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CHARACTERIZATION OF MICROBIALITES AND THE ECOSYSTEM OF STORR'S LAKE, SAN SALVADOR ISLAND

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ABSTRACT. Storr's Lake in San Salvador Island, Bahamas, is an inland, hypersaline lake, harboring lithified, organo-sedimentary structures called microbialites. The physical and chemical properties of the lake water, and the types, distribution, mineralogy and microbial population of the microbialites were investigated during two visits to the lake. The lake level fluctuates depending upon seasonal temperature and rain frequency events. The lake has a pH that fluctuates seasonally between 8.22 and 8.58 along with the salinity, redox chemistry, and composition of chemical constituents. The hypersaline conditions restrict growth of grazing organisms that could feed on the microbial mats found on the surface of the microbialites and the lake floor. However, when the salinity is lowered because of rain events, small fish can be found in the lake. Sporadic gastropod shells also are entombed within the microbialite mounds. Floating or planktonic material including algae, bacteria, dinoflagellates, and organic detritus all contribute to the high-turbidity of the lake water. Five morphologically different microbialites were collected during two visits to the lake from different depths and distances from the shoreline. We identified calcareous knobs, plateau-mushroom, pinnacle mound, and cauliflower-top mushroom shaped microbialites that were described by previous researchers. In addition, we also identified a new morphological type called 'multi-cusate'. The pinnacle mound type was found at the deepest water level along our sampling transect. The microbialite mounds are composed of angular, blocky and needle shaped high-Mg calcite and aragonite, with re-crystallized cyanobacterial filaments and remains of bacteria and exopolymeric substances. Deployment of light detectors at various positions in the lake during a high water level and relatively low turbidity period in 2012 revealed that diffuse sunlight, approximating ~1 to 3% of the surface luminosity, was reaching the bottom of the lake at a depth of ~100 cm. Molecular analysis of single samples from each of the microbial mats revealed that photosynthetic cyanobacteria comprised only 3.5 % and <0.5% of the shallow- and deeper-water microbialites, respectively, suggesting that limited sunlight availability especially in the deeper regions of Storr's Lake probably plays role in controlling the microbial mat diversity. *Chloroflexi* constituted approximately half of the entire bacterial population identified in the deeper water microbialites, and *Deltaproteobacteria*, including several sulfate reducing bacteria, were enriched in the shallow water calcareous knobs (21%) and the cheesecake mat (16%).

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INTRODUCTION

Microbialites are organo-sedimentary deposits formed by the interaction of microbial communities, chemically precipitated sediments and/or detrital particles trapped by the extracellular material produced by the microbial mat (Dupraz and Visscher, 2005). Ancient microbialites are

found in many carbonate rocks with some communities once being actively involved in reef building (Stanley, 2001). Most interpretations of fossil microbialite ecosystems suggest that their growth occurred in shallow water and open marine systems in subtidal to intertidal regions. The size and shape of the microbialite structures are often indicative of the water level and availability of

sunlight (Mann and Nelson, 1989). Modern microbialites are limited in their abundance, but vary from hypersaline (Shark Bay, Australia, Skyring and Bauld, 1990), open marine (The Bahamas, Reid et al., 2000), freshwater lacustrine (Freytet and Verrecchia, 1998), to deep-water methane seeps (Black Sea, Bailey et al., 2009).

The layered mats in microbialites typically consist of the following six communities (Dupraz and Visscher, 2005): oxygenic photoautotrophs dominating the outermost layer of microbialite mats; oxygenic heterotrophs that gain energy from the breakdown of organics; anoxygenic photoautotrophs comprised of purple and green bacteria; anaerobic heterotrophs such as sulfate reducing bacteria (SRB) that oxidize organic material by coupling an electron transfer process with SO_4^{2-} ; sulfide oxidizing bacteria that oxidize reduced sulfur compounds with O_2 or NO_3^- while fixing CO_2 into organic compounds; and fermenters that utilize organic carbon and sulfur in an electron transfer processes. Among the above listed communities, the activity of cyanobacteria, anoxygenic photoautotrophs and SRB can result in net precipitation of carbonate minerals. In theory, any microbial population that is capable of precipitating and trapping minerals, and that can facilitate nucleation could form microbialites (Elizabeth et al., 2011). The phototrophic activity, and in turn carbonate mineral precipitation could be severely affected in microbialites in locations where there is an absence or limited availability of light, such as in deep-methane seeps (Bailey et al., 2009) or in Pavilion Lake (Laval et al., 2000). In such cases, other microbial activities, such as sulfate reduction may play a more dominant role in carbonate production.

Storr's Lake is an inland, hypersaline lake on the Bahamian Island of San Salvador. The lake was formed due to interior flooding of a depression during the late Holocene sea-level rise (Zabielski, 1991). The lake has only limited connectivity with the ocean along a few conduits. Analysis of ostracode assemblages indicate that the lake was

initially hypersaline, followed by a marine breaching and formation of a tidal lagoon, during which the salinity was lowered. Later, marine dune deposition closed the lagoon channel, thus returning the lake back to hypersaline conditions (Teeter, 1995; Zabielski, 1991). Dry seasonal conditions combined with high rates of evaporation result in the present-day hypersaline environment. Planktonic algae, cyanobacteria, and halophilic bacteria are all present as suspended matter in the lake water and inhibit light penetration into the lake water. Secchi disk light penetration measurements indicate that visibility in the lake water is limited to a depth of a few tens of centimeters (Neumann et al., 1989); however, there has not been any quantitative study on the light penetration into Storr's Lake water. The high turbidity would limit the availability of sunlight, and thereby possibly restricting the growth and distribution of the cyanobacterial population that exists on the microbialites.

Storr's Lake harbors lithified microbialites and a thick, leathery microbial mat on the lake floor. A good description of the microbialite types and distribution is provided in Mann and Nelson (1989), and Neumann et al. (1989). Isotope analysis of the carbonate laminations in the microbialites indicates that these structures started developing at around 2310 years ago with a discontinuous growth that averages 160 mm per thousand years (Paull et al., 1992). *Scytonema* was identified as the dominant photosynthetic cyanobacteria in most of the shallow-water microbial mats, in addition to the coccoid *Gloeocapsa*, *Schizothrix*, and *Johannesbaptista* (Pentecost, 1989). A 16S rRNA analysis of the microbial population of the near-shore bulbous crust (calcareous knobs) microbialite shows at least five genera of sulfate-reducing bacteria including *Desulfovibrio*, *Desulfobulbus*, *Desulfococcus*, *Desulfobacter*, and *Desulfobacterium* (Brigmon et al., 2006). The microbial population in the microbial mats associated with the deeper microbialites has not been investigated yet. Submerged microbial mats in

Storr's Lake exhibit a very low rate of photosynthesis and nitrogen fixation compared to open marine microbialites (Paerl et al., 2003). One primary contributing factor for this limitation was suggested to be the stress caused by the hypersalinity. Additionally, we speculate that the limited availability of light could be another reason for the slow growth of cyanobacterial mats. The goal of this paper is to describe the general ecosystem of Storr's Lake, the water chemistry, the types of the microbialites and their distribution, and the microbial population in the mats. The extent of light penetration and its possible effects on the microbialites is also discussed.

