Biophysical and optical determinations of light absorption by phytoplankton *in vivo* & *in situ*.

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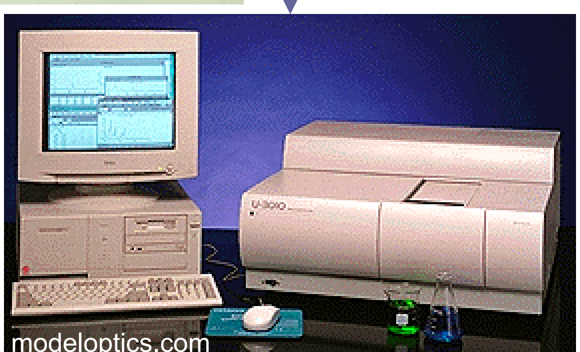
What is the importance of light absorption to algal (PSII) productivity?

• Algae are frequently ‘light limited’ (…rate of absorption determines photosynthesis).

• High resolution biophysical (fluorometric) estimates of PSII productivity are limited by our ability to quantify the rate of light absorption.

Quantifying light absorption

Bio-optical



Bio-physical

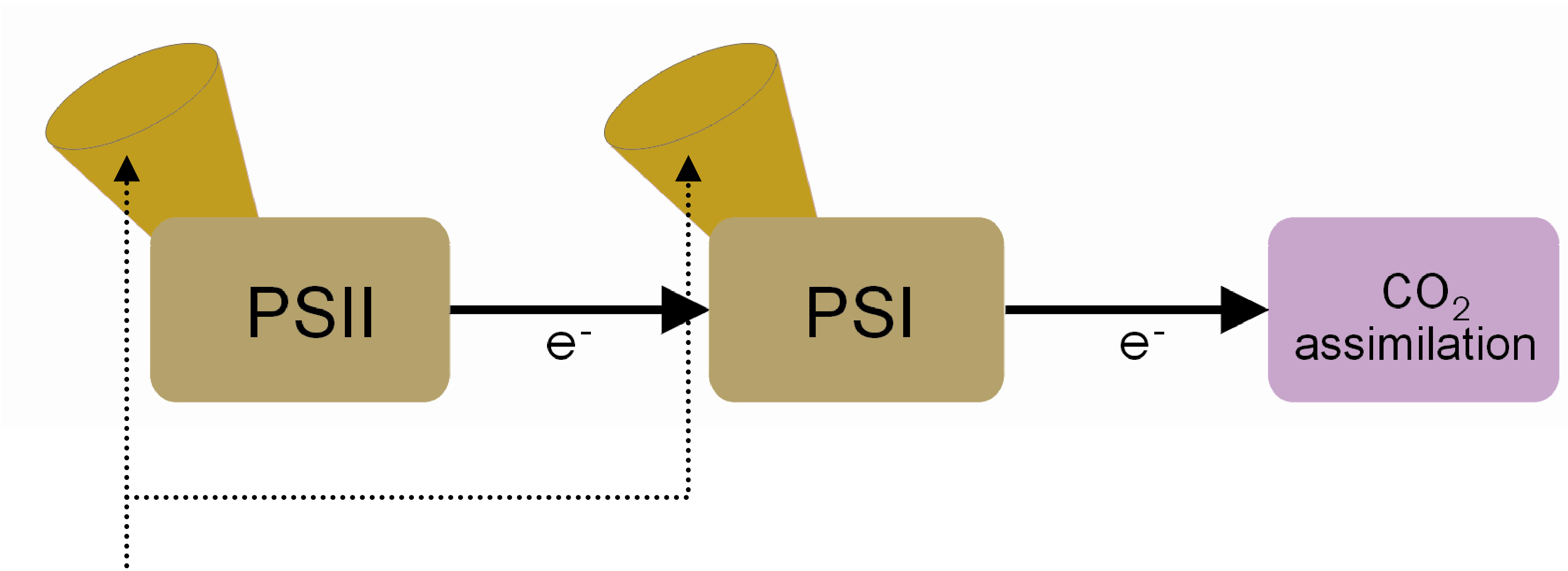
Quantifying light absorption

Outline

(1) Reconciliation of optical- and biophysical-based determinations of light absorption by PSII.

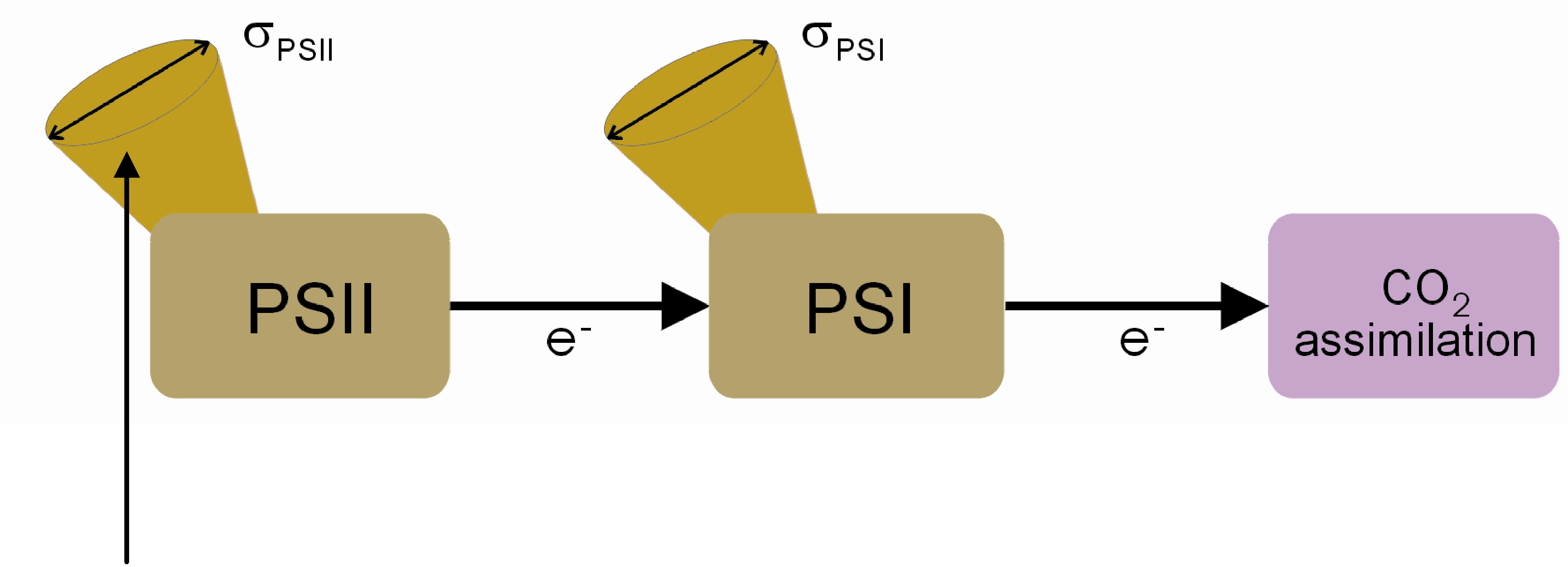
(2) Towards understanding the variability of biophysical-based estimates of light absorption by PSII in situ.

Quantifying light absorption



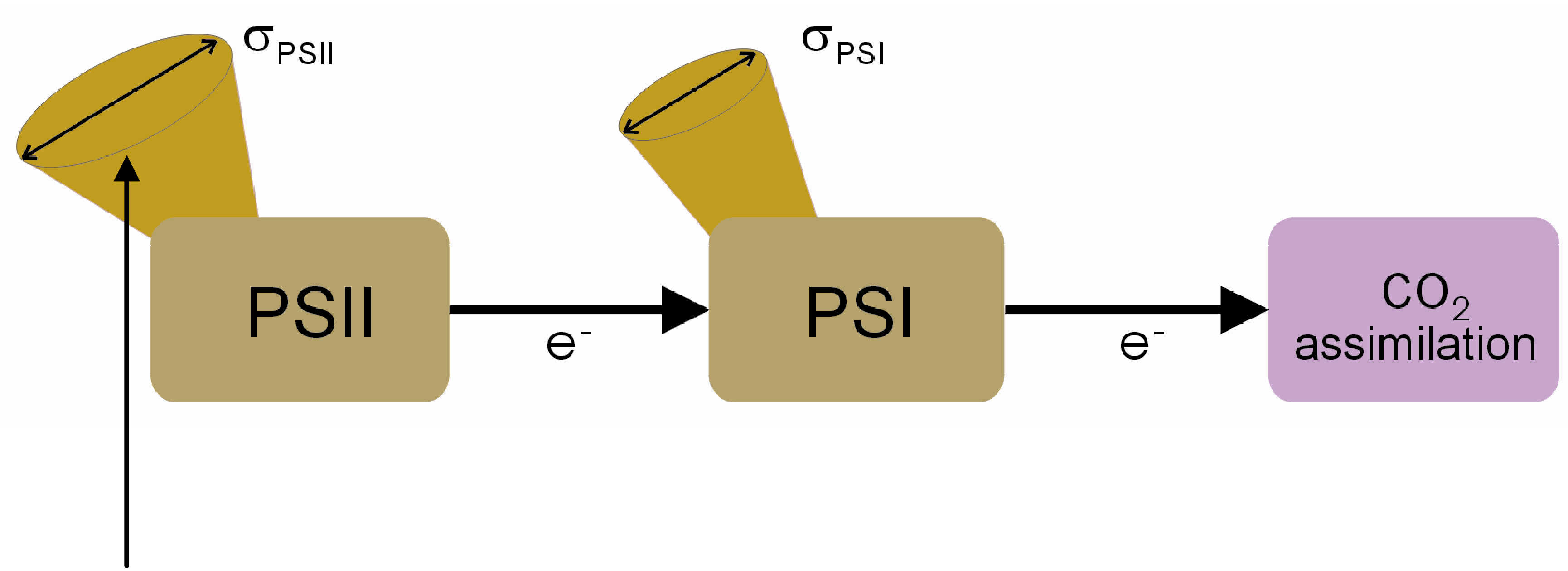
Bio-optical: All potential light absorption by pigments associated with PSII & PSI (expressed relative to the predominant pigment, chlorophyll *a*)

