A re-examination of the mechanism of whiting events: A new role for diatoms in Fayetteville Green Lake (New York, USA)

Short running title: Re-examining whiting events in Fayetteville Green Lake

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ABSTRACT

Whiting events – the episodic precipitation of fine-grained suspended calcium carbonates in the water column – have been documented across a variety of marine and lacustrine environments. Whitings likely are a major source of carbonate muds, a constituent of limestones, and important archives for geochemical proxies of Earth history. While several biological and physical mechanisms have been proposed to explain the onset of these precipitation events, no consensus has been reached thus far. Fayetteville Green Lake (New York, USA), is a meromictic lake that experiences annual whitings. Materials suspended in the water column collected through the whiting season were characterized using scanning electron microscopy and scanning transmission X-ray microscopy. Whitings in Fayetteville Green Lake are initiated in the spring within the top few meters of the water column, by precipitation of fine amorphous calcium carbonate (ACC) phases nucleating on Synechococcus cells (cyanobacteria), as well as on extracellular polymeric substances (EPS), including abundant β-chitin fibrils exuded by centric diatoms. Whiting particles found in the summer consist of 5-7 µm calcite grains forming aggregates with diatoms and their EPS. Simple calculations demonstrate that calcite particles continuously grow over several days, then sink quickly through the water column. In the late summer, partial calcium carbonate dissolution is observed deeper in the water column. Settling whiting particles however reach the bottom of the lake, where they form a major constituent of the sediment, along with diatom frustules. The importance of diatoms and their EPS in whitings at Fayetteville Green Lake is described for the first time here, a largely overlooked mechanism for other whiting events in modern and ancient environments.
1. INTRODUCTION

Fine-grained (micritic) limestone is abundant in the sedimentary record and an important repository for geochemical and isotopic proxy records of Earth history. Despite its importance, the origin of marine mud has been a topic of considerable uncertainty and research for decades (Bathurst, 1966), with both inorganic and biogenic mechanisms being proposed. One source of mud, the apparently spontaneous precipitation of very fine suspended calcium carbonate (CaCO$_3$) particles, called a whiting, has been observed to occur and persist for many days in marine environments, most notably in the Bahamas (Broecker & Takahashi, 1966; Shinn et al., 1989; Robbins et al., 1997; Purkis et al., 2017). Physical disturbance and re-suspension of carbonate sediments (e.g., Boss & Neumann, 1993; Broecker et al., 2000; Morse et al., 2003; Dierssen et al., 2009; Broecker, 2012), chemical precipitation (Brunskill, 1969), and biological mediation (e.g., Robbins & Blackwelder, 1992; Thompson, 2000; Swart et al., 2014; Long et al., 2017) have been proposed to explain the whiting phenomenon.

1.1 Biological hypotheses for the origin of whitings

Among biological mechanisms, a role of photosynthetic microorganisms (in particular, cyanobacteria) has often been invoked for whiting production, supported by the fact that whiting events often coincide spatially and temporally with high abundances of these organisms (Schultze-Lam et al., 1997; Hodell et al., 1998; Thompson, 2000; Dittrich et al., 2004; Dittrich & Obst, 2004). Biological models for the onset of whitings frequently involve the heterogeneous nucleation of CaCO$_3$ minerals on microbial surfaces and extracellular organic materials in supersaturated waters (Robbins & Blackwelder, 1992; Schultze-Lam et al., 1992; Thompson, 2000). Picocyanobacteria in particular, because of their small sizes, offer a larger surface area for CaCO$_3$ precipitation.
nucleation (Robbins & Blackwelder, 1992; Hodell et al., 1998; Dittrich et al., 2004; Dittrich & Obst, 2004). Some studies have focused more specifically on the role of the S-layer of picocyanobacteria of the genus *Synechococcus*. Forming a hexagonally symmetric paracrystalline surface array, the S-layer may act as a template favoring calcite nucleation at the cell surface (Thompson & Ferris, 1990; Schultze-Lam et al., 1992, 1997; Thompson, 2000).

In addition to models based on CaCO$_3$ nucleation on microbial surfaces (“passive” mineralization), cyanobacteria and other photosynthetic organisms might also provoke whiting events by locally increasing supersaturation with respect to calcium carbonates though CO$_2$ uptake (“active” mineralization) (Schultze-Lam et al., 1997; Thompson, 2000; Dittrich & Obst, 2004). Recently, Lisle & Robbins (2016) hypothesized that viral lysis of cyanobacterial cells releases cytoplasmic bicarbonate – which is 23 times more concentrated in the cytoplasm than in seawater – leading to extreme mineral supersaturation in the immediate vicinity of the cells, and enabling homogeneous nucleation of CaCO$_3$.

Other mechanisms for microbial precipitation of CaCO$_3$ minerals have been described in the literature dealing with calcifying microbial mats and microbialites (e.g., Dupraz & Visscher, 2005; Dupraz et al., 2009; Zhu & Dittrich, 2016). Some of these mechanisms, such as bacterial sulfate reduction or anoxygenic photosynthesis, are irrelevant to whiting events, which occur in the oxygenated photic zone of the water column. Others, such as the degradation of microbial EPS locally releasing calcium and carbonate ions, could be relevant to planktonic CaCO$_3$ formation, but it is not clear whether such mechanisms could be playing a role in the onset of whittings. Finally, some cyanobacteria (Benzerara et al., 2014) as well as other bacterial types (Benzerara et al., 2021; Monteil et al., 2021) and microalgae (Martignier et al., 2017) can form amorphous intracellular
calcium carbonate biominerals, but their involvement in whiting events has never been documented.

1.2 A role of diatoms in whitings?

Several studies have described an association of planktonic and benthic CaCO$_3$ precipitation with diatom species in different marine and lacustrine environments (Winsborough & Golubić, 1987; Küchler-Krischun & Kleiner, 1990; Winsborough, 2000; Ehrlich et al., 2016; Fuchs et al., 2016; Long et al., 2017; Gomez et al., 2018). Diatoms can produce large amounts of extracellular polymeric substances (EPS), also called mucilage, playing different roles such as adhesion, motility, protection, and heavy metals detoxification (Urbani et al., 2012; Shnyukova & Zolotareva, 2015). In oligotrophic hardwater lakes, diatoms have been observed in increasing numbers, causing ecological strain (Kirkwood et al., 2007; Novis et al., 2017), and their abundance sometimes coincides with increased sedimentation of CaCO$_3$ (Stabel, 1986; Fuchs et al., 2016). In at least one marine environment (southwest Florida), CaCO$_3$ whiting particles where associated with centric diatoms (Long et al., 2017), but it is unclear whether diatoms or their EPS could be a significant factor in calcium carbonate nucleation in other modern whiting events.

