Concentrating on Carbon Concentration in Algae

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PRECS Phenotypic Plasticity Research Experience for Community College Students

The Question at Hand

Does *Ostreococcus tauri* have a carbon concentrating mechanism?

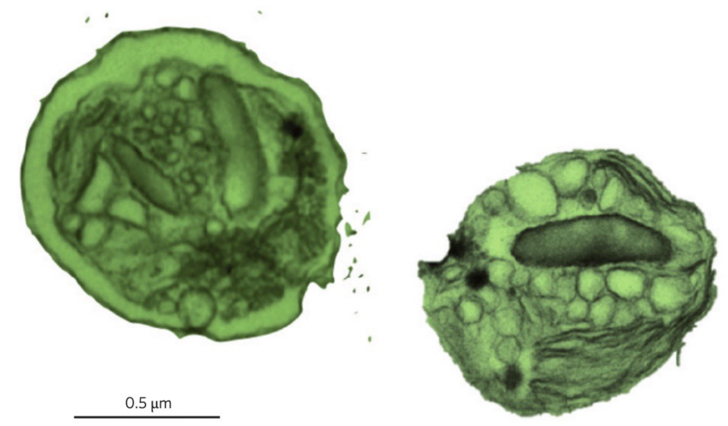

Figure 1: Ostreococcus tauri image provided by Elisa Schaum, University of Edinburgh, to David Hutchins [1]

Carbon Concentrating Mechanisms Improve Photosynthesis Rates in Low-Carbon Environments [2]

CCMs allow the aggregation of carbon near the site of rubisco [2], that way even small amounts of available carbon are being utilized.

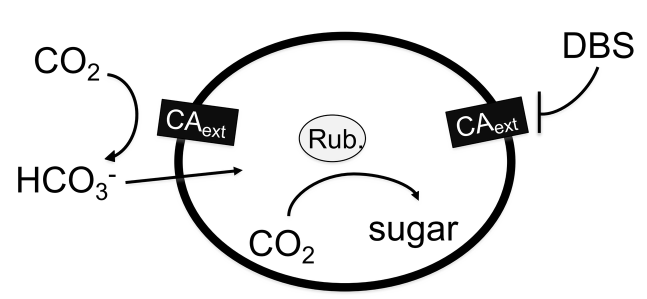

Figure 2: extracellular carbonic anhydrase, a CCM found in Micromonas pusilla, which happens to be inhibited by dextra-bound sulfonamide [3]

Yes, this is important to you!

Albeit somewhat indirectly, but massively nonetheless! Algae are a driving force behind the global carbon cycle [2], they sequester CO_2 in the oceans [4]. Understanding the mechanisms behind the tiny marine alga *O. tauri* gives us a better understanding of a vital global process.

Determine the Presence of a CCM in *O. tauri* Following These Steps:

1. **Determine what light intensity *O. tauri* best photosynthesizes at**
2. **Determine the affinity of *O. tauri* for bicarbonate**
3. **Make a light curve and bicarbonate curve for two *Chlamydomonas reinhardtii* strains**
4. **Compare the *O. tauri* bicarb curve to *C. reinhardtii's* bicarb curves to determine if it resembles that of a CCM-possessing organism or not**

