



Lidya Tarhan received her B.A. degree from Amherst College. The summer after graduation, she held a GSA GeoCorps position as Field paleontologist at Bryce Canyon National Park. She received her M.S. from the University of California, Riverside and is currently enrolled in their Ph.D. program. Dr. Mary Droser is her advisor.

Examining Metazoan-Microbialite Interactions: A Case Study from the Exuma Cays, Bahamas

Lidya Tarhan (University of California, Riverside)

Precambrian carbonates, in contrast to the majority of the Phanerozoic record, are characterized by prolific, widespread and morphologically diverse microbially mediated carbonate buildups. microbialites (Grotzinger and Knoll, 1999; Riding, 2011). Decline in microbialite abundance and diversity during the late Proterozoic and early Phanerozoic is traditionally attributed to the concurrent radiation of burrowing and grazing metazoans (Garrett, 1970; Awramik, 1971, Walter and Heys, 1985). Similarly, the apparent resurgence of microbialites in the wake of Paleozoic and Mesozoic mass extinctions (e.g. Sheehan and Harris, 2004; Mata and Bottjer, 2012) is frequently linked to drastic declines in metazoan reefal communities and increased abundance of microbial communities. Recent work suggests, however, that not only are microbialites relatively common in modern carbonate environments, but they can also host dense and diverse communities of infaunal metazoans. These findings suggest that prevalent interpretations attributing Phanerozoic declines and fluctuations of microbialites to metazoan-mediated exclusion are in need of reevaluation. Moreover, close examination of modern microbialite-metazoan communities will shed light on the effect of metazoan activity upon the formation, early diagenesis and preservation of both modern and ancient microbialite fabrics. In light of growing awareness of the importance of microbialites as hydrocarbon reservoirs, a better appreciation of the role infaunal metazoan communities play in microbialite fabric development may not only significantly augment our understanding of modern and ancient microbialites, but also elucidate the processes involved in the generation of microbialite reservoirs.

On the basis of emerging awareness of the interconnectedness of microbialite and metazoan communities, I propose a detailed study of metazoan abundance and diversity across a spectrum of microbialite types and sedimentary settings at Lee Stocking Island and Little Darby Island, the Bahamas, with the objective of systematically characterizing the relationship between microbial mat-building communities, infaunal metazoan ecology and microbialite fabric development. I will employ a variety of ecological, geochemical, sedimentological and petrographic tools to more closely examine the importance of such factors as sediment stress, energy, exposure; organismal recruitment, settlement, adaptations, tolerances and food supply; and sediment and bottom-water chemistry upon the mutual development of microbialite fabrics and infaunal communities. This work will allow me to test whether spatial, chemical or ecological factors are the predominant control upon microbialite-infauna development. These findings will, in turn, be applied to the ancient microbialite record, to test the feasibility of prevailing models for microbialite decline and metazoan exclusion.

1. Introduction

Extensive study of modern carbonate environments, notably in the Bahamas, and a growing awareness of the importance of modern analogues to ancient sedimentary systems have done much in recent years to improve our understanding of carbonate platform sedimentary and chemical dynamics, which in turn have served as useful working models for Precambrian and Phanerozoic deposits. In the past few decades, exploratory work in the Bahamas has revealed the persistence of microbialites in the open marine settings of Eleuthera Bight (Dravis, 1983) and offshore of the Exuma Cays (Dill et al., 1986). Microbialites are

extensive in the Bahamas, but this appears to be the only modern open marine environment in which they are widespread.

Although our understanding of the sedimentary processes and microbial dynamics involved in Bahamian microbialite accretion have advanced substantially in the last few decades, the relationship between microbialites and metazoan communities in open marine settings remains an unexplored frontier. Earlier work on Bahamian microbialite-metazoan interactions has largely been confined to brief notation of associated fauna, reported in the context of larger sedimentological and diagenetic studies (e.g. Dill, 1991). More in-depth studies of microbial-metazoan interactions (e.g. Farmer, 1992 and references

therein; Konishi et al., 2001; Gingras et al., 2011) have been limited to freshwater or hypersaline systems, where infauna is predominantly composed of insect larvae, limiting their applicability as analogues for ancient marine microbialites. Moreover, most of these studies concern unlithifying microbial mats, unlike the Bahamian and recorded ancient systems, where microbialites undergo progressive lithification (e.g. Planavsky et al., 2009). *In this light, I will undertake a systematic taxonomic and ecological examination of infaunal metazoan communities of various microbialite types in the Exuma Cays of the Bahamas, with the objective of characterizing the relationship between microbialites and metazoan communities.*