1.3 Whiting events at Fayetteville Green Lake

Fayetteville Green Lake (FGL) (NY, United States) is a 53 m deep, permanently stratified lake where annual whitings have been documented. The lake redoxcline at ~20 m water depth separates an upper, wind-mixed, oxygenated mixolimnion from a lower, slightly denser, euxinic monimolimnion (Takahashi et al., 1968). The surface waters of FGL are supersaturated with respect to calcite ($\Omega \approx 10$) (Takahashi et al., 1968), and experience a whiting every spring, when
precipitation of fine-grained suspended calcite transforms the lake color from deep blue to turquoise (Thompson et al., 1990; Schultze-Lam et al., 1997).

Previous investigations of FGL documented the evolution of the whiting over time (Schultze-Lam et al., 1997), and showed that the initiation of the whiting in the spring (May) roughly coincides with an annual cyanobacterial bloom. Carbon stable isotope measurements in the water column and the sediment of the lake further suggest that photosynthesis-driven carbonate precipitation in the mixolimnion is the primary source of carbonate minerals in the sediments (Schultze-Lam et al., 1997; Havig et al., 2017). A model for the onset on the whiting has been proposed in the 1990s, in which *Synechococcus* (micron-sized coccoid cyanobacteria) were the main biological agents driving CaCO$_3$ precipitation. The cyanobacteria are thought to be driving local increases of saturation state from their photosynthetic activity, and acting as sites for mineral nucleation (Thompson et al., 1990; Schultze-Lam et al., 1997). A *Synechococcus* strain isolated from FGL was shown in the laboratory to nucleate calcite crystals at the cell surface, possibly templated on the S-layer (Thompson & Ferris, 1990; Schultze-Lam et al., 1992). Recently, Kamennaya et al. (2020) have shown that *Synechococcus* thriving in the surface waters of FGL produce abundant EPS that can adsorb diverse cations (including calcium) and detach from the cells. However, it is not clear whether or not these cell-free calcium-loaded EPS envelopes play a role in triggering whittings at FGL.

Other recent studies on FGL have focused on thrombolic microbialites growing on the lake shore (DeMott et al., 2020), isotopic fractionation effects associated with microbial calcite precipitation (Chen et al., 2021), as well as carbon and nitrogen biogeochemical cycling (Havig et al., 2017; Fulton et al., 2018), sulfur biogeochemistry (Zerkle et al., 2010; Oduro et al., 2013), and
microbial and geochemical processes at the chemocline and in the monimolimnion (Meyer et al., 2011; Hunter, 2012; Havig et al., 2015; Rojas et al., 2021).

We performed a high-resolution microscopy and spectroscopy characterization of particles suspended in the water column of FGL and collected through the whiting season, as well as of particles from the lake bottom sediments, enabling us to propose new hypotheses for the biological mechanisms involved in whiting events and carbonate sedimentation.

2. METHODS

2.1 Field sampling

Field work was conducted during the spring, summer, and fall of 2018 at Green Lakes State Park, in Fayetteville, NY (United States). Five field trips were executed on April 16, May 31, June 30, August 1, and September 8 of 2018. Samples were collected from a boat stationed near the lake center. Water samples were collected using a peristaltic pump at every 1 m interval for the top ~10 m of the water column. Both filtered (0.2 µm polycarbonate filters) and unfiltered water samples were collected and stored at 4°C. Polycarbonate filters were immediately rinsed with deionized (DI) water, air-dried, and stored for later microscopy analyses. Sediment trap samples analyzed in this study were collected by S. Romaniello (University of Tennessee, Knoxville) between July 12 and July 21, 2017 from 13.5 depth, and stored at -20°C prior to analyses. Samples from the top 4 inches of a Green Lake bottom sediment core were also analyzed. These sediment core samples were freeze-dried for preservation. They have been further described elsewhere (Havig et al., 2015, 2017).
2.2 Scanning Electron Microscopy (SEM)

Suspended particles from the water column and collected on polycarbonate filters, as well as rinsed sediment trap samples, were characterized using scanning electron microscopy (SEM) on a FEI Nova NanoSEM 630 field emission gun SEM. Elemental information was derived from Energy-dispersive X-ray Spectroscopy (EDS) (Oxford Instruments UltimMax detector) to confirm the mineralogy of observed particles. Images were collected at accelerating voltages ranging from 5-15 keV and at working distances down to 3 mm, while EDS analyses were conducted at 15 keV at working distances down to 7 mm. EDS data was processed using the program Oxford Aztec.

Mineral morphology and texture, size, and abundances, as well as diatom abundances were assessed using SEM images generated throughout the whiting season by manual counting on ~700 μm x 700 μm SEM images of the filters. Three types of particles were counted and measured on these large-scale overview images: pennate diatoms, centric diatoms, and carbonate grains. The visual aspect of the carbonate particles was furthermore characterized as either intact or pitted (as indicative of dissolution). Once all particles on these larger images had been counted, the volumetric concentrations of each type of particle was calculated, using the volume of filtered water for each filter and filter area. The areas counted (~0.49 mm²) were assumed to be representative of the entire filters (~490 mm²), which contained all particles from the originally filtered 60 mL samples. Using these values, particle counts from the SEM images were converted into concentrations of particles per milliliter.