METHODOLOGY

Site Description and Sample Collection

The water in Storr's Lake on San Salvador Island (Figure 1) has a characteristic reddish-brown color observed in numerous other lakes in the Bahamas. Microbialites and water samples were collected from different locations in the lake during visits to the island in April 2011 and June 2012. The water level in the lake was slightly higher (~110 cm) on the deepest segment in our transect in 2012

than the previous visit (~100 cm), due to heavy rain events on San Salvador Island during 2012. The year 2011 was relatively dry, with the total rainfall for January recorded at 4.3 cm, while the months of February and March received ~0.1 cm of rain. In comparison, the year 2012 was wetter, with the cumulative rainfall between April 28th and June 2nd totaling to ~29 cm, which is almost twice as much as the average rainfall (~13 cm) for the month of May (Source: Accuweather data). A single transect in the northern segment of lake was made on foot starting from the western shore and proceeding towards the Cactus Island (Figure 1). Our sampling transect was similar to that of Mann and Nelson (1989, section B-B'). Samples of water and microbialites were collected at different locations along the transect (Figure 1C). Five different microbialites were identified and sampled: calcareous knobs, plateau-mushroom, pinnacle mound, cauliflower-top mushroom and multi-cusate. The microbial communities found on the upper surfaces of the microbialites, ranged from few millimeters to ~1 cm thick, and varied from a uniform thickness to being sporadic in their distribution. The leathery 'cheesecake' mats, first described by Neumann et al., (1989) as sediment

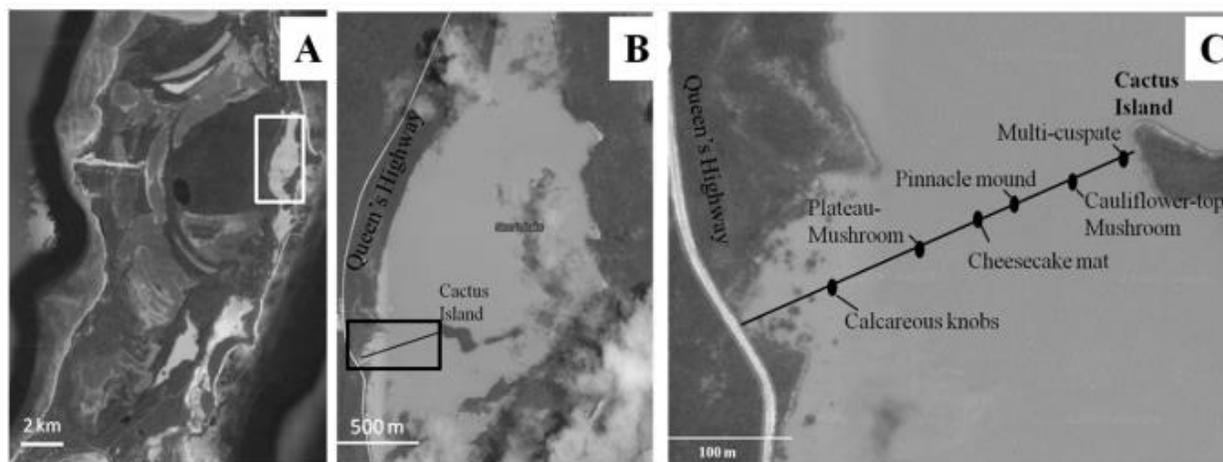


Figure 1. Overview of the collection sites at Storr's Lake. (A) Google map of San Salvador Island with Storr's Lake located along the eastern-margin of the island (white box), Bar = 2 km; (B) Magnified image of white box region depicting the northern section of Storr's Lake. The line indicated within the black box represents the approximate transect along which the water and microbialite samples were collected, Bar = 500 m; (C) Magnified Google map image of the black box in Figure 1B showing the locations where the different microbialites and microbial mats were sampled in 2011 and 2012, Bar = 100 m.

covering the deeper segments of the lake were also collected. For molecular analysis, microbial mat samples from the microbialites were aseptically collected in duplicate (5 -10 g each), stored in a preservation solution (LifeGuard Soil Preservation solution, MoBio Laboratories, Solana Beach, CA, USA) and were kept in a frozen state at all times.

All samples were characterized either on site, at the laboratory in Gerace Research Centre (GRC), or transported to Missouri University of Science and Technology (MS&T) for further analysis. The *in situ* water measurements included pH, redox potential (Eh), light intensity, conductivity, dissolved oxygen, and temperature. Alkalinity was measured by colorimetric titration immediately after returning the samples to shore. The water samples were collected at different depth profiles at locations in the water column above where the microbialite samples were found. The water samples were filtered with a 0.45 µm cellulose acetate syringe filter on-site in the Bahamas and later returned to the MS&T laboratory. Samples destined for inductively coupled plasma-optical emission spectrometer (ICP-OES) cation analysis were acidified with high-purity nitric acid. Major element cations in the water samples were analyzed using a Perkin-Elmer Optima 2000 DV ICP-OES and anions using a Dionex DX-120 Ion Chromatography Unit (IC) at MS&T. Analysis of standards during the experimental runs indicate an accuracy of better

than 99.3% for the pH meter, 97.6% for ICP-OES and 98.0% for IC.

Turbidity-light penetration depths were qualitatively evaluated during the 2012 sampling trip using an underwater video camera and quantitatively measured using a Secchi Disk, a HACH NTU light scattering device, and light intensity loggers from HOBO (Cape Cod, MA, USA). The HOBO light meters were placed at several locations at the bottom of the lake over a period of time ranging from few hours to two days, and the data were logged using HOBOWare program. The buoyant probes were attached to metal spikes using monofilament thread that was then anchored into the bottom sediment of Storr's Lake adjacent to the microbialite. The HOBO detectors were also attached to a floating fishing bobber to allow the units to be relocated and recovered. Weather conditions varied widely during the measuring period from fully sunny conditions to cloudy with occasional rain squalls. Cloudy conditions and/or wind that produced a choppy water surface would tend to minimize light penetration into water during daylight hours.