Quantifying light absorption



Bio-physical: Effective absorption by pigments which deliver photons to PSII photochemistry (expressed relative to the no. of functional RCIIs)

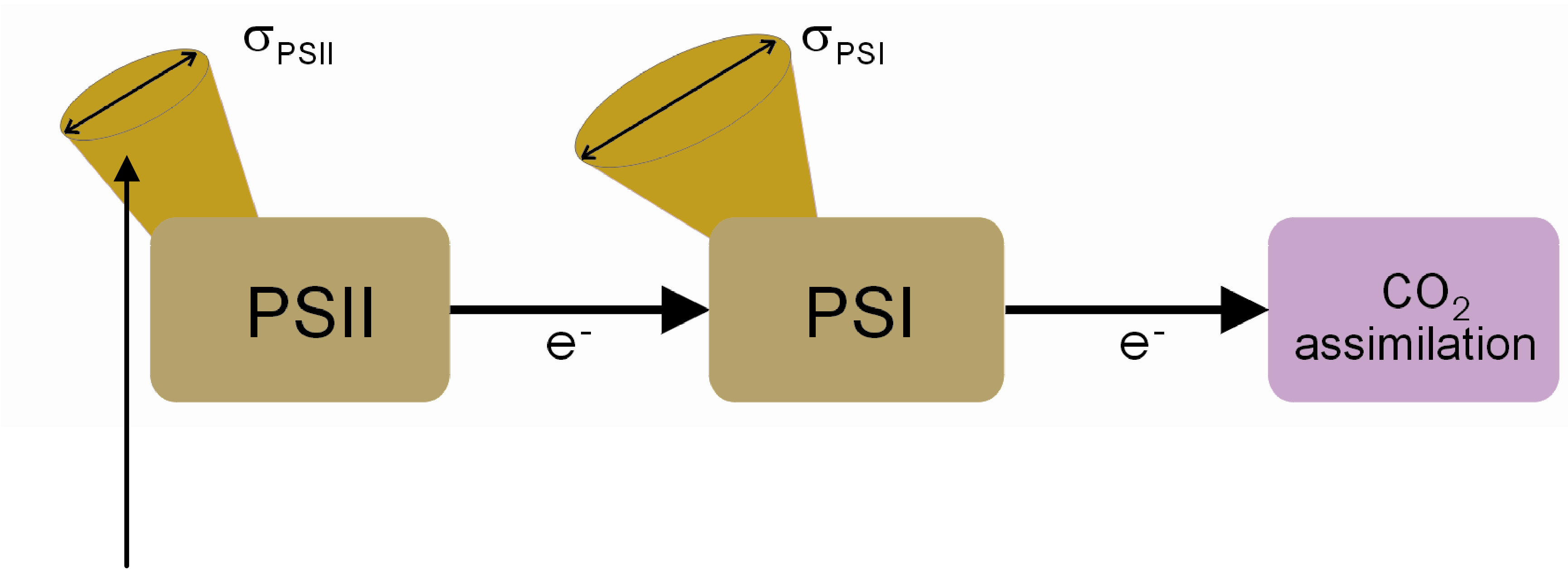
Quantifying light absorption



Bio-physical: Effective absorption by pigments which deliver photons to PSII photochemistry (expressed relative to the no. of functional RCIIs)

- Changes in PSII light harvesting pigments and RCIIs (acclimation)

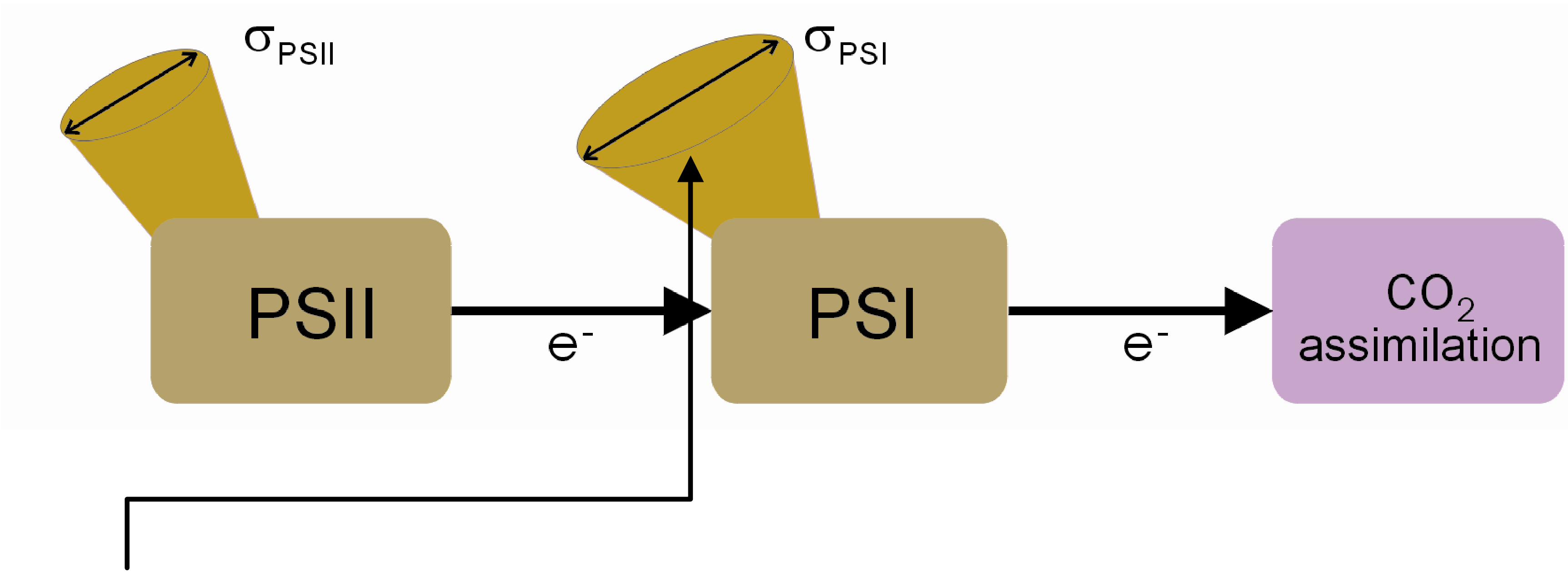
Quantifying light absorption



Bio-physical: Effective absorption by pigments which deliver photons to PSII photochemistry (expressed relative to the no. of functional RCIIs)

- Changes in PSII light harvesting pigments and RCIIs (acclimation)
- Non-photochemical quenching of ex. energy, state transition (ambient light)

