Multidimensional Signatures of Photo-Osmotic Adaptation in *Dunaliella salina*

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Dunaliella salina Biotechnology

[*]DR Smith, RW Lee, JC Cushman, JK Magnuson, D Tran, JW Polle (2010) The *Dunaliella salina* organelle genomes: large sequences, inflated with intronic and intergenic DNA. *BMC Plant Biology*, 10:83

Implications for Dunaliella:

Allows "development of a viable plastid **transformation system** for this model alga… placing *D. salina* in a group of a select few photosynthetic eukaryotes for which complete genome sequences from **all three genetic compartments** are available"*

Mowry Slough: Newark, CA

Implications for alga:

Adaptive halophilicity in *D. salina* provides genetic feedstock for developing **predation-resistant strains** for use in **compromised waters and open systems**

Is *Dunaliella* a suitable biodiesel species?

Lipid yield: 37% organic basis

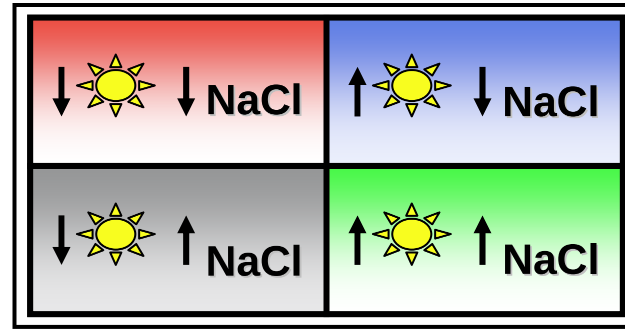
Dunaliella salina **FAME:**

✓ Superb combustion quality (cetane number = 55)
✓ High cold flow
✓ High lubricity - ‘zero’ engine wear scar
✓ Acceptable viscosity
✓ Low sulfur
✓ Low aromatics
✓ Low particulate matter
✓ Carbon neutral
- Poor oxidative stability

Mendoza, H., Martel, A., Jiménez del Río, M. & García Reina, G. (1999) Oleic acid is the main fatty acid related with carotenogenesis in *Dunaliella salina*. *Proceedings of the International Seaweed Symposium* 16: 529-533.

McCormick R., Graboski M., Alleman T., Herring A., and Shainetyson K. (2001) Impact of biodiesel source material and chemical structure on emissions of criteria pollutants from a heavy-duty engine. *Environ. Sci. Technol.*, 35, 1742-1747

Photo-Osmotic Adaptation Matrix



Steady-state Cultures

- lab scale, semi-continuous, gentle aeration constant temperature (27°C), log phase
- 3 PAR levels: 50 µE, 150 µE, 275 µE
- 3 osmotic levels: 0.5M, 1.5M, 2.5M NaCl

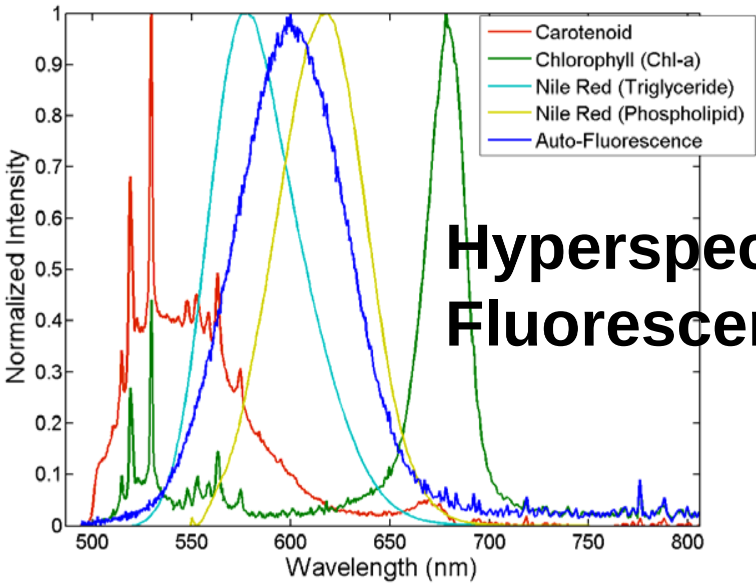
Dynamic (environmentally stressed) Cultures

- Nitrate limitation
- Osmotic shock (+ blocking stretch channels)
- Inhibition of carotenoid biosyntheis (DPA)

