

Salinity Tolerance in Cyanobacteria: evaluating assumptions in ancestral state reconstructions

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November 24, 2022

Abstract

Oxygen first arose in Earth's atmosphere 2.3 billion years ago, but geochemical evidence suggests that small pockets of oxygen may have arisen earlier than the atmospheric rise in oxygen. Cyanobacteria, a modern phylum of bacteria, are believed to have been the driving force behind the oxygenation of Earth's atmosphere, and there are two basic hypotheses about how they caused this major geologic event. There is a hypothesis, called the 'ecological' hypothesis, that suggests cyanobacteria were unable to live in most environments initially, and thus we see the evidence for pockets of oxygen earlier than the atmospheric rise in oxygen. Specifically, the 'ecological' hypothesis says that cyanobacteria originally were unable to swim and couldn't live in saline water, meaning seawater. However, the data for this only considers two possible states for the levels of salinity: freshwater and seawater. We used data from the literature and from experiments to show that the gradient of salinity matters to the ability of cyanobacteria to live in environments, and that we cannot say what salinity levels a cyanobacteria can tolerate based on where they were found alone. See supplemental file for full abstract.

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Introduction: The irreversible oxygenation of Earth's atmosphere occurred at the Great Oxidation Event (GOE) approximately 2.3 billion years ago. However geochemical evidence for localized oxic conditions exists in the geologic record dating back to 3.0 billion years ago. Cyanobacteria are believed to have been the driving force behind the oxygenation of Earth's atmosphere, and there are two basic hypotheses about how they effected this major geobiological transition.

One hypothesis posits that upon the evolution of oxygenic photosynthesis in ancestral cyanobacteria, cyanobacterial productivity dominated the global ecosystem and drove a geologically instantaneous increase in atmospheric oxygen at the GOE (Shih et al. 2017). As a counterpart to this 'origination' hypothesis, a second, 'ecological' hypothesis posits that ancestral cyanobacteria were physiologically restrained to freshwater benthic habitats until they evolved the ability to thrive in marine environments, thereby reconciling early evidence for localized oxidation with the delayed oxygenation of Earth's atmosphere (Lalonde et al. 2015, Sánchez-Baracaldo et al. 2017).

One key aspect of the 'ecological' hypothesis is that reconstructions of the ancestral habitats of early cyanobacteria imply a strong intolerance to seawater salinity levels (≈ 35 ppt; Sánchez-Baracaldo et al. 2017). These ancestral reconstructions of salinity tolerance in Cyanobacteria have been based on the habitats of isolation of modern cyanobacteria and a consideration of salinity tolerance as a binary discrete cyanobacterial trait (Sánchez-Baracaldo et al. 2017).

Ancestral state reconstructions of this kind rely on three assumptions. The first, and least controversial, is that the trait characters and distributions in the extant Cyanobacteria are derived from ancestral characters and distributions. Next, they assume that the distribution of physiological responses in modern Cyanobacteria reflects the potential limits of adaptive responses in ancestral cyanobacteria. And finally, they assume that cyanobacterial traits, once evolved, remain constant over geologic time (Knoll 2004).

Here we provide a meta-analysis of literature data on cyanobacterial salinity tolerance, supplemented by experimental data on key cyanobacterial strains, to evaluate whether salinity tolerance in Cyanobacteria can be accurately modeled as a discrete binary trait.

Results: Our analysis indicates that salinity tolerance cannot be broken down into two discrete categories: freshwater and marine (Figure 1). Additionally, while habitat of isolation sometimes corresponds to the ultimate expression of salinity tolerance (especially for strains isolated from freshwater environments), more often than not, it is a poor predictor of whether a given cyanobacterial strain can survive over a range of salinities. Our experimental data support these results, and in addition show that some cyanobacterial strains retain a phenotypic plasticity that enables them to acclimate to significant changes in salinity, from freshwater to marine and vice versa, on the timescale of weeks.

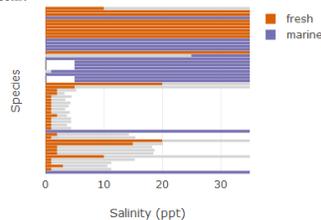


Figure 1: Discrete growth of cyanobacteria across a range of salinities compiled from the literature. Tested ranges are indicated in grey.

Implications: Salinity tolerance is a continuous cyanobacterial trait and should not be treated as a binary discrete one. Recently developed phylogenetic methods allow for a quantitative consideration of the evolution of continuous traits, which when combined with the results reported here, may enable an improved understanding of the original habitats of ancestral cyanobacteria.

References:

- Knoll, A. (2004) *Life on a Lonely Planet: The First Three Billion Years of Evolution on Earth*. Princeton University Press.
- Lalonde, S.V. et al. (2015) *PNAS*, 112.
- Sánchez-Baracaldo, P. et al. (2017) *PNAS*, 114.
- Shih, P.M. et al. (2017) *Geobiology*, 15.

