A Novel Mechanism for Freshwater Reef Growth?

Investigations into the Growth of a New Habitat

Researcher: Nora Covy

Mentor: Paul Moore

Exploration of a new habitat with distinctly reef-like features in South Fishtail Bay of Douglas Lake, Michigan has led to the development of a series of hypotheses regarding the formation of magnetic, tubular features known as cups that are found at the site. The age of the habitat and whether or not the cups are still growing is unknown, but it is nevertheless clear from this project that the cups are distinctive microhabitats. Analysis has shown that the cups contain more organic matter than the surrounding hard substrate, but equivalent amounts of chlorophyll α. The unique chemical and structural composition of the cups could be a product of bacterial activity. Algae and groundwater may also be playing a role in creating altered local conditions at the habitat that contribute to the formation of the cups.

INTRODUCTION

In the summer of 2008, a new habitat known as the Moore Feature was discovered at the bottom of Douglas Lake, Michigan. The discovery of a new habitat with reef-like qualities in a freshwater lake is unusual and could potentially provide new information for the fields of ecology and biology.

The Moore Feature is located off of Grapevine Point, at a depth of approximately 7-18 m. It is unique in its sedimentary composition. There are hard shelves that protrude from a soft substrate and cup-like formations that appear to have biological origins and tend to be grouped in fields. The cups are generally of a tubular shape that either go down into the shelves or protrude from them. Most are a basic circular shape, but more intricate patterns, such as figure eights and ribbons, have also been found. The cups appear to be composed of sand interspersed with small pebbles and are held together by an unknown cement-like substance. Horizontal cross sections have revealed that the cups contain layers of rings.

In mid June of 2009, an underwater camera was set up at the site to record biological activity. No organisms were seen specifically using the cups, but many large predators were viewed at the site including mudpuppies, suckerfish, and pike. This is indicative of the habitat being a biologically productive site with at least four trophic levels.

The goal of the experiment outlined in this paper was to gain further knowledge about the ecological role of the cups with the community structure to complement the information gained from the camera. We also wished to determine how the cups are formed. Cups were compared to the surrounding hard substrate to see how they compared in amount of organic matter and primary productivity. The techniques summarized by Kominoski et al (2007) were used in performing the Ash Free Dry Mass (AFDM) and Chlorophyll α tests.

MATERIALS AND METHODS

Sedimentary samples were collected from the habitat at a depth of approximately 10 m. 6 cups of varying sizes were brought to the surface in ziplock bags. A cork borer with an area of 4.08 cm^2 was used to cut out identically sized disks from the hard substrate that surrounded the cups. The disks were then placed in whirl bags and brought to the surface. Samples were stored in a refrigerator until analysis began.

The Ash Free Dry Mass test was performed using standard procedures. The cork borer was used again to fracture off pieces with areas of 4.08 cm^2 from the cups. The pieces were generally taken from near the rim of the cup, not the base. Samples were placed directly into pre-weighed aluminum pans. The pans were dried in an oven for 24 hours at 65 °C. After the samples were weighed, they were placed in a muffle furnace set at 550 °C for 1 hour. The samples were weighed again after they cooled. The amount of organic material contained in each sample was calculated as the difference between pre- and post-combustion weights.

Chlorophyll α analysis was performed to measure the relative concentrations of chlorophyll. All actions for this procedure were carried out in limited light so the pigments would not degrade. The substrate disks were crushed with a mortar and pestle, mixed with deionized water, and loaded into a syringe. The mixture was then pushed through a filter. The filter papers were carefully removed with forceps and placed in individual test tubes. The cups were thicker than the substrate and therefore had too much hard matter to allow the water to pass through the filter. Instead, a wire brush was used to scrape periphyton off an area equivalent to the cork borer's area. The brush was rinsed with deionized water and the solution was then put into the syringe and passed through the filter. Again, the filter paper was placed into a test tube. Despite this variation in techniques, we believe that the wire brush is effective at removing periphyton and the results should therefore be accurate.