Multi-modal + multi-objective optimization

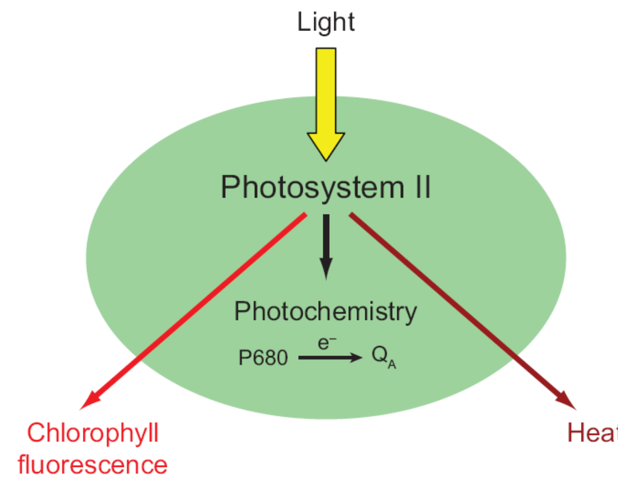
- Reveal correlations among steady-state culture conditions and biochemical, morphological, and photosynthetic observables
- Develop comparative matricies for evaluating impact of environmentally stressed cultures
- Demonstrate optimization strategy for variety of algal bio-products based on growth conditions and end product

Analytical Methods

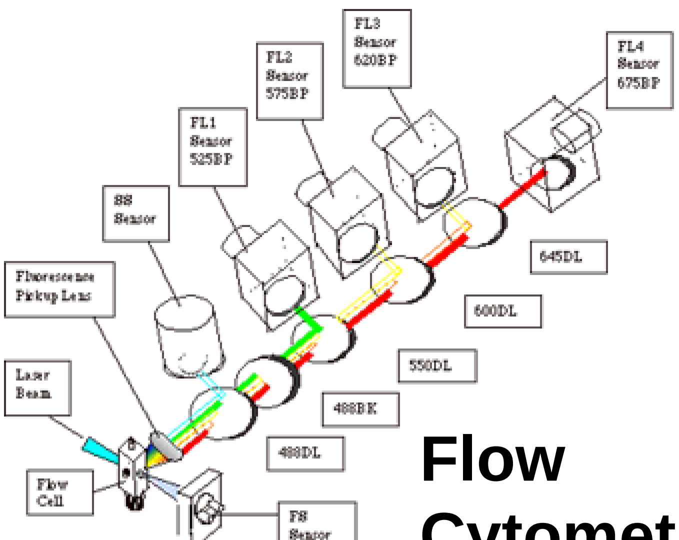


Hyperspectral Fluorescence

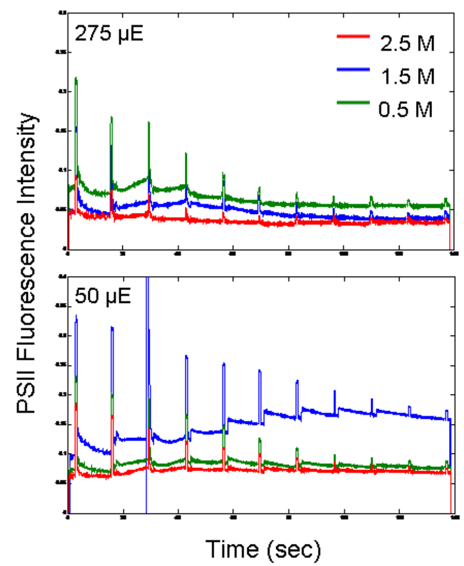
PAM Fluorometry

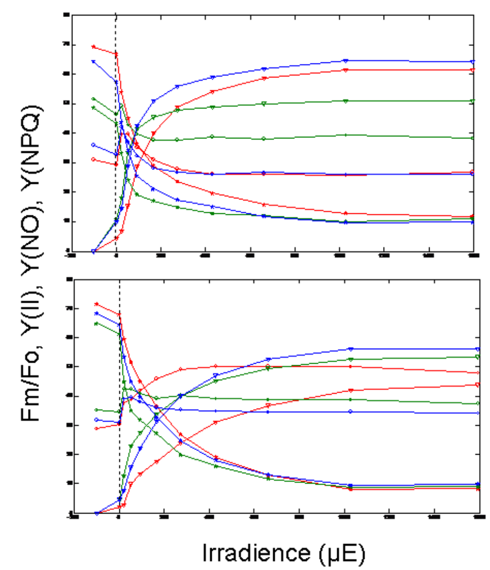


W. Bilger et al., "Determination of the quantum efficiency of photosystem II and of non-photochemical quenching of chlorophyll fluorescence in the field," *Oecologia* **102**, 425-432 (1995).



