Sampling/Counting
Flow cytometry

Sample stream with cells

Stream separates into droplets containing cells that can be sorted

Lasers

Nozzle

Dichroic mirrors

Detectors

Filters

Electronic processing

Dot plot
1 cell = 1 dot

Detector pulse

Peak height

Area

Time

Green fluorescence intensity

Red fluorescence intensity
Next-generation Imaging Flow Cytometry

New instruments, such as the Amnis system, combine flow cytometry with imaging—you get the advantage of having a microscope-like image combined with lasers, counting every particle, etc…
Returning to our box model—we’ve been describing **who** and **how much biomass** is in our “P” box. Now we want to talk about how fast that box is changing....
Phytoplankton Photosynthesis

- RedOx Reactions
- Some more history
- Quantum Yields
- Photosynthetic Units
- Physical Structure
- The Z-Scheme
- The Calvin-Benson Cycle
- Measuring Photosynthesis
• Every pigment has a characteristic Absorption Spectrum
  • Optimal $\lambda$
A Brief History….

• Otto von Warburg, 1920’s

– Insisted that the quantum yield of oxygen evolution (the number of oxygen molecules evolved per photon, or quanta, absorbed) was about 0.25

QUANTUM YIELD: the amount of something produced for a given number of quanta (light photons) absorbed. A yield 0.25 means 4 photons are absorbed for every oxygen molecule produced (1 O₂ for 4 photons, or ¼)
REVIEW:

- Once a cell absorbs sunlight, there are only three possibilities:
  1) Fluorescence
  2) Heat
  3) Photosynthesis

So in our new terms, the QUANTUM YIELD of photosynthesis goes up as the quantum yield of fluorescence goes down (if energy is going to #3, then less energy goes to #1 and #2, and the yield has to decrease)
Emerson and Arnold, 1932, described the PSU. In contrast to von Warburg, their data showed it takes at least 8 photons to produce oxygen.
Emerson and Arnold, 1932, determined that the functional unit of photosynthesis could be called a Photosynthetic Unit, and was made up of about 2400 pigment molecules.

This is the same as our “funnel” that captures light and funnels it down to a bottleneck—if the funnel is blocked, have to produce heat or fluorescence.
Emerson Red-Drop Effect

- 1952, Emerson determined that photosynthesis is maximal when blue AND red light is available.
Photosynthetic Unit (PSU)

• Based on these experiments, determined that:
  – Oxygen is produced by plants using absorbed sunlight—von Warburg said it was a ratio of 1:4 (1 oxygen for 4 photons)
  – It takes about 2400 pigment molecules working as a unit to do this
  – Emerson and Arnold said it’s really a 1:8 ratio
  – Emerson demonstrated that you get MORE oxygen when using two different wavelengths of light
Photosynthetic Unit (PSU)

- To explain these results, Emerson determined that the PSU is made up of 3 distinct units:
  - The ANTENNA (the ~2400 chlorophyll molecules that make up our funnel—in reality this is chlorophyll a plus other pigments)
  - The REACTION CENTER where the electrons are funneled down into…this is called Photosystem II (PSII)
  - Another REACTION CENTER that responds to far-red light; this is called Photosystem I (PSI)
  - The whole complex works together to make up the PSU

Side note: oxygenic photosynthesis requires all of these pieces—anaerobic bacteria can still photosynthesize but don’t produce oxygen because they only have PSI
Growth on CO$_2$ and the Macronutrients N and P

It is convenient (and often necessary) to consider the growth and decomposition of an “average” phytoplankter. Redfield showed strong relationships between elements that were consistent with the growth and decomposition of phytoplankton:

$$\text{C:N:P} \sim 106:16:1 - \text{Termed the Redfield Ratios}$$

$$106 \text{CO}_2 + 122 \text{H}_2\text{O} + 16 \text{HNO}_3 + \text{H}_3\text{PO}_4 \rightarrow (\text{CH}_2\text{O})_{106} + (\text{NH}_3)_{16} + \text{H}_3\text{PO}_4 + 138 \text{O}_2$$

This suggests that there is a cellular “currency” related to photosynthesis (growth) and that we can measure ANY ONE variable to get the other variables....
The Z-Scheme...
The Mn acts as a capacitor, storing up energy from 4 photons and using that to split water—but this only occurs at PSII.
...embedded in a membrane.
Z-Scheme membranes

Stromal side of thylakoid membranes

Photon $\rightarrow$ Chl* $\rightarrow$ PQ $\rightarrow$ I $\rightarrow$ Cyt $\rightarrow$ PC $\rightarrow$ Chl* $\rightarrow$ NADP$^+$ $\rightarrow$ NADPH

Thylakoid Interior

$\frac{1}{2} \text{H}_2\text{O} \rightarrow \text{O}_2$ $\rightarrow$ H$^+$ $\rightarrow$ H$^+$ $\rightarrow$ H$^+$ $\rightarrow$ H$^+$ $\rightarrow$ H$^+$ $\rightarrow$ H$^+$

