Corso di Dottorato di Ricerca in Scienze della Vita e dell'Ambiente, Ciclo XXXVIII

Physiological response to sulphur limitation in three marine microalgae: an evolutionary view

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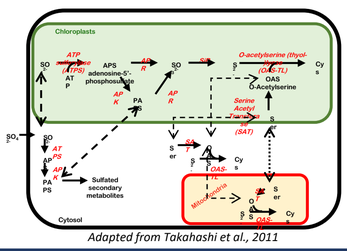
Background

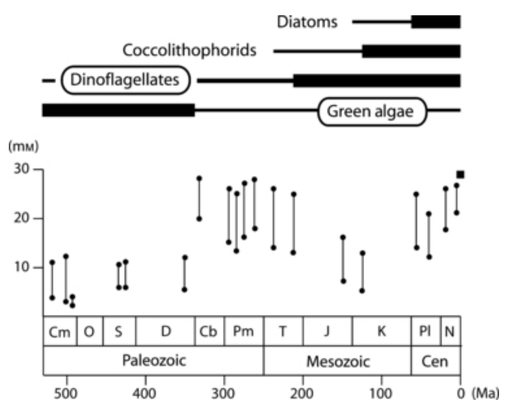
Sulphur (S) is an essential macronutrient for photosynthetic organisms and a key element in multiple cellular processes, including photosynthesis.

How does S limitation influence cell composition and photosynthesis ?



Adapted from Takahashi et al., 2011

Ocean S concentrations and changes in ecological abundances of microalgae



Sulphate concentration and different taxa phytoplankton radiation over geological time. (S. Ratti *et al., 2011)*

Diatoms, Dinoflagellates and Coccolithophorids are the most abundant groups of photosynthetic organisms found in today's oceans, but they're ecological dominance is relatively recent as they radiated only in the Mesozoic supplanting the previous phytoplankton community composed mostly of cyanobacteria and green algae.

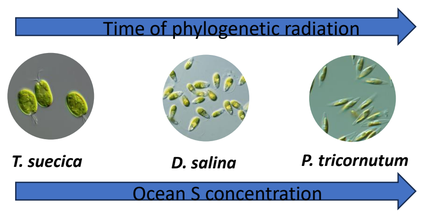
Sulphur facilitation hypothesis

The rise of sulphate concentrations in early Mesozoic oceans may have facilitated the change in ecological abundances (Ratti *et al.,* 2011*)*.

Aim

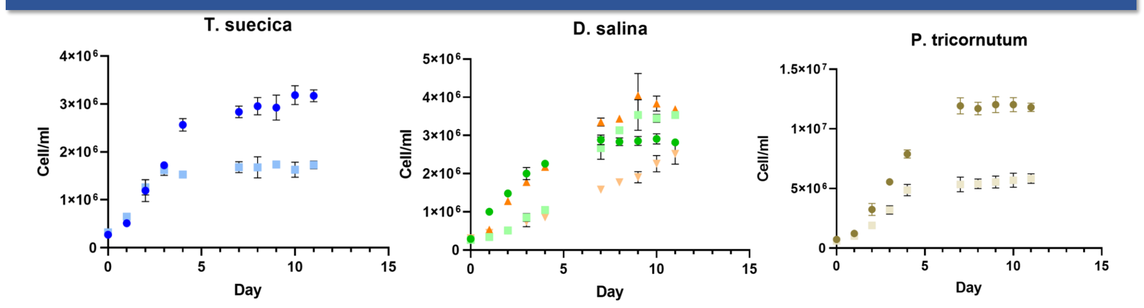
We chose two Chlorophyta species, ***Tetraselmis suecica*** *and* ***Dunaliella salina****,* and the diatom ***Phaeodactylum tricornutum*** based on biotechnological interest and phylogeny.