Flow Cytometry





21 Measured Parameters

1) Specific Growth Rate (S.G.R, FC)
2) Cell volume (FC)
3) [Carotenoid]/cell (FC)
4) [ChlA]/cell (FC)
5) [ChlB]/cell (FC)
6) [Lipid]/cell (FC: Nile Red)
7) Cellular complexity (FC)
8) Cellular stability (FC: debris/cell)
9) Motility (FC: depth)
10) Intracellular pH (pH_{int}, FC: SNARF)
11) Medium pH (pH_{ext}, pH meter)
12) Intracellular calcium ($[Ca^{2+}]_{int}$, FC: Fura-Red)
13) Nitrate consumption ($\Delta[NO_3^-]$, I.S.E)
14) NAD(P)H consumption (ΔNAD(P)H, dPAM)
15) Maximum photosynthetic efficiency (Fm/Fo, dPAM)
16) Effective photosynth. eff. @ 50μE (Y(II) 50, RLS-PAM)
17) Effective photosynth. eff. @ 275μE (Y(II) 275, RLS-PAM)
18) Non-photochemical quenching @ 50μE (Y(NPQ 275, RLS-PAM)
19) Non-photochemical quenching @ 275μE (Y(NPQ) 50, RLS-PAM)
20) Non-protective non-photochemical quenching @50μE (Y(NO) 50, RLS-PAM)
21) Non-protective non-photochemical quenching @275μE (Y(NO) 275, RLS-PAM

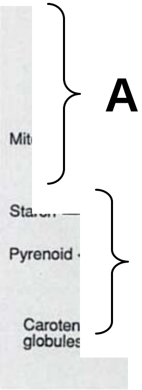
Biomass productivity (1–2)

Algal bioproducts (3–6)

Ease of harvest parameters (7–9)

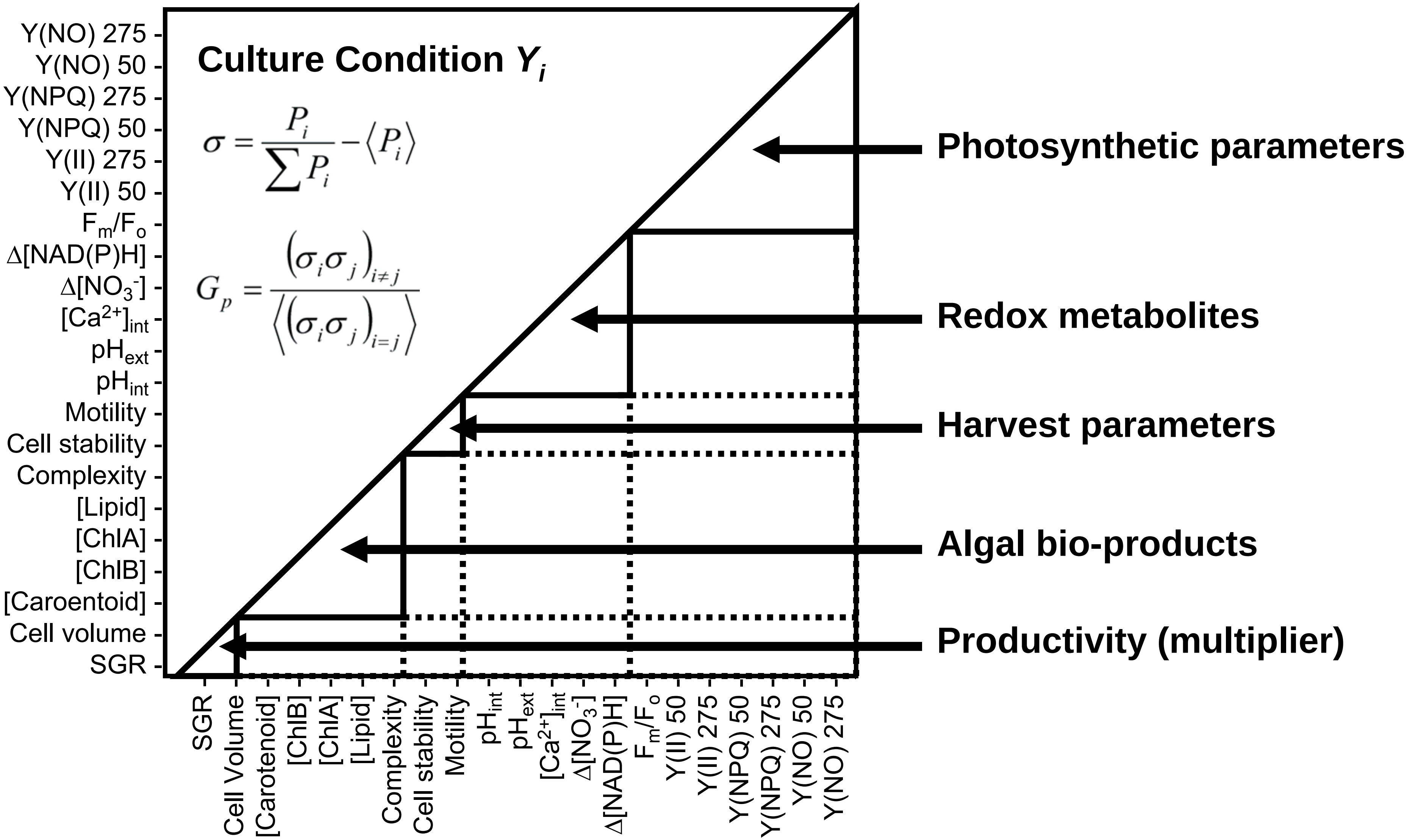
Basic metabolic parameters (10–14)