2.3 Scanning Transmission X-ray Microscopy (STXM)

2.3.1 STXM data acquisition and processing
Scanning Transmission X-ray Microscopy (STXM) was used to collect spectroscopic data on the sub-micrometer scale distribution and speciation of carbon and calcium in minerals and associated organics. STXM analyses were performed on beamline SM at the Canadian Light Source, Saskatoon, SK, operating with a 35 nm zone plate (ZP) on samples collected from the water column in April 2018. Another beamtime at the STXM beamline 5.3.2.2 (operating with a 25 nm ZP) of the Advance Light Source, Berkeley National Lab, CA, was used to analyze sediment trap and sediment core samples. For STXM, particulate materials were centrifuged, rinsed with deionized water, and deposited onto silicon nitride windows (Norcada).

STXM data acquired at the C K-edge and Ca L$_{2,3}$-edge were processed in aXis2000 (Hitchcock, 2012). Transmission images were first converted to optical density (OD). At the C K-edge, organic carbon maps were generated by subtracting an OD image at 280 eV (below the C K-edge) from another at 288.2 eV (absorption energy of 1 s→$\pi^*$$_{C=O}$ electronic transitions in amide groups). Calcium maps were obtained at the Ca L$_{2,3}$-edge by subtracting an OD image at 343 eV (below the Ca L$_3$-edge) from another at 349.3 eV (energy of the Ca L$_3$-edge).

X-ray absorption near edge structure (XANES) spectra were extracted from image stacks according to the procedure described by Cosmidis & Benzerara (2014). The Stack Fit tool of aXis2000 was used to extract and map the distribution of representative components in image stacks. Linear background removal was performed on XANES spectra in the 270-283 eV energy range below the C K-edge and the 340-346 eV energy range below the Ca L$_{2,3}$-edge.

2.3.2 Determination of $I_C/I_{Ca}$ ratios

XANES spectra at the C K-edge and Ca L$_{2,3}$-edge were used to obtain a semi-quantitative measure of elemental ratios of carbon versus calcium in the samples. The $I_C/I_{Ca}$ ratio is defined as the ratio of the areas under the spectra in the 280-310 eV region versus the 345-354 eV region.
(Fig. 1a). $I_C/I_{Ca}$ ratios measured on different calcium-bearing reference samples with known elemental compositions showed good correlation with C/Ca molar ratios (Fig. S2) ($R^2 \sim 0.95$) (Fig. 1b). The reference compounds included carbonate minerals (amorphous calcium carbonate, aragonite, benstonite, calcite, dolomite), calcium-phosphate minerals (francolite, hydroxyapatite), and *Escherichia coli* cells, and have already been described elsewhere (Benzerara et al., 2004; Cosmidis & Benzerara, 2014; Cam et al., 2015; Cosmidis et al., 2015a, 2015b).

$I_C/I_{Ca}$ ratios were used to distinguish calcium carbonate mineral phases (empirically defined as $I_C/I_{Ca} < 3.3$) from organic materials with adsorbed Ca$^{2+}$. The presence of X-ray absorption features at 290.3 eV and around 301.5 eV was furthermore used to discriminate calcium-carbonate minerals from organic matter (absorbing in the 285.0-288.7 eV range) (Brandes et al., 2010) (Fig. 1a). A more quantitative assessment of carbonates versus organic carbon concentrations was not attempted due to the fact that the intensity of the X-ray absorption signal 290.3 eV (1s → π* electronic transitions in carbonate groups) in carbonate minerals depends on the orientation of the crystals with respect to the X-ray beam (Metzler et al., 2008).

### 2.3.3 Determination of calcium carbonate crystallinity: splitting ratios at the Ca L$_{2,3}$-edge

Calcium carbonate mineral phases were identified by comparison with reference spectra at the Ca L$_{2,3}$-edge. The crystallinity of calcium carbonates was furthermore quantified using calculated splitting ratios ($SRL_2$, $SRL_3$), which provide a measure of the crystal field splitting at the Ca L$_2$ and L$_3$ edges, as defined in Politi et al. (2008). Typically, the Ca L$_{2,3}$-edge spectra of amorphous calcium carbonates (ACC) have poorly split L$_2$ and L$_3$ peaks, while the spectra of crystalline phases such as calcite display higher $SRL_2$ and $SRL_3$ splitting ratios characteristic of well resolved split peaks (Politi et al., 2008) (Fig. 1c).
3. RESULTS

3.1 Types and abundances of whiting particles

SEM was used to image and quantify the abundance of solid particles collected on filters at different depths in the FGL water column throughout the 2018 whiting season. Sampled particles primarily consist of centric and pennate diatoms, carbonate minerals grains, microbial cells, and extracellular organic materials (Figs. 2, S1). Calcium carbonate minerals are not visible in SEM images in April but are present in June, with highest abundances in the 3 m sample, showing the shallow location of the whiting in the water column (Fig. 3). Both centric and pennate diatoms increase in numbers as the summer progresses, peaking in June, while the abundance of carbonate minerals peaks in August. Unfortunately, abundances of microbial cells (e.g. cyanobacteria) cannot be quantified using SEM images, due to their small sizes and low density to the electron beam. Both calcium carbonate minerals and diatoms sink deeper in the water column with time, evidenced by counts showing decreasing abundance in the shallow water column and increasing abundance at greater depth through the summer. The aspect of carbonate minerals changes through time, as depicted in Figure 2a-c: early carbonate grains (June) appear smooth, while later carbonate grains (August) develop pitted, rough outer surfaces as well as rounder shapes. However, the average size of suspended carbonate grains remains relatively constant with time (~5-7 μm in length) through the whiting. Carbonate grains and diatoms are found within a mesh of long organic filaments, ~100 nm thick, likely corresponding to β-chitin fibrils produced by some centric diatoms (Herth & Barthlott, 1979; Gügi et al., 2015; Novis et al., 2017). These fibrils are also observed aggregated with carbonates and diatoms in sediment trap samples collected at 13 m depth in July 2017 (Fig. 2f, S2). Some centric diatoms from the water column samples appear to be extruding EPS materials through pores of their silica valves (Fig. 1d). The brightness of this exuded material
in SEM images suggests that it might be associated with heavy elements such as absorbed metallic cations.

