"N, 86° 56' 50.46"W) and quantify rates of limestone (calcite) dissolution. Cenote San Carlos is a coastal cave system hypothesized to be actively forming through geochemical mixing of fresh and saltwater, and possibly from chemoautotrophic bacterial activity that generates H₂S where organic carbon collects at the fresh-saline density boundary (halocline). To observe total dissolution rates and variation across water layers, three pairs of calcite crystals were prepared by cleaning in a 1mol HCl acid solution. Crystal mass was measured to 0.0005 g, and dimensions were measured with calipers to 0.1 mm to relate mass loss to exposed surface area. All crystals were zip-tied into 3 mm mesh bags. Pairs were used to obtain duplicate results and control for mass loss in travel.

I dived Cenote San Carlos on January 2, with explorer Jason Richards, and we placed the three sets of calcite crystals above (12 m), within (16 m), and below the halocline (17.5 m). The halocline was observed at 15-17 m, consistent with observations in nearby Sistema Crustacea (Figure 1B) (Haukebo, 2010). The halocline was cloudy with traces of H₂S and contained white cm-scale globules at 15-16m.

August 2016

In order to distinguish between dissolution occurring from interactions in the mixing zone and local acidity generated by microbes on rock surfaces, I prepared a second set of calcite crystals by the same methods, but enclosed one crystal in each pair in a 0.1 μm membrane bag. Bags were sterilized by autoclave at 120°C for 15 min.

I returned to San Carlos with Christine Lowe, a local cave diver and instructor. We retrieved the crystals set in January (except for the pair below the halocline, which wasn't found), and hung the sterile/non-sterile pairs at similar depths on the dive line with plastic markers. Water samples for ion composition and conductivity/DO/pH/temp profiles were obtained at each site. Conductivity increased abruptly at 14m from 9800 μs to 19000 μs. Dissolved oxygen dropped from 8.5 mg/L at the entrance to 0.4 at 14 m and remained near zero even in the saline layer at 18m. Temperature was 25°C in the freshwater and 28°C in the saline water. pH ranged from 7.3 in the freshwater zone to 5.7 at and below the halocline, although typically seawater is alkaline. Water at and below the halocline was moderately undersaturated with respect to calcite (Langelier index -2). Surface water was unusually high in Nitrates and Lithium, possibly related to fertilizer use on the ranch above the cave.

Mass differences of the crystals retrieved were 2.22-2.41 x 10⁻³ g above the halocline, 3.15-3.74 x 10⁻³ g within the halocline. The mass lost by those above the halocline was approximately 66% of that lost at the halocline. A control set placed in a cave pool of a different cave system known to be supersaturated with respect to calcite also exhibited mass losses of 1.96 x 10⁻³ g and 7.41 x 10⁻⁴ g, likely a result of mishandling in travel. Due to the fragility of the calcite crystals, this experiment was repeated using 6 pairs of cut limestone blocks for a minimum of 1 year. Edwards Limestone from Austin, TX was cut into 2.5 cm cubes, cleaned with HCl, dried at 100°C for 1 hour, and then measured to 0.1 mm and weighed to 0.0005 g. These were paired in 3 mm mesh bags to be placed above, within, and below the halocline.

Aubri JENSON
June 2016

Lewis and Clark Fund for Exploration and Field Research
Evidence of Microbially Mediated Dissolution in a Submerged Cave

January 2017

Another dive was done with Christine Lowe to place pairs of limestone blocks, retrieve samples, and take photos. Two of the sterile/non-sterile pairs were left in the cave to extend time for dissolution and achieve more conclusive results. The pair below the halocline was removed for preliminary results. One pair of limestone blocks was set at 12 m, one pair at 18 m, and remained in four pairs were set between 14 and 17m to account for variation within the halocline.

Water samples and profiles were taken again at 15 m, 17 m, and 20 m. Salinity changes and halocline depth were similar to observations in August. Dissolved Oxygen was about 8 mg/L at the entrance and throughout the freshwater layer, and dropped abruptly to 0.3 mg/L at and below the halocline. pH was near 8 at the entrance and through the freshwater layer, but remained near 7 at and below the halocline, which was significantly higher than in August. Temperature was near 30°C at the cenote entrance, but remained near 25°C below 2m, including in the saline zone below 17 m. Undersaturation with respect to calcite was estimated within and below the halocline (Langelier index -1).

