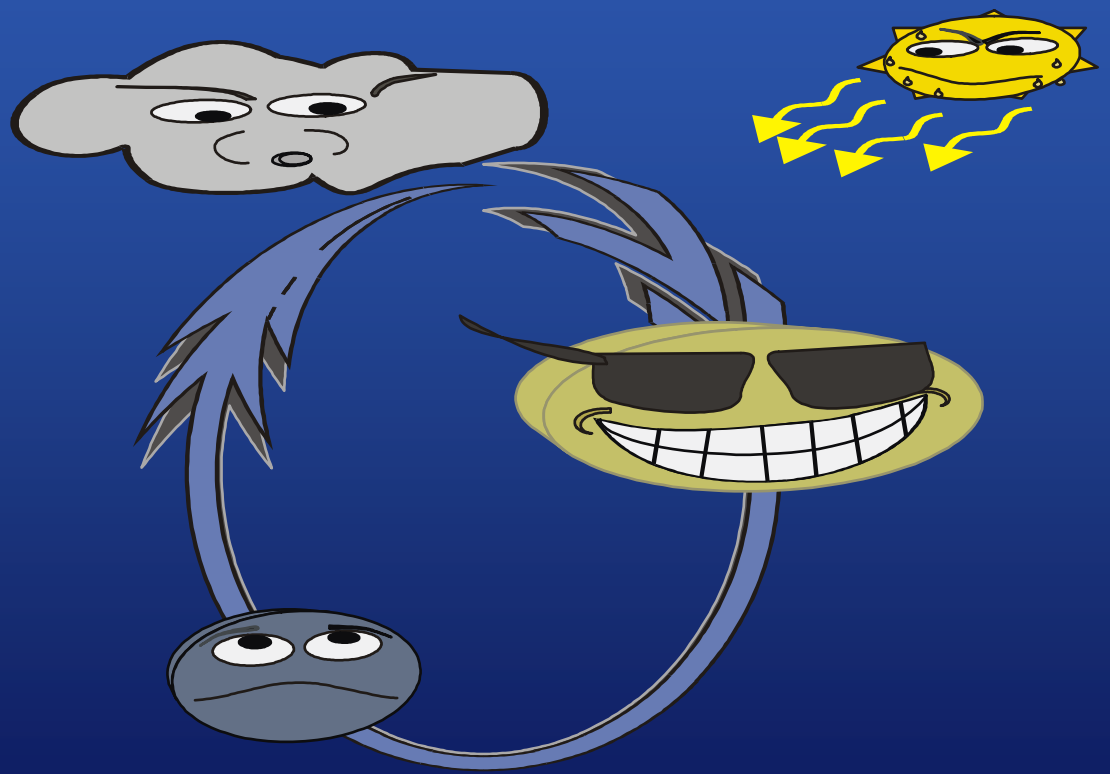
Photoacclimation of the diatom *Asterionella formosa* in a simulated vertically mixed water column



John Zastrow

Photoacclimation

- ***Changes in photosynthetic apparatus which result in cells being better able to utilize their light climate***
- **Response to changes in light intensity and quality (spectral distribution)**
- **Distinct from diel cycle**
- **Occurs within the lifespan of a single cell (is not adaptation)**
- **Changes in morphology and biochemical composition varies with algal group**

Strategies of acclimation

- Change number & density of thylakoid membranes, adjust amount and ratio of photopigments, move chloroplasts
- Increase size of photosynthetic units (PSUs) embedded in thylakoid membrane, eg. Add chlorophyll *a* molecules (increases efficiency, but not capacity of photosynthesis)
- And/or increase number of PSUs eg, adds chlorophyll *a* and electron transport capacity (can increase efficiency, but primarily raises capacity)

Responses to light intensity

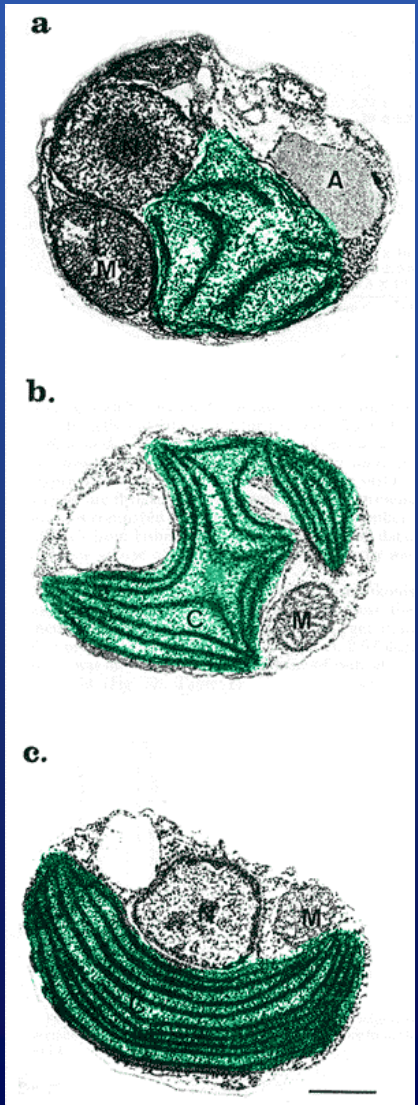
Increasing light

- Decrease chl a
- Decrease photon capture efficiency per unit chl a (α^B)
- Increase photon capture capacity (P^B_{max})
- slower

Decreasing light

- Increase chl a
- Increase photon capture efficiency per unit chl a (α^B)
- Decrease photon capture capacity (P^B_{max})
- faster

Microscopic analysis



High light acclimated

40 hours after transition

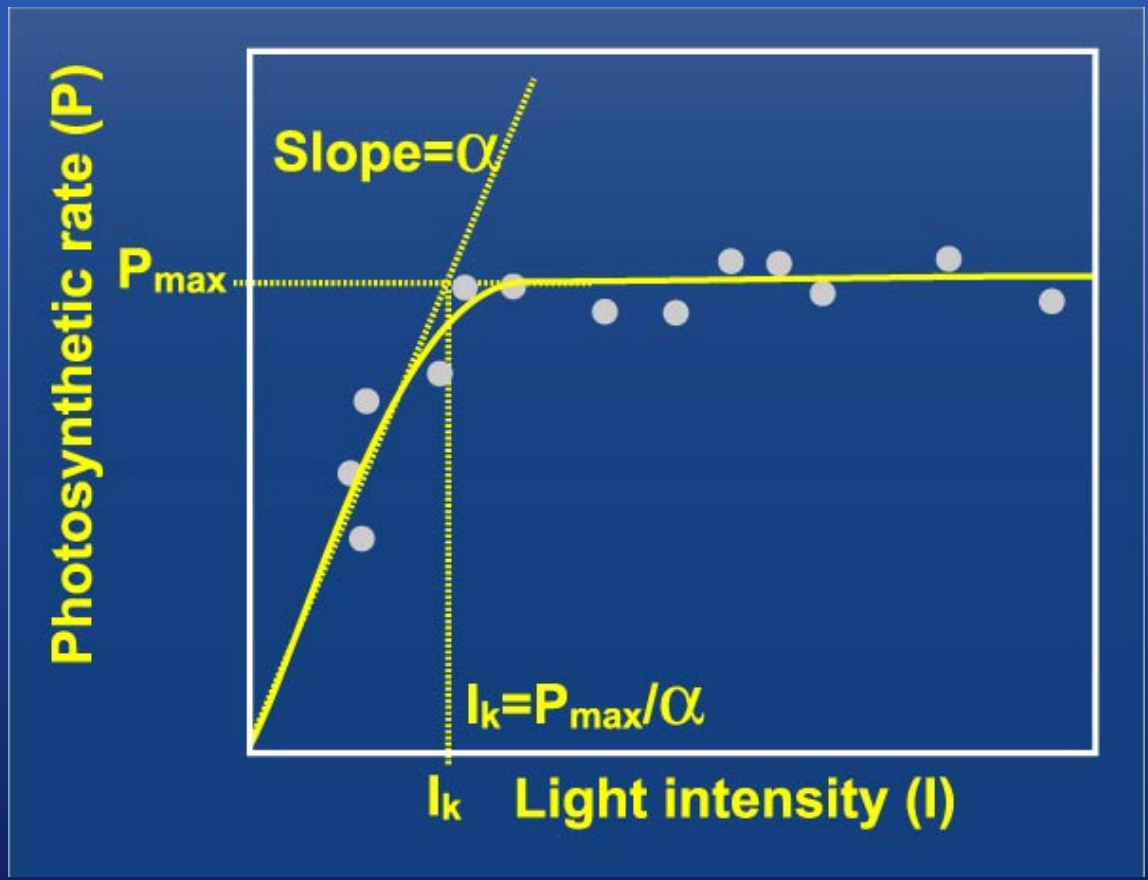
Low light acclimated

- Changes in chloroplast after transition from high light to low light in *Nannochloropsis sp.,* a marine *Chlorella*-like cell
- Increase in the number of thylakoid membranes after 40 hours
- Complete acclimation state shows increased thylakoid density with more PSUs in the membranes than high light cells

