KECK GEOLOGY CONSORTIUM

PROCEEDINGS OF THE TWENTY-FIFTH ANNUAL KECK RESEARCH SYMPOSIUM IN GEOLOGY

April 2012
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INVESTIGATING ENDOLITHIC ALGAE PROLIFERATION USING STABLE CARBON ISOTOPES IN BOULDER STAR CORAL

CORNELIA CLARKE, Pomona College
Research Advisor: Robert Gaines

INTRODUCTIOIN

Coral reefs are important ecosystems under threat. Corals create the framework for reefs by precipitating calcium carbonate skeletons, in turn hosting vibrant and diverse communities. Corals are colonial animals; each coral head is composed of hundreds of polyps, each polyp with symbiotic zooxanthellae that produce sugars for the coral in return for nutrients and shelter. This symbiotic relationship, under conditions in the coral's physiological range, works quite well—corals produce calcium carbonate three times faster in the light than in the dark due to the zooxanthellae's role in carbon cycling in the coral (Gattuso et al., 1999) (Fig. 1). The photosynthetic and calcifications reactions complement each other well, and the coral is able to dictate the rate of each by controlling the amount of reactants for each process.

Figure 1. Coral polyp anatomy (left) and carbon transport scheme (right). The boxes on the left are expanded to cross sections on the right. These cross sections, although only shown as one line on the left, are actually two tissue layers and space in between. On the right, the top equation is the equilibrium of carbonate in ocean water, with the percent abundance of each carbon component. The solid lines for the bicarbonate (HCO_3-) through the tissues represents active transport of the bicarbonate, while the dotted lines for the carbon dioxide (CO_2) represent the diffusion of CO_2 through tissue layers. The black solid ovals are carbonic anhydrase (CA), an enzyme that mediates the reactions with CA over the arrows. The oval with a Z represents the zooxanthellae, and the large unfilled oval is the calcium/proton antiporter, which provides the skeletonizing tissues with the calcium needed to make calcium carbonate ($CaCO_3$).

