NAT. CROAT. | VOL. 21 | Suppl. 1 | 43–46 | ZAGREB | September 30, 2012

THE DYNAMICS OF COMMUNITY STRUCTURE WITHIN A SUBMARINE COASTAL SPRING WITH AN ANCHIALINE SOURCE

James R. Garey* & Damian M. Menning

University of South Florida, Department of Cell Biology, Microbiology and Molecular Biology, 4202 E. Fowler Ave ISA 2015, Tampa, FL 33620 U.S.A.

Double Keyhole Spring is a unique coastal submarine spring with complex and dynamically changing hydrology and water chemistry. It periodically reverses direction of flow, with accompanying changes in water chemistry and microbial communities. Although these transient changes follow the tidal cycle, longer term seasonal changes have also been detected.

Key words: community structure, anchialine, spring, estuary, geochemistry

INTRODUCTION

Double Keyhole Spring is a brackish coastal submarine spring on the west-central coast of Florida. This tidally influenced spring discharges into an estuarine pond at low tide. At high tide, the flow often reverses, allowing estuarine water to enter the spring conduit to a depth of at least 41 m. The source of the spring is an anchialine breakdown room located 300 m from the entrance accessible by divers. Isabella Spring is a freshwater spring that discharges into a freshwater pond located 2 km inland from Double Keyhole Spring. It is tidally influenced and likely has an anchialine source which is not accessible by divers. Both springs periodically reverse flow, so in Double Keyhole Spring, saltwater from the Gulf of Mexico flows in at high tide while at Isabella Spring, fresh pond water flows in at high tide. We are interested in understanding the hydrology, biodiversity and water chemistry of these related spring systems because they are both tidally influenced and connected to the aquifer. We hypothesize that microbial communities in the two springs are distinct from one another and change on two time scales: (1) a short term cycle with respect to tide-related flow reversals and (2) longer term changes due to seasonal differences in the surface water and aquifer discharge.

METHODS

We used Hydrolab datasonds, Acoutic Doppler Velocimeters (ADV) and more detailed chemical analysis of water samples to characterize the hydrology and basic water chemistry of the systems. We have also collected water samples by SCUBA for detailed molecular analysis of the community structure of the water column bacteria, Archaea and microbial eukaryotes using length-heterogeneity PCR (lh-PCR) (Yi-Guo *et al.*, 2011). Water column macrofauna were not observed. PRIMER v6

* garey@usf.edu

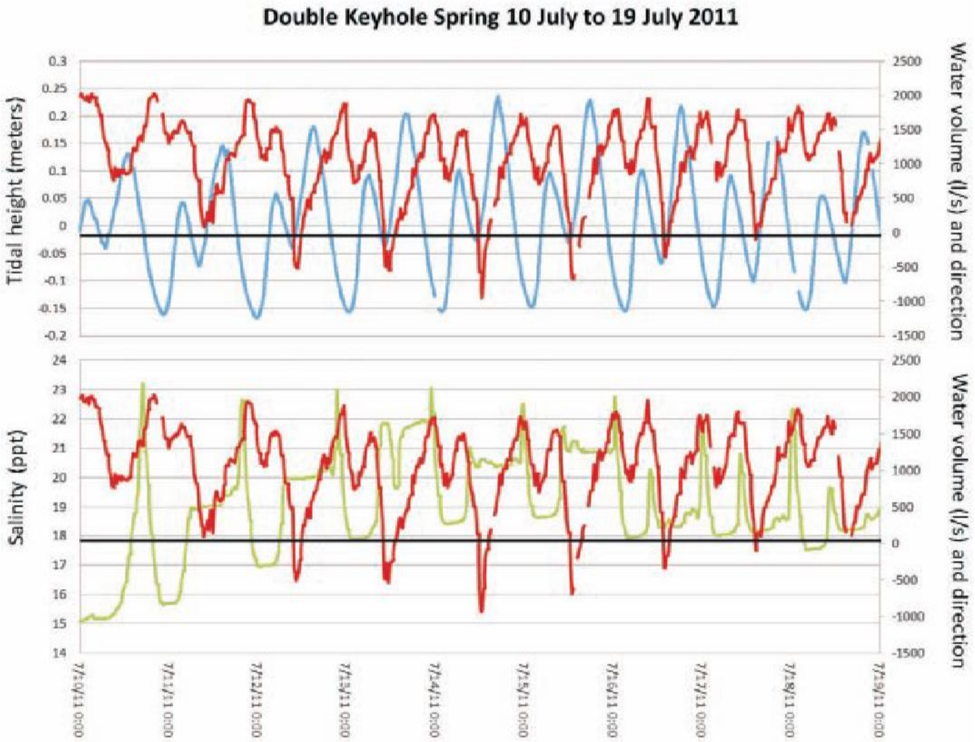

Fig. 1. Top – Datasonde profiles indicating the relationship between tides (blue) and discharge volume (red) at Double Keyhole Spring. Negative volumes (below dark black line) indicate flow reversals. Bottom – Datasonde profile indicating the relationship between increased aquifer discharge (red) and increases in salinity (green). Gaps in volume data represent errant spikes in the data set caused by excess flocculent material in the water column. Gaps in the tidal data represent data loss by the government data collection site.

(Primer-E Ltd., UK) software was used to analyze the community structure data and correlate it to chemical properties of the water. This study was carried over a one year period with data collected every two to three months. All samples were collected in replicates of five.