From Fisher, et al. 1998

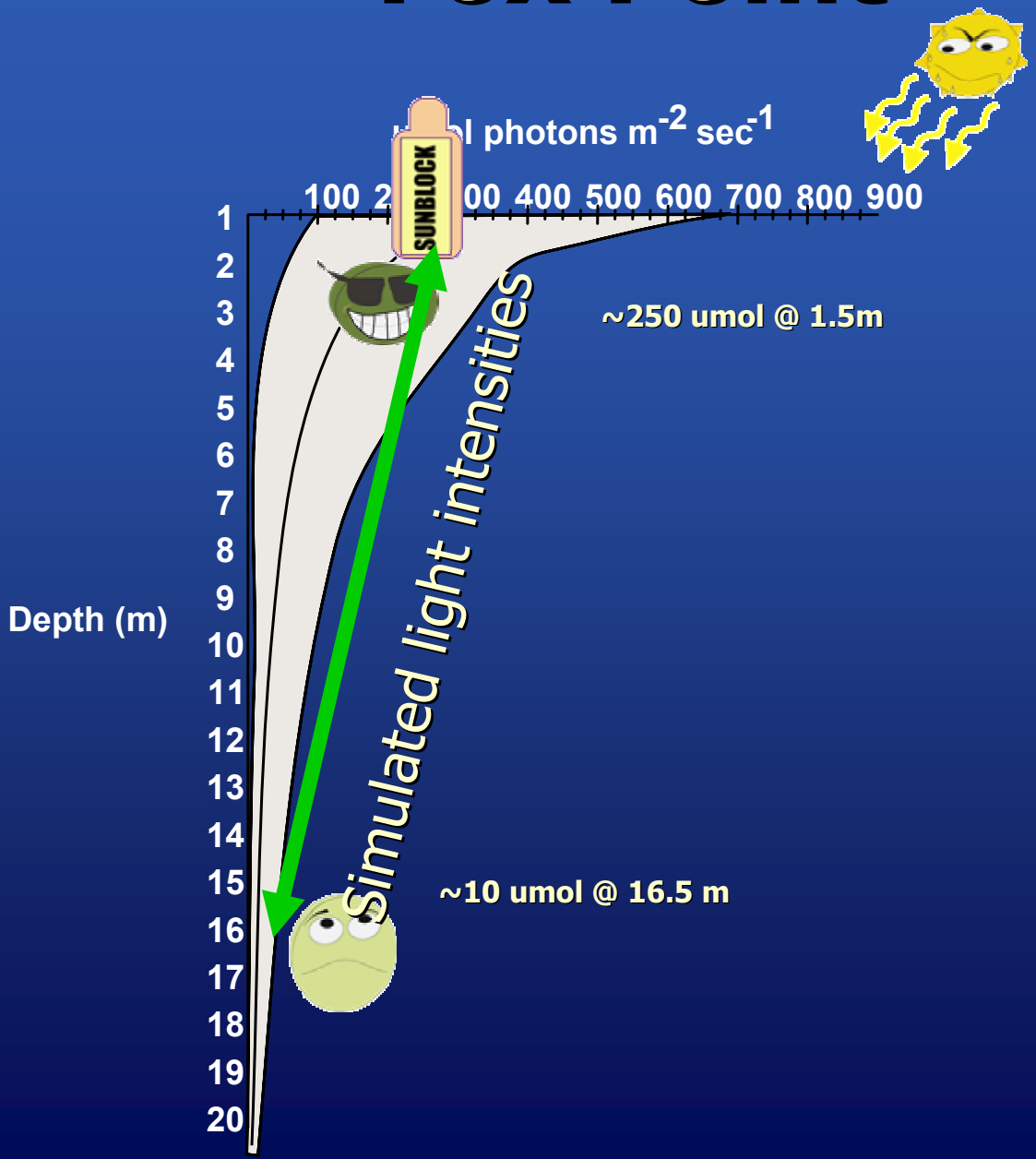
Photosynthesis v irradiance

- **P measured by net reduction (fixation) of $^{14}CO_3$**
- **Higher α = better able to use low light**
- **Higher P_{max} = able to use more photons**
- **Higher I_k = acclimated to use high light**



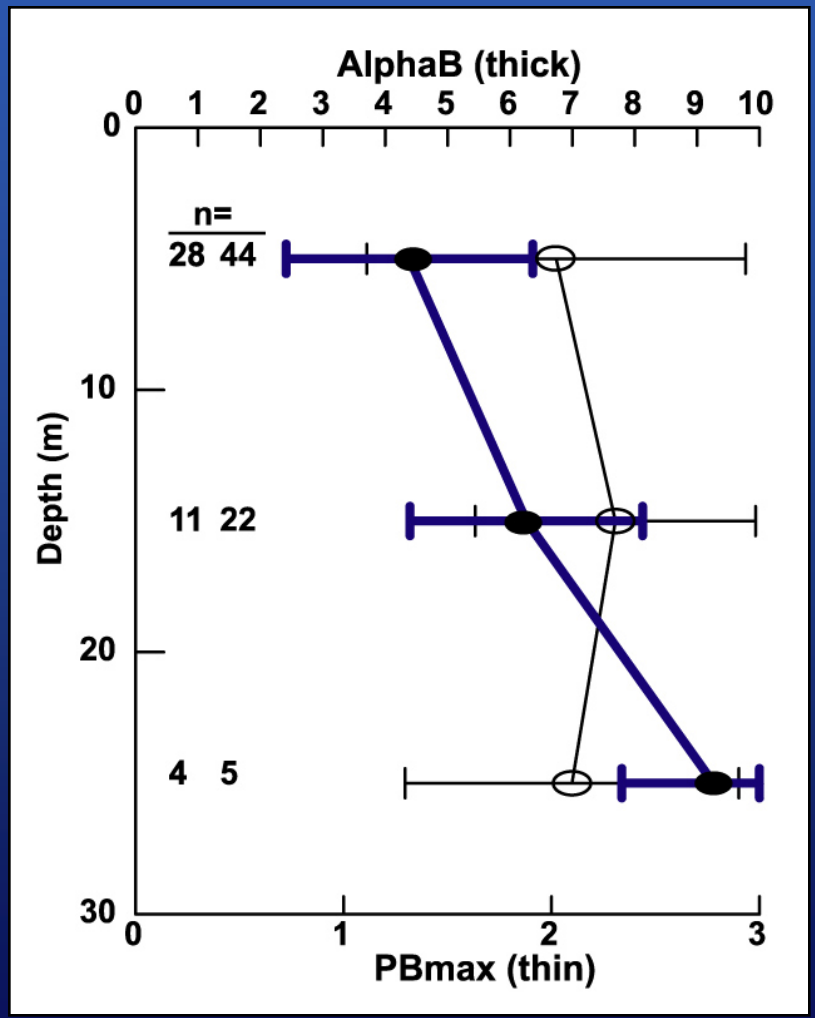
Intensity=photosynthetically available radiation (μmol photons m^{-2} sec^{-1} 400⇒700 nm**)**

Fox Point

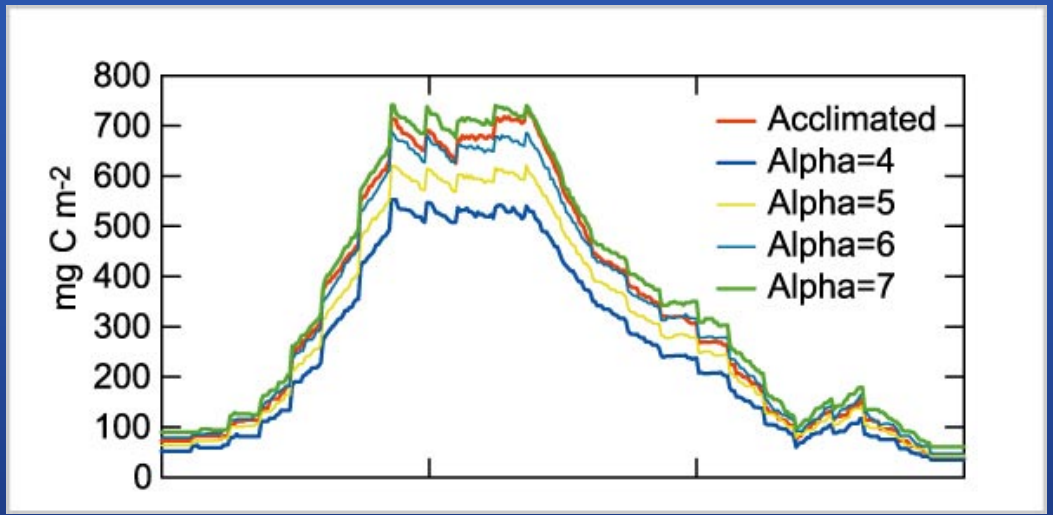

l photons m-2 sec-1
100 2 00 400 500 600 700 800 900
SUNBLOCK
~250 umol @ 1.5m
Simulated light intensities
~10 umol @ 16.5 m
Depth (m)
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20