To determine the settling rate of the particles in Storr's Lake, a sample aliquot of water was measured for turbidity at regular intervals under darkened laboratory conditions at ambient room temperature. The water sample was placed in a clean glass vial and allowed to remain undisturbed in the turbidometer for a six month period. The

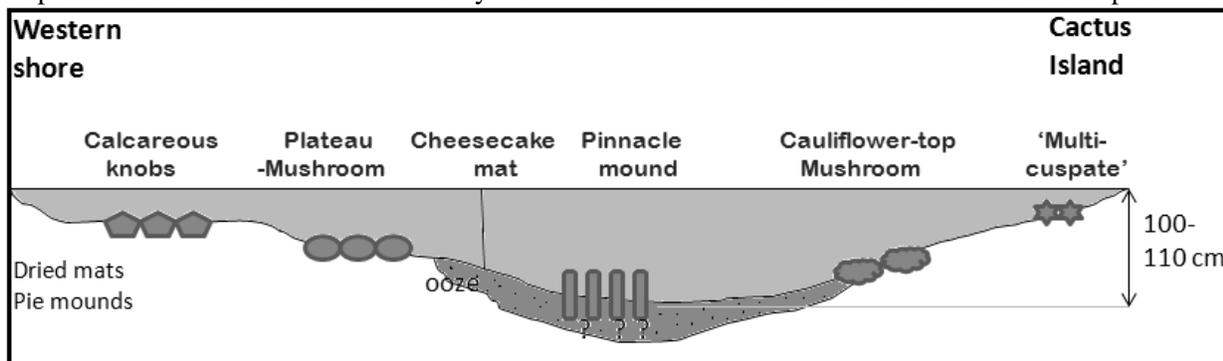


Figure 2. The distribution of the microbialites in Storr's Lake showing locations from which the microbialites and water samples were collected. See Figure 1 for location of sampling transect. The exact depth of the pinnacle mound penetration into the sediment substrate is not known and hence is represented by question mark.

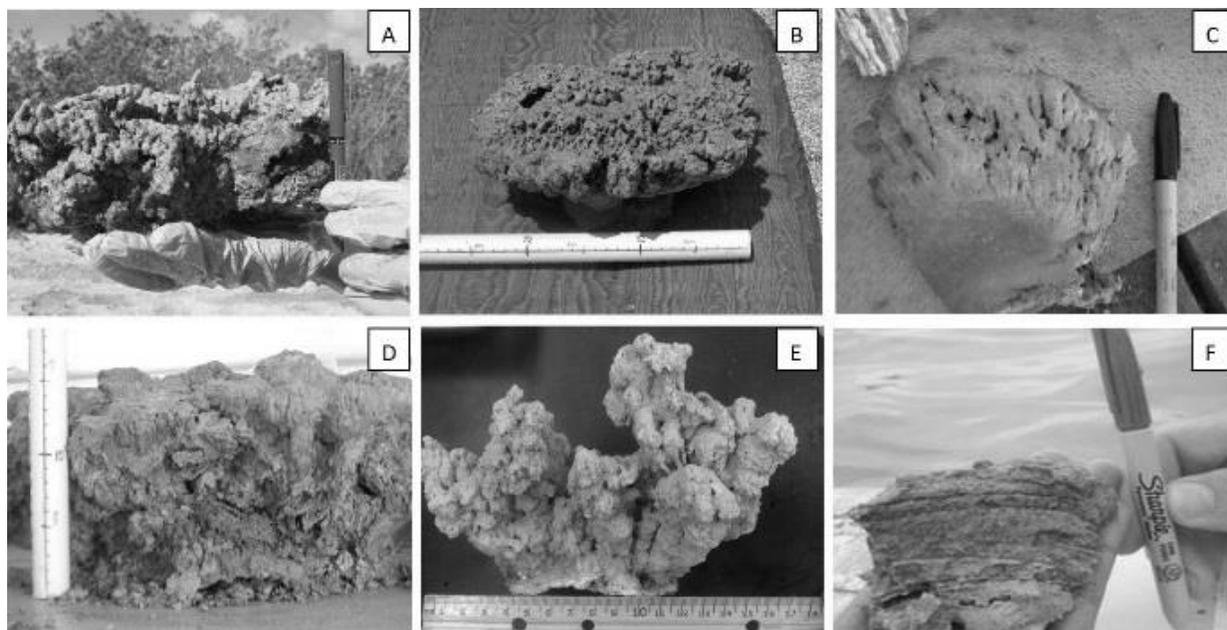


Figure 3. The different microbialite types (A-E) and the non-lithified and layered, 'cheesecake' microbial mat (F) collected from Storr's Lake. A. calcareous knobs, B. plateau-mushroom, C. pinnacle mound, D. cauliflower-top mushroom shaped, and E. multi-cusped type. The pen used in A is ~12 cm, the PVC pipe in B and D and the ruler in E are marked in cm intervals, the marker used in C and F is ~15 cm.

turbidity values were then compared against the settling rates for smectite and illite clay standards that were mixed separately with a 5% seawater mixture.

Microscopy and X-Ray Diffraction Analysis

Scanning electron microscopy (SEM) using a Hitachi S4700 instrument was used to investigate the crystals and grain structure of the microbialites. The EDS energy dispersive spectral tool in the SEM was used to obtain semi-quantitative elemental composition of the crystals. The suspended material in the lake water was also examined by using an optical microscope. The mineralogy of the microbialites was investigated using the X-ray Diffraction (XRD) technique using crushed samples that had been rinsed with deionized water to remove soluble salts and low-density organics, dried at 105°C, and analyzing using a PANalytical X'Pert Pro Multi-Purpose X-Ray Diffractometer.

Microbial Diversity Analysis

To determine the microbial mat community composition in the microbialites of Storr's Lake, a 16S rRNA based gene analysis by using multiplex barcoded pyrosequencing method was performed. Genomic DNA was isolated (UltraClean™ Soil DNA Isolation Kit, MoBio Laboratories, Solana Beach, CA, USA) from microbial mats associated with calcareous knobs, plateau-mushroom, cauliflower-top mushroom and pinnacle mound microbialites, as well as the non-lithifying cheesecake microbial mat. The extracted DNA was shipped to MOgene, LC, St. Louis, MO, USA for sequencing. MoGene quantified the DNA by using a Quant-iT Picogreen dsDNA Assay kit (Invitrogen, Carlsbad, CA) and polymerase chain reaction (PCR)-amplified by using barcoded primers targeting the V1-V3 region of the 16S rRNA gene. The bacterial primers used were the 27-Forward AGRGTTTGATCMTGGCTCAG (Weisburg et al., 1991) and 518-Reverse CGTATTACCGCGGCTGCTGG (Muyzer et al., 1993).

