Diel changes in the expression of a marker gene and candidate genes for intracellular amorphous $CaCO_3$ biomineralization in *Microcystis*.

Bruley et al.

This manuscript describes laboratory experiments with a strain of toxic bloom forming cyanobacterium, *M. aeruginosa* PCC 7806 in order to clarify the molecular basis for internal calcium carbonate accumulation. The study focuses on rRNA transcriptomics, particularly that of the expression of *ccyA* gene and its upstream and downstream neighbors in a light-dark incubation. Based on their observations, the authors show a diel expression pattern, correlation with CCMs. They also use Foldseek to assign functions to hypothetical proteins, encoded for by neighboring genes on the same DNA strand. If assumptions are correct, this indicates that specific transporters and specific carbon concentrating mechanisms are activated (note to the authors: you may be aware of this but do not mention in the manuscript that many CCM exist in cyanobacteria; Kupriyanova et al. 2011) when the calcium concentrating gene is also turned on (at night). Although the authors are speculative in their interpretation of some observations, they also provide novel insights based on elegant experiments, especially the description of neighboring gene functions.

This reviewer found a few issues somewhat puzzling:

The observations are clear and interpretations somewhat speculative but persuasive, this reviewer is not convinced that identical general expression patterns of genes would be found in species that do not contain the *ccyA* gene. The absence of a proper control is weakness of this manuscript.

Growth of the strain was carried out in full strength BG-11, something generally prevented in carbonate precipitating experiments due to artefacts cause by e.g., high phosphate concentrations (Rivadeneyra et al. 2006, 2010).

If energy is required for “biomineralization” (lines 79-80), why is the strongest gene expression for *ccyA* observed at the end of the dark period?

Although the Gaëtan et al (2022) shows illustrations of calcium inclusions in some but not all *Microcyctis* spp. obtained from several lakes in France and Spain, it is still not clarified which fraction of a natural bloom has calcium concentrating capabilities. Furthermore, by far the majority of lakes around the globe have calcium concentrations far lower than those found in full-strength BG-11 (Weyhenmeyer et al. 2009).

The authors refer to an earlier paper from the same group that hypothesizes that calcium carbonate (ρ = 2.71 $g.cm^{-3}$) would be part of buoyancy regulation. This do not make much sense, as it would increase the sinking rate, and thus removal from the photic zone. In fact, Gu et al. (2020, 2023) demonstrated that the presence of calcium

(in concentrations below but close to that of full strength BG11) induce massive exopolymeric substance production. There is no report in this manuscript if exopolymers were formed.

Walter et al. (2016) demonstrated a role of calcium in regulating carbonic anhydrase in *Anabaena* sp., perhaps a complicating factor if this exists in the *Microcystis* strain used in this study? On the same topic, but a different issue is the impact of the culture conditions on gene expression. Why did the authors choose 11 hr dark/13 hr light (which, by the way, is not diurnal as stated in line 101, but diel, as correctly stated in the title of the manuscript).

Why would a Na^+-expelling antiporter (ApNhaP) typically found in halotolerant cyanobacteria be of functional importance to Ca^{2+} accumulation?

It was not clear to this reviewer what “normalized counts” used, e.g., in Figs 1 and 7 refer to. Normalized to what? The use of statistics was useful and thorough in this manuscript, especially since some observations seem barely significant (Table 1, the day night transcriptome numbers).

I suggest that the authors use be more specific when making certain statements (e.g., line 22 “many phylogenetically diverse”; lines 46, 66 how “widespread?, 67 “several” – how many exactly; line 83 “ one third of the publicly released genomes” but how many are published?, etc.) and tone down some other statements (the presumed importance in (bio)geochemical cyles; the cell may accumulate but then lyse after a bloom, so it would merely be another transport mechanism; line 63 “massively sequester”; line 66 “ widespread”; line 78 *potential* “environmental importance, etc.). The definition of “biomineralization” in line 46 is ambivalent and weak – directly/indirectly? Induce, produce…?

The authors introduce in line 36, abstract and in later in the text the “CoBaHMA” domain. For those of us who have read the Benzerara 2022 paper may recall the introduction of this abbreviation, but given the importance the authors give to the potential function and thus importance to the calcium concentrating mechanism, the may consider providing a brief explanation.