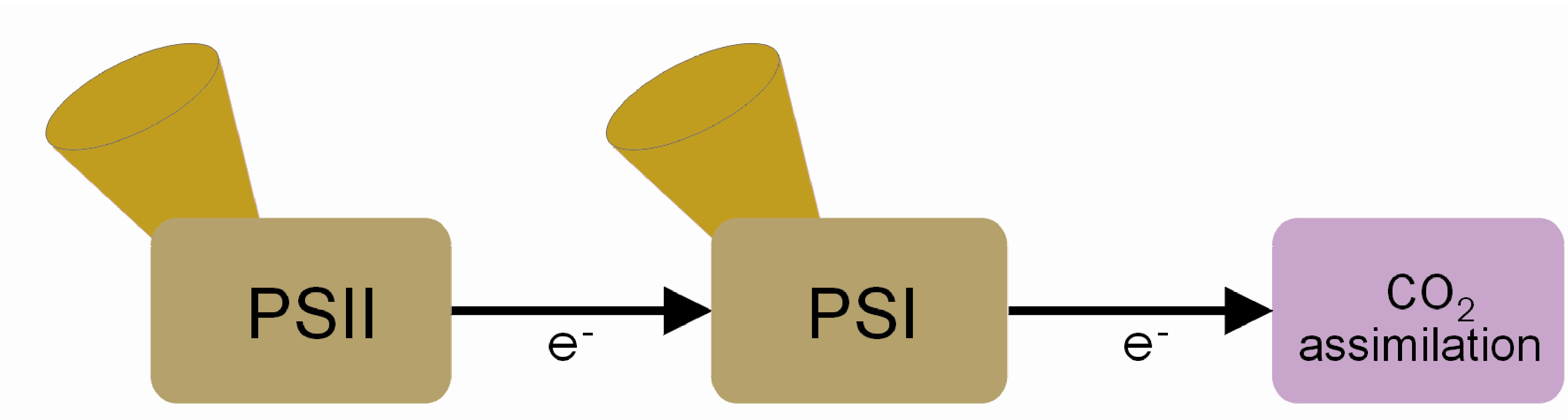
Quantifying light absorption



Bio-physical: Effective absorption by pigments which deliver photons to PSII photochemistry (expressed relative to the no. of functional RCIIs)

- Coincidental changes in σ_{PSI}: state transitions
- Cyclic electron flow around PSI: pigments, RCIs or state transition?

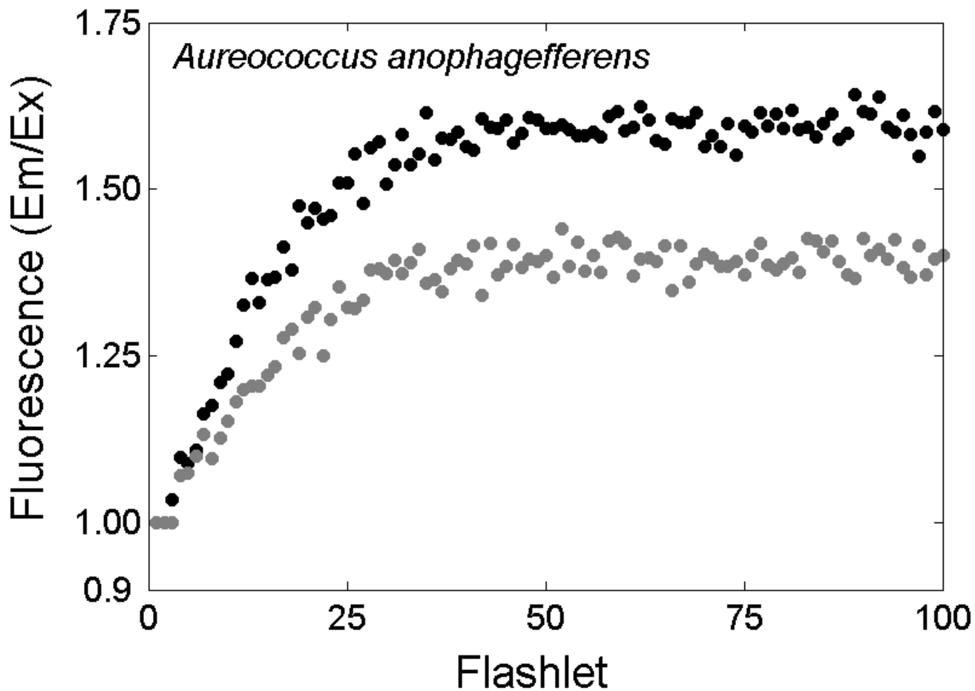
Quantifying light absorption



To what extent can we reconcile biophysical and optical absorption estimates for mechanistic PSII productivity models?

- 11 spp. from 8 algal divisions grown under 18 & 300 mmol photons m^{-2} s^{-1}
- Natural samples (Celtic & Irish Seas, SW England)

1. Biophysical absorption by PSII: FRR fluorescence



Fitting the biophysical model of Kolber *et al.* (1998) yields σ_{PSII} (m^2 mol RCII^{-1} • 10^{-4})

'Low light' (20-80 μmol m^{-2} s^{-1})
'High light' (300-400 μmol m^{-2} s^{-1})

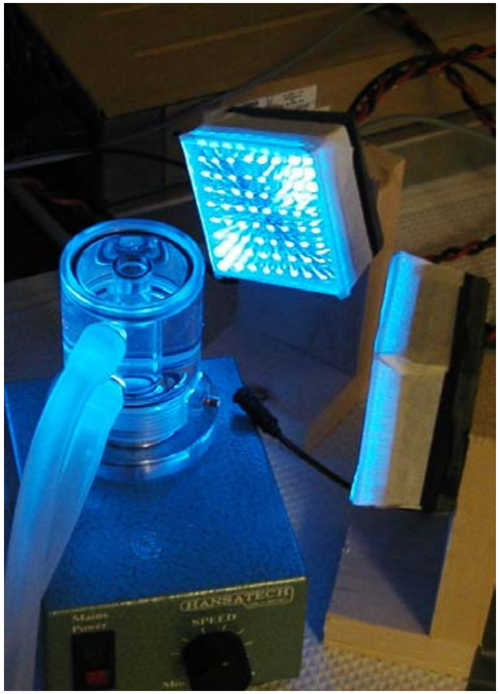
1. Biophysical absorption by PSII: FRR fluorescence

	σ_{PSII} (m^2 mol $RCII^{-1} \bullet 10^{-4}$)
Aureococcus anophagefferens	874 - 671
Chaetoceros muelleri	211 - 284
Dunaliella tertiolecta	172 - 208
Emiliania huxleyi	298 - 349
Prorocentrum minimum	361 - 547
Pycnococcus provasolii	758 - 606
Thalassiosira weissflogii	157 - 207
Rhodomonas salina	246 - 223
Storeatula major	211 - 175
Synechococcus spp. 1479/9	92 - 141
Synechococcus spp. WH7803	162 - 226

Max. difference between spp.	**9.5**
Max. difference between growth PPFDs	**1.5**

1. Biophysical absorption by PSII: n_{PSII}

n_{PSII} = mol RCII (mol chl*a*)$^{-1}$



O_2 flash yield technique
(Falkowski *et al.* 1981:
Plant Physiol. 68)

LED system
(Suggett *et al.* 2003:
Eur. J. Phycology 38)

Low sensitivity of O_2 electrode requires
highly concentrated algal solution
(> 0.75 g m^{-3} chl *a*)

1. Biophysical absorption by PSII: n_{PSII}

	σ_{PSII} (m^2 mol $RCII^{-1}$ • 10^{-4})	$1/n_{PSII}$ (mol chl *a* mol $RCII^{-1}$)
Aureococcus anophagefferens	874 - 671	951 - 724
Chaetoceros muelleri	211 - 284	591 - 521
Dunaliella tertiolecta	172 - 208	742 - 538
Emiliania huxleyi	298 - 349	650 - 538
Prorocentrum minimum	361 - 547	535 - 431
Pycnococcus provasolii	758 - 606	938 - 666
Thalassiosira weissflogii	157 - 207	584 - 556
Rhodomonas salina	246 - 223	510 - 472
Storeatula major	211 - 175	520 - 445
Synechococcus spp. 1479/9	92 - 141	279 - 241
Synechococcus spp. WH7803	162 - 226	294 - 221
Max. difference between spp.	**9.5**	**3.4**
Max. difference between growth PPFDs	**1.5**	**1.4**

1. Biophysical absorption by PSII: a^{chl}_{PSII}

	σ_{PSII} (m^2 mol $RCII^{-1} \bullet 10^{-4}$)	$1/n_{PSII}$ (mol chl *a* mol $RCII^{-1}$)
Aureococcus anophagefferens	874 - 671	951 - 724
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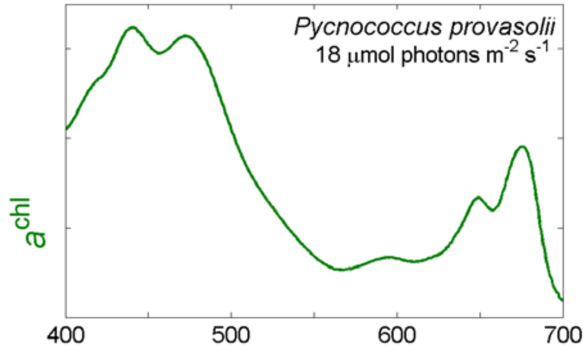
$$a^{chl}_{PSII}\ (m^2\ mg\ chl\,a^{-1}) = \sigma_{PSII} \bullet n_{PSII} = (0.003 - 0.014)$$