**3.2 Calcium carbonate mineralogy and calcium association with cells and organics**

**3.2.1 Pre-whiting samples from the FGL water column (April)**

Samples collected from the FGL water column at 8 m depth in April 2018 (before the onset of conspicuous calcium carbonate precipitation) were analyzed using STXM at the C K- and Ca L$_{2,3}$-edges. Abundant centric diatoms were observed, confirming SEM results, along with spherical and rod-shaped microbial cells (Fig. 4). The spherical cells are relatively small (~0.8 µm in diameter), and likely correspond to cyanobacteria of the genus *Synechococcus*, the dominant autotrophs in Green Lake (Thompson *et al.*, 1990; Schultze-Lam *et al.*, 1997; Kamennaya *et al.*, 2020). Diatoms and bacteria are found amidst extracellular organic material, forming either fibrils or thin films, and interpreted as EPS produced by microbial cells and/or diatoms. Note that this EPS material, although visible on STXM transmission images, do not always appear on organic carbon maps, likely due to their extreme thinness (possibly 10 nm or thinner; Svetličić *et al.*, 2013) which might prevent the obtention of a proper focus of the X-ray beam and good absorption signal.

C K-edge XANES spectra obtained on *Synechococcus* cells exhibit a main peak at 288.2 eV (amide groups in peptides), with smaller peaks at 285.0-285.5 eV (aromatics and unsaturated carbon), and shoulders at 286.6 eV (ketonic and phenolic groups), 287.4 eV (aliphatics, phenols and/or ketones), 288.5 eV (carboxylic groups), 289.4 eV (hydroxyl groups, ethers, and/or C=O groups in nucleic acids), and 290.3 eV (carbonate groups) matching previously published bacterial spectra (e.g. Benzerara *et al.*, 2004; Chan *et al.*, 2011; Cosmidis *et al.*, 2015a) (Fig. 5).
In contrast, the C K-edge spectra of the EPS show a main peak at 288.5 eV (carboxylic groups), consistent with a composition dominated by acidic polysaccharides (Lawrence et al., 2003; Chan et al., 2009; Mitsunobu et al., 2014), with smaller peaks and shoulders around 285.0-285.5 eV (aromatics and unsaturated carbon), 287.4 eV (aliphatics, phenols and/or ketones), 288.2 eV (amide groups), and 289.4 eV (hydroxyl groups, ethers, and/or C=O groups in nucleic acids).

Note that peaks at 288.5 and 289.4 eV are also consistent with the presence of β-chitin (Lehmann et al., 2009), which composes the EPS fibrils extruded by many centric diatoms (Herth & Barthlott, 1979; Novis et al., 2017).

At the Ca L2,3-edge, STXM shows the presence of abundant calcium on EPS films and diatoms, in particular on perforations of the frustules, from which EPS are typically exuded (Herth & Barthlott, 1979) (Fig. 6). Relatively minor amounts of calcium are detected on microbial cells (see calcium maps on Fig. 4), where this element is only present as adsorbed Ca2+, identifiable by a weak absorption signal at the Ca L2,3-edge (IC/Ca ratios 10-50) and absence of strong peaks at 290.3 eV and 301.5 eV (corresponding to carbonates) at the C K-edge. The surfaces of the *Synechococcus* cells are enriched in adsorbed calcium compared with their interiors, which can be determined by comparing the intensity of the absorption signal at the C K-edge and Ca L2,3-edge on the XANES spectra extracted from a cell interior (labelled S1) and cell surface (labelled L) on Figure 7. For instance, for the *Synechococcus* cell in Fig. 7a, IC/Ca = 46 on the cell interior and IC/Ca = 17 on the cell surface. The calcium enrichment of the cell surface may be indicative of Ca2+ adsorption on *Synechococcus* S-layers, as described in previous studies (Thompson & Ferris, 1990; Schultze-Lam et al., 1992).
Calcium is furthermore enriched on EPS films, forming irregularly shaped dense clots (white arrows on Fig. 4), displaying low $I_C/I_{Ca}$ ratios ranging from 0.5 to 2.5, as well as intense X-ray absorptions at 290.3 eV and ~301.5 eV, and thus interpreted as calcium carbonate minerals. At the Ca L$_{2,3}$-edge, their calculated splitting ratios are SRL$_3$ ~1.2-1.4 and SRL$_2$ ~ 1.3-1.4, matching a reference amorphous calcium carbonate (ACC) (Fig. 8). ACC in the pre-whiting samples is also found as small (<500 nm) mineral grains located on or nearby *Synechococcus* cells (phases mapped in blue in Fig. 7). These ACC phases were not identified on SEM images, possibly due to their small sizes.

Calcium is also concentrated in round-shaped areas within the EPS films, measuring ~1 µm in diameter (open arrows in Fig. 4d,f; Fig. S3a). Due to their weak signal at the Ca L$_{2,3}$-edge as compared with ACC grains described above, they likely correspond to adsorbed Ca$^{2+}$ on organic material rather than to calcium-carbonate minerals. No XANES spectra were acquired on these objects, preventing calculations of $I_C/I_{Ca}$ ratios. These round shaped calcium enrichments may correspond to “bag-like” EPS envelopes issued from *Synechococcus* cells, which can dissociate from the cells and have high Ca$^{2+}$ adsorption capacity (Kamennaya et al., 2020).

**3.2.2 Whiting sample (sediment trap)**

STXM analyses were performed on whiting particles collected in a sediment trap placed in the FGL water column at 13.5 m depth between July 12 and July 21, 2017. The sample contains abundant calcium carbonate particles, measuring up to 7 µm in length, and identified as calcite based on the similarity of their Ca L$_{2,3}$-edge XANES spectra with that of a reference calcite (Fig. 9, S4), in agreement with previous studies of whitings at FGL (Thompson et al., 1990; Schultze-Lam et al., 1997). Calculated $I_C/I_{Ca}$ ratios for these calcite grains range between 2.5 and 3.3, and splitting ratios at the Ca L$_{2,3}$-edge are SRL$_2$ ~ 1.6-1.7 and SRL$_3$ ~1.8-2.0 (Fig.
8), consistent with crystalline calcite (Politi et al., 2008). C K-edge XANES spectra obtained on the calcite grains sometimes display absorption peaks at 288.2-288.5 eV (amides and carboxylics), suggesting that organic compounds may be adsorbed onto or incorporated within the mineral particles (Fig. 9d).