The PCR mixture included 1X Master mix (MOLZYM Mastermix 16S Basic, Molzym, Bremen, Germany), 0.2 μ M each of the barcoded forward and reverse primers, 0.51 U Taq DNA polymerase (MolTaq, Molzym), 30 ng of the template DNA, and DNA-free water for a final volume of 25 μ L. Conditions for PCR were 96°C for 5 min, 25 cycles of 95°C for 10 sec, 56°C for 30 sec and 72°C for 30 sec, followed by a single cycle of 72°C for 5 min. The amplicons were then purified with a 1.8% agarose gel purification run (QIAquick Gel Extraction kit, Qiagen, Chatsworth, CA). The purified amplicons were examined with an Agilent Bioanalyzer DNA 7500 (Agilent, Santa Clara, CA) to determine the concentration and assess the size of the products. The amplicon libraries were normalized, pooled and then pyrosequenced with Titanium chemistry (454 GS-FLX, Roche, Branford, CT, USA). The bioinformatics software program, QIIME was used for processing, cluster analysis, and classification of the raw barcoded sequences (Caporaso et al., 2010). High quality reads were separated based on the barcode sequences and analyzed for taxonomy by comparison to reference database Greengenes (DeSantis et al., 2006).

RESULTS AND DISCUSSION

Microbialite Morphology and Distribution

Five different types of microbialites (calcareous knobs, plateau-mushroom shaped, pinnacle mound, ‘multi-cusate’ type, and cauliflower-top mushroom shaped stromatolites) were collected during both visits, along with water samples from each of these sites collected at different lake depths (Figure 2). Mann and Nelson (1989) and Neumann et al. (1989) previously described four microbialites from the lake. Proceeding from the shore, thrombotic pie mounds that periodically emerged above the lake level and exposed to dry conditions when the water level was low. The next microbialite zone occurred at locations further into the lake, where the bottom

of the lake began to rise slightly in an otherwise continuous slope. This region contained crumbly, calcareous knobs as tall as 15 cm (described as bulbous crust by Neumann et al., 1989). Continuing lake-ward, the bottom suddenly drops at which point, flat-topped plateau-mushroom stromatolites that have a rounded and hard calcified head structure attached firmly to the stalk-shaped base were identified. These deeper-water structures were never visible below the water surface due to the high turbidity of the lake water and had to be located by touch. The heads of these plateau types stick out above the dense, slimy, sedimentary ooze on the lake bottom. At the deeper locations, columnar pinnacle mounds (club-shaped) that were up to 70 cm in length were identified. Neumann et al. (1989) also sampled three different types of the microbial mats in the lake: ectoplasmic pie mounds, an offshore mat that extended up to the calcareous knobs, and a thick, leathery, ‘cheesecake’ microbial mat that was present between and sometimes on top of the deeper-water microbialite structures.

We were able to identify calcareous knobs, plateau-mushroom shaped (and the closely resembling cauliflower-top mushroom) microbialites, and pinnacle mounds in 2011, and calcareous knobs, plateau, and a new morphological type called ‘multi-cusate’ in 2012. The calcareous knobs had heads projecting from the calcified base, with the knobs being thick, but sometimes crumbly, and containing a slimy microbial mat (Figure 3A). Plateau-mushroom shaped (Figure 3B) were identified at a water depth of ~ 80cm or deeper and the pinnacle mounds (Figure 3C) were found in even deeper portions of the lake at ~ 100 cm. Morphologic features of the plateau-mushroom type varied only slightly from the cauliflower type, with the top crust of the head having a reticulated structure that was more brittle than that exhibited by the cauliflower type (Figure 3D). The plateau type had a visible gap between the more reticulated top crust and the compacted segment located directly below the crust that was variable in height (~ 0.2 cm and 1.0 cm). This

microbialite exhibited a highly compacted base with a prominent stalk (also highly cemented) attached to the lake sediment segment, above which, the lithification lacked any prominent, horizontally stratified layers. The abundance of microbialite coverage in the deeper portions of the lake bottom was minimal. Towards Cactus Island, at a depth of about 46-50 cm, another microbialite (we termed 'multi-cusplate'), which was morphologically different from calcareous knobs or plateau-mushroom type owing to its more distinguished protrusions and sharper edges, was identified (Figure 3E). The multi-cusplate microbialites were distributed fairly continuously, but disappeared closer to Cactus Island in shallow water. The lake bottom between the location of multi-cusplate microbialites and Cactus Island was characterized by gelatinous sediment. A well-laminated, thick, leathery, 'cheesecake' microbial mat was found distributed on top of the ooze in most locations (Figure 3F). The distribution of the microbialites in Storr's Lake appears to be controlled more by the water depth rather than tidal influence (Mann and Nelson, 1989).

Water Analysis

A comparison of the lake water parameters measured during the two visits to the lake is shown in Table 1. The values shown are averages of at least 6 to 15 measurements with their standard deviation. The seawater values are measurements made from ocean water collected from Dim Bay at the beach opposite to the Salt Pond, located directly south of Storr's Lake. The water samples were collected from locations directly above the microbialites. The water depth measured at the deepest point in our transect increased from ~1 meter in April 2011 to ~1.1 meters in June 2012, due to the rain events that occurred preceding the second trip. The decreases observed in conductivity and dissolved ion content between our sampling intervals of 2011 and 2012 reflect this difference in rainfall. The conductivity (salinity) during 2011 and 2012 were 92.1 mS/cm (138 ppt) and 42.7

mS/cm (64 ppt), respectively. We also measured the water conductivity during cursory visits to the island in May of 2004, 2005 and 2009. The conductivity values during these three years were 98.3 mS/cm (~66 ppt), 108 mS/cm (~72 ppt) and 40.6 mS/cm (~26 ppt), respectively. The lowest recorded salinity-conductivity value that has ever been reported in the lake was our measurement in May 2009, when the pH and Eh were 8.38 and -0.81 mV, respectively. The May 2009 visit was immediately preceded by a heavy rainfall event.

The pH of the lake varied from an average of 8.22 (± 0.05) in 2011 to 8.58 (± 0.08). The redox potential (Eh) measured in milliVolts (mV) indicated that the lake water was mildly reducing with values of -60 (± 2) and -80 mV (± 12) during both time periods. The turbidity of the lake water was high, ranging between an average of 169 (± 15) nephelometric turbidity units (NTU) in 2011 and 96 (± 12) NTU in 2012. Alkalinity values averaged 178 and 151 mg/L for the 2011 and 2012 seasons respectively, values that were ~35 to 60% higher than local seawater. The concentrations of major elements analyzed by ICP-OES and IC are displayed in Table 1. While calcium, magnesium, potassium, and magnesium are reported, the silica, aluminum, manganese, and iron were all below the detection limit of 10 ppm for the samples that needed to be diluted 100-fold due to the high salinity. The chloride and sulfate contents measured in 2012, were higher (21% and 31%, respectively) than seawater.

A comparison of the parameters along any given vertical water column revealed only small variations with depth. As an example, the values obtained for water samples collected above the plateau-mushroom type microbialite in 2012 displayed near constant pH values at 8.56 at all depths, while the Eh values gradually became more reducing with depth (-86.6 to -88.1 mV). The turbidity values increased from 76 NTU for the shallow surface-water, to 88 NTU at 50 cm and then

decreased to 81 NTU at the deepest portion (104 cm).