1. Optical absorption by PSII: a^{chl}_{PSII}



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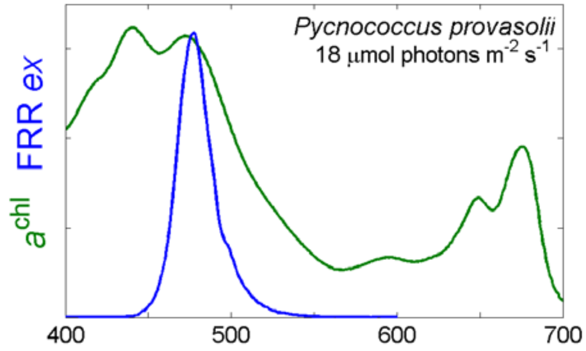
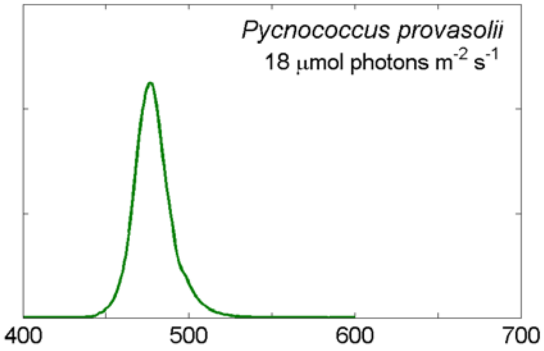


1. Optical absorption by PSII: a^{chl}_{PSII}

1. Effective optical absorption (relative to FRR *ex*)



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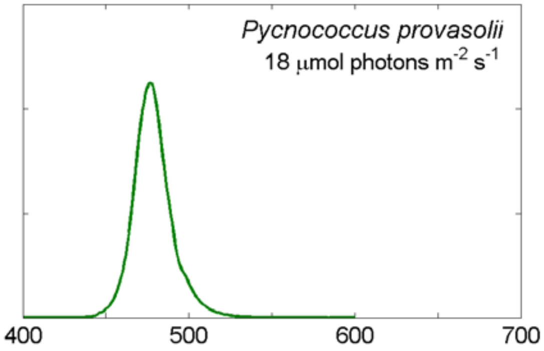

2. Proportion of light absorbed by ‘photochemically active’ pigments

1. Optical absorption by PSII: a^{chl}_{PSII}

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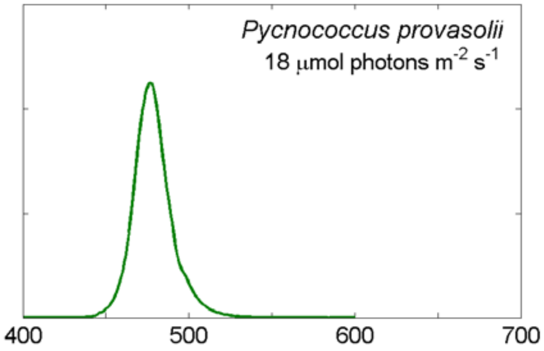
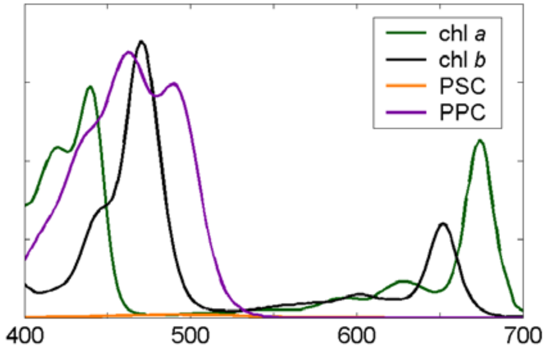
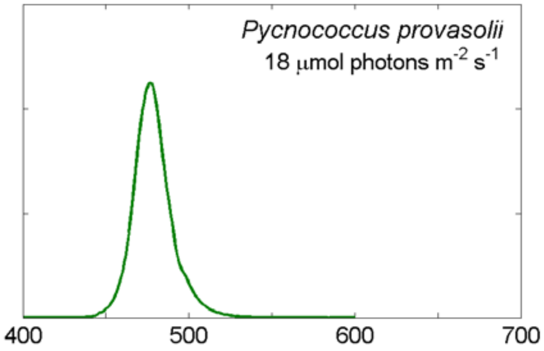




1. Optical absorption by PSII: a^{chl}_{PSII}

1. Effective optical absorption (relative to FRR *ex*) →



2. Proportion of light absorbed by 'photochemically active' pigments (adjusted to FRR *ex*) → 0.59 (59%)

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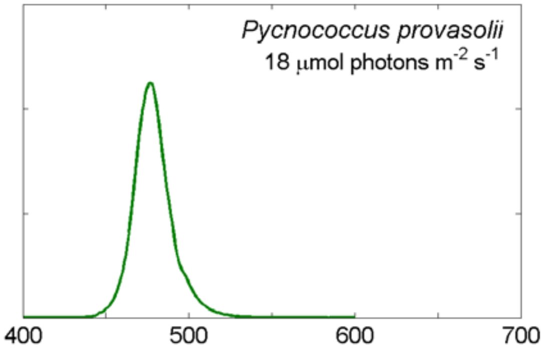

2. Proportion of light absorbed by 'photochemically active' pigments (adjusted to FRR *ex*)

0.59 (59%)

3. Proportion of light absorbed by only PSII

1. Optical absorption by PSII: a^{chl}_{PSII}

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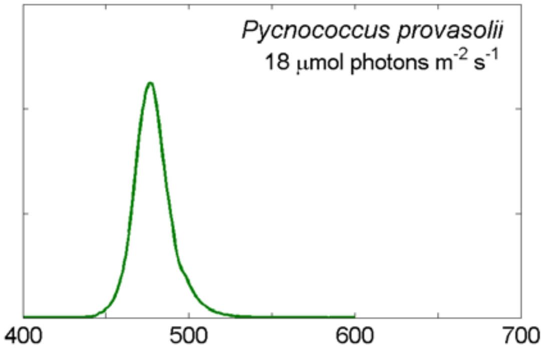

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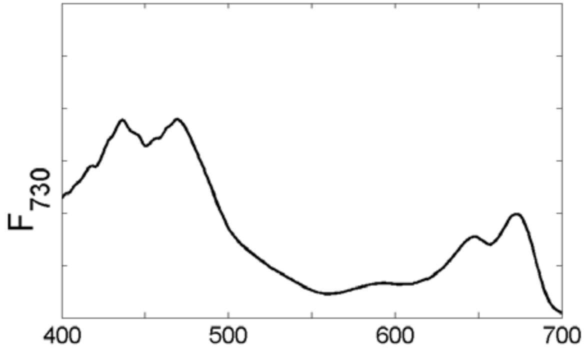

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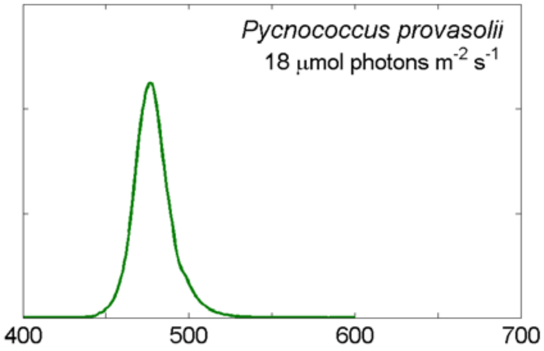

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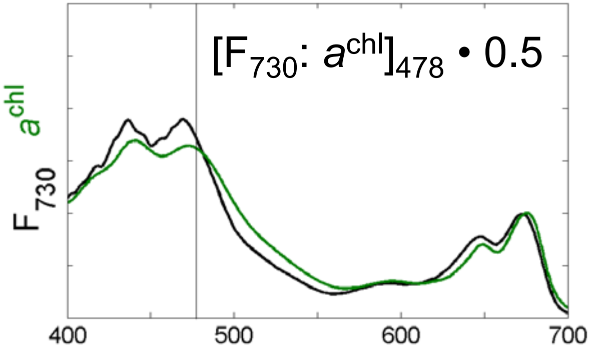

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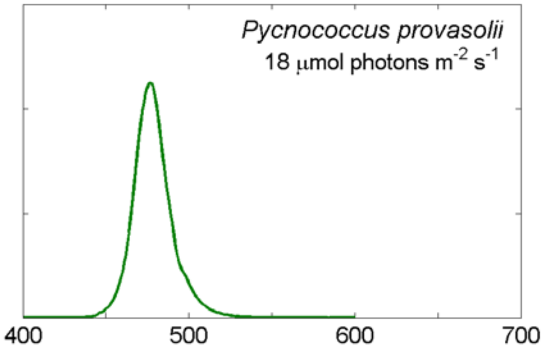
1. Effective optical absorption (relative to FRR *ex*) →