2. The Exuma Cays, Bahamas

The Exuma Cays are situated on the eastern margin of the Great Bahama Bank and at the western margin of the Exuma Sound, at the interface and zone of intense mixing between the warm, salty waters of the carbonate platform and the colder waters of the Atlantic (Dill, 1991). Microbialites occur commonly in subtidal (channels or sandy embayments) and intertidal settings throughout the Exumas (Reid et al., 1995). Bahamian microbialites exhibit considerable diversity in physical, chemical and biotic microenvironments, notably degree and duration of exposure, sediment stress, framework construction (i.e. fabric) and macroalgal and metazoan colonization (Reid et al., 1995; Shapiro et al., 1995).

Bahamian lithifying microbial mats include diverse communities of autotrophic and heterotrophic bacteria and both single-celled and multi-cellular eukaryotic algae (e.g. Stolz et al., 2009; Reid et al., 2011). However, the overall structure of the mats (and thus microbialite fabric) is controlled largely by the interactions of a framework-building cyanobacterium (e.g. the oscillatoriaceans *Schizothrix* and *Microcoleus* or the nostocalean *Dichothrix*) with detrital carbonate flux. Therefore, reconstruction of the dominant processes of microbialite fabric formation is reliant upon understanding of the dynamics of the framework cyanobacterial community (e.g. Reid et al., 2000; Visscher et al., 2000).

Preliminary findings from Highborne Cay and Little Darby Island suggest that certain microbialites are associated with abundant and diverse populations of infaunal metazoans. Equally intriguing, these fauna-rich microbialites occur within meters of fauna-poor microbialites, suggesting that neither macroenvironmental factors nor patterns of metazoan settlement significantly influence variability in microbialite infaunal populations or fabric development. However, further fieldwork is needed in order to verify preliminary findings and to investigate additional localities, including a new locality at Little Darby Island, as well as subtidal microbialites off of Lee Stocking Island (the home of the famed "giant stromatolites" [Dill et al., 1986] that first put Bahamian microbialites on the map). *The core of this proposed work consists of rigorous testing of these initial observations and deciphering the mechanisms controlling the abundance of microbialite-hosted infauna.*

3. Objectives

The Exuma Cay microbialites offer an exciting opportunity to resolve metazoan-microbialite interactions in modern normal marine settings. The preliminary results obtained from Highborne Cay and Little Darby Island are compelling but incomplete. These results require replication and further study is needed to elucidate the mechanisms controlling infaunal abundances. Specifically, additional work is required to determine 1) whether the coexistence of microbialites and diverse metazoan communities persists across a variety of environments, 2) whether this relationship is observed across a broad range of microbialite morphologies and cyanobacterial framework-builders or whether it is confined to microbialites constructed by particular cyanobacteria (e.g. *Schizothrix*, *Dichothrix* and *Microcoleus*), 3) what is the chemical relationship between microbialites and metazoan infauna and 4) what is the relationship between the infaunal community and microbialite fabric development.

Samples collected during pilot field studies were taken from microbialites accreting in the intertidal zone and subtidal sandy embayments off of Highborne Cay and Little Darby Island, respectively and rich infaunal metazoan communities were observed in both environments. However, microbialites occur

across a wide range of settings throughout the Exuma Cays. For instance, it is unknown whether the microbialites in the high-energy (currents up to 150 cm/s [Dill, 1991]) subtidal channels offshore of Lee Stocking Island host metazoan communities comparable to those of Highborne Cay and Little Darby Island. Investigation of this possibility is especially pertinent in light of the fact that the stromatolites of Lee Stocking Island are commonly touted as ‘Precambrian analogues’ (Dill et al., 1986). Exploration of further microbialite-bearing localities like Lee Stocking Island is necessary to determine to what extent environmental conditions may permit or exclude microbialite colonization by infauna. Microbialites of a previously undescribed morphology (tentatively attributed to the oscillatoriacean cyanobacterium *Phormidium*) were also recently discovered growing in peritidal sand flats along the northwestern shore of Little Darby Island. Investigation of possible microbialite-hosted infaunal communities in these periodically exposed, quiet water settings may therefore help to clarify the role of sedimentological factors in the dynamics of microbialite-metazoan communities and microbialite fabric development.