A comparison of the turbidity values measured in the Storr's lake water sample and clay

mineral samples (illite and smectite) mixed separately with 5% synthetic seawater is shown in Figure 4. The turbidity values indicated that the lake water sample took around 179 days to decrease

Parameters	March 31- April 01, 2011 15:00 (EST)	N	June 16-20, 2012 15:00 (EST)	N	Seawater-June 16, 2012 14:00 (EST) N=1
Max. Water Depth	~ 1 m	8	~1.1 m	6	-
pH	8.22 (± 0.05)	15	8.58 (± 0.08)	10	8.26
Eh (mV)	-60 (± 2)	15	-80 (± 12)	5	-55
Alkalinity (mg/L as CaCO ₃)	178 (± 8)	6	151 (± 1)	6	113
Temperature (°C)	27.5 (± 1.0)	15	28.7 (± 1.2)	10	28
Conductivity (mS/cm)	138 (± 4.5)	15	64 (± 1.3)	10	53.9
Salinity (ppt)	92.1 (± 3.0)	15	42.7 (± 0.9)	10	36.0
Dissolved Oxygen (mg/L)	3.86 (± 1.07)	15	-	-	4.70
Turbidity (NTU)	169 (± 15)	13	96 (± 12)	8	5
Calcium (ppm)*	990 (± 32)	15	503 (± 15)	8	386
Magnesium (ppm)*	2,873 (± 84)	15	1,676 (± 43)	8	1,361
Sodium (ppm)*	25,133 (± 275)	15	14,422 (± 280)	8	11,811
Potassium (ppm)*	926 (± 27)	15	520 (± 15)	8	417
Chloride (ppm) [†]	-	-	24,075 (± 624)	4	19,000
Sulfate (ppm) [†]	-	-	3,910 (± 94)	4	2,700

Table 1. Water parameters measured from Storr's lake during the two sampling visits showing the average values of 6 to 15 measurements with their standard deviation in parentheses. The number of measurements for each parameter in each year is represented by 'N'. Calcium, magnesium, potassium and sodium concentrations were analyzed using ICP-OES (*), while chloride and sulfate were analyzed using IC (†). Anions were not analyzed for the 2011 water samples. Anion concentrations for seawater are reported from Hem (1985).

from 121 to 8 NTU. In contrast, the illite and smectite clay standards suspended in the seawater solution, each took ~94 days to go from ~450 to <1 NTU. Clays tend to have slow settling rates due to their nature of forming colloidal particles when mixed in an aqueous medium, and thus serve as good proxy for comparing with Storr's lake water, although no clays were detected in the Storr's Lake water or sediments during this study.

The reddish brown coloration in the lake is likely caused by the (i) presence of abundant particulate halophilic alga and bacteria (Mann and Nelson, 1989), which are usually enriched in reddish carotenoid pigments, or (ii) dissolved organic tannins brought in by the brackish water draining into the surrounding land regions. Several other lakes and ponds on San Salvador Island are also turbid and possess the same characteristic reddish-brown color. It is possible that processes similar to Storr's Lake occur in those water bodies as well. Several mechanisms may be responsible for the longevity of the suspended turbidity particles in Storr's Lake. The particles may remain

in a suspended state due to surficial electrostatic repulsion. Bacteria, algae and most organic material have a net negative surface charge, which causes particles to repel and thereby cause each other to remain in a suspended state. Planktonic microorganisms that are photosynthetic will seek sunlight for their metabolism, and thus swim upwards in a water column during daylight hours. Neumann et al. (1989) suggested that oxygen gas released from the gelatinous thrombolitic mounds carries some of the mat material from the lake floor to the surface. A similar process could be presumed to occur with the deep water microbial mats and could involve other gas sources, such as methane to provide positive buoyancy. Additionally, high wind activity seen in the lake environment could possibly agitate the water. It should be noted that the lake experiences only minimal tidal exchange with the adjacent ocean due to limited connectivity to the ocean, though abnormally high or low tides could influence the lake parameters (Mann and Nelson, 1989). In addition to the above factors, freshwater inflow during rain and storm events could also

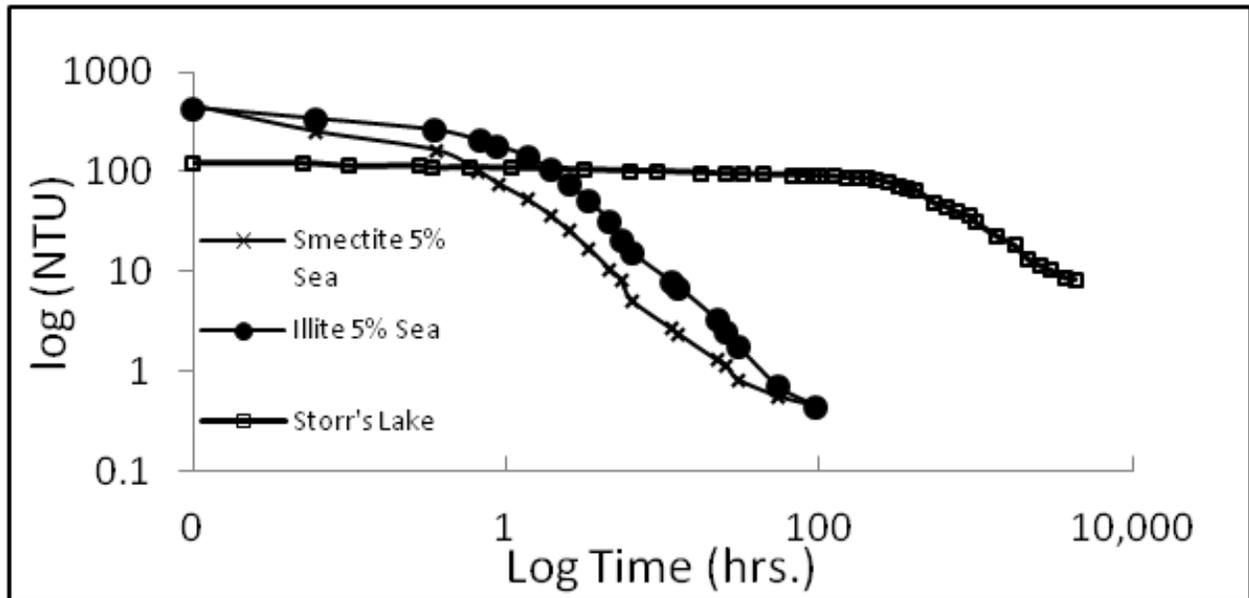


Figure 4. Turbidity measurements in a controlled laboratory environment in the absence of light, showing settling rates of organic particles in Storr's Lake water relative to clay-mineral standards for smectite (from Wyoming) and illite (from illite-bearing shale, Fithian, Illinois). The clays were suspended in a 5% synthetic seawater solution. The relatively flat horizontal settling trend at the beginning of each test reflects the time taken for the suspended particles to move from the top of the water column in the vials to below the level of the light scattering detector. NTU, Nephelometric Turbidity Units.

significantly affect the documented changes seen in turbidity, pH, salinity and other water parameters.