The calcite grains are forming aggregates also containing centric diatoms and organic material, present either as fibrils, or as organic particles surrounding the minerals. Synechococcus cells were not observed in the sediment trap sample. The C K-edge XANES spectra of the organic fibrils and particles display main peaks at 288.5 eV (carboxylic groups) and 288.2 eV (amide groups), with smaller peaks or shoulders at 285.0 and 285.5 eV (aromatics and unsaturated carbon), 286.6 eV (ketonic and phenolic groups), 287.4 eV (aliphatics, phenols and/or ketones), 289.4 eV (hydroxyl groups, ethers, and/or C=O groups in nucleic acids). This composition is similar to that of EPS and diatom β-chitin fibrils previously described in pre-whiting samples from the water column, with an increased contribution from amide groups which may be derived from proteins issued from decomposing microbial cells. The organic fibrils and particles display high $I_C/I_{Ca}$ ratios (73-605) and no absorption signal from carbonates at the C K-edge, showing that calcium is only present as minor amounts of adsorbed Ca$^{2+}$.

Similarly, the diatoms display C K-edge XANES spectra consistent with cellular material with main absorption features characteristic of amides and carboxylic groups and absence of strong carbonate signal, with relatively high $I_C/I_{Ca}$ ratios (e.g. $I_C/I_{Ca} = 61$ for the diatom fragment in Fig. S4) consistent with the presence of adsorbed Ca$^{2+}$ only.

### 3.2.3 Sediment core samples

STXM analyses of sediment core samples from the bottom of FGL are presented in Figure 10 as well as Supplementary figures S5 and S6. The sediment contains abundant calcium
carbonate grains, identified as calcite based on their Ca L\textsubscript{2,3}-edge spectroscopic signature, and similar in shape and size with those observed in the whiting samples. This observation is consistent with isotopic studies suggesting that carbonate precipitation in the oxic zone of the lake is the primary source of carbonate in the sediments (Havig et al., 2017). I\textsubscript{C}/I\textsubscript{Ca} ratios measured on the sediment calcite particles range between 2.1 and 2.6, and splitting ratios at Ca L\textsubscript{2,3}-edges are SRL\textsubscript{3} ~ 1.9-3.2 and SRL\textsubscript{2} ~ 1.8 and 2.2 (SRL\textsubscript{2}) (Fig. 8), indicating increased crystallinity compared with the whiting calcite particles from the sediment trap. C K-edge XANES spectra obtained on these sedimentary calcite particles display peaks at 288.2-288.5 eV, again showing possible adsorption of incorporation of organic molecules.

Abundant diatoms, sometimes fragmented, are also observed in the sediment. Diatoms are mostly centric, with fewer pennate forms (e.g., Fig 10c, S5). The diatoms are associated with higher amounts of calcium compared with diatoms from the sediment trap, as visible on STXM calcium maps. This abundance of calcium is reflected in low I\textsubscript{C}/I\textsubscript{Ca} ratios ranging from 3.4 to 7.5. Combined with strong absorption signals at 290.3 eV at the C K-edge (see in particular Fig. S6b), low I\textsubscript{C}/I\textsubscript{Ca} ratios indicate the presence of fine calcium carbonate phases associated with the diatoms frustules. Calculated splitting ratios at the Ca L\textsubscript{2,3}-edge range between those of ACC and calcite (SRL\textsubscript{2} ~1.3-1.6 and SRL\textsubscript{3} ~1.1-1.5), corresponding to either ACC or poorly crystalline calcite.

Organic matter in the sediment samples is found in diatoms and rare extracellular fibrils (Fig. S5), as well as dense organic-rich particles found around diatoms and calcite grains, measuring up to ~5 µm (see one of these large organic particles in Fig. S6). These organic particles have a C K-edge signature similar to that of organic particles in the sediment traps.
4. DISCUSSION

4.1 Biological mechanisms in the FGL whiting

The whiting at FGL corresponds to the conspicuous precipitation of calcite crystals in the shallow water column of the lake, first observed at ~3 m depth in June (although find ACC grains are present associated with organics are present as early as April). Calcite saturation indices of ~1 at the surface of FGL (Takahashi et al., 1968; Havig et al., 2015) can be compared to indices of ~0.3 in waters of the Bahama Banks (Broecker & Takahashi, 1966), where marine whittings are regularly observed. Previous work has suggested that variability in saturation state leads to measurable changes in whiting precipitation rates (Morse et al., 2003), suggesting that precipitation rates at FGL have the potential to be fast in comparison to marine whittings. It is unclear whether or not photosynthetic organisms such as cyanobacteria and diatoms play a role in calcite precipitation through CO$_2$ uptake and localized alkalinization. Indeed, calcite abundances (peaking at ~4 m depth in August) are uncorrelated with maximum abundances of diatoms (at ~8 m in June) (Fig. 3), and previous studies found no correlation in space and time between peak abundances of calcite and Synechococcus (Schultze-Lam et al., 1997).

However, it is obvious that organic-mineral interactions involving microbial cells and EPS play a crucial role in calcium carbonate mineralization at the onset of the whiting. In April, STXM analyses reveal fine ACC phases covering abundant EPS derived from diatoms (Fig. 6), as well as cyanobacterial cells (Synechococcus) (Fig. 7). The precipitation of ACC minerals is likely facilitated by the adsorption of Ca$^{2+}$ on organic surfaces such as Synechococcus cells (Fig. 7), diatom EPS exuded from apertures of the silica valves (Figs. 2d, 6), and possibly “bag-like” empty EPS envelopes detached from Synechococcus cells (Fig. 4d,f) (Kamennaya et al., 2020). A significant portion of the diatom EPS likely consists of β-chitin fibrils, found associated with
calcite particles later in the summer (Figs. 2, S2). This interpretation is supported by the fact that microorganisms degrading chitin have been described in the FGL monimolimion (Rojas et al., 2021).