It has long been recognized that microbialites in the Exumas occupy a morphological continuum far more complex than the simple dichotomy of ‘laminated’ and ‘clotted’ (Reid et al., 1995; Planavsky and Ginsburg, 2009). Further characterization of microbialite mesofabric is requisite if we are to come to a better understanding of the relationship between microbialite fabric and infaunal community structure. As the preliminary results from Highborne Cay and especially Little Darby Island indicate, the community of cyanobacterial framework builders appears to have some bearing upon infaunal community development that transcends gross microbialite mesostructure (e.g. ‘laminated’ vs. ‘clotted;’ see Shapiro, 2000). The Little Darby microbialites, in spite of their ‘stromatolitic’ (laminated) mesostructure, possess a rich and diverse infauna comparable to that of the Highborne Cay thrombolites. This suggests that we need to turn to the microscopic community of mat-builders in order to better decipher the differences between fauna-rich and fauna-poor microbialites in the Exumas. Therefore, I will examine as wide and varied a range of microbialite morphologies as possible, in order to discover relationships among the microstructure of individual microbialites. *A closer examination of the bacterial framework-building community of lithifying microbial mats may shed important light upon the formation of microbialite fabrics and help to highlight differences between fauna-rich and fauna-poor microbialites.*

Moreover, toxicity assessment will be employed to determine if metazoan-poor microbialites at Highborne Cay and Little Darby are the result of chemical exclusion by toxic strains of cyanobacteria, thereby testing for biochemical controls on microbial fabric development. A recent area of interest in the microbiological community is the extent and effect of toxicity among benthic cyanobacteria. As highlighted in recent studies (e.g. Golubic et al., 2010; Mankiewicz et al., 2003), toxicity in benthic cyanobacteria can be trophically transferred to and concentrated in metazoans (e.g. ciguateric fish and giant clams), with potentially fatal results to human consumers. It is unknown whether any of the lithifying, mat-forming cyanobacteria responsible for the Exumas microbialites include toxic strains and to what extent these may be associated with infaunal communities. Toxicity in cyanobacteria is diverse at both the species and sub-species level; morphological features traditionally used in taxonomic study are not much use for the assessment of toxicity (Golubic et al., 2010). Moreover, expression of toxicity may be environmentally-driven; not all genetically toxic strains may produce toxins in all environments (Golubic et al., 2010). Analytical work is needed to determine whether the lack or dearth of infauna in certain *Schizothrix*-dominated microbialites may be toxin-induced. Traditional enrichment methods (e.g. extraction of microbialite organic matter, placed in culture with known infaunal species or typical toxicity assay templates, such as the nematode *Caenorhabditis elegans* [Swatloski et al., 2004]) or standard screening for specific toxic compounds may shed new light upon the potential toxicity of Bahamian microbialite framework-building cyanobacteria.

Lastly, morphological-ichnological and petrographic work may do much to clarify the role of metazoan infauna in the formation and early diagenesis of microbialite fabrics. It has been suggested that metazoan-mediated modification may be one cause of thrombolitic fabric development in modern microbialites

(Planavsky and Ginsburg, 2009). However, the extent to which metazoan activity may be implicated in the development of other fabrics has been largely unexplored. In addition to thin section work, traditional macro-ichnological methods (e.g. Curran and Martin, 2003), previously unapplied to microbialites, such as creating polyester resin casts by infilling burrows with liquid resin, may help to determine the degree to which infaunal activity can influence microbialite fabric development, for instance the relationship between burrows and clots, as well as more generally between burrows and micro-porosity.

Continued exploration of microbialite-metazoan communities across a wide range of microbialite types, microbial framework communities and sedimentological settings will reveal the extent and diversity of microbialite-hosted metazoan communities. Additionally, in-depth chemical, taxonomic, petrographic and ichnological work will help pinpoint the mechanisms responsible for the abundance and diversity of infauna in various microbialite-metazoan systems.

4. Materials and Methods

Sediment samples will be collected for faunal, chemical and petrographic analysis from microbialites at Highborne Cay, Little Darby Island and Lee Stocking Island. Analyses will be performed *in situ*, at Little Darby Island Field Station (maintained by the Rosenstiel School of Marine and Atmospheric Science, University of Miami) and at the University of California, Riverside.

For faunal analysis, sediment samples will be carefully extracted by knife from the unlithified upper portions of each microbialite and placed intact in a plastic bag. Standard techniques (e.g. Higgins and Thiel, 1988) will be employed to assess metazoan abundance and diversity. Microbialite samples will be gently crushed and all living macro- and meiofauna will be extracted at 10x magnification under a binocular microscope. All macro- and meiofauna will be taxonomically identified, described and photographed. A 10% magnesium chloride solution will be used as a relaxant and a 10% buffered formalin solution (transferred after 24 hours to 70% ethanol) will be used as a preservative to allow for further taxonomic work. Taxon-specific and total abundances for each microbialite sample will be normalized to sediment volume. Further, a portion of each sediment sample will be retained and homogenized (powdered) to measure total organic carbon (TOC) concentration, in order to test for a bulk organic substrate control upon infaunal abundance. TOC contents will be determined by the difference between total carbon by combustion (at 1450° C) and total inorganic carbon by acidification using an ELTRA carbon/sulfur determinator at the University of California, Riverside.