Comparison of Water Data with Previous Studies

Mann and Nelson (1989) reported a water level fluctuation of 16 cm between 1985-1986 for Storr's Lake, suggesting periods of high rates of evaporation during the months December to April, followed by wetter conditions between May and November. They observed only minor stratification of lake waters, noting salinities of 92, 93 and 93.5 ppt at surface, 30cm and 60 cm depths, respectively. McNeese (1988) noted that the salinity in the lake ranged from 70 to 100 ppt during the months of December to May. Brigmon et al. (2006) presented a more thorough water chemistry analysis of water samples collected from six locations close to the shore at depths between 0.1 and 0.5 m. The dissolved oxygen content reported from their study varied from 0 to 5 mg/L. Their measured cation and anion concentrations varied among locations, with the Na, K, and Ca values having an average of 844 ± 279 , 201 ± 56 and 33 ± 3 g/L, respectively. The sulfate and chloride concentration also averaged about 110 (± 25) and 684 (± 91) g/L, respectively. The temperature recorded during their sampling period ranged from 32°C to 42°C, indicating warm conditions and thus higher concentration of dissolved solids resulting from lake water evaporation.

Fowler (2011) analyzed water samples from four different locations and depths in the relatively shallower southern section of Storr's Lake during June 2010. The average salinity and temperature of the lake was ~60 ppt and ~40°C, respectively, with the pH ranging from ~8.1 to ~8.6. The warm conditions during the time of Fowler's sampling generally caused the ionic concentration to be higher than our measurements in 2011 and 2012. Fowler reported averaged values of 661 ppm Ca, 6,466 ppm Mg, 26,616 ppm Na, 45,050 ppm Cl, and 6,204 ppm SO₄. Our average water Ca concentration was higher than Fowler's in 2011, but

lower in 2012 (Table 1). The other elements always were higher in Fowler's measurements. It is possible that the southern sections of the lake being shallower than the northern sections would be more strongly influenced by evaporation and therefore exhibit higher ionic concentrations.

Light Penetration

The Secchi disk values were measured at two locations where water depth was a maximum of 40-46 cm (June 2012). The white portion of disk was not visible below depths of 17-26 cm and the black portion disappeared below depths of 15-20 cm. The turbidity reported by Neumann et al. (1989) with Secchi disk measurements taken during December 1987 was 46 cm. The HOBO pendant loggers used in our study gave a more quantitative description of the amount of light penetrating through the water column. A preliminary light penetration survey was first conducted using a HOBO logger attached to a PVC pipe with depth markings in centimeters. This arrangement provided a mechanism to rapidly take readings at multiple locations and depths while traversing the lake in relatively short time periods of 10 to 15 minutes. Near the location of the plateau-mushroom microbialite, the measured light intensity dropped from 608 lumens per square feet (lumens/ft²) at the shallow surface (~5 cm), to 37 lumens/ft² at ~72 cm. At ~100 cm, the light intensity ranged from 0-3 lumens/ft². Sediment stirred up by our movement in the lake may have contributed to these low readings at the bottom of the lake.

The long term, day-night profile, of one stationary HOBO light detector over a 5-day period in June 2012 is shown in Figure 5. The probe was initially set adjacent to the bulbous crust (calcareous knob) microbialite at a depth of 40 cm during the afternoon of recording Day 1. The weather was mostly cloudy during this day and the probe recorded a maximum signal of ~400 lumens. The probe was left in this same location for Day 2, where a maximum reading of ~1150 to 1200

lumens was reached during a day that was only partly cloudy. The probe was removed from the water and exposed for a short time to daylight conditions during Day 3, where a maximum reading of ~20,000 lumens was obtained. The probe was then placed adjacent to a plateau-mushroom microbialite at a depth of about 100 cm and kept in this position for two days over a period characterized by weather patterns that changed from partly cloudy on Days 3 and 4 to scattered rain on Day 5. The light intensity during Days 4 and 5 at the plateau-mushroom position varied from between 200 to 600 lumens, or between 1 to 3% of the surface light intensity. The penetration of any amount of sunlight to these depths was unexpected given the high turbidity of the lake water.

The light that reaches the bottom is scattered between particles as it penetrates through the water column. In one recent study, cyanobacterial mats showed maximum photosynthetic efficiency under light-limiting conditions (Al-Najjar et al., 2012). During times of solar high irradiance, considerable energy is used by cyanobacteria in dissipating the excess light energy. The actual process of photosynthesis is thus reduced during excess light conditions such that the activity rates are comparable to those occurring at low light conditions. Also, the availability of abundant suspended organic material in Storr's Lake could help certain cyanobacteria to metabolize even under dark conditions either by fermentation or heterotrophic consumption (Richardson and Ragoonath, 2008). Additionally,