Photo-synthetic parameters (15–21)

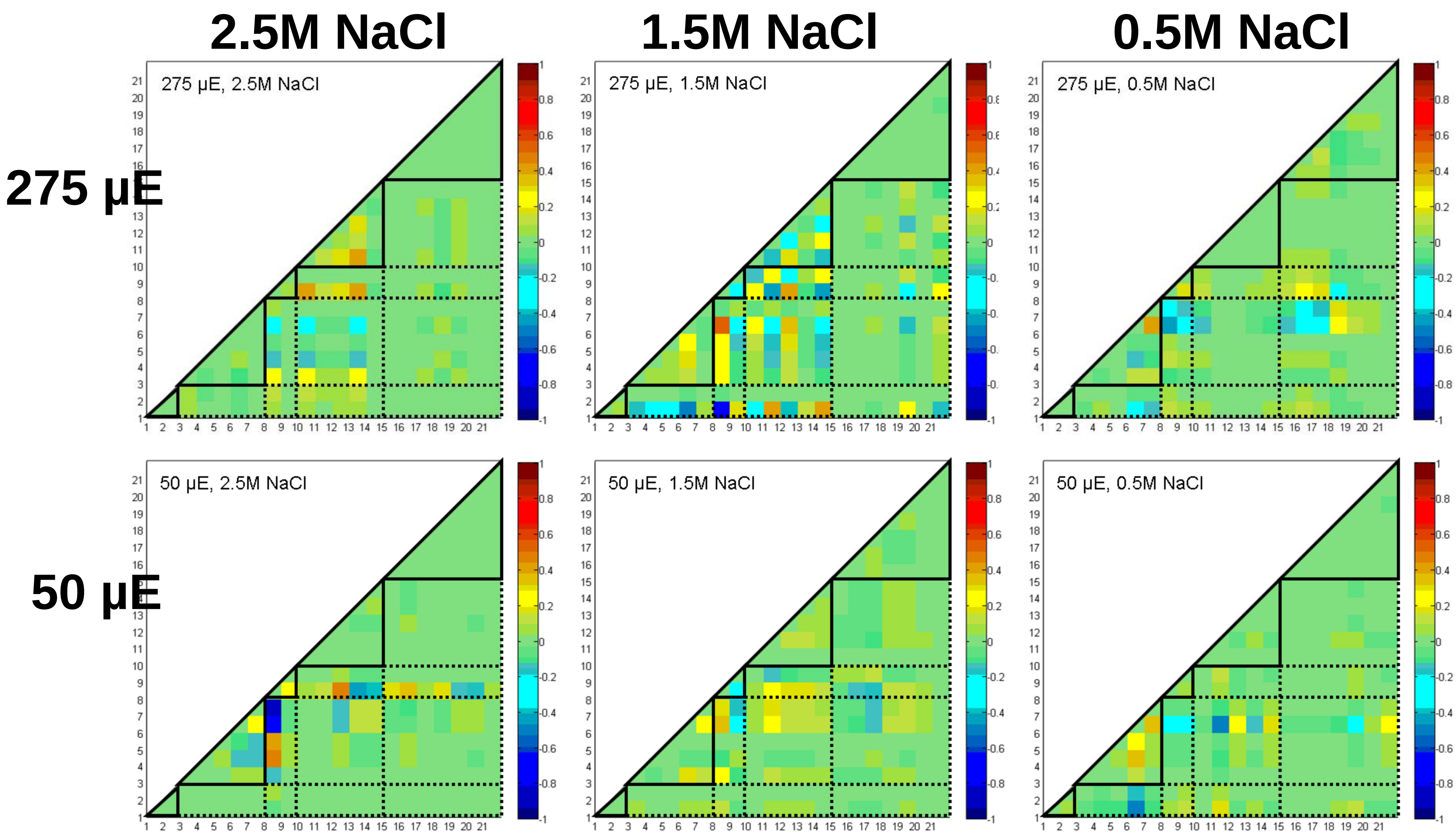


Optimization Method 1: Correlating Observables

(pair-wise for each culture condition at steady-state)