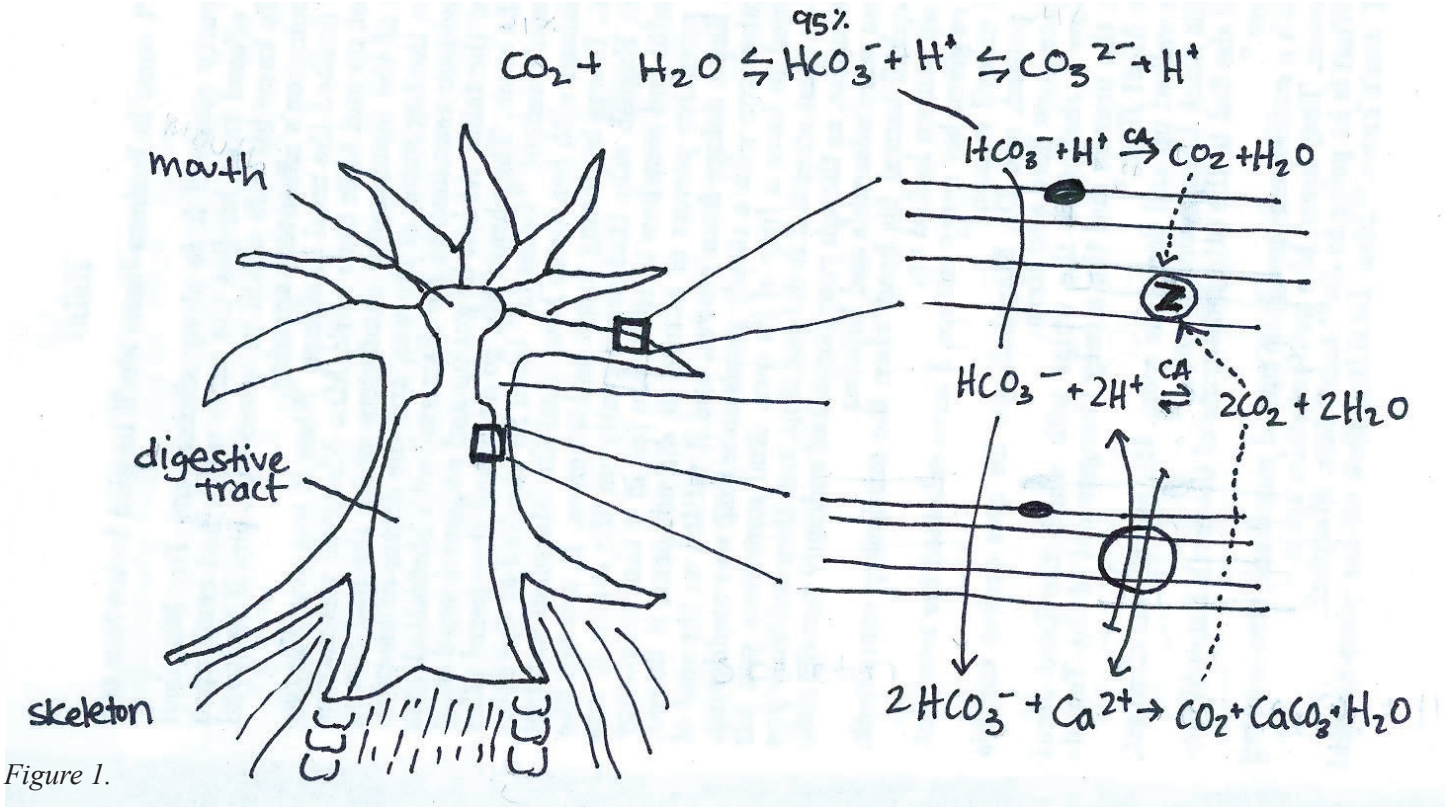

Figure 1.

Precipitating $CaCO_3$ produces CO_2 that is used for photosynthesis, driving the calcification reaction forward. CA mediates the amount of HCO_3- and CO_2 depending on the demand for each component between these two processes of calcification and photosynthesis. There is a shared pool of carbon between the calcification and photosynthesis tissue layers where metabolic carbon isotope fractionation can occur.

However, when the coral is stressed by conditions over physiological limits, such as high temperature, the relationship between the coral and the zooxanthellae degrades. The zooxanthellae's photosynthetic pathway breaks down at high heat, producing radical oxygen species (ROS) (Wooldridge, 2010). ROS are essentially toxins, damaging lipids and proteins. The coral evicts the zooxanthellae as self-defense, leading to coral paling, characterized by the loss of color of coral polyps, as photosynthetic pigments in zooxanthellae provide much of the color of polyps. Coral bleaching is the total loss of zooxanthellae, which turns the corals bright white. Corals can survive bleaching if conditions are otherwise supportive and if the temperature drops back down again allowing the coral polyps to re-recruit new zooxanthellae (Brown and Cole, 2003). The loss of zooxanthellae is an extreme stress reaction in corals.
Due to global warming, sea surface temperatures are rising, a troubling environmental change for corals, as corals are living on the edge of their heat tolerance. Experiments in the 1920s and 1930s found that corals have a physiological limit of heat tolerance only a degree or two away from the summer high temperatures where they live (Brown and Cole, 2003). Bleaching events have increased over the past 30 years, increasing coral mortality (Brown and Cole, 2003). The mass bleaching event in 1997- 1998 resulted in massive coral fatality in reefs in 47 countries (Fitt et al., 2001).

When corals experience metabolic shifts, the isotopic fractionation of carbon changes. When zooxanthellae are active, the photosynthetic enzymes select for ^{12}C to fix into sugars. This leads to an enrichment of ^{13}C in the shared carbon pool, and an enrichment of ^{13}C in the carbonate skeleton (Fig. 1). When photosynthesis is not occurring as much, more ^{12}C is available and the skeleton has a lighter isotopic fractionation. In high light, the carbon isotopes in coral skeletons are heavier than in low light (Grottoli, 2000).

Corals also host a suggested symbiotic species of photosynthesizing algae in their skeleton. Receiving less than 2% of solar radiation, these algae still manage to thrive in the corals despite the low light (Halldal, 1968). They are not major components in the coral— the algae have 6% of the photosynthetic activity of the zooxanthellae (Schlichter et al., 1997). They are dubbed endolithic algae because they bore into the carbonate skeleton, living in the carbonate skeleton. As the coral grow upwards, the algae grow with it, leaving behind evidence of their occupation through their borings into the carbonate. Usually, populations of endolithic algae are not dense enough to be seen in the skeleton in cross section by naked eye, but sometimes the algae experiences a bloom and can be seen as a millimeter to centimeter thick green band (Ralph et al., 2007). These endolithic algal blooms are then preserved in the skeleton thanks to the lack of oxygen, slowing decay (Kanwischer and Wainwright, 1967) (Ingalls et al., 2003). Green bands can thus be seen in coral cores from 50 or more years ago (Carilli et al., 2010).