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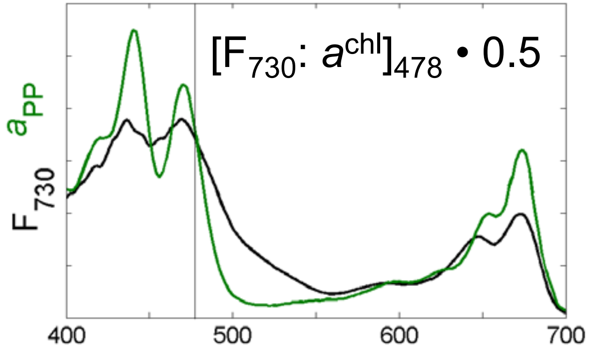

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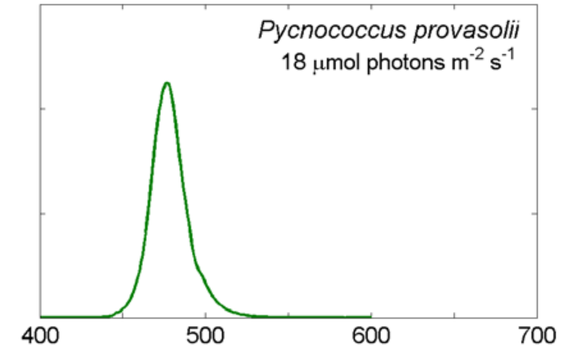
1. Effective optical absorption (relative to FRR *ex*)



2. Proportion of light absorbed by 'photochemically active' pigments (adjusted to FRR *ex*)

0.59 (59%)

3. Proportion of light absorbed by only PSII

0.55 (55%)

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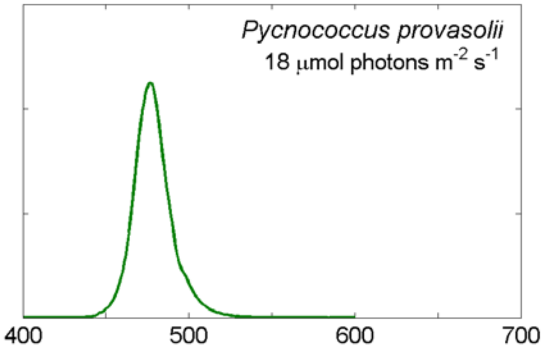
1. Effective optical absorption (relative to FRR *ex*)



2. Proportion of light absorbed by 'photochemically active' pigments (adjusted to FRR *ex*)

0.59 (59%)

3. Proportion of light absorbed by only PSII

0.55 (55%)

$$a^{chl}_{PSII}\ (m^2\ mg\ chl a^{-1}) = 1 \cdot 2 \cdot 3$$

1. Optical absorption by PSII: a^{chl}_{PSII}

Range from 11 spp.

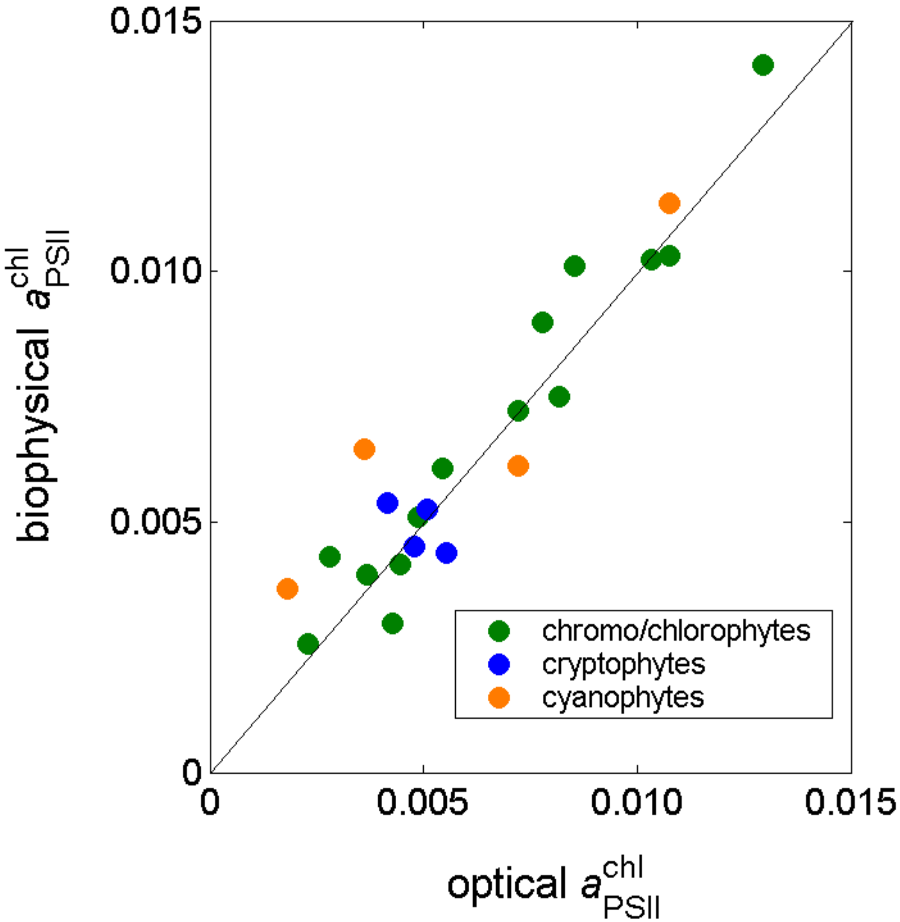
1. Effective optical absorption (relative to FRR *ex*) → 0.007 – 0.058

2. Proportion of light absorbed by 'photochemically active' pigments (adjusted to FRR *ex*) → 42 – 98%

3. Proportion of light absorbed by only PSII → 9 – 57%

a^{chl}_{PSII} (m^2 mg chla^{-1}) = 0.002 – 0.013

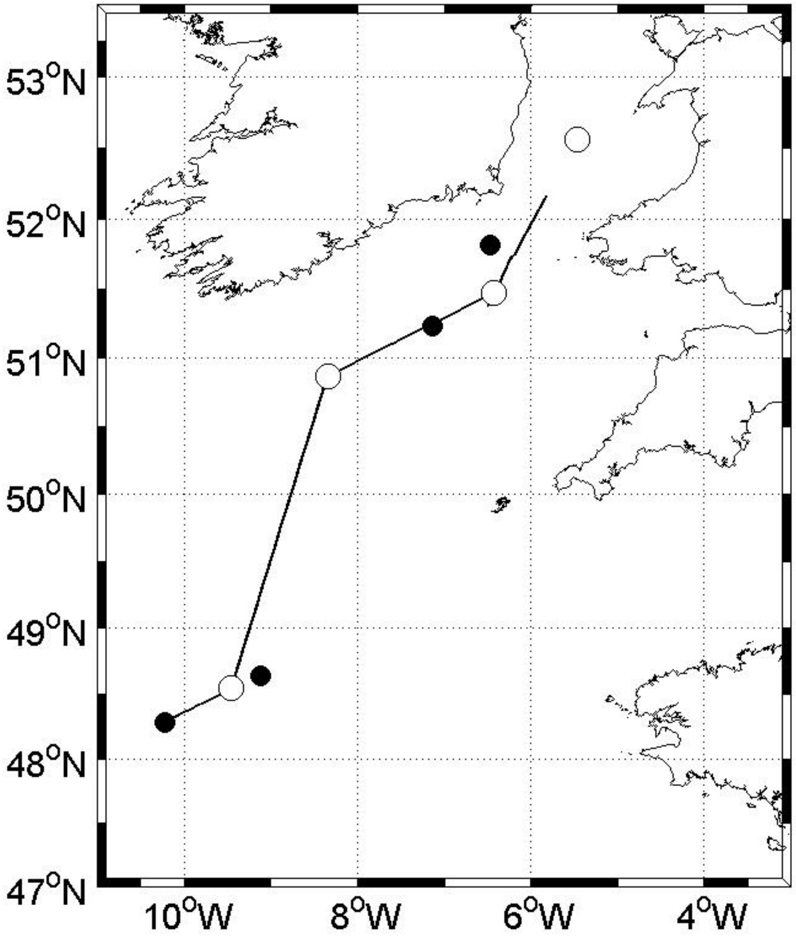
1. Biophysical & Optical a^{chl}_{PSII} compared - laboratory



$y = 1.039x$
($r^2 = 0.874$, $n = 22$, $p<0.001$)