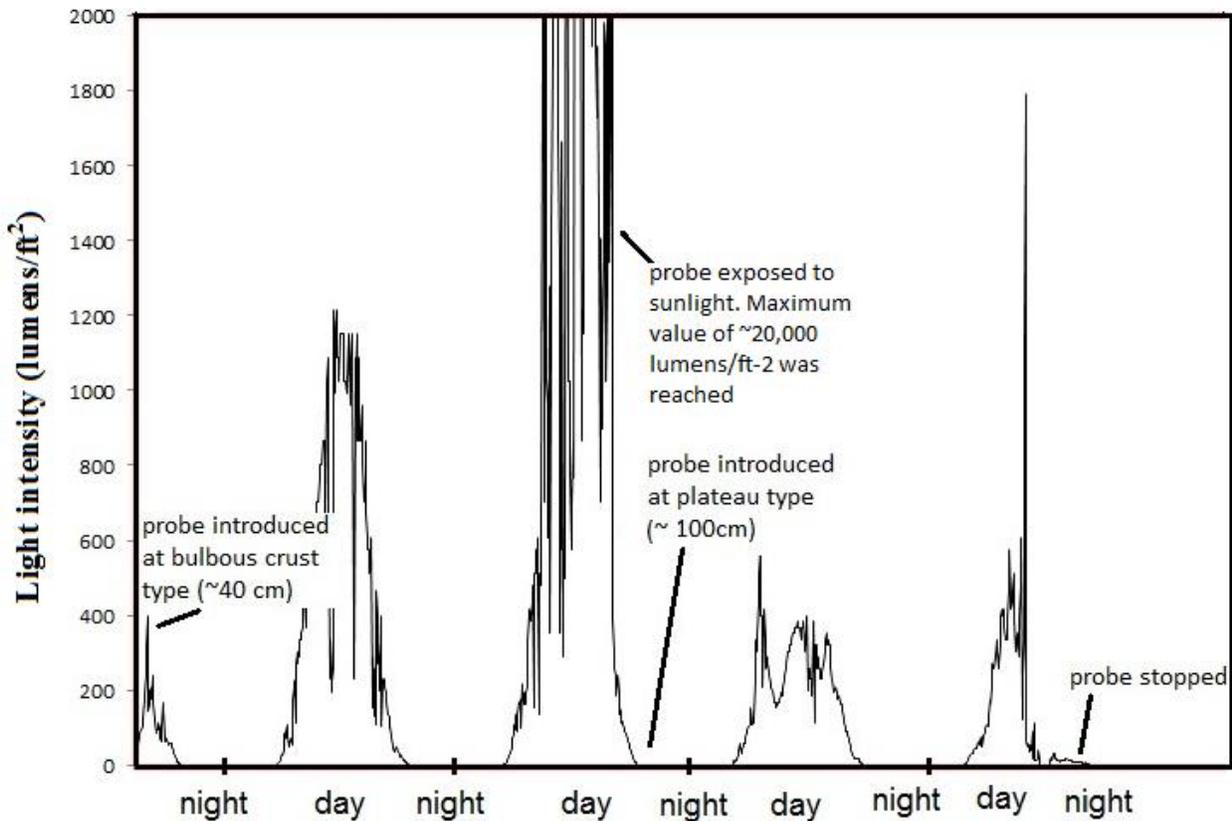


Figure 5. Light intensity data collected using HOB0 logger anchored into the bottom sediment and set at a depth of the microbialite heads. Total light intensity including aerial exposure spike up to ~20,000 lumens was observed. The graph shows the day and night cycles. Left side displays two-day pattern at a depth of 40 cm near the head of the bulbous crust (calcareous knobs) microbialites. Right side displays two-day pattern at plateau-mushroom microbialite region (~100 cm depth).

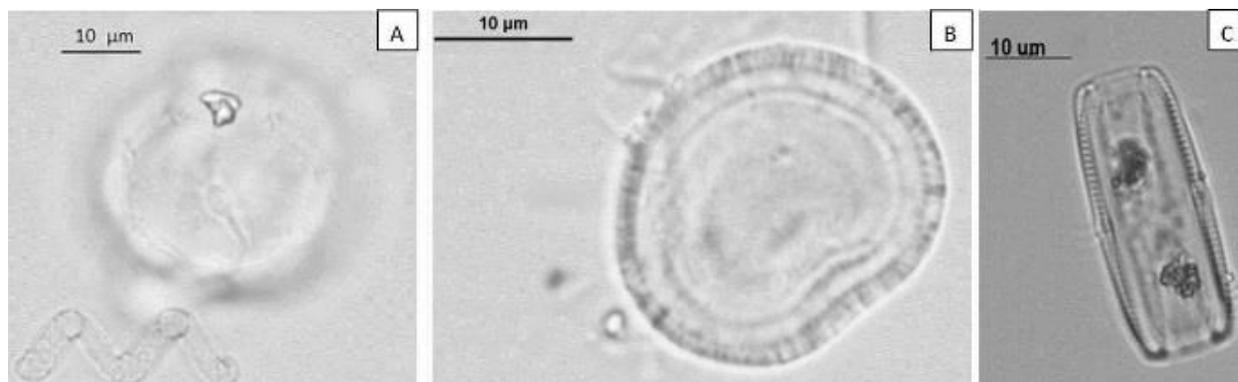


Figure 6. Optical microscopy images of some of the suspended microorganisms that contribute to the high turbidity of Storr's Lake water. A. dinoflagellate (center) with a spiral alga (bottom), B. circular microalgae, C. diatom.

the wavelengths of the penetrating light are also important; for a cyanobacterial photosynthetic community to function, it must receive light of wavelengths between 400nm to 700nm (Jørgensen et al., 1987). However, some cyanobacteria communities are capable of surviving in wavelengths of the extended spectrum of visible light (ultraviolet and infrared), having developed specialized pigments and enzymes capable of using these extended wavelengths of light (Castenholz and García-Pichel, 2000; Kuhl et al., 2005). The light probe used for our analysis measured a full array of wavelengths over 200 to 1100 nm, but does not differentiate between the different wavelengths. It is possible that the microbial community at the deeper locations could be photosynthetic or at least sporadically active during periods of low turbidity. A robust light penetration study, such as measuring the photosynthetically active radiation is essential for determining the amount of light that could be actually used for photosynthesis. An alternative mechanism that has been associated with carbonate precipitation in microbial mats is the activity of sulfate reducing bacteria (Visscher et al., 2000). The sulfate concentration in the lake is higher than that of seawater (Table 1), indicating that sulfate is available for chemotrophic cycling in this environment.

Mineralogy and Microscopic Analysis of the Microbialites and Lake Water

The mineralogy of five different microbialite types analyzed by XRD showed that high-Mg calcite was the dominant mineral found in all the samples. In addition, aragonite was also found in three of the five microbialite types: plateau-mushroom, pinnacle mound and multicusate.

Scanning electron microscopy of the microbialites revealed a variety of internal morphological features. The microbialites were dominated by calcium carbonate crystals of irregular or rhombohedral morphology. The crystals within the plateau-mushroom and pinnacle mound microbialite exhibited occasional needle shaped structures consistent with the presence of aragonite as detected by XRD analysis (Figure 7A). The crystals exhibit a random orientation (Figure 7B) with possible cyanobacterial or heterotrophic bacterial filaments encrusted with the calcium carbonate. Exopolymeric substances (EPS) produced by the bacteria probably play an important role in the mineralogy and random orientation of crystal growth. The SEM images show fossilized remains of diatoms and EPS (Figure 7A and C). Empty fossil-mold remains of coccoid bacteria are also seen in the Storr's Lake microbialites (Figure 7B-white arrow). Gastropods, probably from the genera *Cerithidea*, *Bulla* or *Cerithium*, identified in Storr's and surrounding lakes (Neumann et al., 1989; Hagey and Mylroie, 1995), and diatoms in the microbialite structure

indicates that extragenous biologic material can be also incorporated in the microbialites.