Observed photoacclimation

- Collection of observed parameters for mid-spring at Fox Point from 1986-1997
- P^{B}_{max} does not change with depth, but $Alpha^{B}$ acclimates to lower light with depth
- Evidence of acclimation, but no knowledge of physical mixing rates



Importance of photoacclimation



		g C m-2 year-1	Scenario	Difference from observed (g C)	Basinwide impact (kg C year-1)	Impact to fish food (kg C year-1,10% conversion)	Impact to fish (kg C year-1,10% conversion)
Basin area (km)(GLIN)	57,800	118.5	Observed				
Assume 70% area > depth than euphotic zone	40,460	90.7	Alpha=4	27.8	1,124,788,000	112,478,800	11,247,880
Deep lake area (m)	40,460,000,000	104.4	Alpha=5	14.1	570,486,000	57,048,600	5,704,860
		116.6	Alpha=6	1.9	76,874,000	7,687,400	768,740
		127.2	Alpha=7	-8.7	-352,002,000	-35,200,200	-3,520,020

Mixing in prior studies

Type of simulation	Complete mixing time	Reference
Experimental	~ 0.1 h	Harris & Lott (1973)
Experimental	0.05 h	Jewson & Wood (1975)
Experimental	~ 3 h	Kremer & Nixon (1978)
Experimental	1-1.5 h	Marra (1978)
Mathematical	~3 d	Platt & Gallegos (1980)
Mathematical	4.6 – 460 d	Falkowski & Wirick (1981)
Experimental	0.14 – 1.3 h	Gallegos & Platt (1982)
Experimental	2 - 6 d	This study

From Lewis, Cullen and Platt (1984)

Objectives

- Detect and characterize photoacclimation under conditions somewhat like Fox Point station
- Determine a rate of transport through the light gradient that exceeds *A. formosa'a* ability to acclimate
- Relate results to conditions found in Lake Michigan

Methods

Natural assemblage

BACILLARIOPHYCEAE (DIATOMS)

CENTRALES

- Discineae
  - **–Aulicosira spp.**
  - –Cyclotella spp.
  - –Stephanodiscus spp.
- Soleniineae
  - –Rhizosolenia eriensis

PENNALES

- Araphidineae
  - **–Tabellaria spp.**
  - **–Fragilaria spp.**
  - **–Asterionella formosa**
  - **–Synedra spp.**
- Biraphidineae
  - –Navicula spp.
  - –Amphora spp.
  - –Cymbella spp.
  - –Nitzschia spp.
  - –Cymatopleura solea

CHLOROPHYTA (GREEN ALGAE)

TETRASPORALES

- –Elakatothrix spp.
- –Gloeocystis spp.

ULOTRICHALES

- –Ulothrix spp.

CHLOROCOCCALES

- –Actinastrum spp.
- **–Ankinastrum sp.**
- –Crucigenia spp.
- –Franceia spp.
- –Golenkinia spp.
- –Lagerheimia spp.
- –Oocystis spp.
- –Pediastrum boryanum
- –Scenedesmus spp.

ZYGNEMATALES

- –Mougeotia sp.

DESMIDS

- –Cosmarium sp.
- –Staurastrum sp.

CYANOPHYTA

- –Anabaena spp.
- –Chroococcus spp.
- –Coelosphaerium sp.
- –Gloeocapsa sp.
- –Lyngbya sp.
- –Microcystis spp.
- –Spirulina major

PYRRHOPHYTA

- –Ceratium hirundinella spp.
- –Peridinium spp.

CHRYSOPHYTA

- **–Dinobryon spp.**

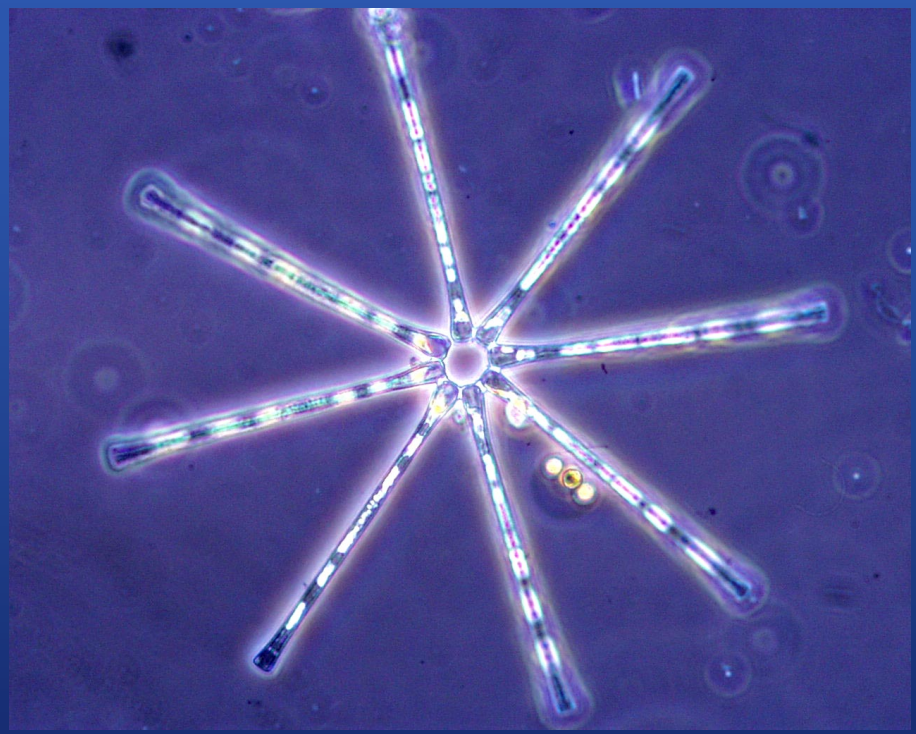
CRYPTOPHYA

- –Cryptomonas spp.
- **–Rhodomonas sp.**

Taxa encountered during 1976 at Fox Point; Ristic, 1977

Study species

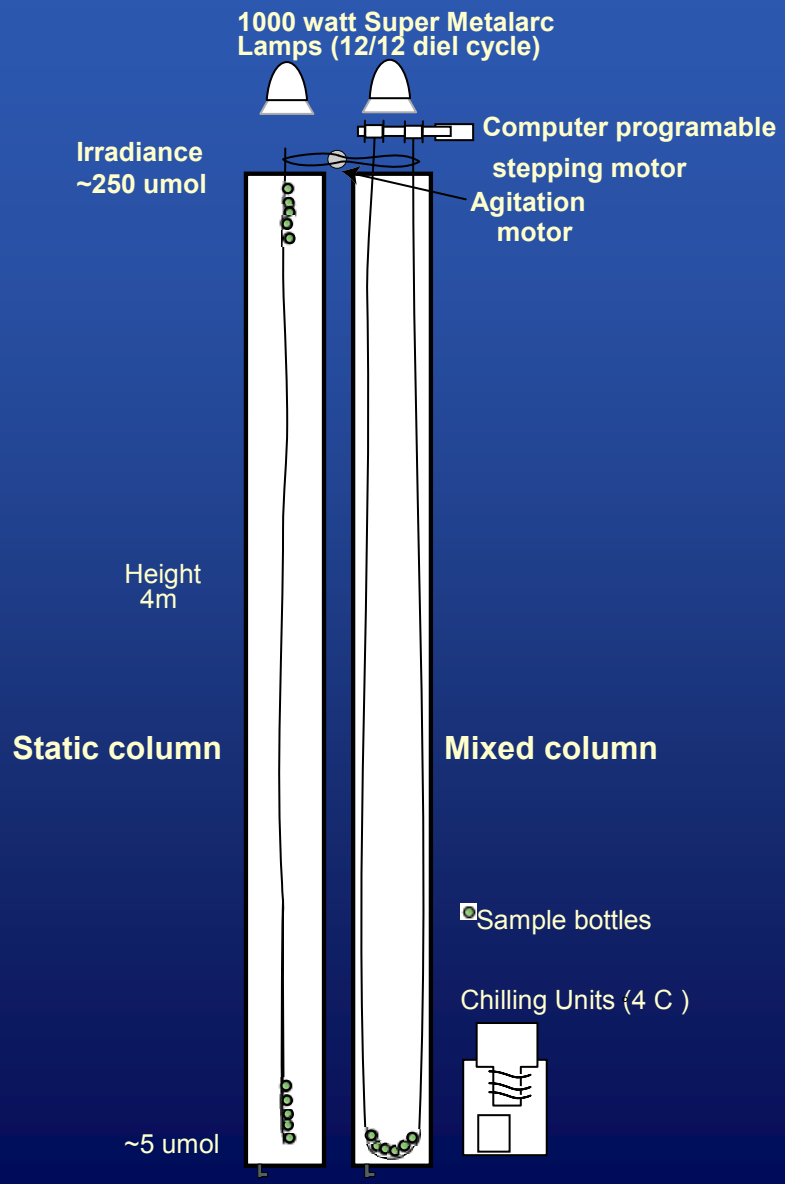
- *Asterionella formosa*
- Represent diatoms found in L. Michigan in spring
- Able to conduct cell counts in particle counter versus visual counting
- Easy to culture