The potential toxicity of *Schizothrix* framework-builders in fauna-poor microbial mats will be assessed by means of standard culture work and screening for known toxins. Following procedures outlined by Laurent et al. (2008), collected samples will be placed in plastic bags, sealed underwater and vigorously agitated to dislodge cyanobacteria. The resulting solution will be successively sieved up to 45 µm. The retentate will be cultured and introduced to cultures of living microbialite-extracted metazoans and *Caenorhabditis elegans* to assess potential toxicity. *Schizothrix*-dominated microbial mat samples from fauna-poor microbialites will also be collected for toxicity assay work. Fresh bulk microbial samples will be physically isolated from sediments and mixed with deionized (DI) water to form an organic-rich slurry. The slurry will be lyophilized and the resulting dry bulk sample will be sent to a commercial laboratory (e.g. Green Water Laboratories/CyanoLab) for toxicity assessment – specifically screening for aplysiatoxins (the only toxins currently known to be associated with any species of *Schizothrix*) via liquid chromatography mass spectrometry (LC-MS) (e.g. Hamilton et al., 2002).

Sedimentology, cyanobacterial framework and algal community will also be observed under magnification and recorded for each collected sediment specimen. Lithologic replicate samples will be retained in order to determine macro- and micro-porosity, respectively. Replicate microbialite samples will also be collected for neo-ichnological study. Samples will be thoroughly impregnated with polyester

resin (e.g. Curran and Martin, 2003); the resulting internal molds will be used to assess the extent of burrow development, burrow interconnectedness and relationship to original microbialite fabric.

5. Broader Impacts

The proposed work in the Exumas will do much to clarify the enigma of metazoan-microbialite relations. Previous modern microbialite studies have dismissed, overlooked or only briefly noted a metazoan presence without attempting further analysis or discussion. Yet, as preliminary findings have demonstrated, coexistence of microbialites and infaunal metazoans is not a rarity in the Exumas and the mutual development of microbialite and metazoan communities merits further investigation. A fuller understanding of the nature and causes of this interconnectedness is vital if we are to address the energy needs of today's society. The importance of microbialites as hydrocarbon reservoirs is receiving growing attention in the petroleum industry, with the recently discovered lower Cretaceous deposits in the offshore-Brazil Santos Basin providing the foremost example of a significant microbialite reservoir. A clear understanding of the processes involved in fabric development in carbonate reservoir rocks is a necessary step in the development of an exploration strategy. The manner in which metazoan diversity and abundance influence the formation, early diagenesis and preservation of modern microbialite fabrics holds important implications for our understanding of both modern and ancient microbialites, as well as of microbialite reservoir development.

Moreover, our growing awareness of microbialite-metazoan associations indicates that prevailing theories for the post-Precambrian demise and periodic resurgence of microbialites in post-extinction intervals are in need of significant revision. The healthy persistence of microbialites, in conjunction with grazing, boring and burrowing metazoans, in modern open marine settings suggests that supposed end-Proterozoic declines in microbialite abundance and diversity cannot be attributed to concomitant radiations of metazoan phyla alone. Likewise, brief resurgences of microbialites in reefal settings in the wake of events of profound biotic crisis during the Paleozoic and Mesozoic cannot be causatively linked to contemporaneous declines in certain metazoan guilds; the fluctuating shape of the microbialite record throughout earth history cannot be simply laid at the door of metazoans. However, with a better comprehension of the nature of microbialite-metazoan interactions at minute spatial and temporal scales, we can begin to extrapolate our modern observations to an improved and uniformitarian understanding of ancient processes.

