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The Effect of Authentic Sinusoidal Light Variation on Growth and Productivity of Microalgae

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Project Goals: The overarching goal of this project is to understand and manipulate fundamental molecular mechanisms involved in maximizing growth rate and lipid accumulation in diverse classes of microalgae under authentic diurnal conditions to enhance production capabilities for biofuels. The aim is to fill two critical knowledge gaps in microalgal cultivation by addressing fundamental cellular issues that govern growth and fuel molecule accumulation in conjunction with realistic climate-simulated cultivation: 1) The insufficient understanding of the effect of metabolic topology on cellular carbon partitioning and its regulation with regards to productivity, 2) the understudied effect of the diurnal and cell cycles on cellular metabolism, resource allocation, and resource mobilization under simulated production conditions.

Our team has focused on a comparative analysis of metabolic pathway topology in two classes of microalgae, diatoms and chlorophytes, as well as addressed the disconnect between laboratory cultivation and outdoor cultivation under sinusoidally-varying light intensity conditions. Microalgal performance under laboratory conditions commonly does not correlate with outdoor productivity because outdoor sinusoidal light intensity variation and diurnal temperature changes are not mimicked. Microalgae must accumulate defined amounts of energy, energy-storage compounds, and biochemical constituents to replicate. *Typically, cells are not continuously "growing" throughout the day, but they alternate periods of enlargement (G1 cell cycle phase) and division (S and G2+M phases).* Microalgal cultures commonly synchronize their division processes, dividing once or twice per day at specific times. We monitored gene transcript changes during synchronized cell cycle progression in the diatom *Thalassiosira pseudonana*, and identified cell cycle stage-specific dependence on nutrients. Energetic, nutrient, and metabolic processes vary throughout the cell cycle, and understanding the relationship between these parameters and times of day should have a significant impact on productivity, providing, for instance, insight into optimal times for addition of nutrients and harvesting.

We evaluated the response of the diatom *Cyclotella cryptica* to sinusoidal variation of daylight intensity and temperature on a 12:12 l:d cycle. Cell cycle synchronization occurred, with cells undergoing the division process in S and G2+M phases for a substantial portion of the day. There was no strict correlation between OD750, typically used as a proxy for biomass, and cell number. Small changes in OD750 occurred at night, suggesting minimal respiratory losses. Timing of chloroplast division and changes in cell volume related to the growth and division process. Variation in triacylglycerol (TAG) levels suggested accumulation prior to a maximal period of membrane synthesis, then additional accumulation of a pool prior to nighttime. Because productivity is affected by photosynthetic performance, we examined the photoadaptive response by tracking changes in photosynthetic and photoprotective pigment levels. Continuing investigation should lead to a fuller understanding of the effect of diurnal conditions and synchronized cell cycle progression on growth and productivity.