Thylakoid becomes acidic
Non-Cyclic Photophosphorilation (Z-Scheme)
Cyclic Photophosphorylation

- Only PS I used
- NO O2 formed
- Produces ATP
Dark vs. Light Reactions

**Light Reactions**: Require light energy to run

**Dark Reactions**: technically don’t require light, but they stop very quickly in the dark
Measuring Photosynthesis

If you understand the biochemical basis for photosynthesis (the Z-scheme, the relationship between heat, light, fluorescence, oxygen production, and carbon consumption), then there are a number of ways to estimate growth (photosynthesis)....
Measuring Photosynthesis

- Change in biomass
- Change in $\text{H}_2\text{O}, \text{O}_2$
- Change in $\text{CO}_2$
- Production of Heat
- Production of Fluorescence
Measuring Productivity

• Oxygen bottles measure Gross and Net Production

• $^{14}$C measures something between gross and net production
# Photosynthesis Measurements

<table>
<thead>
<tr>
<th>Method</th>
<th>PROS</th>
<th>CONS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>14C</strong></td>
<td>• Easy to use</td>
<td>• Requires a bottle</td>
</tr>
<tr>
<td></td>
<td>• Tracer method</td>
<td>• Deck vs. In situ?</td>
</tr>
<tr>
<td></td>
<td>• Measures carbon</td>
<td>• Radioactive!</td>
</tr>
<tr>
<td></td>
<td>• Cheap</td>
<td>• Neither gross nor net productivity</td>
</tr>
<tr>
<td></td>
<td>• Very Sensitive</td>
<td></td>
</tr>
<tr>
<td><strong>Oxygen</strong></td>
<td>• Measures GPP</td>
<td>• Not easy to do</td>
</tr>
<tr>
<td></td>
<td>• Not complicated</td>
<td>• Not very sensitive</td>
</tr>
<tr>
<td></td>
<td>• Instantaneous</td>
<td>• BIG assumptions</td>
</tr>
<tr>
<td><strong>Fluorescence</strong></td>
<td>• No bottle</td>
<td>• Instrumentation</td>
</tr>
<tr>
<td></td>
<td>• First principles</td>
<td></td>
</tr>
</tbody>
</table>
## Photosynthesis Measurements

<table>
<thead>
<tr>
<th>Method</th>
<th>PROS</th>
<th>CONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-Oxygen</td>
<td>• VERY sensitive</td>
<td>• Requires a bottle</td>
</tr>
<tr>
<td></td>
<td>• GPP, NPP, R</td>
<td>• Very Expensive, hard to do</td>
</tr>
<tr>
<td></td>
<td>• Very Sensitive</td>
<td></td>
</tr>
<tr>
<td>Heat</td>
<td>• Really cool!</td>
<td>• Lab only</td>
</tr>
<tr>
<td></td>
<td>• Non-invasive</td>
<td>• VERY difficult!</td>
</tr>
<tr>
<td></td>
<td>• First principles</td>
<td>• Instrumentation</td>
</tr>
<tr>
<td></td>
<td>• Incredibly simple</td>
<td>• Space, Time-scale dependent</td>
</tr>
<tr>
<td>Biomass</td>
<td>• Community NPP</td>
<td>• Not a rate!</td>
</tr>
</tbody>
</table>
Now that we can measure photosynthesis, how do we turn that into equations that can be used in our model?
Photosynthesis versus Irradiance Curves
Photosynthesis versus Irradiance Curves

\[ P_{\text{max}} \]

\[ E_k \]

\[ \alpha \]

\[ \beta \]
For a Photosynthesis versus Irradiance curve (PvsE), Ek is the special point where the cell goes from LIGHT LIMITATION to BIOCHEMISTRY LIMITATION… at first, as you increase the light, photosynthesis increases linearly (more light = more oxygen). At some point, you can’t push electrons through the Z-scheme any faster (the funnel is backing up) and the curve flattens out because the Calvin-Benson Cycle can’t go any faster…
Phytoplankton **adapt** to the light environment by changing the amount and type of pigment per cell, which changes the light reactions (the dark reactions are controlled primarily by cell size, nutrients, and temperature)
RED is low-light adapted
BLUE is high-light adapted

Under low light, it is advantageous to maximize light absorption (steep initial slope) but it's a waste of energy to build up a big "back end" capacity so you saturate quickly

(upper panel—normalized to the amount of chlorophyll—red line has more chlorophyll; lower panel—how fast the cells are actually growing)
Summary

• There are many ways to measure photosynthesis, but in all cases we are simply tracking how fast the cell processes sunlight into organic material

• For modeling purposes, it is convenient to describe the processes using P vs. E curves

• Low-light adapted cells (plants) have more pigments, and sacrifice total capacity (dark reactions) for sensitivity to low light

• High-light adapted cells have fewer pigments and more dark reaction components, so don’t do well at low light can handle more photons (high light) by not letting the “funnel” back up

• Nutrient limitation primarily impacts the dark reactions—can’t use all the light energy, so you back up the funnel