Gastropods and ostracods assemblages and their correlation to the salinity and other paleoenvironmental conditions of the aquatic environments on San Salvador and other Bahamian islands have been studied in detail (Zabielski, 1991; Hagey and Mylroie, 1995; Noble et al., 1995). Gastropods such as *Cerithidea* may graze on the microbial mats (Garrett, 1970). In Storr's Lake, the hypersaline conditions probably keeps such grazers to limited numbers, thus allowing the formation and development of the microbialites. During the low salinity and high lake level period of our 2012 visit, we observed that the lake was inhabited by an unidentified fish species (Figure 8). Dissection of a single individual revealed stomach and intestine contents that included acid soluble carbonate fragments. Such an occurrence indicates that the collected individual fish had been feeding on the hardened structures of the microbialites prior to its capture. To our knowledge, no previous occurrence of fish in Storr's Lake has been reported. Therefore, the gastropods and fish that inhabit the lake may be opportunistic colonizers that populate the lake water during periods of relatively low salinity, only to migrate or die when higher salinity periods return.

Analysis of the suspended particles in Storr's Lake water samples under an optical microscope showed that the turbidity is caused by

floating biological material consisting of various algae including dinoflagellates, cyanobacteria, diatoms, and halophilic bacteria (Figures 6A and B). This was consistent with earlier description of the lake water by Mann and Nelson (1989). Several nearby lakes also have similar planktonic microbial communities. For example, the spiral shaped algae (Figure 6A) was identified as *Spirulina*, from nearby Stout's Lake, San Salvador Island (Elliott, 1992). Several diatoms, such as *Navicula* (Figures 6C and 7C) were also identified in Storr's Lake. These and other phytoplanktons, such as *Oscillatoria*, *Coccochloris*, *Chroococcus*, *Gleocapsa*, *Lyngbia*, and *Scytonema* were identified in the nearby Little and Great Lakes, Stouts Lake and also in Storr's Lake (Marshall, 1982; Neumann et al., 1989; Pentecost, 1989; Elliott, 1992). All species could occur as floating material in the water, while also existing on the microbial mats covering the microbialites and lake-bottom sediments.

Microbial Diversity Analysis

Preliminary results obtained from sequencing the five different microbial mat samples from Storr's Lake indicated that the microbial population was diverse and varied greatly within one mat and another. The photosynthetic cyanobacterial population in the overall microbial community was very low with a maximum of ~3.2% in the shallow calcareous knobs to as low as

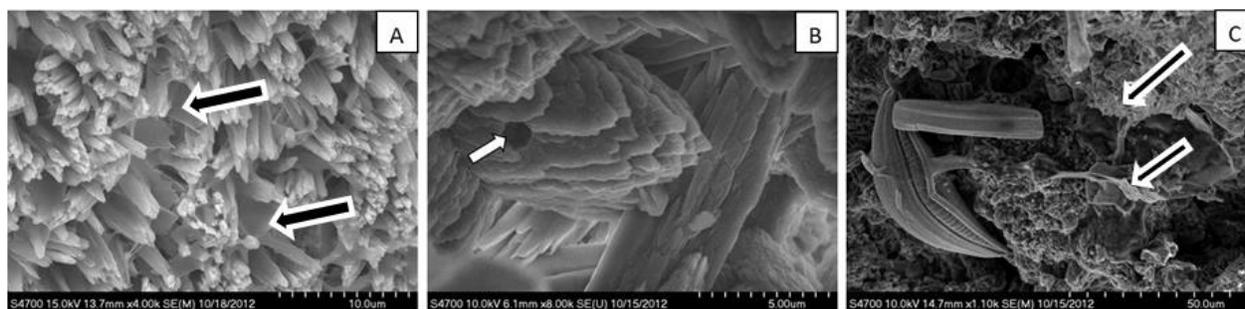


Figure 7. SEM images of the microbialites from Storr's Lake. A. Acicular crystals found in the lower section of plateau-mushroom type microbialite, probably indicating aragonite. Remains of EPS material are indicated by black arrows; B. Pinnacle mound microbialite showing random crystal orientation of calcium carbonate minerals. White arrow indicates position of an empty mold of coccoid bacteria; C. Diatoms and remains of EPS (black arrows, also in 7A).

~0.01% in the pinnacle mounds. Several of the bacterial communities were unknown or unidentified in the different mats, for example, ~36% of the total population was unknown in the case of cheesecake microbial mat. The majority of the known population fell into families of *Dehalococcoidaceae*, *Desulfobacteraceae*, and *Spirochaetaceae*. *Chloroflexi* constituted approximately half (~46%) of the entire bacterial population identified in both the deeper-water cauliflower-top mushroom and pinnacle mound mats, while calcareous knob, plateau-mushroom and cheesecake types had only 16 to 20% *Chloroflexi*. The family *Dehalococcoidaceae* of the phylum *Chloroflexi* was common in all of the mats. Members of *Dehalococcoidaceae* include a variety of anaerobic to aerobic, and photosynthetic (green-sulfur bacterium) microorganisms. The class, *Deltaproteobacteria* includes several sulfate reducing bacteria, and these were enriched in the shallow water calcareous knob (21%), and the cheesecake mat (16%). The lowest percentage of *Deltaproteobacteria* occurred in the deeper-water pinnacle mound (5%) and cauliflower-top mushroom (6%) mat types. The sulfate-reducing bacteria family, *Desulfobacteraceae*, dominated the recovered *Deltaproteobacteria* sequences. For example, 16% of the 21% of *Deltaproteobacteria* were represented by *Desulfobacteraceae*.

It is thus evident that cyanobacteria, though limited, are still present in the microbial mats. Other photosynthetic populations, such as the anaerobic green-sulfur bacteria survive even in the light-limited deeper-water waters of the lake. Though the presence of key carbonate mineral precipitating microbial communities including anaerobic phototrophs and sulfate-reducers (except for cyanobacteria) were identified in the mats, whether these microorganisms are actively contributing to the precipitation on the microbialite head is not certain. In the absence of cyanobacteria, carbonate mineralization can potentially occur with the activity of SRBs and anoxygenic phototrophs (Visscher et al., 2000; Dupraz and Visscher, 2005).

CONCLUSIONS

Our investigation of the Storr's Lake ecosystem provides valuable data to the existing information on the lake's unique water chemistry, ecosystem and microbialite communities. Salinity and other chemical parameters fluctuate widely as demonstrated by our measured salinity values ranging from 92.1 ppt in April 2011 to 26 ppt in May 2009. Our May 2009 salinity measurement was the lowest ever recorded for the lake and was lower than the salinity of water in the adjacent ocean water. Our extensive light penetration study in the lake indicates that low amounts of sunlight can reach the bottom, in spite of the high turbidity of the water. Preliminary molecular analysis of the microbialitic mats revealed that photosynthetic cyanobacteria represented only a small fraction of the entire microbial population. Several members of *Chloroflexi* and *Deltaproteobacteria* were present in the microbial mats indicating that anoxygenic photosynthesis and heterotrophic activity dominate the mat environment. Continued investigation of the lake water and the microbialites is essential to clearly understand the effects of water chemistry on the microbial population and *vice versa*, and further to determine if any seasonal, spatial and temporal trends are observed in the water chemistry and the microbial population in response to changing climate and sea level conditions.

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