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The Context:

- Cyanobacteria drove Great Oxidation Event (GOE, 2.3 Gya)
- Did they evolve oxygenic photosynthesis just before GOE or long before?
- Some geochemical evidence says benthic lacustrine oxidations before GOE
- Ancestral state reconstructions (ASR) sometimes support freshwater origin which matches this evidence

The Question:

- ASR provides look into the past
- To date ASRs of cyanobacteria have viewed salinity as binary discrete trait
- Results for discrete don't always agree, maybe the answer is in the middle?

The Results:

- Discrete mapping gives fresh ancestor ~75% of runs and marine ancestor ~25% (Fig 1)
- Using simple assignment of optima (Fig 2) versus optima from distributions (Fig 3) yields similar results (estuarine origin)
- The continuous models show that the modern taxa seem to lose influence on the state of the ancestral nodes quickly
- These cases are still simplifying the reality of salinity tolerance to a large degree, in reality, growth can occur across a range of salinities (Fig 4)

References:

Bano and Siddiqui (2004) *Pakistan Journal of Botany* 36 (1).
Batterton and van Baalen (1971) *Archiv fr Mikrobiologie* 76 (2).
Brand (1984) *Estuarine, Coastal and Shelf Science* 18.
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Sánchez-Baracaldo et. al. (2017) *PNAS* 114.
Shih et. al. (2017) *Geobiology* 15.
Uyeda et. al. (2016) *PLoS One* 11 (9).

Figure 1: All-rates-differ model ASR mapping of salinity as a discrete trait based on habitat of isolation. Probabilities of node states shown in pie charts. Tree and character mapping from Uyeda et. al. 2016

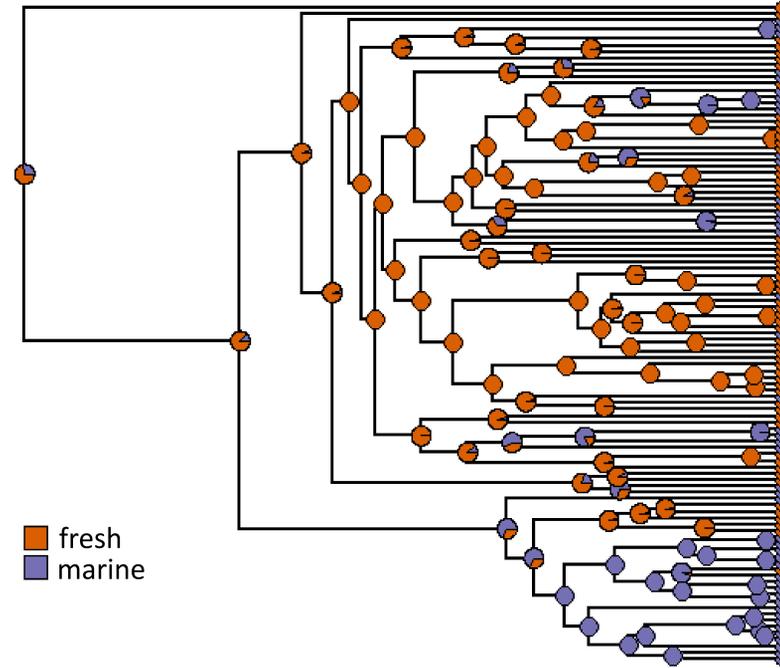


Figure 2: Maximum likelihood ASR of salinity as a continuous trait with optimum salinity of growth determined from habitat of isolation. Tip values assigned as fresh = 0 ppt (blue), marine = 35 ppt (orange).

