**Single Turnover Variable Chlorophyll Fluorescence**

* Use a single-turnover variable chlorophyll fluorescence (ST-ChlF) approach to evaluate the loss of synchronization of the s-state cycle1
* Acclimate cells to the dark and monitor their fluorescence as they’re exposed to a series of short, high-intensity flashes:
  + Begin with diatoms in a dark regulated state for at least 60 seconds. In the dark, we can assume:
    - All RCII are open (electrons have been passed further down the pathway)
    - Any of the non-photochemical quenching processes have been fully reversed, so heat loss is minimal
    - Therefore, the maximum amount of energy absorbed will be partitioned to photochemistry, so we get minimum fluorescence
  + Deliver a series of short, high-intensity flashes using the single-turnover technique
    - Delivers a rapid series of sub-saturating flashes on the order of microseconds
    - As PSIIs absorb light, electrons are passed downstream onto QA, which effectively reduces the pool of available electron acceptors
    - Therefore, the photochemistry pathway can’t accept any more energy so the fluorescence yield reaches a maximum value
    - At this time, we get maximum fluorescence
* Each flash delivers one photon to each RCII and corresponds to one step in the cycle
* Every 4 flashes correspond to one s-state cycle (in ideal system with no recombination)
* Can derive derive Fv/Fm, which is the maximum quantum yield of photochemistry in PSII
  + In other words, the maximum fraction of light energy that can be partitioned to the photochemistry pathway under the given environmental conditions
    - Or maximum amount of energy that can be productively extracted by absorption of one photon

A recombination event = 1 PSII slipping in their s-state cycling

* A given sample has countless PSII
  + As more and more fall out of sync, the overall fluorescence signal is scrambled since they’re all in different s-states (no more 4 step oscillation)2

**Spectral Analysis**3

* Fast Fourier Transform
  + Extract frequency data in the form of a wave function4
* Should get a wave with a wavelength of 4 flashes, and an amplitude that decreases as more and more individual PII fall out of sync
* See how long the waves have a significant amplitude = how long the overall sample is remaining in sync

**Temperature**

* Alter temperature using temp-controlled cuvette (diagram)
* Temperatures: range between 0 & 22 C
  + Expect low-temperature suppression of wasteful paths
  + Recombination is temp-sensitive5
    - Less occurrences at lower temps, seen by more synchronous cycles

**Interval**

* Changing the frequency of photon delivery: 1, 2, 4, 8, 16s
  + Analogous to different light levels
  + As flash spacing increases, there is more competition between recombination and electron transport
    - Expect fewer synchronous cycles

**Psychrophilic & Temperate Taxa**

* Comparing these across taxa under common conditions lets us see how/if psychrophilic taxa have evolved less wasteful (more efficient) photosynthesis to cope with the low light at the poles

References

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