RESULTS AND DISCUSSION

ADV data from Double Keyhole Spring indicates a second magnitude spring with a maximum flow 2,083 L/s and an average flow of 936 L/s (Fig. 1). Similar values were measured in the winter. The water is brackish, ranging from 15–23 salinity (PSS). This outflow volume does not appear to change between summer and winter (Fig. 1). Double Keyhole Spring reverses flow approximately 5% of the time, periodically allowing estuarine surface water into the system. We have detected this flow as deep as 41 m but have not yet determined the maximum depth of the intrusion events. The flow reversals coincide with most but not all daily high tide events. We did not collect ADV data at Isabella Spring but have observed flow reversals when diving at high tide at that location. Isabella Spring discharge usually has a salinity of less than 1 with occasional increases as high as 8.

Estuarine water intrusion events at Double Keyhole Spring are accompanied by changes in water temperature, dissolved oxygen and pH (Fig. 1). Increased salinity correlates with low tide and increased water outflow from the anchialine source. This is supported by hydrology and water chemistry measurements within and around Double Keyhole and Isabella Springs. Sulfate, alkalinity and pH change significantly over time within both Double Keyhole and Isabella Springs. Samples collected within Double Keyhole Spring at different depths ranging from 10 m to 41 m along the conduit on the same day have nearly identical chemical properties.

Molecular analyses of microbial prokaryote and microbial eukaryote environmental DNA indicates that the community structure at Double Keyhole is significantly different from Isabella Spring (Fig. 2). In general, the difference appears likely due to the brackish nature of Double Keyhole Spring compared to the freshwater flow in Isabella Spring. The community structure within Double Keyhole Spring during flow reversal events is very distinct from the community structure during normal outflow. The community structure at normal outflow correlates best to oxygen concentration (0.521 Spearman Correlation), while during flow reversal the community structure correlates best with pH (0.871 Spearman Correlation). Although we did not collect data at Isabella Spring during a flow reversal event, community structure correlates best with oxygen concentration (0.554 Spearman Correlation) during normal outflow, similar to that of normal outflow at Double Keyhole Spring.

Microbial community analyses at both Isabella and Double Keyhole Springs demonstrate that long term changes in community structure occur during normal discharge (Fig. 2). At Double Keyhole Spring, although the community structure during flow reversal is different than during normal outflow, both display long term, perhaps seasonal variation (Fig. 2).

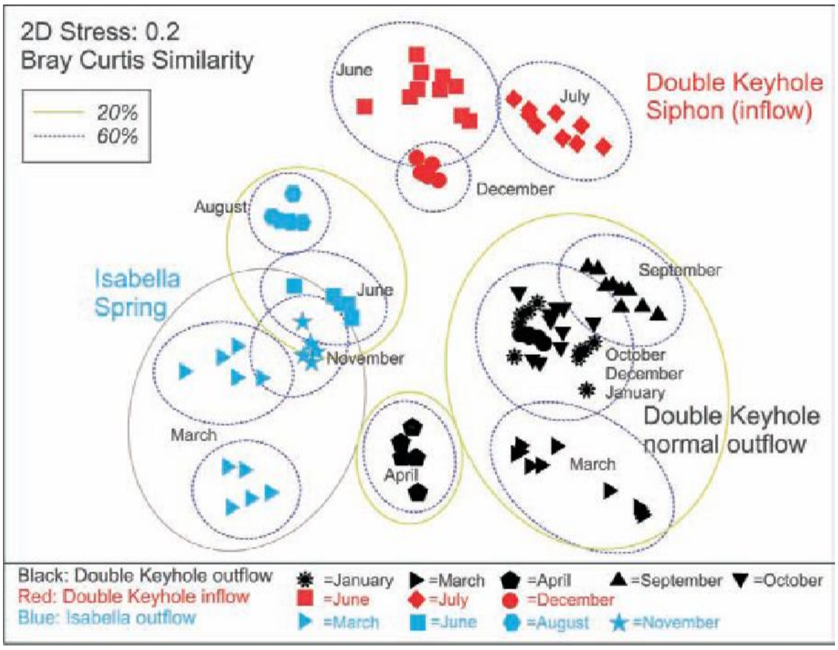

Fig. 2. Multidimensional Scaling Analysis of microbial communities within water column samples. Symbols near each other are more similar in community structure than those more distant. Each symbol represents one of five replicate samples from lh-PCR analysis of microbial prokaryote and eukaryotes. Samples from Double Keyhole outflow (black) vary from month to month but are distinct from Double Keyhole inflow (red) and samples from Isabella Spring (blue) are distinct from all Double Keyhole samples.

CONCLUSIONS

1. Double Keyhole Spring and Isabella Spring have distinct community structures and water chemistry. Community structure during normal outflow is most correlated to oxygen concentration at both springs.

2. At Double Keyhole Spring the community structure is dependent on the direction of water flow which varies with the tidal cycle.

3. Both Double Keyhole and Isabella Spring have microbial communities that change from month to month which could reflect changes in the season.

FUTURE GOALS

We are in the process of analyzing sediment samples and water samples from both springs and nearby estuary sites. We plan to continue short and long term monitoring of these systems to characterize the biogeochemistry of the source water in Double Keyhole Spring through DNA, chemical, and stable isotope analysis in order to determine how the output of these systems affects the near shore Gulf of Mexico ecosystem.

REFERENCES

Yi-Guo, H., Bo, Y. & Tian-Leng, Z., 2011: Diversity of anammox bacterial community in the deep--ocean surface sediment from equatorial Pacific. Applied Microbiology and Biotechnology **89**, 1233–1241.