Effect of S limitation on: → ***Growth*** → ***Cell Composition*** → ***Photosynthesis***



Hypothesis:
Members of Groups that radiated in oceans characterized by Low S concentrations should be able to better adapt to low S availability

Growth

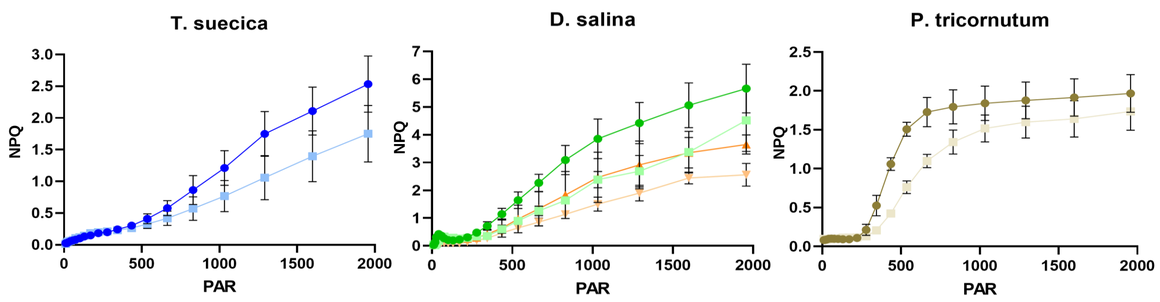


Growth is differently affected in S-limited conditions (S-lim) according to the species: in ***T. suecica*** S-lim does not affect the exponential growth phase but the final cell concentration, in ***D. salina*** growth is slowed down in the exponential phase and in the final cell concentration at high salinity, similarly to ***P. tricornutum***

Cultures were sampled in the mid exponential phase to characterize their photosynthesis and cell composition

Photosynthesis

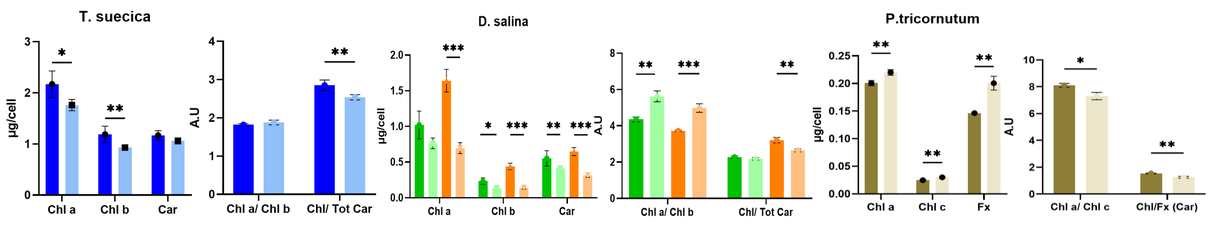
In vivo fluorescence analysis



NPQ: Non-Photochemical Quenching mechanisms dissipate excess energy that would otherwise damage the Photosystems.
Changes in NPQ suggests that changes in Regulation of the Photosynthetic apparatus occurred

T. suecica and ***D. salina*** show **smaller NPQ** amplitude at higher intensities in S-lim conditions. ***P. tricornutum*** shows lower NPQ in the middle range intensities

Pigment quantification

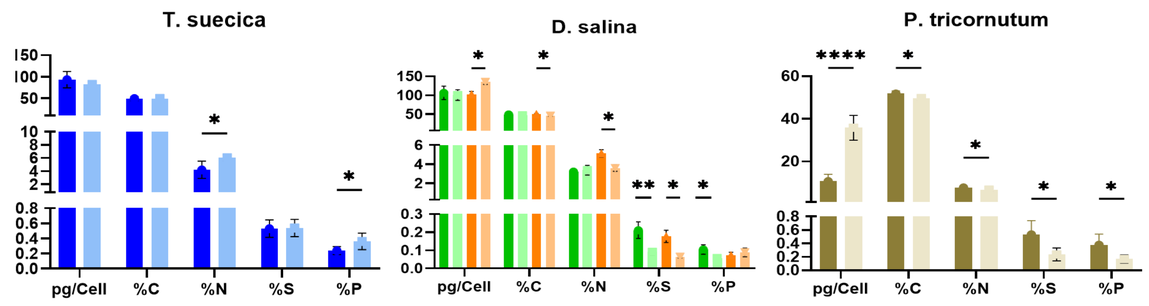


S-lim caused a shift in pigment content. The two green algae reduced the cellular pigments content, while ***P. tricornutum*** in S-lim displayed bigger cells and had slightly more pigments per cell. Changes in pigments ratios also suggest changes in the photosynthetic apparatus were also qualitative.