At the molecular level, adsorption of Ca\(^{2+}\) ions can occur on negatively charged functional groups such as deprotonated carboxylic groups in acidic polysaccharides and proteins. In FGL waters, at pH 6-8, carboxyl groups exist in a deprotonated state (Beveridge, 1981), and photosynthetic CO\(_2\) uptake may result in local pH increases surrounding cyanobacteria and diatoms, further facilitating deprotonation and calcium binding. Likewise, β-chitin molecules forming an important part of diatom EPS present C=O, O–H, and N–H groups as well as oxygen atoms with affinity for calcium ions, and chitin has been previously described as a nucleating agent for both amorphous and crystalline calcium carbonate biominerals (Ehrlich, 2010).

While a role of *Synechococcus* S-layers in calcium carbonate nucleation and templating has been proposed in earlier studies (Thompson & Ferris, 1990; Schultze-Lam et al., 1992), implication of diatom EPS in whiting at FGL is proposed here for the first time. Recent studies have described spatial associations of centric diatoms with calcium-carbonates precipitates in the water column of Lake Stechlin (Geramany) (Fuchs et al., 2016) as well as in a southwest Florida whiting event (Long et al., 2017). In the second example, amorphous and crystalline CaCO\(_3\) particles were observed along the diatom girdle bands, which was explained by the unique surface chemistry and chemical microenvironment in this region of the diatom frustule associated with cell division.

It is not clear why the role of diatoms and exuded EPS has been overlooked in previous studies of the whittings at FGL. The high abundance of diatoms in the lake sediments (Figs. 10, S5, S6) indicate that they have been thriving in past decades, and their presence is mentioned in several
older articles (Culver & Brunskill, 1969; Thompson et al., 1990; Schultze-Lam et al., 1997). Nanoparticulate ACC nucleating on diatom EPS in the early spring may have been unnoticed in the past due to unsuitable observation techniques. It is also possible that whiting nucleation mechanisms at FGL have evolved over time, with varying contributions of cyanobacteria versus diatoms through the lake’s recent history. Predominant mechanisms of calcium carbonate nucleation may shift depending on the microbial community dynamics in the water column, as a result of changes in climatic conditions and/or in the nutrient status of the lake.

In the summer, during the peak of the whiting, calcium carbonate is found as larger (up to ~7 µm) crystalline calcite grains, aggregating with diatoms as well as EPS materials including abundant β-chitin fibrils (Figs. 2, 9). It is unclear whether the whiting calcite particles correspond to the crystallization and growth of ACC particles observed in pre-whiting samples, although ACC is a common precursor phase to crystalline calcite in biological precipitation systems (Weiner et al., 2005). In particular, ACC is a precursor phase for CaCO$_3$ mineralization on microbial EPS (Enyedi et al., 2020; Shiraishi et al., 2020). Intermediate phases between nano-ACC particles and calcite crystals measuring several micrometers in lengths were not observed, which may be due to fast calcite growth in supersaturated solutions (see next section of the discussion).

In the sediment trap sample (July), organic materials and diatoms associated with the calcite particles display very small amounts of adsorbed Ca$^{2+}$ as compared with pre-whiting samples (see high $I_C/I_{Ca}$ values in Figure 8). Low amounts of calcium adsorbed on organics and diatoms in this whiting sample may thus suggest that calcite minerals grown at the expense of Ca$^{2+}$ initially adsorbed on biological materials.
4.2 The fate of whiting particles: calcite growth, partial dissolution, and sedimentation

The end of the whiting season is marked by a decline in calcite mineral abundances in the water column in September (Fig. 3). Although the surface waters of the lake remain supersaturated with respect to calcite in the late summer (Brunskill, 1969), the termination of the whiting may be linked with the decline in abundances of photosynthesizing organisms in shallow waters with time, leading to a shortage of organic surfaces for calcium carbonate heterogeneous nucleation.

The duration of the whiting is additionally controlled by the settling rate of mineral particles. Settling rates of mineral grains exponentially increase with size, as shown by Gibbs et al. (1971). This phenomenon results in a dramatic increase in settling rate as minerals grow longer than 10 µm in length. Based on our assessment of calcite grain sizes, average whiting calcite grains (5-7 µm in length) have a settling rate of ~1 m/day in still waters (based on calculations from Gibbs et al., 1971). Particles likely have somewhat longer residence times in the turbulent mixolimnion than these still-water settling rates imply, and smaller particles likely remain suspended in the upper portion of the lake for longer, contributing to the light scattering by carbonate grains, giving the lake its turquoise color. However, minerals in the mixolimnion likely grow quite fast (up to 2 µm/day, based on saturation state rate studies conducted by Wolthers et al., 2012), sinking faster as they grow, and ultimately fall out of the water column, settling in the sediments. Thus, a mineral can quickly grow to 5-7 µm in length over the span of 2-3 days, sinking as it grows. A mineral 1 µm in size at the surface will sink <0.5 m in a day but grow to a size of 3 µm over the same span of time. Small minerals, on the order of <5 µm in size, are not found suspended in the water column, likely because the growth rate of these grains is fast following nucleation on organic templates. Minerals larger than 15 µm were not found suspended in the water column, consistent with their calculated very short residence time. All told, the residence time of a mineral grain in...
the upper 10 m of the lake is estimated to be less than 7 days when taking into account combined
growth and sinking. As demonstrated above, calcite particles are furthermore frequently found in
aggregates formed by diatom frustules and EPS, which would also increase settling rates. In order
to support the continued growth and settling of mineral grains, nucleation of new minerals must
be continuous throughout the duration of the whiting event.

Abundance of calcite grains in the FGL sediments (Figs. 10, S5, S6) suggests that settling
whiting particles reach the bottom of the lake despite some dissolution in the mixolimnion in the
late summer (as evidenced by corroded grain surfaces below ~8 m depth in August and September;
Figs. 2, 3, S1). Calcite dissolution below ~8 m is explained by the development of slightly
understaturated conditions (Havig et al., 2015), possibly due to cumulative respiratory CO₂ build-
up through the summer. The sedimented calcite particles display similar shapes and sizes to those
found in the water column, but they are slightly more crystalline (Fig. 8b). Calcite in the sediments
is found in association with diatom frustules and organic materials, which can be coated by
important amounts of calcium (Fig. 8a), reflecting the higher concentrations of dissolved Ca²⁺ in
the lake monimolimnion (Havig et al., 2015). Calcite grains and diatoms are the main components
of FGL bottom sediments, confirming previous studies, and suggesting that these sediments record
biogeochemical signals (such as carbon isotopic signatures) from the lake surface waters (Havig
et al., 2017).
5. CONCLUSION

This microscopy study of the annual whiting at Fayetteville Green Lake highlights the role of photosynthetic microorganisms (cyanobacteria and diatoms) and their exuded polymers (EPS) in calcite mineralization through nucleation of an ACC precursor phase. This work describes a previously overlooked role of diatoms and their EPS in calcite precipitation, a mechanism that may be relevant to different types of environments experiencing whiting events.