10.0 mL of 90% acetone with Mg^{2+} buffer were added to each test tube to dissolve the filter paper. The tubes were mixed thoroughly on a vortex machine and subsequently placed in a sonicator for 20 minutes. The samples were left in a freezer for 48 hours. After this time they were brought back to room temperature and mixed again on the vortex machine. They were analyzed using a TD-700 fluorometer. Approximately half of the sample solution was poured into a cuvette which was wiped with a kim wipe before being placed in the machine for the reading. The cuvette was rinsed with acetone between each use and rinsed again with a small portion of the next sample that would be read.

Results

A significant difference was found between the cups and the substrate. A one-way MANOVA test ($F_{2,9}$= 5.643) gave a p value of 0.026. Subsequent post-hoc tests using Tukey Honest Significant Difference showed that there was a significant difference between cups and substrate for organic material (Fig. 1), but not for chlorophyll α (Fig. 2). The cups had almost twice as much biomass as the surrounding substrate and the two sample groups showed a highly significant difference (p= 0.006). The standard error was 0.028. We are 95% confident that the average cup organic matter was between 0.181 g and 0.306 g and the average substrate organic matter was between 0.043 g and 0.169 g. The substrate had a slightly higher average amount of chlorophyll α than the cups, but there was not a significant difference between the two sample groups (p= 0.311). The standard error for chlorophyll α was 13.200. We are 95% confident that the average concentration of chlorophyll α for cups was between 16.321 μg/L and 75.14 μg/L and the average concentration of chlorophyll α for substrate was between 36.238 μg/L and 95.062 μg/L.

Discussion

The results of this project indicated that the cups have a significantly larger amount of organic matter than the substrate at the Moore Feature. However, the cups and the substrate contain the same concentration of chlorophyll. The difference between the biomass of the cups and substrate is therefore unaccounted for. Several hypotheses have been suggested to explain what is contributing to the large amount of organic matter found on the cups. The first is that red or yellow algae could be growing preferentially on the cups. These alga use phycobillins and xanthophylls, two photosynthetic pigments that we did not test for but are common at lower light levels. Previous research has shown that the three major algal species present at the Moore Feature are a green algae, *Chladophora profunda*, and two species of epiphytic blue-green alga, *Gomphonema*, and *Heteroleibleinia* (Graeff 2008). However, this research did not look directly at the algal growth on the cups, but was generalized over the site and variation in microhabitats may have been overlooked. The second hypothesis is that the cups are heavily colonized by unique bacterial species that are not present in high numbers on the hard substrate. Finally, the cups may have been formed through biological processes which left a large amount of organic material as part of their basic composition.

During this project several unexpected, but highly relevant observations were made that are important general knowledge concerning the defining features of the habitat, but also may help explain how the cups were formed and why they are biologically significant. The first observation was the difference in appearance between the cups and substrate after they had been combusted during the AFDM test. When they were first collected, the samples looked highly similar. The cups and substrate were both dark russet and brittle. The only major observable differences were in basic shape, thickness and texture. A layer of fuzzy growth was located on the rims of the cups that was not present on the substrate. Post-combustion, the substrate samples had changed into a light brown, sandy composition. The cups had remained generally intact, however, and were a dark red-brown. We assumed that the red coloration was due to the presence of iron and further investigation led us to discover that the cups were magnetic.

Iron is found in high concentrations throughout the habitat, but seems to be found as hematite only within the cups. Iron exists in a variety of forms and changes between forms are based on a variety of factors, the most influential being redox potential and pH (Fig. 3). These factors are subject to a large degree of change from day to night and season to season. The conditions at the Moore Feature are in a range that allows three different forms of iron to exist with slight fluctuations. Most iron exists as Fe^{2+}, but an increase in oxygen levels or pH could lead to conversion to hematite or siderite. We believe that localized photosynthesis on the cups may be increasing the oxygen levels and causing formation of hematite.

The second observation that is of great consequence to the work on the Moore Feature was that the insides of the cups appear to be slightly cooler than the surrounding substrate. Preliminary work showed that the temperature inside the cups is approximately 2 °C cooler than in the surrounding substrate. We believe this is indicative of groundwater entering the site. Pressure from the hill on Grapevine Point may be forcing groundwater influx into the lake at weak points in the lake bottom. If this is true, groundwater could be playing a significant role in forming the unique features that are observed at the Moore Feature. There have been many examples in nature of tubular structures forming through bubbling precipitates leaving mineral deposition rings on a growing structure (Stone and Goldstein 2004). The groundwater could be forming the cups directly through deposition rings. This does not appear to be entirely likely since we have not observed the cups bubbling and the cups are wide in diameter and variable in shape. A more likely explanation is that the groundwater is altering local conditions and creating a unique environment for different bacterial and algal species to exist. These localized species may be responsible for the formation of the tube-like structures.