Making a Light Curve

Step 1: Grow Healthy *O. tauri* Cultures

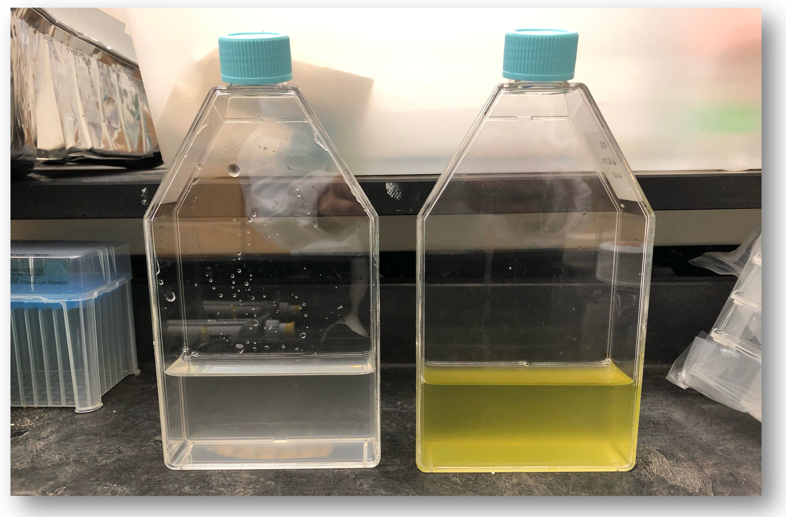

Figure 3: Healthy (right) and unhealthy (left) cultures of O. tauri. There was some difficulty with spontaneous culture crash, and later a contaminant resurfaced, eventually infecting all cultures.

Step 2: Measure Oxygen Evolution

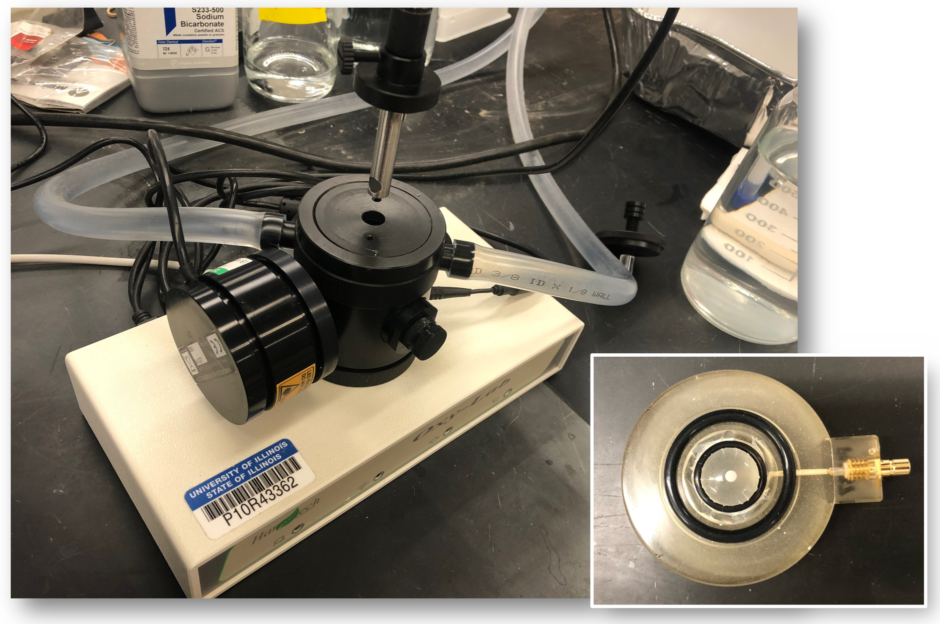

Figure 4: Fully assembled oxygen electrode chamber (left) and the electrode disc itself (right).

Step 3: Struggle to Get a Light Curve

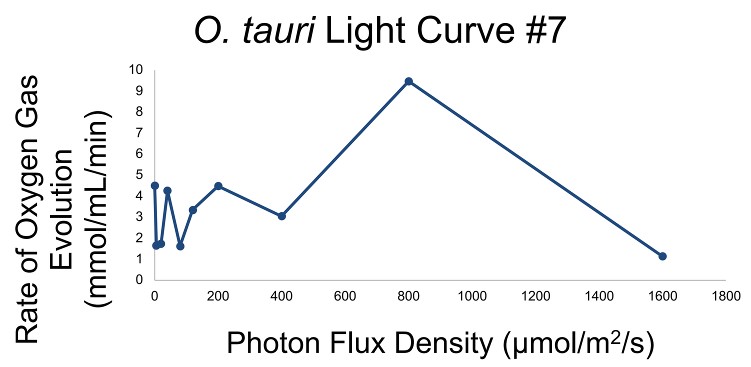

Figure 5a: my 7th attempt at a light curve, which did not result in much of a curve. The main error was using an incorrectly sized o-ring when setting up the electrode. Other conditions included spinning down 25mL of cells to 1.5mL, then adding 900µL of the concentrated cells into the electrode chamber with 100µL of 0.1M sodium bicarbonate solution.