Micrograph by Patrick Eberland, REU 2000

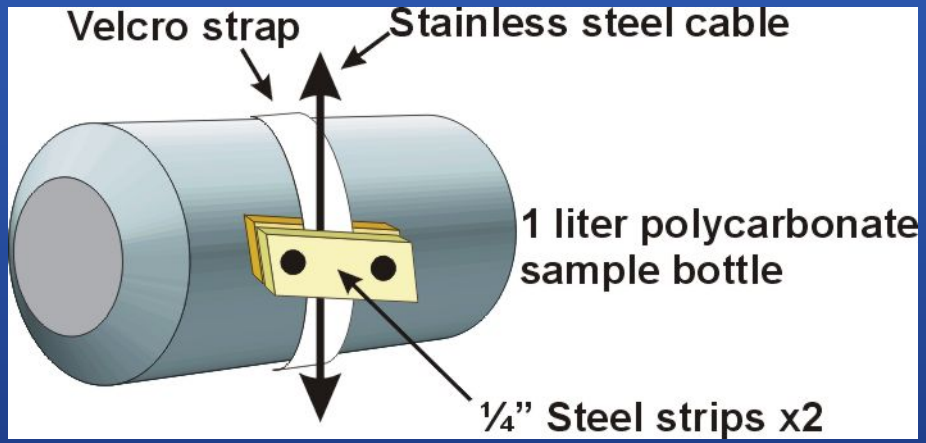
Incubation columns

- Rate of travel controlled by programmable stepping motor
- **MIX** samples are in motion. **TOP** and **BOT**tom samples are fixed in position in static column
- Samples in **WALK** treatment remained in growth chamber
- Bubbles to keep columns homothermal and increase light attenuation



Exposure vessels

- Each treatment (TOP, BOT, MIX, WALK) in each experiment used 15 bottles and 3 were removed as replicates for each sampling
- Bottle diameter meant each treatment spanned significant amount of the light gradient
- Self-shading by the bottles



Column incubation methods

- **Replicate bottles within each column under same conditions (12/12 L/D, 4°C)**
- **Samples removed at 24 hour intervals throughout incubation and assayed for acclimation response**
  - Measured light dose, cell count, pigment content and ratios, photosynthetic parameters
- **Process repeated at different mixing rates (Period = 2 and 12 days)**

Total daily light dose

$(Intensity)^{0.25} = HLIR$

$HLIR = a*PAR^{\wedge b}$

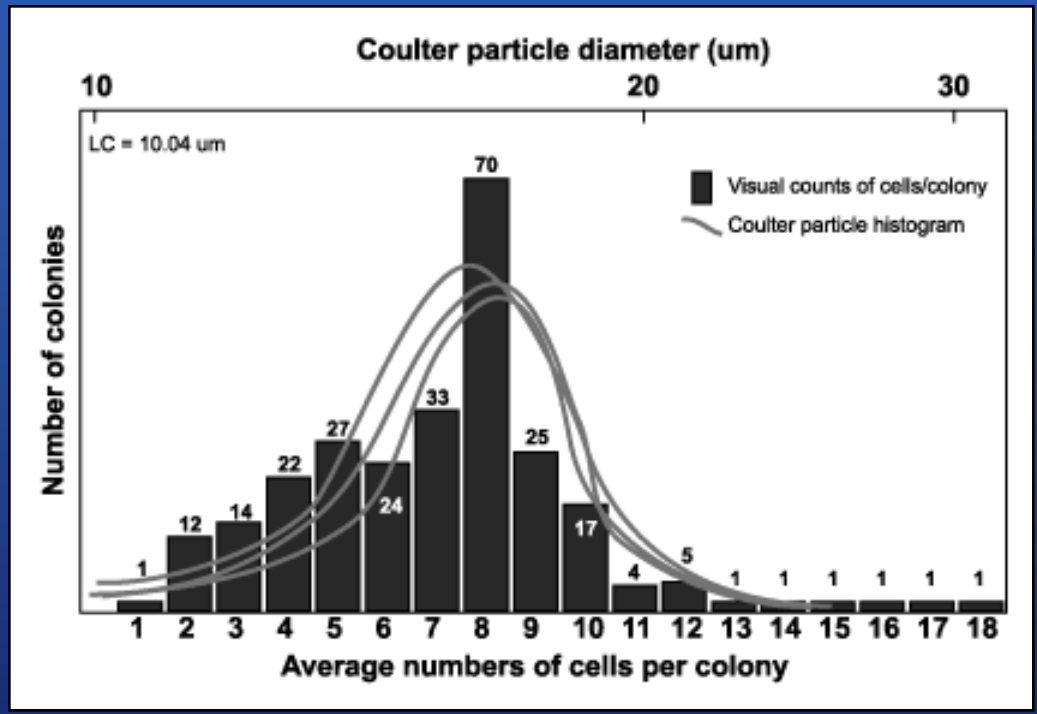
$PAR = (HLIR/0.832)^{(1/0.253)}$

$TDLD\ (24\ hours) = (PAR * D) / 1x10^{6}$

- Conversion from lumens to PAR used empirical relationship for the columns and data from an intensity logger attached to the bottles
- For the TOP and BOT and WALK treatments, TDLD is calculated from constant PAR
- D is the duration between recordings in seconds

Cell counts

- All counting done with 3-4 replicate counts per sample using Coulter particle counter
- Coulter particle size histogram was binned to generalize cells/colony determined from microscope counts