The sterile/non-sterile pair of calcite blocks that was removed from 17 m showed mass losses of 1.7×10^{-3} (sterile) and 1.7×10^{-2} (non-sterile). These results are consistent with acceleration of dissolution occurring on the rock surface, at a rate an order of magnitude higher. Under a Scanning Electron Microscope, it appears possible that bacteria lodging in crevices could chemically erode and deepen fractures where they locally increase pH. Further work will need to be done, as this is only one data point. All samples remaining in San Carlos will be removed in August 2018. The additional time will allow for stronger conclusions as the mass differences are very slight and may be influenced by handling. Preliminary results suggest a maximum dissolution potential resulting in a wall retreat of 3 mm per 1,000 years, or a 1m increase in radius over 30,000 years. This is about 3 times slower than current assumptions based on geochemical modeling alone. It is possible that geochemical mixing effects are hindered by the presence of additional ions, and that microbial activity, while theoretically slower is sufficient to enlarge passages over geologic time.

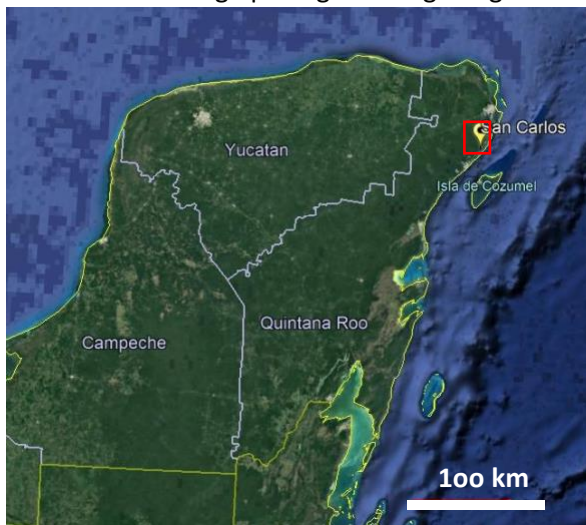


Figure 1A

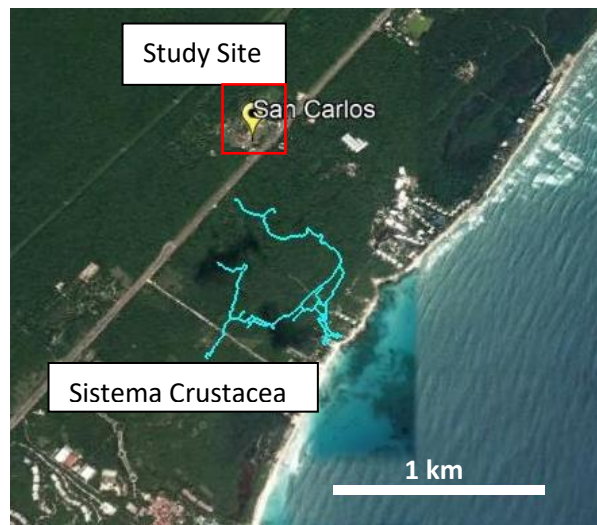


Figure 1B

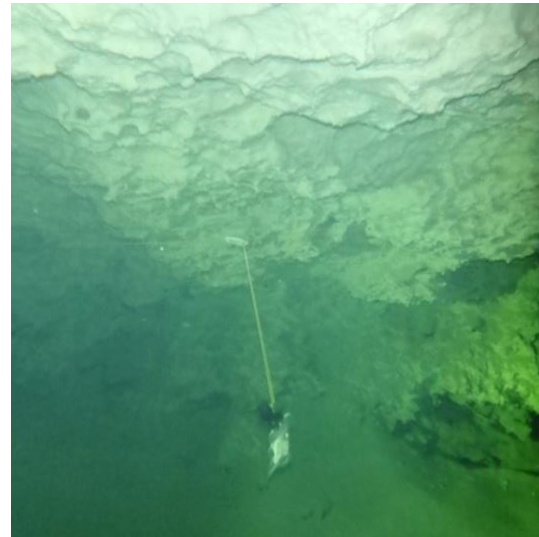
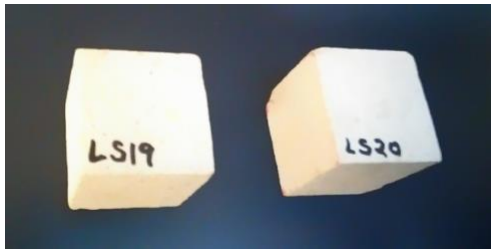
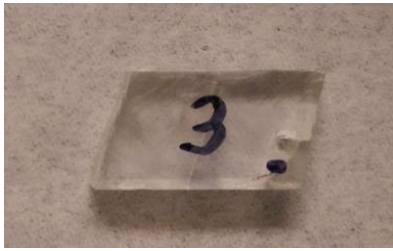