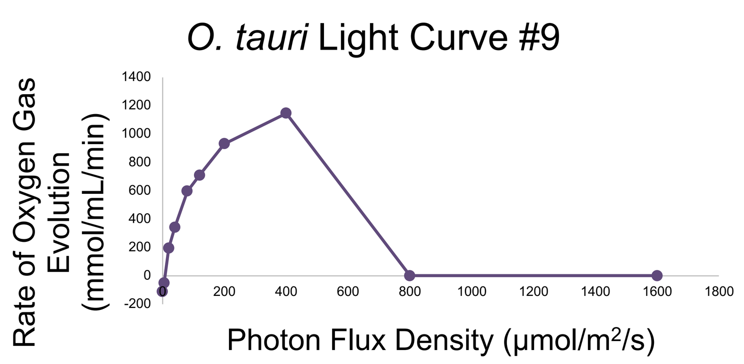

Figure 5b: my 9th light curve attempt, which only required one more adjustment before getting an acceptable one. This was done by spinning down 50mL of cells to 1 mL, then adding 900µL of the concentrated cells into the electrode chamber with 100µL of 0.5M sodium bicarbonate solution. The result was a max at 400µmol of light followed by an abrupt crash.

Step 4: Measure Chlorophyll

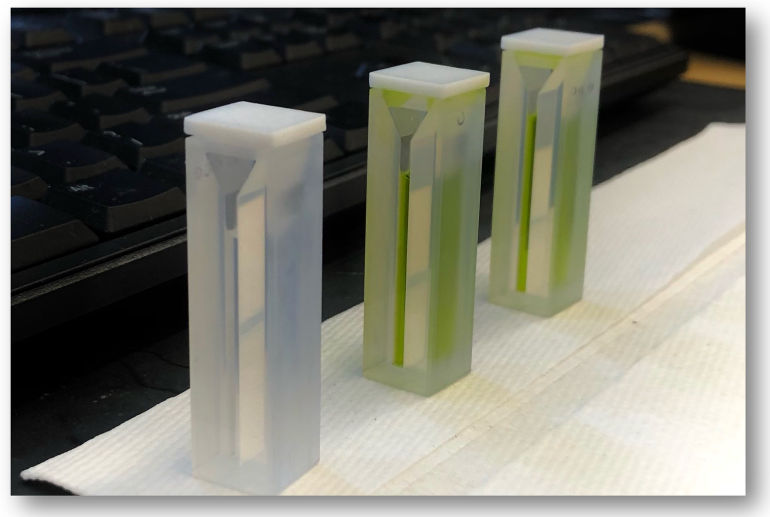

Figure 6: Using spectrophotometry on the sample used in a light curve allows oxygen evolution to be put into the context of chlorophyll concentration.