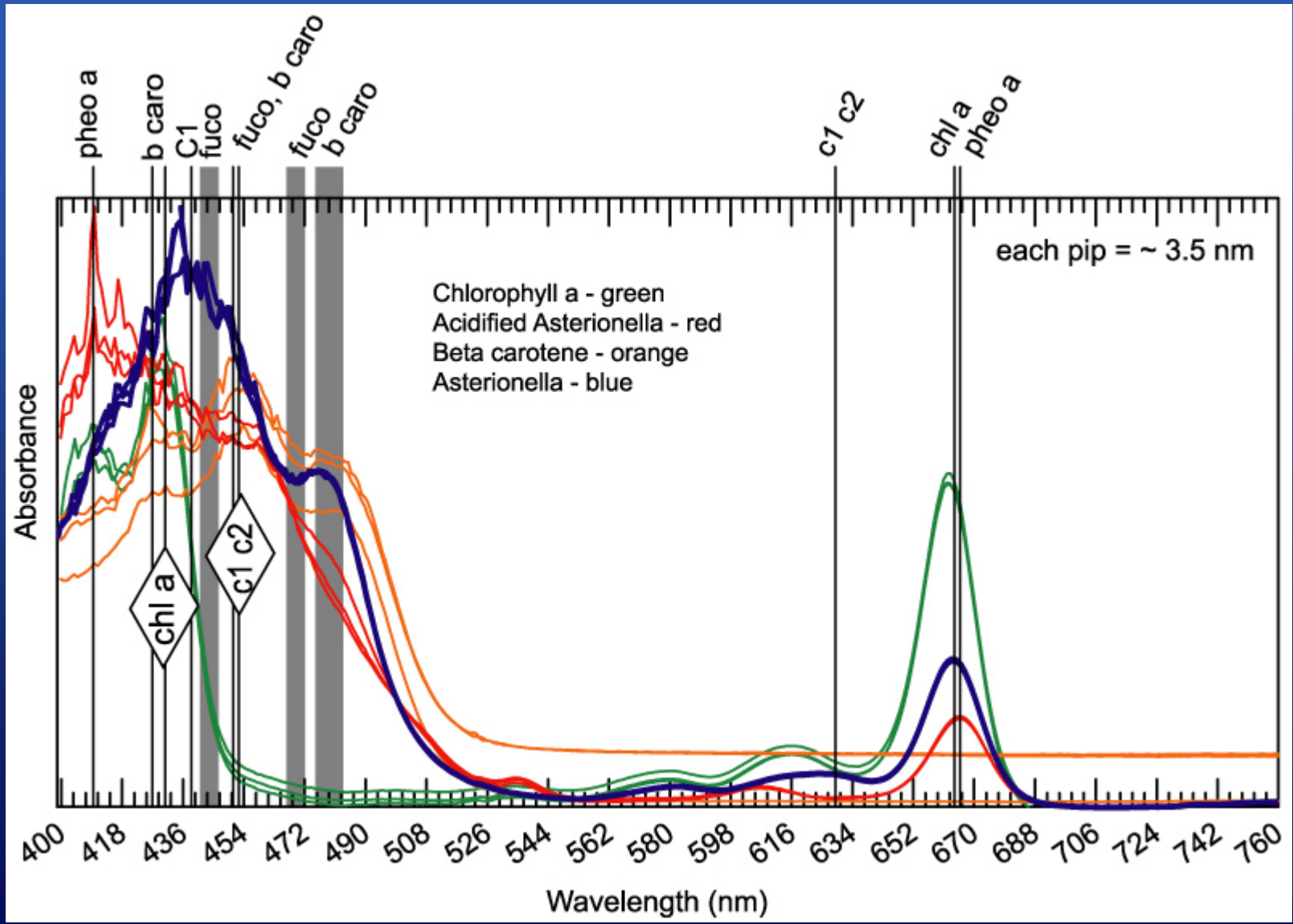


8 cells/colony is very common in *A. formosa*

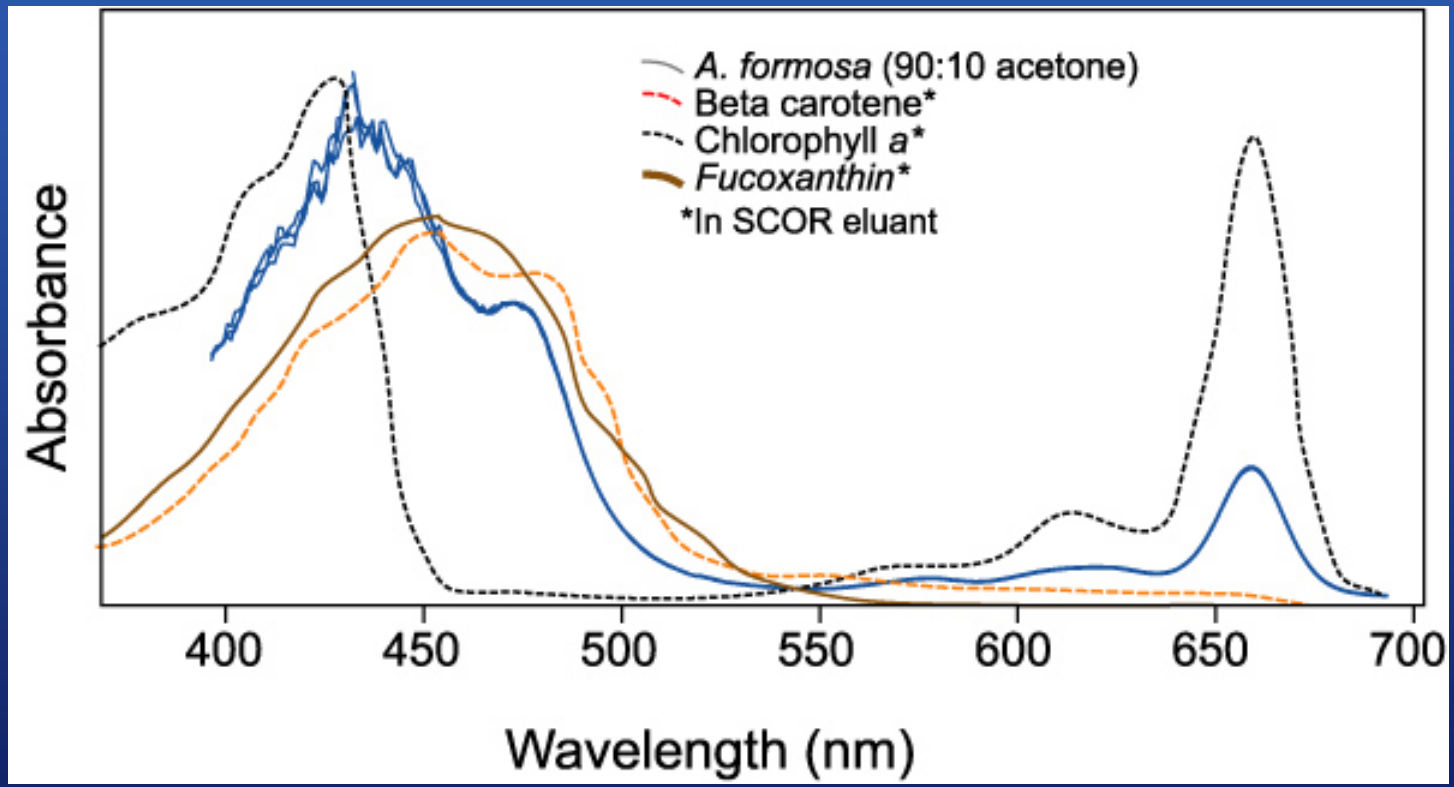
Photosynthetic modeling

- Used Jassby and Platt, 1976 formulation throughout study
- Fitted with SYSTAT, then with dedicated application (Fee)
- All parameters are cell-specific, eg. $P_{cellmax}$=μg ^{14}C (20,000 cells)$^{-1}$ μmol photons hour^{-1}
- Or cellular chlorophyll-specific, eg. $P^{B}_{cellmax}$=μg ^{14}C (pg Chl *a* cell^{-1}) μmol photons hour^{-1}

In vitro pigment absorption



Fucoxanthin absorbance



Source: Jeffrey, S. W., R. F. C. Mantoura, et al. (1997). Phytoplankton pigments in oceanography. Paris, United Nations Educational, Scientific and Cultural Organization.

Pigment analysis

- *In vitro* pigment concentration determined through spectrophotometric analysis
- Chlorophyll *a*, *c1*+*c2* determined through derivation of Jeffrey and Humphrey's trichromatic equations
- Carotenoids were calc'd from absorbance maxima (Mantoura 1983; Rowan 1989)
- Fucoxanthin-like absorbance

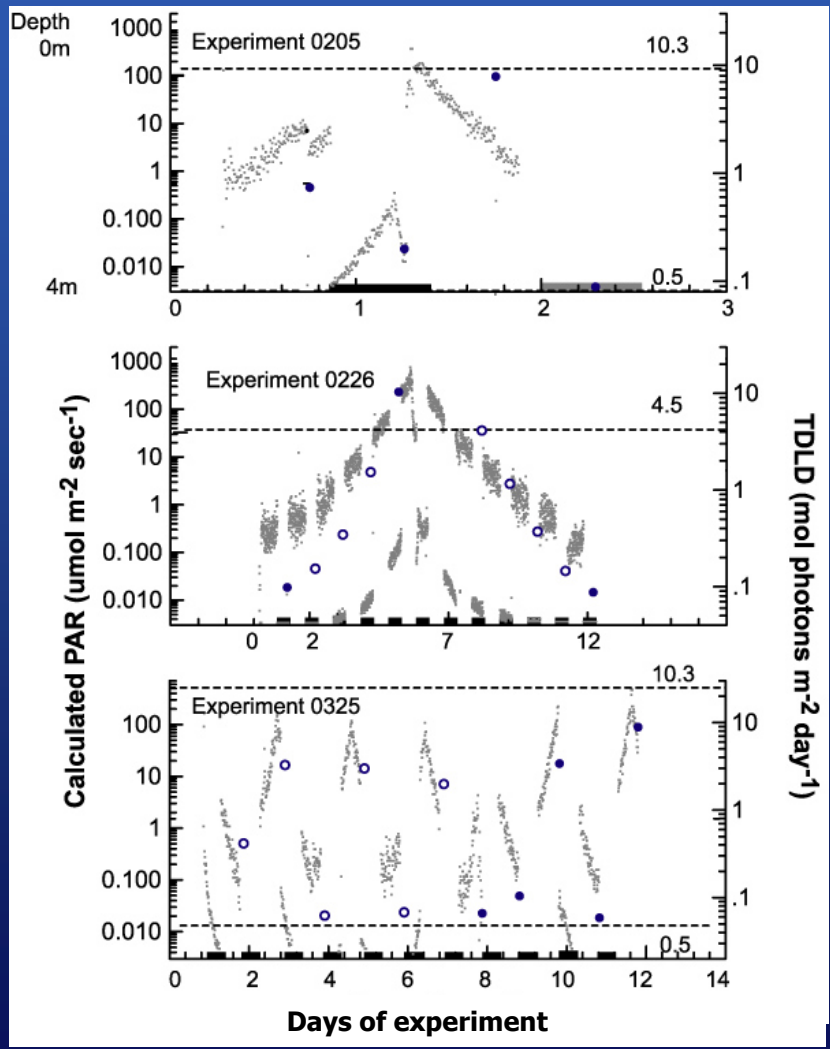
  CELLFUCO= (ABS 444+446+449+467+469+471+473) / CELLCOUNT
- β-carotene-like absorbance