Figure 2a (top): A calcite crystal placed in Cenote San Carlos in August 2016 to measure a relative rate of dissolution. The same type of crystals were placed in sterile/non-sterile pairs in January 2017.

Figure 2b (bottom): Limestone blocks placed at various depths in January 2017, and will be retrieved in August 2018 to allow maximum time for dissolution. **Figure 2c (right):** A sterile/non-sterile pair hanging on the dive line within the halocline, to be removed in August 2018.

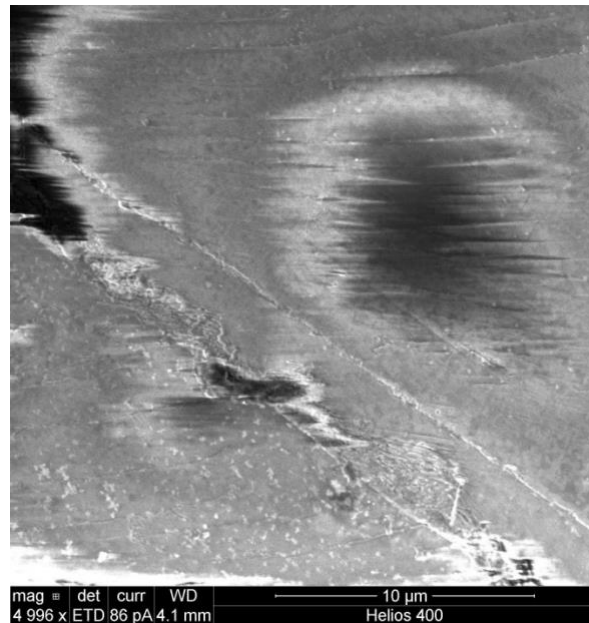
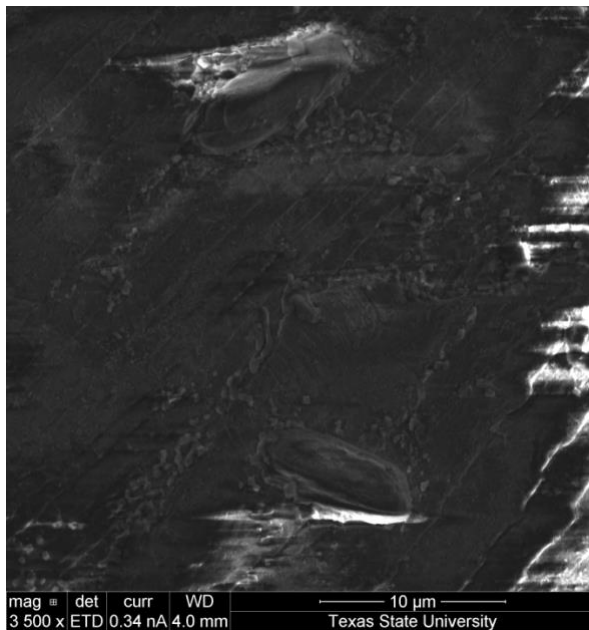


Figure 3a (left): Scanning Electron Microscope (SEM) image of a crystal exposed to bacterial activity. Surface etching may occur where colonies are present in crevices. **Figure 3b (right):** SEM image of a crystal encased in sterile 0.1µ mesh, showing a relatively smoother surface. Additional imaging will be done on the crystals and limestone blocks currently in the cave.