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8. FIGURES AND CAPTIONS

Figure 1. STXM methods for the calculation of $I_{C}/I_{Ca}$ ratios and splitting ratios (SRL$_2$ and SRL$_3$). (a) XANES spectra of several reference compounds spanning the C K- and Ca L$_{2,3}$-edges, and calculated $I_{C}/I_{Ca}$ ratios. $I_{C}/I_{Ca}$ is calculated as the ratio of the areas under the curve in the 280-310 eV region versus the 345-354 eV region (shaded areas). Calculated $I_{C}/I_{Ca}$ ratios are noted (numbers in brackets). The C K-edge spectra of carbonate minerals typically display strong absorption peaks at 290.3 eV and broad absorption bands around 301.5 eV. Some of the mineral
references contain minor amounts of organics, detectable as small peaks in the 285.0-288.7 eV region. ACC: amorphous calcium carbonate; CHA: carbonated hydroxyapatite (francolite). (b) Plot showing the correlation between $I_C/I_{Ca}$ ratios and measured C/Ca molar ratios for different calcium-bearing reference minerals. ACC: amorphous calcium carbonate; CHA: carbonated hydroxyapatite (francolite), HA: hydroxyapatite. (c) Ca L$_{2,3}$-edge spectra of a reference calcite and amorphous calcium carbonate, showing the method for the calculation of splitting ratios, quantifying calcium carbonate crystallinity (see Politi et al., 2008).
Figure 2. SEM images of whiting particles from the FGL water column. (a) Smooth-surfaced calcium carbonate mineral interlaid with organic fibrils, characteristic of peak whiting (3 m depth, June). (b) Pitted calcium carbonate grains and centric diatoms (8 m depth, August). (c) Increasingly pitted and rounded carbonate grain, along with pennate diatom (8 m depth, September 8). (d) A centric diatom with organic material (EPS) extruding from the valve pores (3 m depth, June 30). The brightness of the extruding material is indicative of heavy elements associated with the EPS (possibly adsorbed ions). (e) A centric diatom, attached to a carbonate mineral grain with organic fibrils (3 m depth, June). (e) Sediment trap whiting material (July 2017, 13 m depth) showing carbonate minerals within a dense mesh of organic fibrils. White arrows in (a), (d), and (e) point to possible microbial cells (Synchococcus).
Figure 3. Particle types and abundances at different depths in the water column of FGL before and throughout the whiting season, as counted from SEM images. Note that abundances of carbonate grains have been divided by ten, and that sample collection depth vary for different sampling times. The percentage of pitted carbonate particles (relative to smooth grains) is indicated (brackets).
Figure 4. STXM analyses of pre-whiting particles from the FGL water column (April, 8 m depth). Images (left) were acquired at 288.2 eV. The corresponding maps (right) show the distribution of organic carbon (red) and calcium (cyan). Whites squares show the locations of the image stacks displayed in Fig. S3 and Fig. 7. White arrows show the location of irregularly shaped calcium-rich clots within EPS films (identified as ACC minerals), while open arrows show micron-scale, round calcium enrichments that may correspond to Ca$^{2+}$ adsorbed on empty EPS envelopes of *Synechococcus* cells. Note that EPS films and fibrils visible on STXM images do not appear on organic carbon maps, which may be due to excessive thinness (see main text).
Figure 5. STXM analyses of diatom EPS and *Synechococcus* cells from a FGL pre-whiting sample (April, 8 m depth). (a) Image obtained at 288.2 eV. A centric diatom, surrounded by EPS film, is visible. (b) Close-up on the area depicted by a square in (a). *Synechococcus* cells are present within the EPS film. (c) Organic carbon map. (d) Calcium map. (e) XANES spectra representative of calcium-rich clots within the EPS film (green) and *Synechococcus* cells (red). C K-edge spectra (middle panel): vertical lines correspond to the absorption energies of different functional groups (see main text). Ca L$_{2,3}$-edge spectra (right panel): vertical lines correspond to the position of the main peaks in the reference amorphous calcium carbonate (ACC) spectrum (grey). The calcium-rich clots are identified as calcium-carbonate phases based on the presence of strong X-ray absorption at 290.3 eV and 301.5 eV. Their calculated splitting ratios are SRL$_3$ ~ 1.2 and SRL$_2$ ~ 1.3, corresponding to ACC.
Figure 6. STXM image and maps of a centric diatom and associated EPS in a FGL pre-whiting sample (April, 8 m depth). (a) Image at 288.2 eV. (b) Organic carbon map. (c) Calcium map. Calcium is particularly enriched on the EPS material around the diatom, and on the perforations of the frustule (from which EPS are typically exuded).
Figure 7. STXM analyses of *Synechococcus* cells, EPS and calcium-rich grains in a FGL pre-whiting sample (April, 8 m depth). (a) and (b): maps showing the distribution of different components extracted from image stacks. S1, S2: *Synechococcus* cells; L: outer layer of a cell; E: EPS; C1,C2: calcium-rich grains. (c) Corresponding XANES spectra spanning the C K- and Ca L_{2,3}-edges. (d) C K-edge XANES spectra normalized at 320 eV. Vertical lines correspond to energy positions of the main absorption features of different function groups (see main text). (e) Ca L_{2,3}-edge spectra, normalized at 349.2 eV (energy of the Ca L\_3 peak). Vertical lines correspond to the energy positions of the main absorption features of amorphous calcium carbonate (ACC). Both the calcium-rich grains and EPS have spectroscopic signatures consistent with ACC, showing that ACC is nucleating at the surface of *Synechococcus* cells, and as nanophases on EPS.
Figure 8. $I_{Ca}/I_{Ca}$ ratios and splitting ratios (SRL$_3$, SRL$_2$) for different types of samples from the FGL water column and sediment. Ratios calculated from reference spectra for calcite and amorphous calcium carbonate (ACC) are also plotted for comparison. (a) $I_{Ca}/I_{Ca}$ ratios: Pre-whiting *Synechococcus* cells and ACC correspond to the water column sample collected in April at 8 m depth. Whiting calcite, diatoms, and organic matter (OM) correspond to sediment trap samples (July, 13.5 m depth). Note that sediment trap OM $I_{Ca}/I_{Ca}$ values range from ~73 to > 600 (off chart). Sediment calcite, diatoms and OM correspond to sediment core samples. (b) SRL$_3$ versus SRL$_2$ plot for calcium carbonate particles from the pre-whiting water column (April, 8 m depth) (ACC), the sediment trap (whiting calcite) and the sediment core (sediment calcite). Only particles with $I_{Ca}/I_{Ca}$ ratios smaller that 3.3, identified as calcium carbonate minerals, are plotted on this chart.
Figure 9. STXM analyses of settling whiting particles collected in a sediment trap (July 2017, 13.5 m depth). (a-c) Images at 288.2 eV (left) and maps of organic carbon (red) and calcium (cyan) (right). The interior of CaCO₃ grains sometimes appear black on calcium maps due the excessive thickness of the grains, causing saturation of the X-ray absorption signal. The white box in (a) represents the location of the image stack from which spectra shown in (d) were extracted. (d) XANES spectra representative of a CaCO₃ grain, an organic fibril, and a dense organic carbon particle in (a). C K-edge spectra (middle panel): vertical lines correspond to the absorption energies of different functional groups (see main text). Ca L₂,₃-edge spectra (right panel): vertical lines correspond to the position of the main peaks in a reference calcite (Calc.) spectrum (black). Reference Ca L₂,₃-edge XANES spectra for aragonite (Arag.) and amorphous calcium carbonate (ACC) are also shown.
Figure 10. STXM analyses of particles from a FGL sediment core at 0-2 inches (a,b) and 2-4 inches (c) depths below the lake floor. (a-c) Images at 288.2 eV (left) and maps of organic carbon (red) and calcium (cyan) (right). The locations from which XANES spectra shown in (d) were extracted are indicated: c1 (calcite grain), o1-o2 (organic material) and d1-d4 (diatoms). (d) Corresponding XANES spectra. C K-edge spectra (middle panel): vertical lines correspond to the absorption energies of different functional groups (see main text). Right panel: Ca L_{2,3}-edge spectra.
Supporting Information