  CELLBCARO=(ABS 449+453+475+477+480) / CELLCOUNT

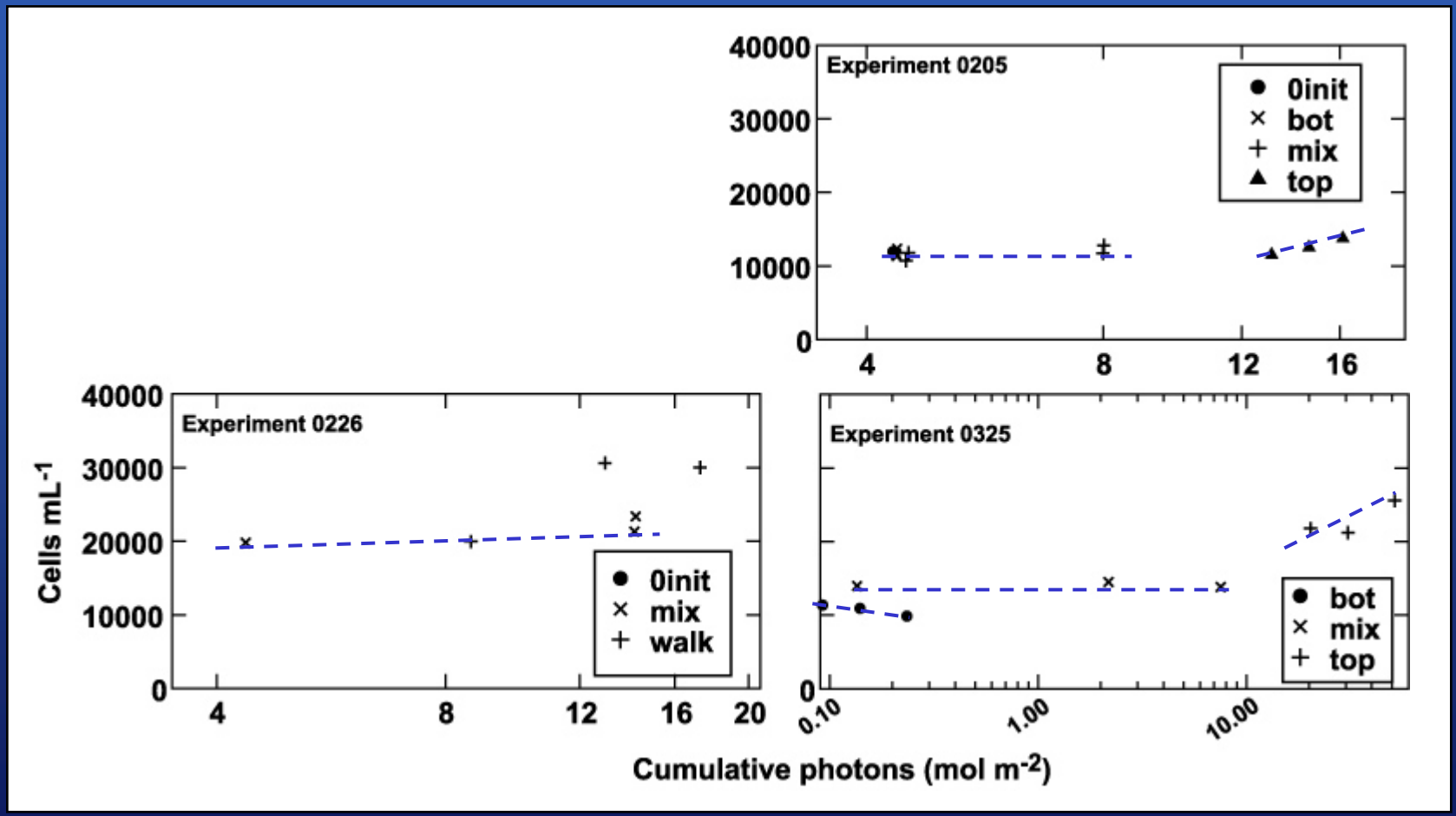
Results

Light exposures

- Experiments 0205 and 0325 had MIX sampled that traversed the light gradient every 24 hours.
- 0226 traversed every 144 hours (6 days) and WALK treatment
- 0325 had an entrainment acclimation of 7 days before sampling began

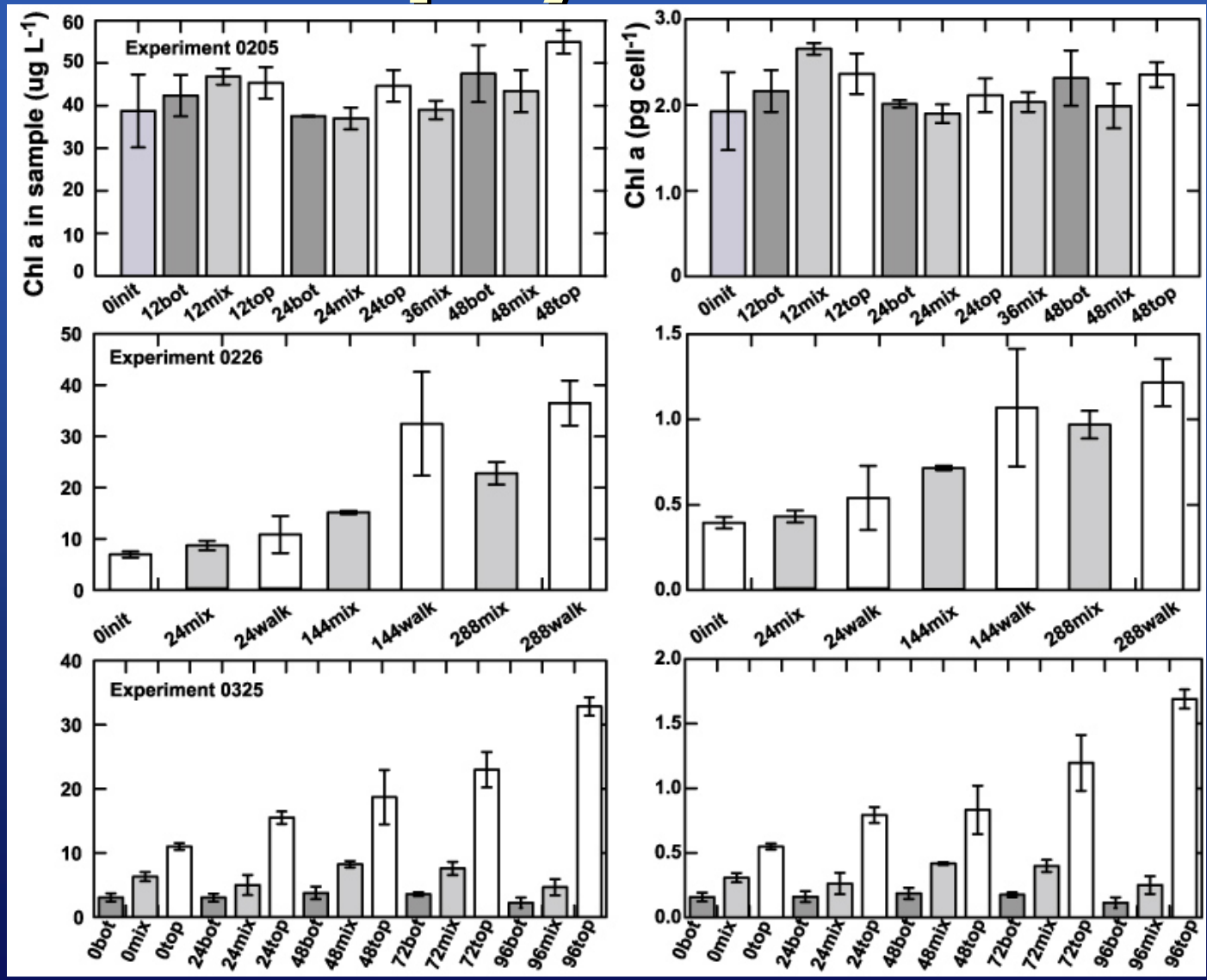


Cell growth and light history



Chlorophyll *a*

- 0205: little change in any treatment over 48 hours. (2.0-2.5 pg cell^{-1})
- 0226: both treatments increased, though WALK achieved steady state higher than MIX
- 0325: after acclimation, TOP continued to increase, BOT and MIX did not increase