Step 5: Replicate an Acceptable Curve

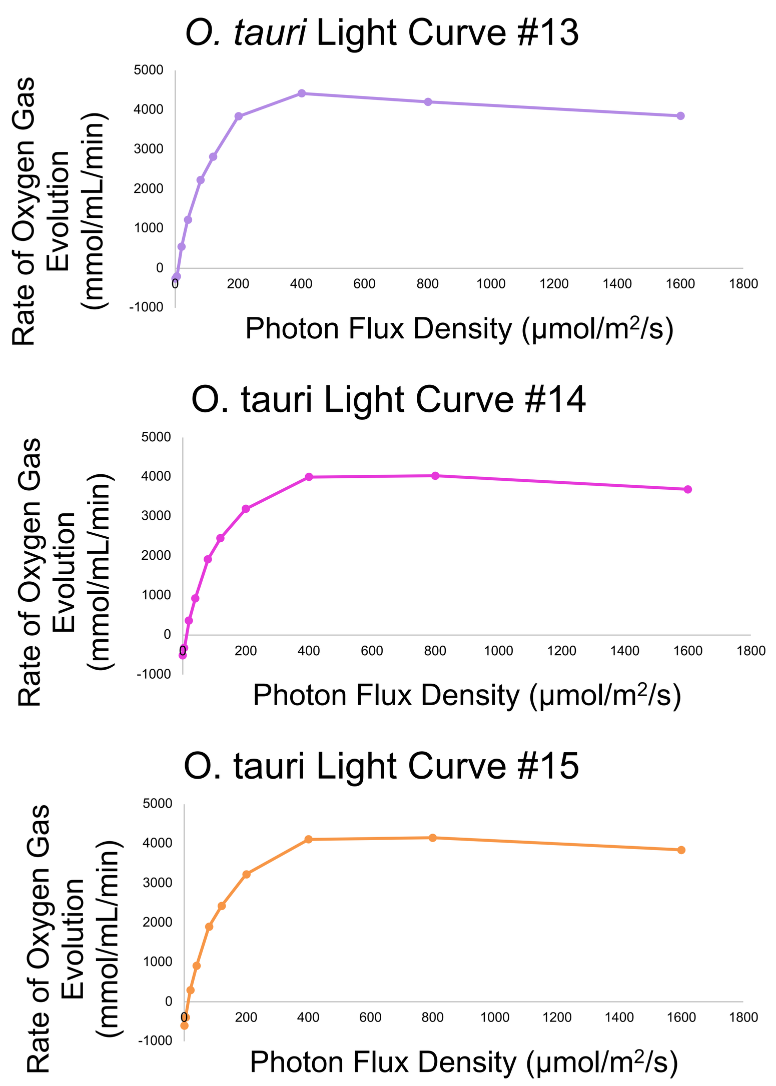

Figure 7: light curves 13 through 15, successful replicates. Spinning down 25mL of cells to 1mL solved the problem of maxing out at 400µmols of light from the last round. All other conditions mentioned in figure 4 remained the same.

Step 6: Use Excel Wizardry

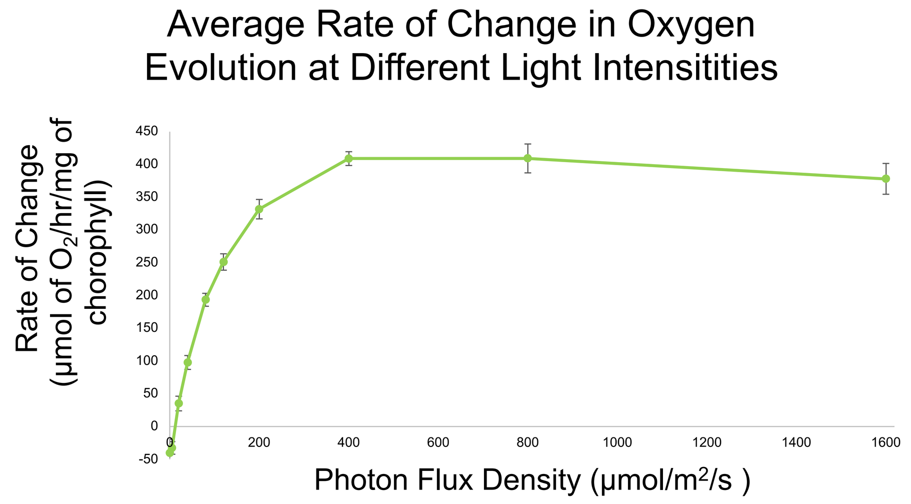

Figure 8: The relationship between oxygen gas evolution and photon flux density. Graph is composed of three technical replicates with error bars that represent positive and negative standard deviation. Reaction conditions were a 25℃ water bath, 10% bicarb concentration (v/v) and an average of 17.09µL of chlorophyll per mg of sample.

Conclusion

O. tauri best photosynthesizes between 400µmol and 800µmol of light.

What's Next?

- Run a test bicarb curve at 400µmol, 600µmol, and 800µmol of light, then conduct three more bicarb curves at whichever intensity yields the highest oxygen gas production
- If there is a CCM, determine what the molecular mechanisms are behind it by experimenting with inhibitors of different parts of known CCMs

References

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Acknowledgments

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