REFERENCES

- Awramik, S.M., 1971: *Science*, v. 174, p. 825-827.
- Curran, H.A. and Martin, A.J., 2003: *Palaeogeography, Palaeoclimatology, Palaeoecology*, v. 192, p. 229-245.
- Dill, R.F., Shinn, E.A., Jones, A.T., Kelly, K. and Steinen, R.P., 1986: *Nature*, v. 324, p. 55–58.
- Dill, R.F., 1991, in Osborne, R.H., ed., *From Shoreline to Abyss*: SEPM, Society for Sedimentary Geology, Tulsa, Oklahoma, p. 147–171.
- Dravis, J.J., 1983: *Science*, v. 219, p. 385–386.
- Farmer, J. D., 1992, in Schopf, J. W., ed., *The Proterozoic Biosphere*: Cambridge, Cambridge University Press, p. 1348.
- Garrett, P., 1970: *Science*, v. 169, p. 171-173.
- Gingras, M., Hagadorn, J. W., Seilacher, A., Lalonde, S. V., Pecoits, E., Petrash, D., and Konhauser, K. O., 2011: *Nature Geoscience*, v. 4, no. 6, p. 372-375.

- Golubic, S., Abed, R.M.M., Palińska, K., Pauillac, S., Chinain, M. and Laurent, D., 2010: *Toxicon*, v. 56, p. 836-841.
- Grotzinger, J.P. and Knoll, A.H., 1999: *Annual Review of Earth and Planetary Sciences*, v. 27, p. 313-358.
- Hamilton, B., Hurbungs, M., Jones, A. and Lewis, R.J., 2002: *Toxicon*, v. 40, p. 1347-1353.
- Higgins, R.P. and Thiel, H., 1988, *Introduction to the study of meiofauna*, Smithsonian Institution Press, Washington D.C.
- Konishi, Y., Prince, J. and Knott, B., 2001: *Hydrobiologia*, v. 457, p. 39-47.
- Laurent, D., Kerbrat, A.-S., Darius, H.T., Girard, E., Golubic, S., Benoit, E., Sauviat, M.-P., Chinain, M., Molgo, J., Pauillac, S., 2008: *Harmful Algae*, v. 7, p. 827–838.
- Mankiewicz, J., Tarczynska, M., Walter, Z., Zalewski, M., 2003: *Acta Biologica Cracoviensia*, v. 45, p. 9–20.
- Mata, S. A., and Bottjer, D. J., 2012: *Geobiology*, v. 10, no. 1, p. 3-24.
- Planavsky, N.J. and Ginsburg, R.N., 2009: *Palaios*, v. 24, p. 5-17.
- Planavsky, N.J., Reid, R.P., Andres, M., Visscher, P.T., Myshrall, K.L. and Lyons, T.W., 2009: *Geobiology*, v. 7, p. 566-576.
- Reid, R.P., MacIntyre, I.G., Browne, K.M., Steneck, R.S. and Miller, T., 1995: *Facies*, v. 33, p. 1–17.
- Reid, R.P., Visscher, P.T., Decho, A.W., Stolz, J.F., Bebout, B.M., Dupraz, C., MacIntyre, I.G., Paerl, H.W., Pinckley, J.L., Prufert-Bebout, L., Steppe, T.F. and Des Marais, D.J., 2000: *Nature*, v. 406, p. 989–992.
- Reid, R.P., Foster, J.S., Radtke, G. and Golubic, S., 2011, in Reitner, J., ed., *Advances in Stromatolite Geobiology: Lecture Notes in Earth Sciences 131*, Springer-Verlag, Berlin, p. 77-89.
- Riding, R., 2011, in Reitner, J., ed., *Advances in Stromatolite Geobiology: Lecture Notes in Earth Sciences 131*, Springer-Verlag, Berlin, p. 29-74.
- Shapiro, R.S., Aalto, K.R., Dill, R.F. and Kenny, R., 1995, in Curran, H.A., and White, B., eds., *Terrestrial and Shallow Marine Geology of the Bahamas and Bermuda: Geological Society of America Special Paper 300*, p. 139–156.
- Shapiro, R.S., 2000: *Palaios*, v. 15, p. 166-169.
- Sheehan, P.M. and Harris, M.T., 2004: *Nature*, v. 430, p. 75-78.
- Stolz, J., Reid, R. P., Visscher, P., T., Decho, A. W., Norman, R. S., Aspden, R. J., Bowlin, E. M., Franks, J., Foster, J. S., Paterson, D. M., Przekop, K. M., Underwood, G. J. C., and Prufert-Bebout, L., 2009: *Atoll Research Bulletin*, v. 567, p. 1-29.
- Swatloski, R.P., Holbrey, J.D., Memon, S.B., Caldwell, G.A. Caldwell, K.A. and Rogers, R.D., 2004: *Chemical Communications*, v. 6, p. 668-669.
- Visscher, P.T., Reid, R.P. and Bebout, B.M., 2000: *Geology*, v. 28, p. 919–922.
- Walter, M. R., and Heys, G. R., 1985: *Precambrian Research*, v. 29, p. 149-174.