Pigment correlations

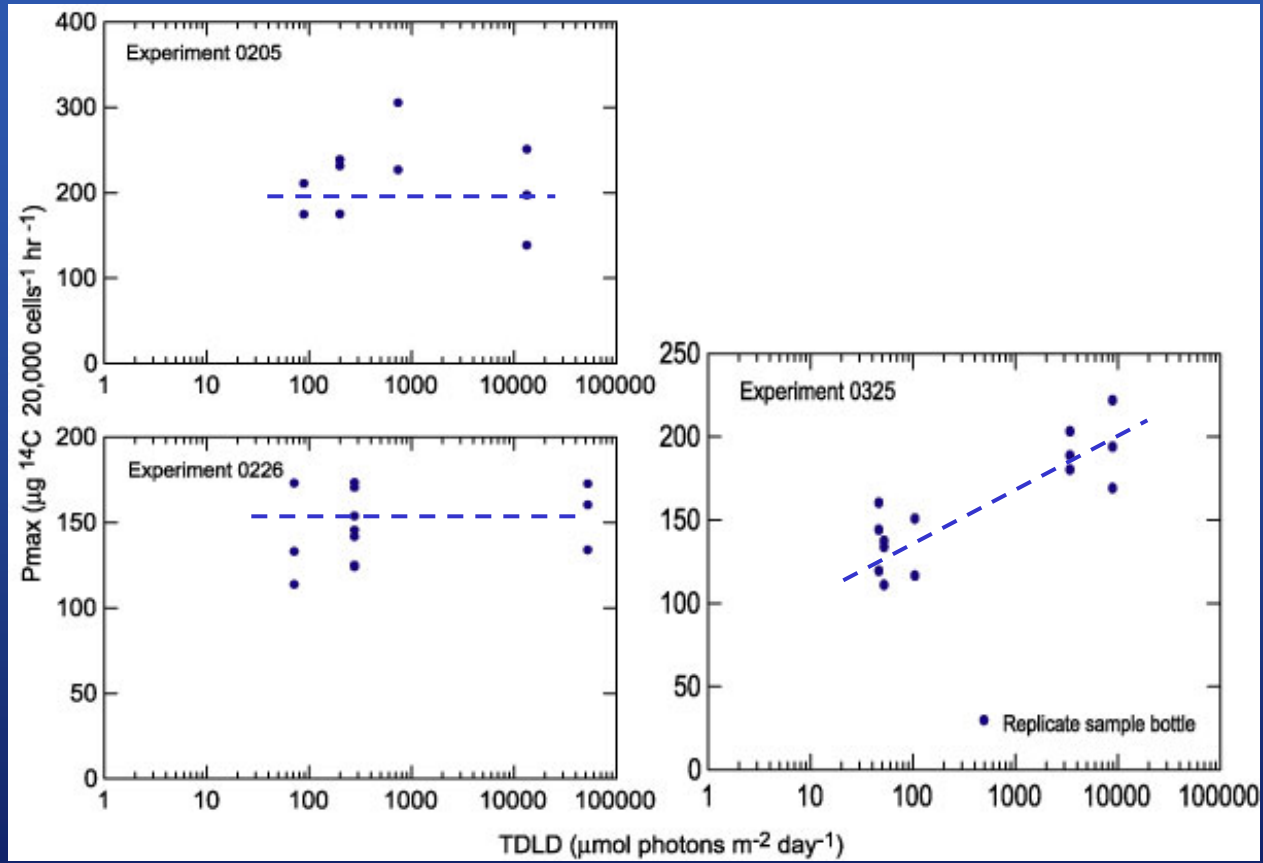
- Chlorophyll *a*, β-carotene & fucoxanthin generally correlated for all experiments.
- No evidence of photoprotective response or chromatic acclimation
- The exceptions don't follow a trend

Appendix C. Pearson correlations					
Experiment	Treatment	Cellular chl a : cellular fucoxanthin	Cellular b carotene : cellular fucoxanthin	Cellular pheophytin : cellular chl a	Cellular chl c1+c2 : cellular chl a
0226	00init	1.00	1.00		
0226	24mix	0.57	0.93		
0226	24walk	1.00	1.00	0.73	-0.10
0226	144mix	0.96	1.00	0.54	1.00
0226	144walk	1.00	1.00	1.00	1.00
0226	288mix	0.70	0.70	0.65	0.11
0226	288walk	0.98	1.00	0.73	1.00
0325	0bot	0.97	1.00	0.98	0.99
0325	0mix	-0.60	1.00	1.00	-0.19
0325	0top	0.96	1.00	0.63	0.87
0325	24bot	1.00	1.00	-1.00	1.00
0325	24mix	1.00	1.00	1.00	1.00
0325	24top	1.00	1.00	-1.00	1.00
0325	48bot	1.00	1.00	-1.00	1.00
0325	48mix	0.90	0.95	0.90	0.42
0325	48top	0.88	0.98	0.86	0.92
0325	72bot	0.82	0.90	0.51	0.97
0325	72mix	1.00	1.00	0.73	0.99
0325	72top	0.94	0.81	0.92	0.85
0325	96bot	0.89	1.00	1.00	-0.93
0325	96mix	1.00	1.00	0.82	0.99

0205 left off, but follows similar relationships

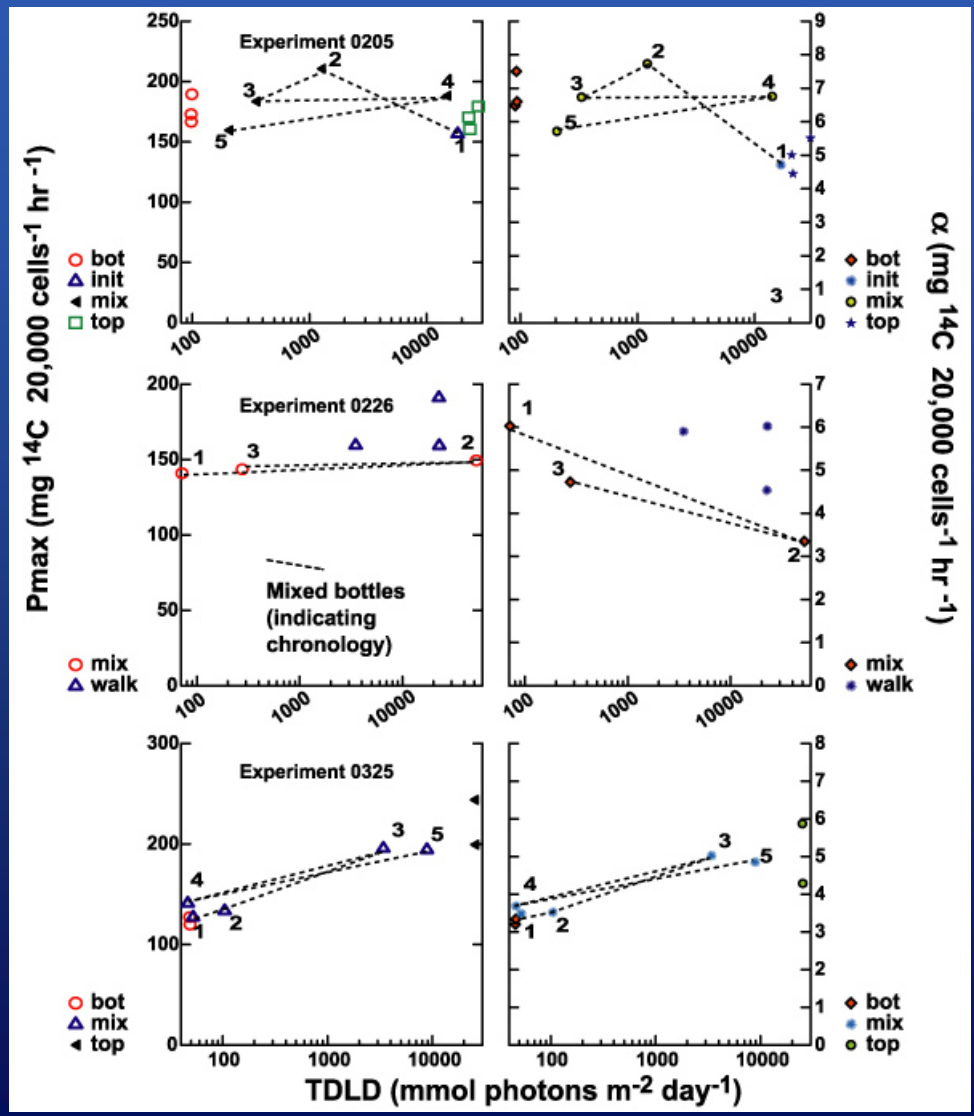
Pcellmax by TDLD

P parameters for the replicate bottles of the MIX treatments versus their 24 hour light history