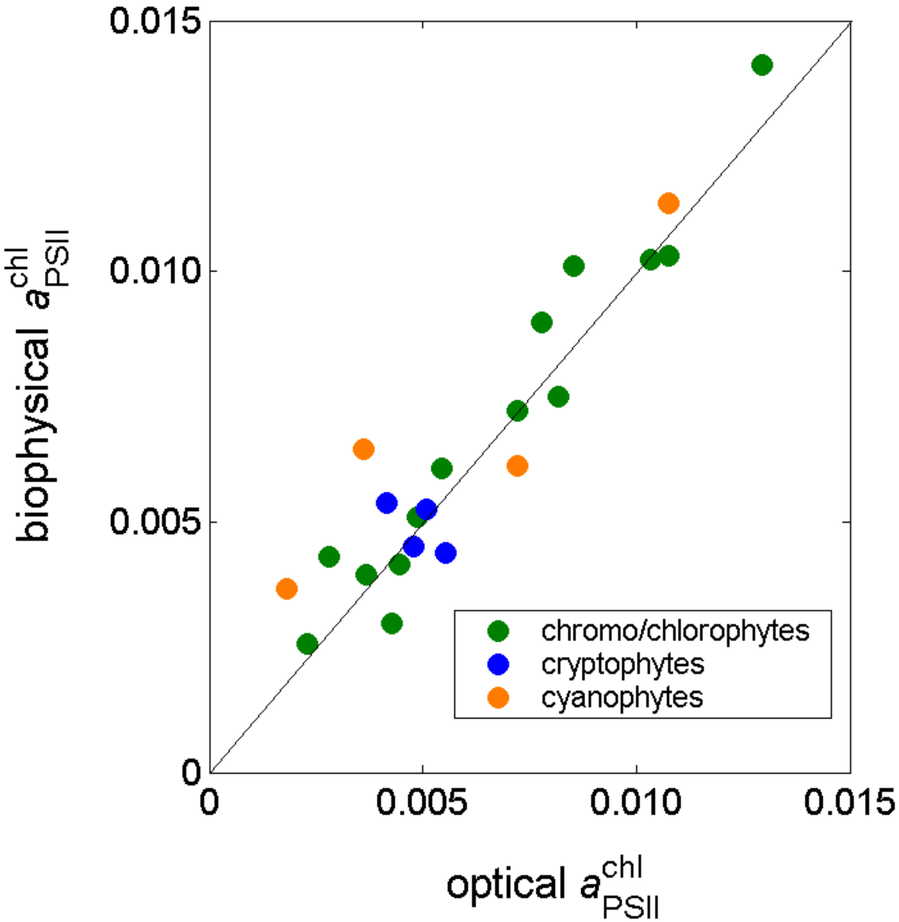
1. Biophysical & Optical a^{chl}_{PSII} compared - field

JR98 Celtic & Irish Seas, August 2003



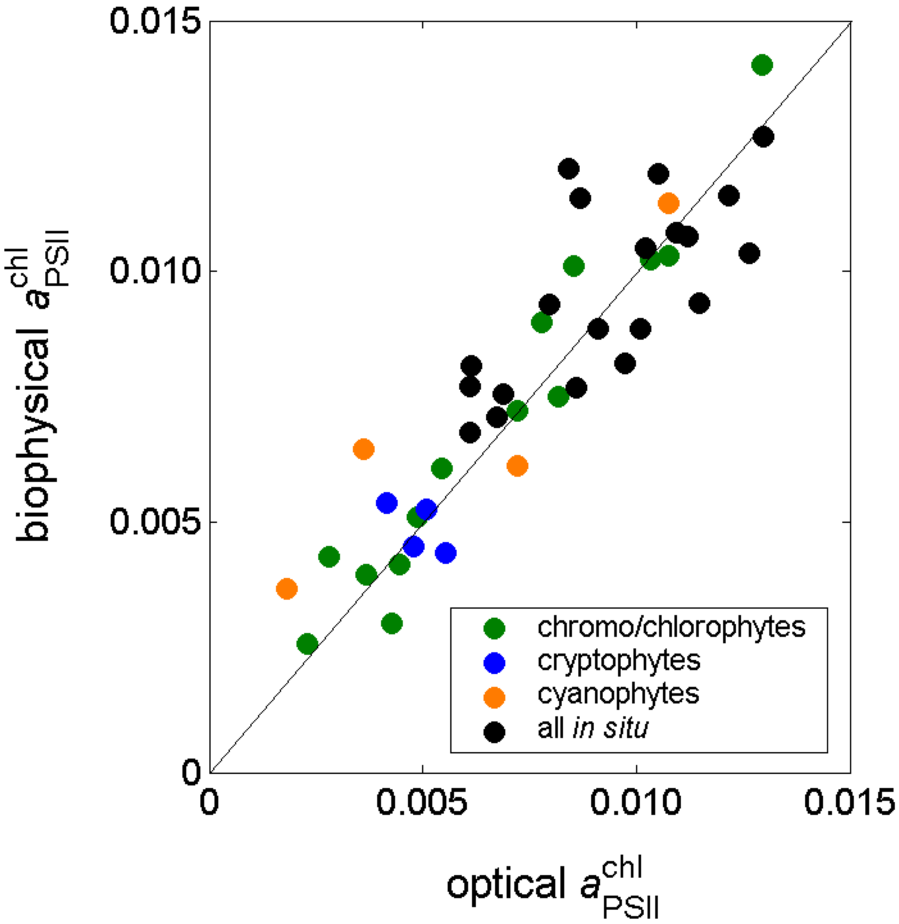
Variable	Range	Units
[chl *a*]	0.34 - 2.38	mg m^{-3}
σ_{PSII}	363 - 690	m^2 mol $RCII^{-1} \cdot 10^{-4}$
$1/n_{PSII}$	456 - 746	mol chl*a* (mol $RCII)^{-1}$
a^{chl} (FRR *ex*)	0.009 – 0.019	m^2 (mg chl*a*$)^{-1}$
% *a* by PP	55.4 – 86.9	%
% *a* by PSII	33.2 – 47.5	%

1. Biophysical & Optical a^{chl}_{PSII} compared - field



y = 1.039x
(r^2 = 0.874, n = 22, $p<0.001$)

1. Biophysical & Optical a^{chl}_{PSII} compared - field



y = 1.039x
(r^2 = 0.874, n = 22, p<0.001)

y = 1.004x
(r^2 = 0.324, n = 20, p<0.01)

1. Biophysical & Optical a^{chl}_{PSII} compared - conclusions

• Biophysical and optical approaches yield comparable rates of light absorption (*provided* several variables are measured)…more confidence in biophysical measurements.

• Assumed values for n_{PSII} or proportion of light absorbed by PSII are a significant source of error.

Factor of difference using assumed relative to measured

Assumption		Eukaryotes	Cyanophytes
assume $[2.0$ *or* $3.3 \cdot 10^{-3}] \cdot [(F_v/F_o)/1.8]$	n_{PSII}	± 1.3	- 4 to 7
assume 0.5	% *a* by PSII	± 1.4	+ 2 to 6

1. Biophysical & Optical a^{chl}_{PSII} compared - conclusions

• Biophysical and optical approaches yield comparable rates of light absorption (*provided* several variables are measured)…more confidence in biophysical measurements.

• Assumed values for n_{PSII} or proportion of light absorbed by PSII are a significant source of error. How does this contribute to the overall error in estimating PSII productivity?

• We still need 'indirect' measurements…Do n_{PSII} and % total absorption by PSII vary significantly in nature and is the variability systematic (can it be predicted)?

Suggett et al. (2003). Eur. J. Phycol. (38)
Suggett et al. (in press) Limnol. Oceanogr: Methods

2. Variability of biophysical a^{chl}_{PSII} - σ_{PSII}

σ_{PSII} reflects the amount of light absorbed for photochemistry:

1. 'Photochemical': 'Non-photochemical pigments'
2. Transfer efficiency of various pigments to chlorophyll a pigment-protein complex
3. RCIIs that are available/functional for linear e^- flow

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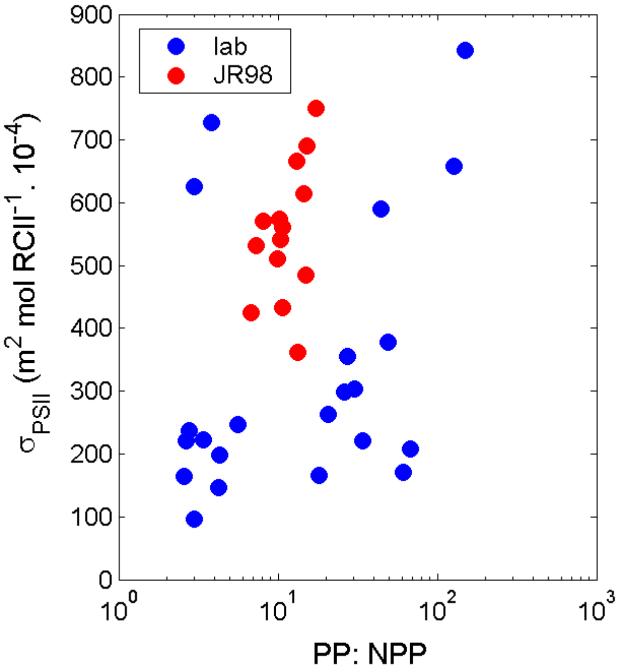