Carotenoids have roles in **photoprotection** and **ROS scavenging.**
As a trend, all analysed species decreased the **(Chl/Car) (Chl/Fx) ratio.** i.e., they accumulate more car per chl in S-lim

Composition

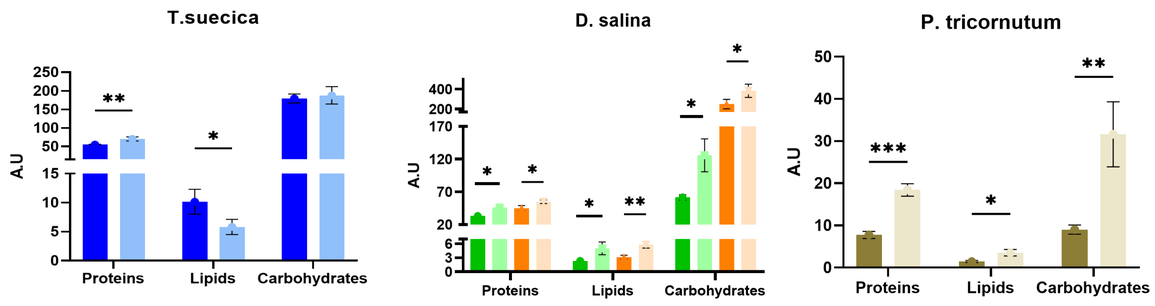
Elemental Composition



Lower cellular S content confirms that the cells are Sulphur limited

In ***D. salina*** grown at 1.5M NaCl and ***P. tricornutum****, S-lim* causes the cells to grow bigger.
P. Tricornutum was the most impacted in composition and **Cell size**

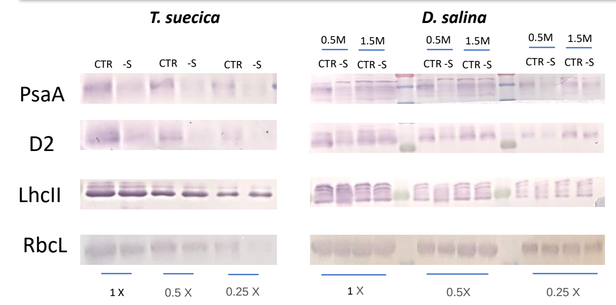
Macromolecular Composition



The cells modify their macromolecular composition in a species-specific manner

T. Suecica appear to be the least affected in macromolecular composition, while S-lim cells in ***P. tricornutum*** accumulate significantly more macromolecules due to their increase size

Immunoblotting



PsaA: Core subunit of Photosystem I

D2: Core subunit of Photosystem II

LhcII: Light harvesting complex of PSII

RbcL: Rubisco Large subunit

Through **immunoblotting** we evaluated in ***T. suecica*** and ***D. salina*** changes in the content of **Proteins** relevant to the photosynthetic process

The Immunoblot confirms that there are **changes in the photosynthetic apparatus,** with a reduction of PS core subunits in S-lim. **RbcL** is evaluated as a proxy for the **C fixation reactions of photosynthesis**

Conclusions

All species were able to **acclimate to the tested conditions** through **resource reallocation** in different macromolecular pools and **photosynthesis modulation** in a species-specific manner. The species response to S-lim were in **accordance with the sulphur facilitation hypothesis**: with ***P. tricornutum*** the member of the **most recently radiated** group as the **most impacted** and ***T. suecica***, member of the **earliest diverging group** as the **least impacted**.