The cause of the green bands is still unknown, but several groups have theorized that the endolithic algae bloom when they receive more light, usually precipitated by coral paling (Highsmith, 1981). Evidence for this relationship is that endolithic algae can be spotted on bleached corals (Rodríguez- Román et al., 2006) and the algae can adapt to higher light conditions, given sufficient time (Fine et al., 2005). A symbiotic relationship has also been suggested for the algae (Odum and Odum, 1955), supported by findings that endolithic algae can transport products of photosynthesis to the corals (Schlichter et al., 1995). Endolithic algae may thus act as symbiotic organisms to corals, assisting corals through paling episodes by giving their hosts sugars.

I am testing the hypothesis that the green bands occur during coral paling by investigating the carbon isotopes of the coral skeleton. I predict that in years with green bands, the carbon isotopes will be lighter than in previous and subsequent years without green

bands, due to decreased photosynthetic activity.

METHODS

Sample Collection

We collected all *Montastraea annularis* corals over a two-week period in the summer in US Virgin Islands National Park, St. John (see Hubbard and Parsons-Hubbard, this volume). We cut corals into several slabs. I scanned each slab on a flatbed scanner, creating a record of green band locations in each coral sample. We also imaged the corals with X-radiography to reveal density banding to date the coral.

Figure 2. Cross section of a coral with 4 green bands (blue arrows). Most corals did not have more than 2 green bands.

Scanning Electron Microscopy

The endolithic algae were identified as *Ostreobium* through the use of scanning electron microscopy to view to boring patterns (Lukas, 1974). We cut coral sections with green bands from the slabs, capturing a section of the band and the area around it, and impregnated them with resin. I then used 7% hydrochloric acid for one minute to dissolve the carbonate on the surface to expose the resin in relief. I carbon coated the exposed surface and loaded into Pomona College's scanning electron microscope to capture images of the borings. I compared the images to previous studies to determine the species represented (Budd and Perkins, 1980).

Carbon and Oxygen Isotopes

I sonicated coral slabs in water for thirty minutes to remove organic material and then dried them overnight at room temperature. I powdered and collected samples for isotopic analyses by spot drilling with a hand dremel with a 1/16-inch diameter diamond drill bit. Samples were taken along corallite walls to a depth of 1-2 mm from areas within annual density bands below, in, and above green band zones. Three corals with 14 green bands total were sampled. The UC Riverside FIRMS mass spectroscopy laboratory performed the $\delta^{13}C$ and $\delta^{18}O$ analyses.

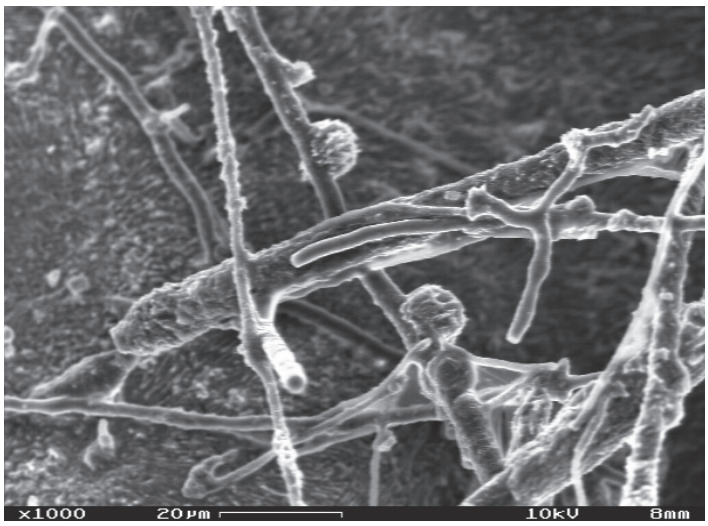

Figure 3. SEM image of an etched resin coral. The Ostreobium borings are the smooth thin tubes.

RESULTS AND DISCUSSION

53% of the corals had at least one green band (Figure 2). The green bands are common but are not ubiquitous. A green band was found right underneath the surface for a live coral collected, indicating that the green bands occur below the surface of the coral, unlike other corals where the algae are sometimes millimeters below the surface (Hartmann et al., 2010). The algae present in the green bands are Ostreobium constrictium, an endolithic algae species often found in the Caribbean Sea (Fig. 3) (Lukas, 1974). Unfortunately, the isotope data is taking longer than expected, so I do not have it.

CONCLUSION

Green bands are common in *Montastraea annularis*, similar to other coral species. The green bands in *M. annularis* are made primarily by Ostreobium, as in other coral species (Lukas, 1974). I hope to have the isotopic data to present at the symposium.

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