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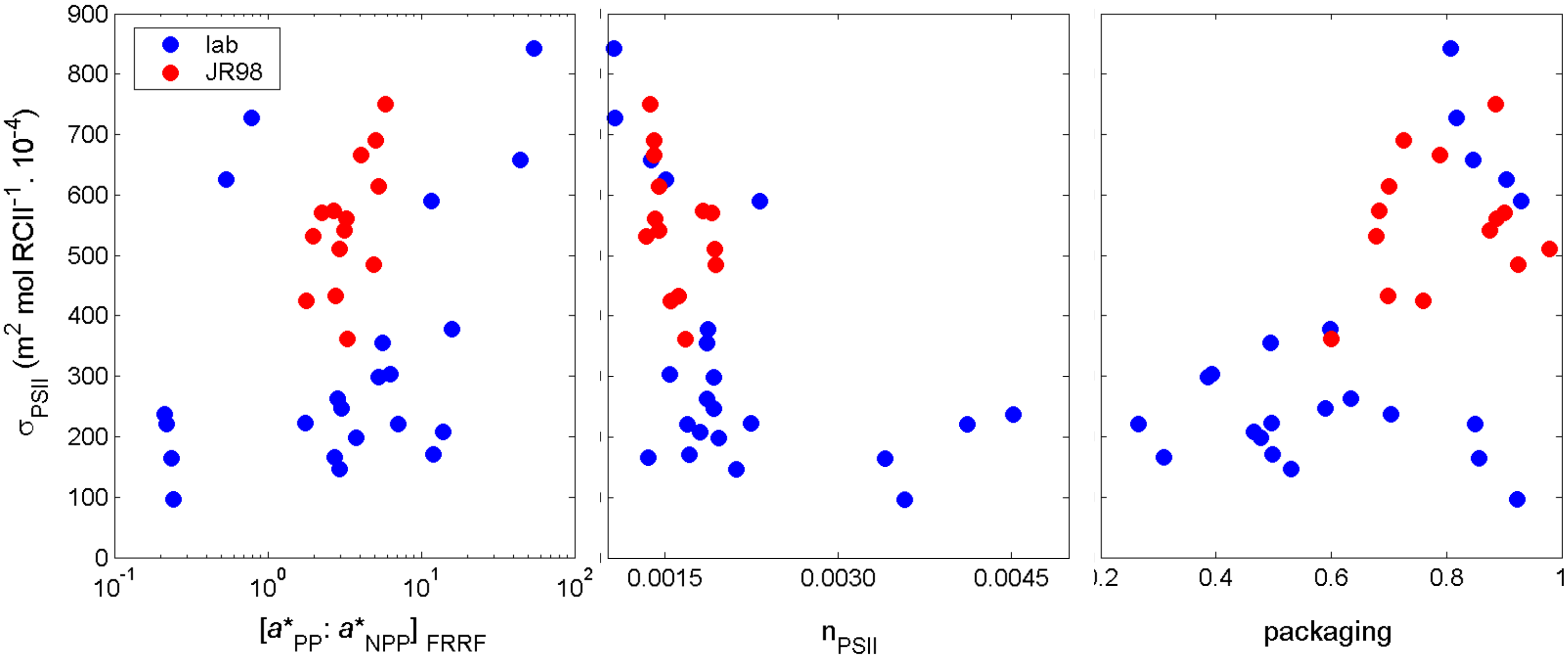
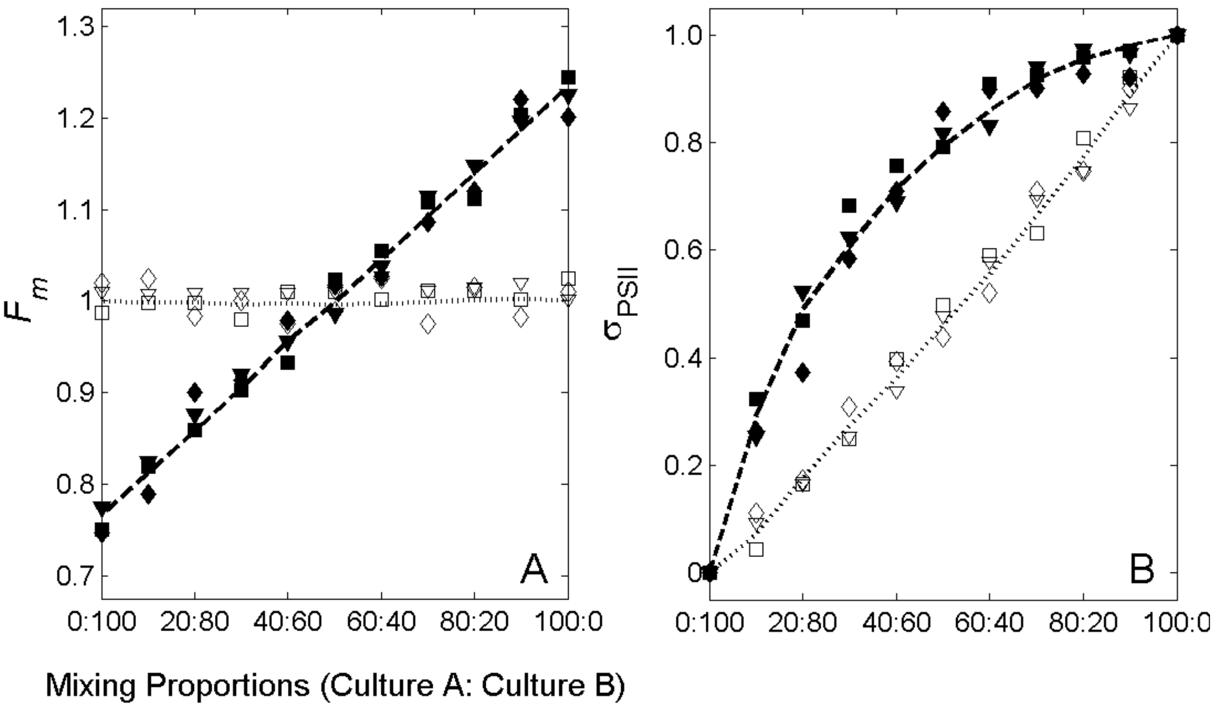


Forward stepwise regression explains >75% of σ_{PSII} variability within and between taxa (with pigment packaging being the greatest predictor of σ_{PSII}.

BUT the remaining variability - error? transfer efficiencies? (What is a photochemcially active versus a non-photochemically active pigment?).

2. Variability of biophysical a^{chl}_{PSII} - σ_{PSII}

Largest variability in σ_{PSII} is observed between taxa. Therefore, changes in σ_{PSII} can be explained by alterations in phytoplankton community structure.



