

52. Responses of the Widespread Green Alga *Micromonas* to Elevated CO₂ at Different Levels of Phosphorus Availability

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Project Goals: To develop a system biology approach to the study of the widespread marine alga *Micromonas* and use it to investigate gene function, pathways and consequences of environmental perturbations on primary production.

Marine phytoplankton are responsible for about 50% of global carbon fixation. Changing environmental conditions such as ocean acidification, warming, increased stratification of the water column and reduced supply of nutrients to the upper mixed layer will likely alter phytoplankton physiology and carbon fixation. While increased carbon availability can cause increased rates of carbon fixation, such a response might be counteracted by intensified nutrient limitation. In order to predict changes in biogeochemical cycles a mechanistic understanding of the physiological response of phytoplankton to both individual and combined environmental perturbations is required.

Here, we focus on the response of the photosynthetic picoeukaryote *Micromonas* to the synergistic effects of high CO₂ and diminished phosphate, as would be expected from reduced surface-ocean mixing. *Micromonas*, a broadly distributed unicellular green alga, has already increased in some arctic regions related to climate change. Moreover, it is related to land plants and provides a simplified model system for understanding plant evolution. Here, we performed continuous culturing experiments with *Micromonas* sp. RCC299 under phosphate replete (control), phosphate limited and phosphate re-fed conditions by utilizing a novel culturing system developed at MBARI. Because multi-factorial stresses are closer to real-life scenarios, the combined effect of phosphate availabilities and elevated CO₂ are being explored. Real-time dissolved oxygen, pH, cell size, photosynthetic parameters and growth rate were monitored. Besides these physiological responses, transcriptomic and proteomic responses to different phosphorus and carbon availabilities are being investigated by using stranded RNAseq and high-throughput proteomics. The preliminary results indicate that sustained phosphate limitation decreases growth rates ($p < 0.01$) and results in increased cell size ($p < 0.05$) of *Micromonas*. While CO₂ availability had only minor effects on its physiology, these effects were dependent on the phosphorus availability. Elevated CO₂ caused increasing growth rates only at high phosphorus availability and an increase in cell size only at low phosphorus availability.

The global proteomic approach generates an extensive list of proteins that directly linked to phosphate deprivation responses and phosphate resupply responses. 434 differentially expressed proteins (absolute z-score difference 2) were identified. Among those, 262 proteins were more abundant in P-replete condition and 172 proteins were more abundant in the P-deficient condition. Under phosphorus limited condition, proteins involved in starch metabolism, glycolysis and major CHO metabolism, as well as several other pathways were up-regulated, while proteins involved in protein synthesis, protein modification and targeting, and protein degradation were down-regulated. This indicates a sophisticated response to

phosphorus deficiency that strongly affects cellular carbon metabolism. These joint analyses will allow us understanding the phosphorus and carbon physiology of ecologically important microbial species and permit a better prediction of how marine ecosystems may respond to environmental change.

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