A re-examination of the mechanism of whiting events: A new role for diatoms in Fayetteville Green Lake (New York, USA)
Figure S1. SEM images of FGL water column particulate samples collected on filters throughout the 2018 whiting season. Collection times (months) and depths are indicated.
**Figure S2.** SEM images of FGL whiting particles collected in a sediment trap (July 2017, 13.5 m depth).
Figure S3. STXM analyses of pre-whiting particulate material from the FGL water column (April, 8 m depth). (a) Image obtained at 288.2 eV and corresponding map showing the distribution of organic carbon (red) and calcium (cyan). (b) Map obtained on the area depicted by a rectangle in (a), showing a *Synnechococcus* cell in red, EPS in blue, and ACC particles (green). (c) XANES spectra representative of the *Synnechococcus* cell (in red), EPS (in blue) and ACC particles (in green). C K-edge spectra (middle panel): vertical lines correspond to the absorption energies of different organic functional groups (see main text) and carbonate groups (at 290.3 eV). Ca L_{2,3}-edge spectra (right panel): vertical lines correspond to the position of the main peaks in amorphous calcium carbonate. XANES spectra acquired on the EPS (blue spectra) show the presence of adsorbed Ca^{2+} but very weak carbonate signal, while strong carbonate and calcium peaks in the green spectra indicate the presence of calcium carbonate grains, identified as ACC based on splitting ratios (SRL_3 ~ 1.2, SRL_2 ~ 1.4).
Figure S4. STXM analyses of settling whiting particles collected in a sediment trap (July, 13.5 m depth). (a-d) Images at 288.2 eV (left) and maps of organic carbon (red) and calcium (cyan) (right). The interior of CaCO$_3$ grains sometimes appear black on calcium maps.
due the excessive thickness of the grains, causing saturation of the X-ray absorption signal. The white boxes represent the locations of the image stacks from which spectra shown in (e) were extracted. (e) XANES spectra representative of CaCO$_3$ grains in (a) and (d), a dense organic carbon particle in (d), organic fibrils in (a), (c) and (d), and a diatom in (a). Note that for the fibril in (c), the XANES spectrum was only acquired at the C K-edge. C K-edge spectra (middle panel): vertical lines correspond to the absorption energies of different functional groups (see main text). Ca L$_{2,3}$-edge spectra (right panel): vertical lines correspond to the position of the main peaks in the reference calcite (Calc.) spectrum (black). Reference Ca L$_{2,3}$-edge XANES spectra for aragonite (Arag.) and amorphous calcium carbonate (ACC) are also shown.
Figure S5. STXM analyses of particles from a FGL sediment core (0-2 inches). (a) Image at 288.2 eV (left) and maps of organic carbon (red) and calcium (cyan) (right). (b) Close-up on the area depicted by a white box in (a). Left: image at 288.2 eV. Right: map showing the distribution of different components extracted from an image stack: a calcite grain (blue), a pennate diatom (green), and an organic filament (red). (c) Corresponding XANES spectra. The vertical lines in the C K-edge spectrum (middle panel) correspond to energy positions of the main absorption features of different function groups (see main text).
Figure S6. STXM analyses of particles from a FGL sediment core (2-4 inches). (a) Image at 288.2 eV (left) and maps of organic carbon (red) and calcium (cyan) (right). The white arrow points to a dense organic-rich particle. (b) XANES spectra representative of different types of objects in (a): a calcite grain, an organic particle, and two different diatoms. The vertical lines in the C K-edge spectrum (middle panel) correspond to energy positions of the main absorption features of different function groups (see main text).