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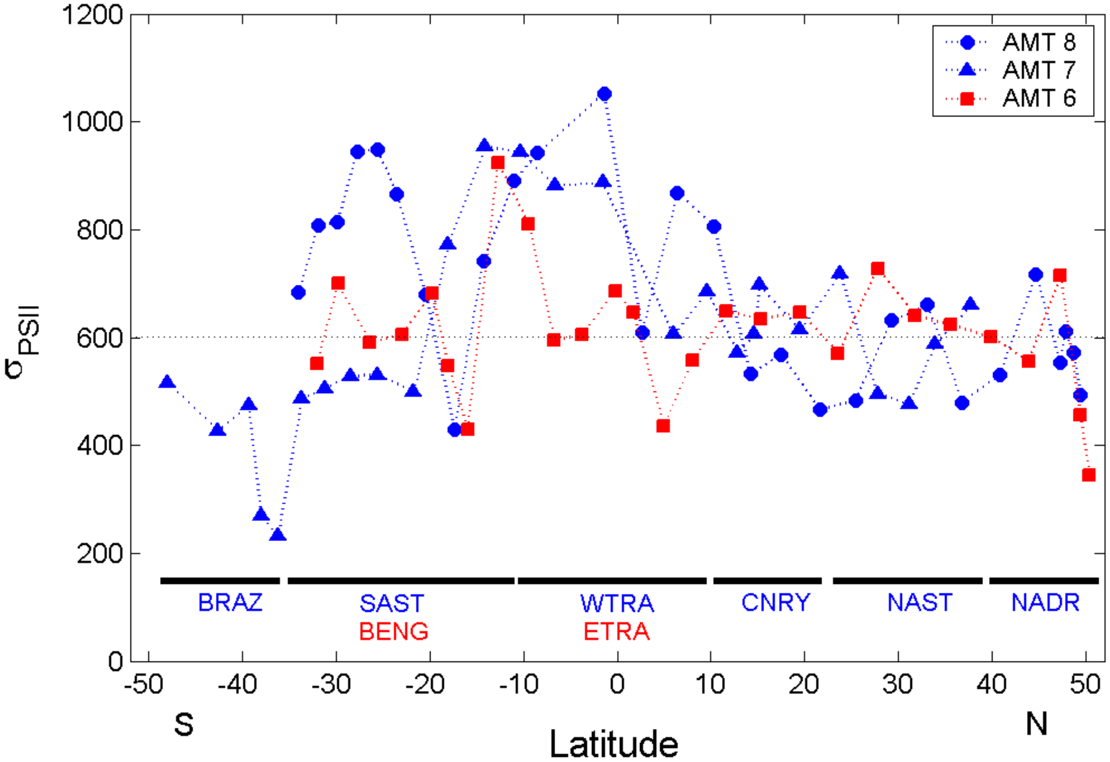
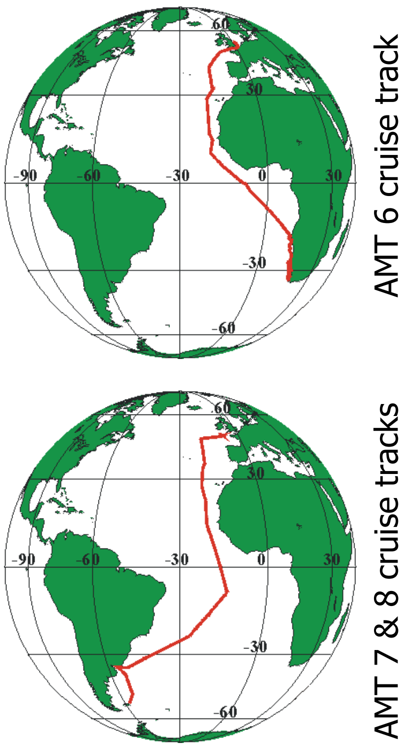
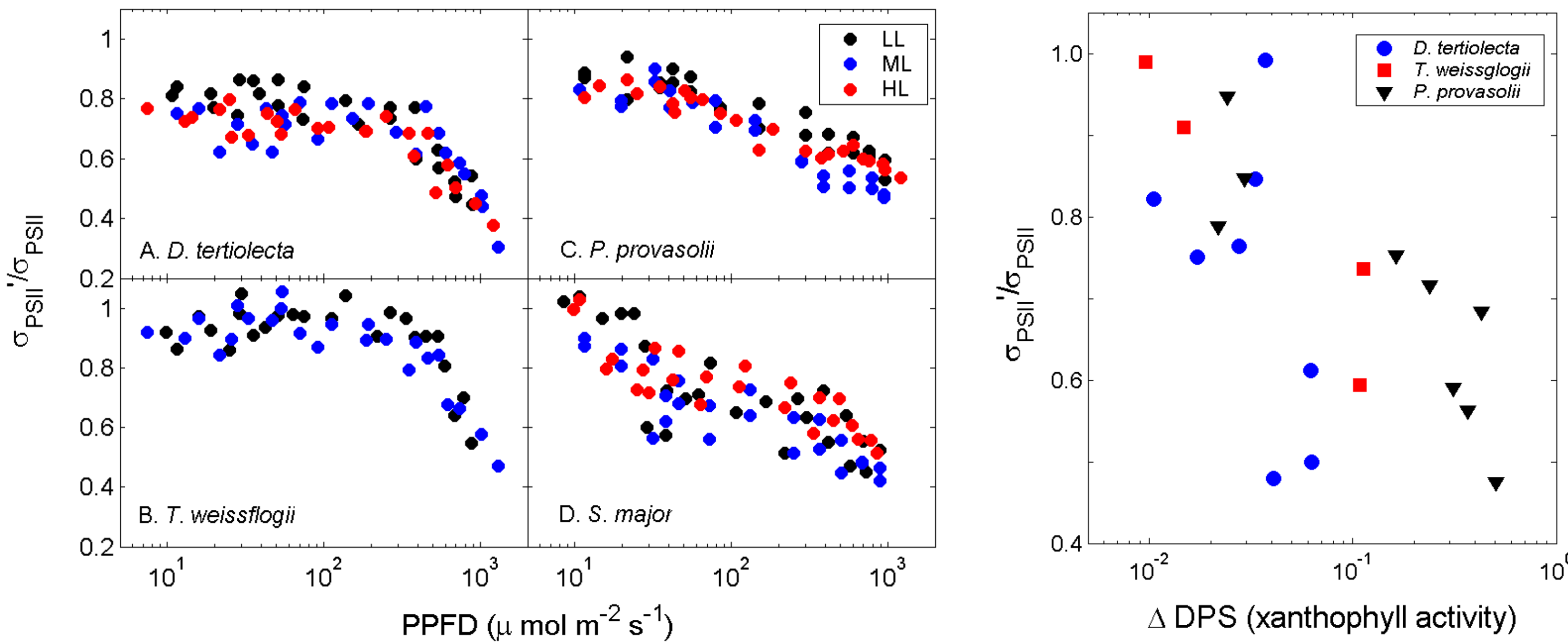




Are the greatest changes of σ_{PSII} in nature the result of shifts in the predominant phytoplankter?

2. Variability of biophysical a^{chl}_{PSII} - σ_{PSII}'

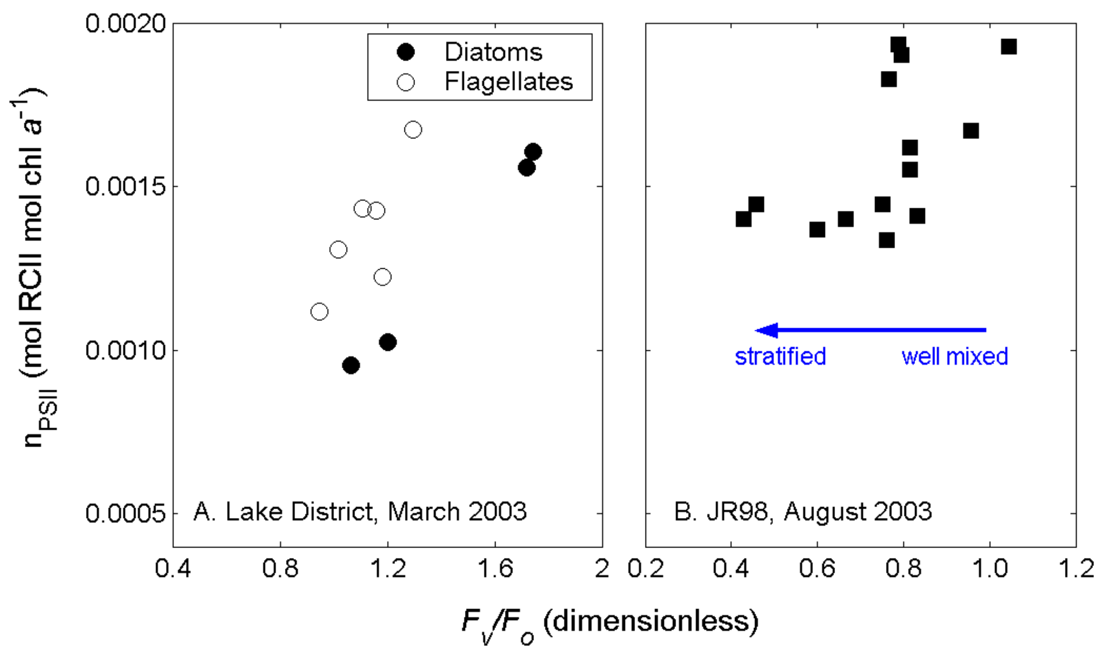
σ_{PSII} also varies under ambient light from non-photochemical quenching of fluorescence in the antenna bed.



Therefore, σ_{PSII} (σ_{PSII}') should be considered:
Routine use in mechanistic PSII productivity models (removing some of the concerns of an appropriate blank?).
Building a highly sensitive light absorption component into acclimation models.

2. Variability of biophysical a^{chl}_{PSII} - n_{PSII}

Again, expect largest variations between phytoplankton communities. However, variation from RCII availability will also be significant
(Growth acclimation - Δchl *a* > ΔRCII; Growth limitation Δchl *a* < ΔRCII)



Measurements of n_{PSII} currently limited by ability to measure changes in O_2
(Important for accuracy of PSII productivity models).
Also, provides a direct measure of the min. quantum requirement of O_2 evolution (However, n_{PSII} is a measure of net O_2 evolution…)

Future Perspectives...

σ_{PSII} is a highly useful parameter for understanding algal growth. However, we need a better understanding of:

(a) How pigments operate (eg. transfer efficiencies of various pigment compliments).

(b) Control of σ_{PSII} in cyanobacteria.

(b) Environmental dependence of PSI (σ_{PSI} and RCIs) that may act to alter σ_{PSII} but more importantly the energy available for photochemistry.

n_{PSII} is a fundamental parameter to place biophysical absorption into a relevant environmental context. Therefore,

(a) Understand the variability of n_{PSII} from environmental change/multiple limitation.

(b) Accurate n_{PSII} from O_2 (or CO_2?) must quantify factors that alter the min. quantum requirement for O_2 evolution and intracellular O_2 consumption….What do our 'productivity' estimates using fluorescence currently mean.

Acknowledgements

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