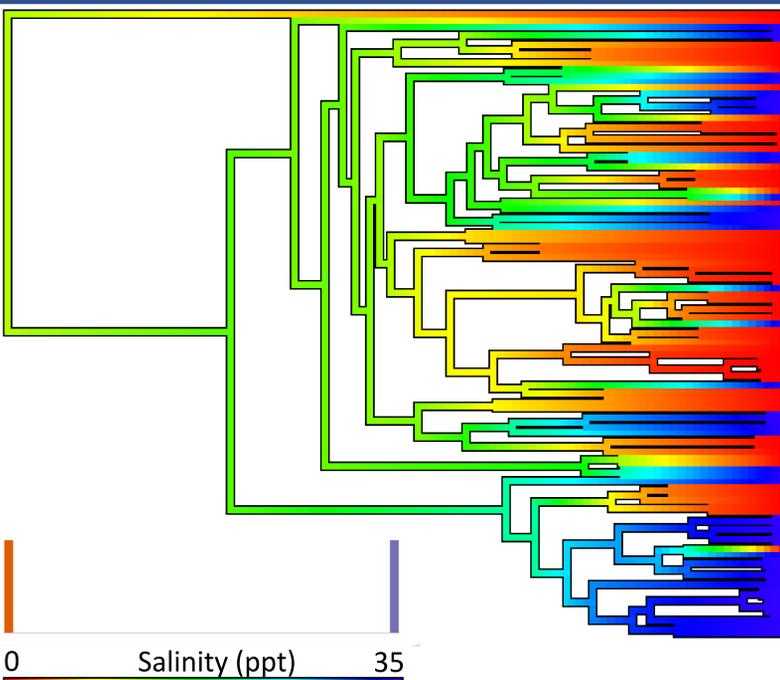
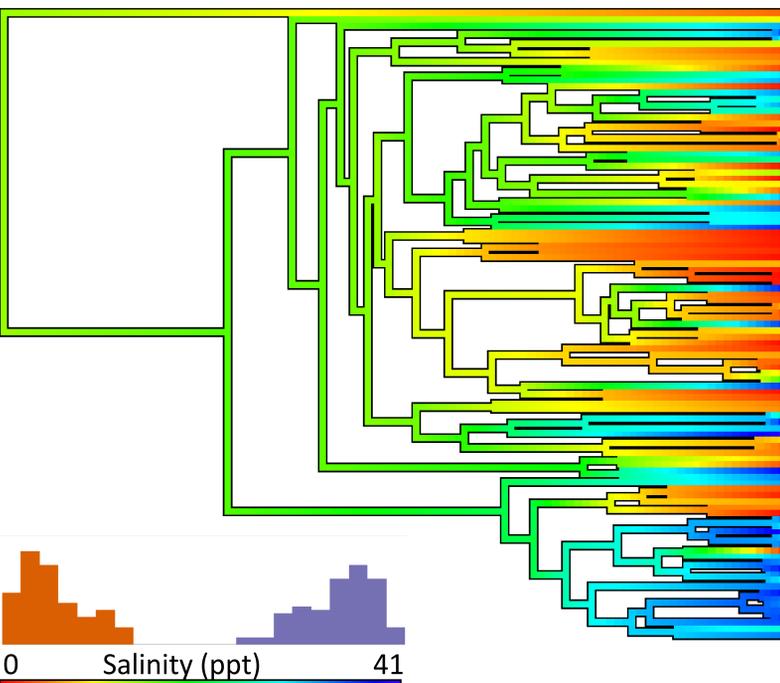


Figure 3: Maximum likelihood ASR of salinity as a continuous trait. Fresh salinities determined from bounded distribution (blue), marine from normal distribution with mean of 35 ppt (orange).



The Takeaway: Switching to continuous models of salinity produces estuarine ancestor. Using continuous models allows for the inclusion of more biological reality in future models.

Future Directions:

- Use known salinity optima for strains
- Use additional parameters from reaction norms
- Investigate influence of reaction norm on adaptation to changes in salinity

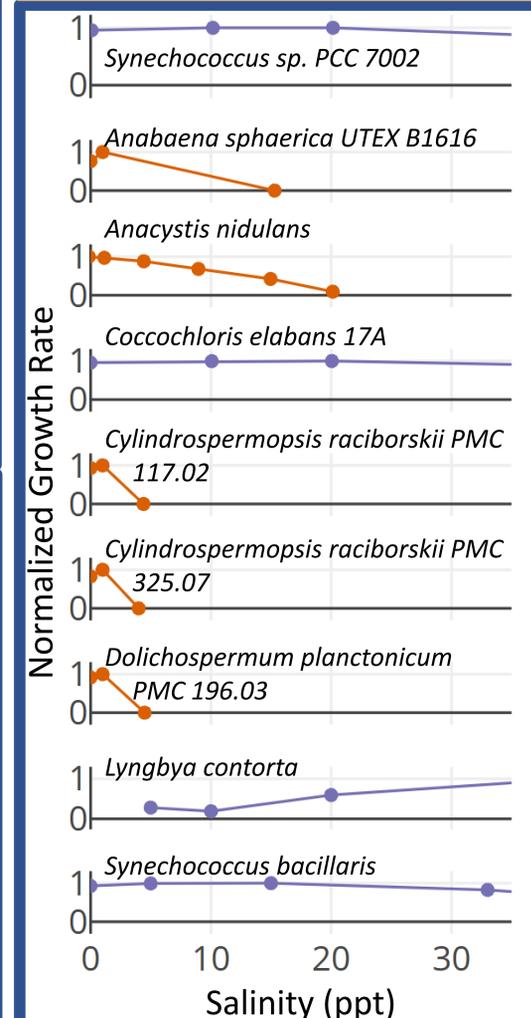


Figure 4: Growth of cyanobacterial strains across freshwater to marine salinity