Conclusion

Using all of the knowledge gathered as a result of this project, we have created several theories on cup formation. First, influx of groundwater may be influencing the growth of the cups either through deposition or altering of local conditions. Second, concentrated hematite formation in the cups may be linked to localized photosynthesis. Finally, the large biomass of the cups may be indicative of high numbers of bacteria. Fe- reducing bacteria as well as polysaccharide producing bacteria have been documented (Chaudhuri et al 2001; Lovely and Philips 1988; Bell et al 1988; Chan et al 2004) and may be contributing both to the formation of hematite and to the creation of the tube-like structures. Although we are unsure of the method, we believe that the cups are being formed by biological means which leads us to conclude that the Moore Feature is a biotic freshwater reef, the first of its kind to be documented.

A great deal of further research is required to fully understand this unique habitat. One of the first steps is ascertaining whether groundwater is entering the Moore Feature and, if it is, analyzing its water chemistry. It also would be useful to compare the concentrations of phycobillins and xanthophylls between the cups and the substrate to determine if different algae types account for the difference in biomass. A general survey of the microbes and algal species present would also be incredibly valuable to work on the habitat. Finally, determining the age of the cups and whether or not they are still growing would contribute greatly to our knowledge base. One proposed experiment would be to locate a site where groundwater is suspected to be entering the lake, clearing it of cups, and observing it over time to see if any physical changes occur.

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Appendix

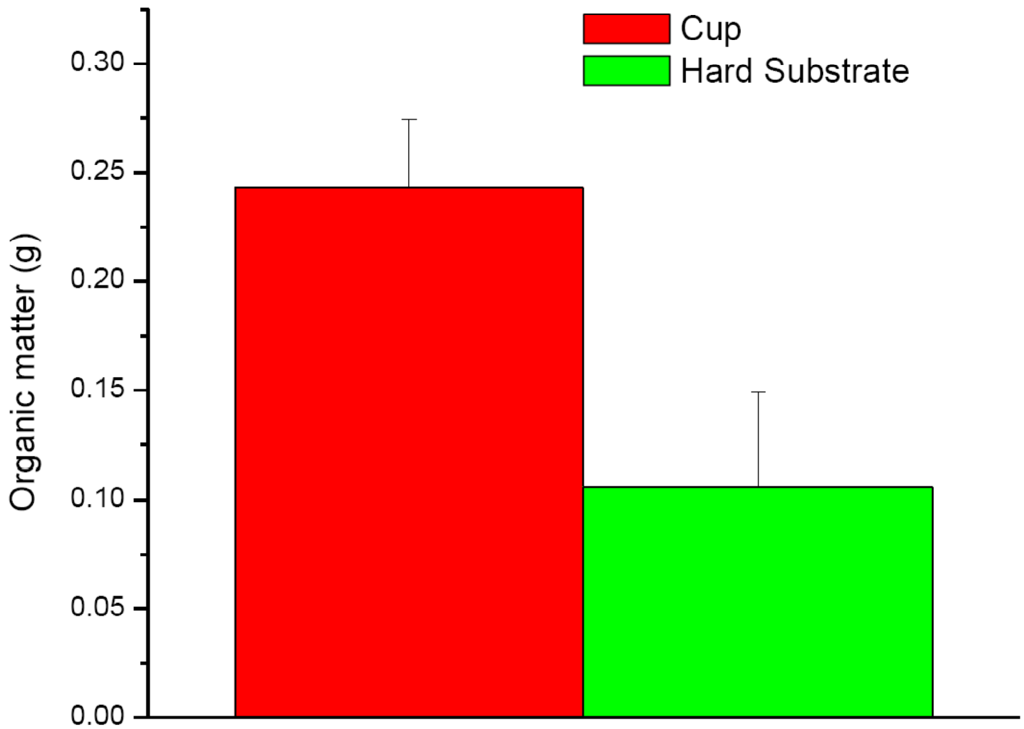

Fig. 1 Relative amounts of biomass for cups and the surrounding hard substrate, as determined by Ash Free Dry Mass.