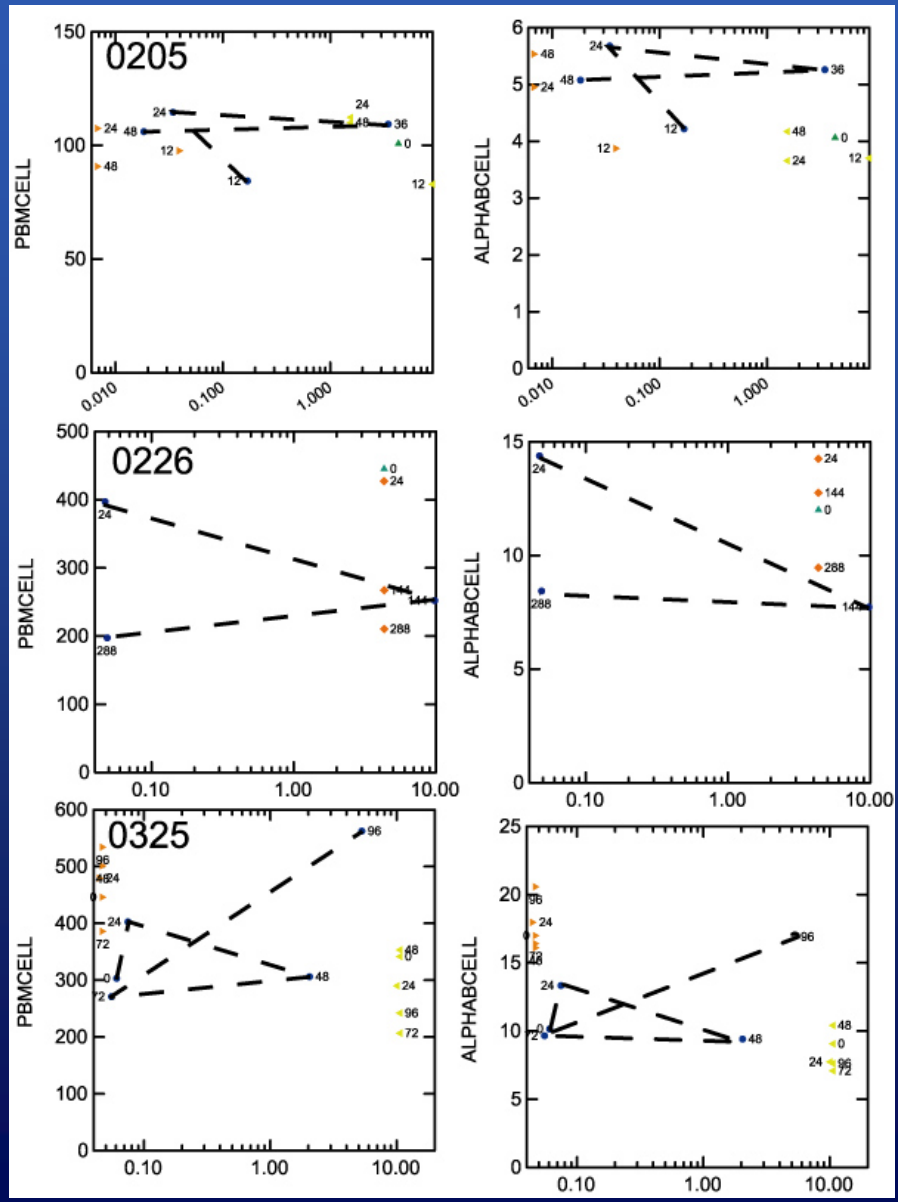
TDLD & cell-sp parameters

- Experiments 0205, 0226 did not provide clear evidence of acclimation
- Both parameters in experiment 0325 oscillate according to 24 hour light history
- 0325 cell-chlorophyll specific parameters have opposite slopes. Chlorophyll increased against the rules



TDLD & cell-chla-sp parameters

- Perhaps correcting for chlorophyll content would clear things up
- Nope. Even fewer coherent responses to light history



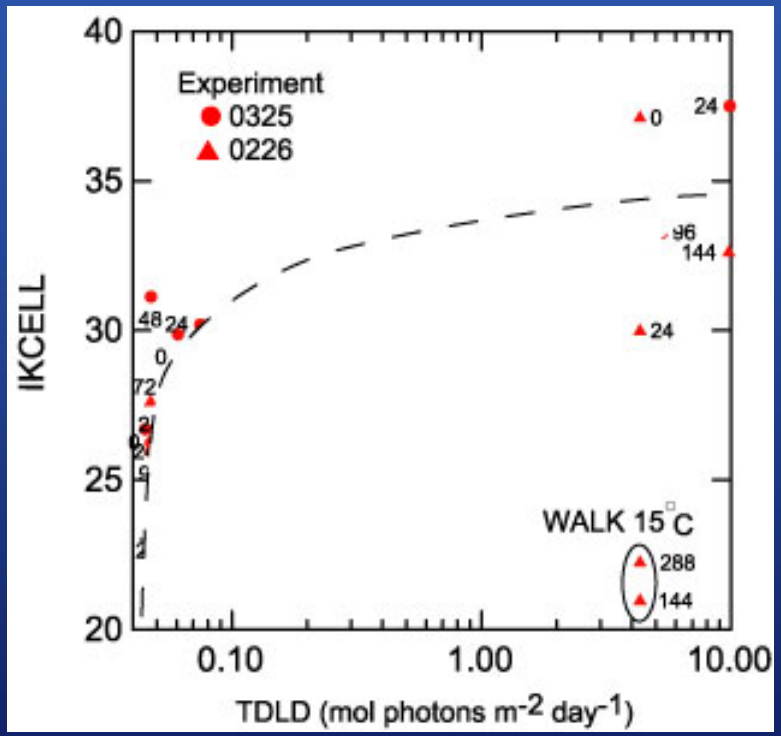
Parameter : TDLD regression

- Regression of cell-specific parameters against TDLD
- 0325 was highly correlated with light history
- Need more "n"

Experiment/test	F-ratio	P	n
0205 α_{Bcell}	2.24	0.17	5
0226 α_{Bcell}	0.84	0.40	3
0325 α_{Bcell}	10.32	**0.006**	**5**
0205 PBm_{cell}	1.35	0.27	5
0226 PBm_{cell}	0.04	0.84	3
0325 Pbm_{cell}	4.22	*0.06*	**5**
0205 Ik_{Bcell}	0.25	0.63	5
0226 Ik_{Bcell}	0.89	0.39	3
0325 Ik_{Bcell}	12.92	**0.003**	**5**

Ik_{cell}

- Makes you want to fit a line through the points…
- All you can say is that Ikcell increases with light history



Numbers are hours into experiment

Conclusions

- **Even under slow mixing, acclimation was not confirmed (0226)**
- **0325 indicated acclimation to TDLD**
  - and/or secondary cyclic light regime overlaid with diel cycle (Legendré, Prezelin)
- **After 7 days at <0.01 TDLD (10 umol ≅ 16.5 m FP) BOT samples were unable to acclimate further. Implications for Lake Michigan?**

Further work

- How does acclimation get manifested during calm periods interrupted by episodic events (geek speak for storms)?
- What role does UV play?
- How does temperature (cold) influence ability to photoacclimate?

Acknowledgements

- Arthur Brooks
- Harvey Bootsma
- Craig Sandgren
- Joyce Witebsky
- Carmen Aguilar
- Russell Cuhel
- Lisa Belli
- Jen Garrow
- Anna Kuester
- Jerry Liu
- Maurizio Murru
- John Niebler
- Steve Robillard
- Stephanie Schroeder
- The staff of the WATER Institute and Biological sciences
- ….A gazzlion other friends and family

Fidoplankton

Supported in part by The Great Lakes Cruising Club Paul Frederick Memorial Fellowship