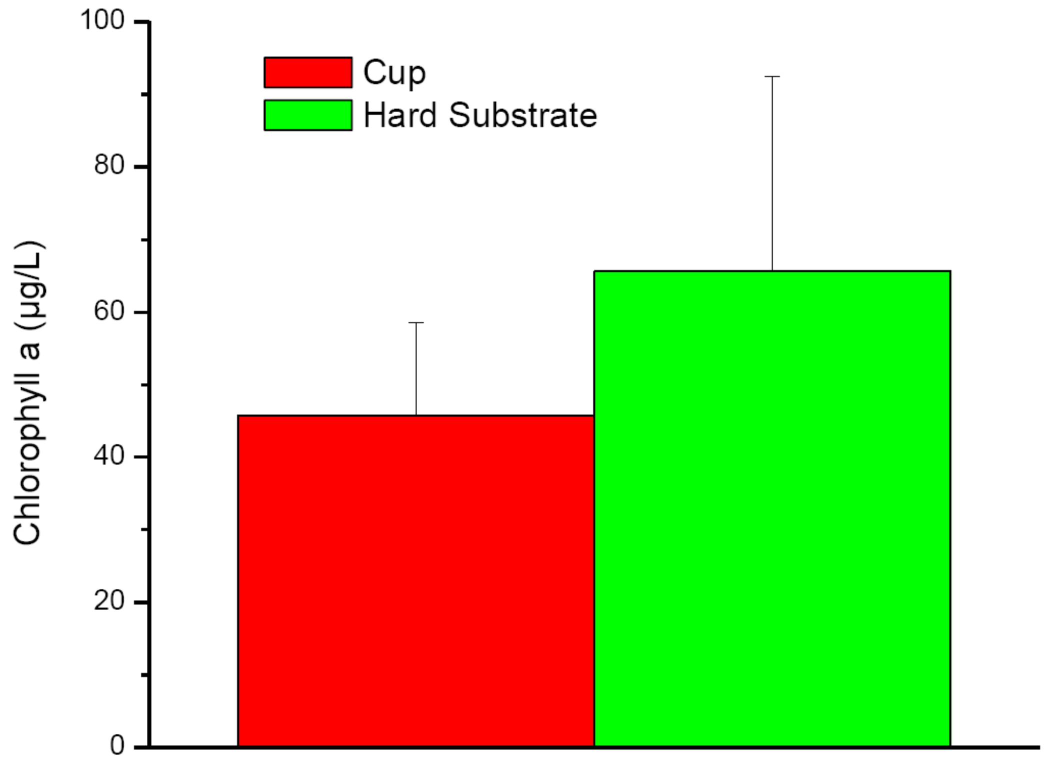

Fig. 2 Relative concentrations of chlorophyll α for cups and surrounding hard substrate.

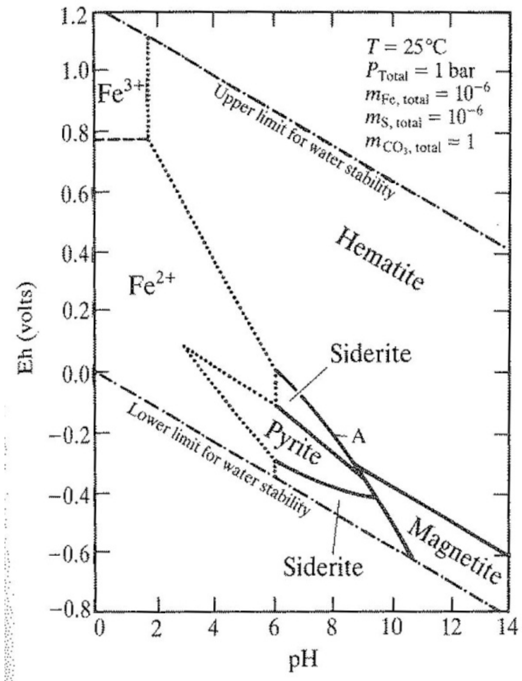

Fig. 3 Eh-pH diagram showing fields of common iron minerals. Dotted lines represent equilibrium between hematite, siderite, and pyrite. (Reprinted with permission from Garrels and Christ 1965.)