Correlations among observables, by growth condition



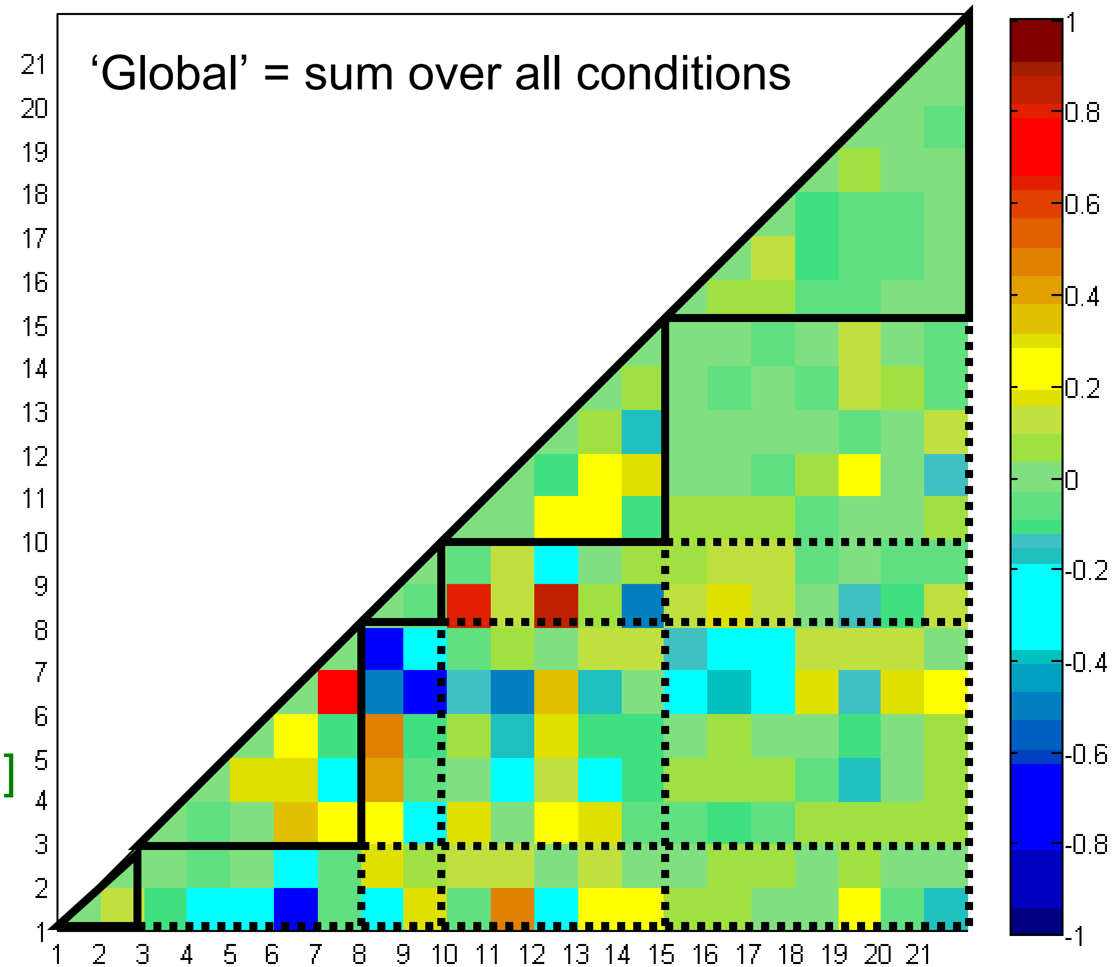
Global Correlations Among *Dunaliella salina* Observables

Positive correlations:

[12,8]: $[Ca^{2+}]_{int}$ • pH_{int}
[7,6]: [Lipid] • complexity
[10,8]: pH_{int} • motility
[11,1]: pH_{ext} • SGR
[8,5]: motility • [ChlA]
[12,6]: $[Ca^{2+}]_{int}$ • [Lipid]
[18,6]: Y(NPQ) 50 • [Lipid]
[20,6]: Y(NO) 50 • [Lipid]

Anti-correlated parameters:

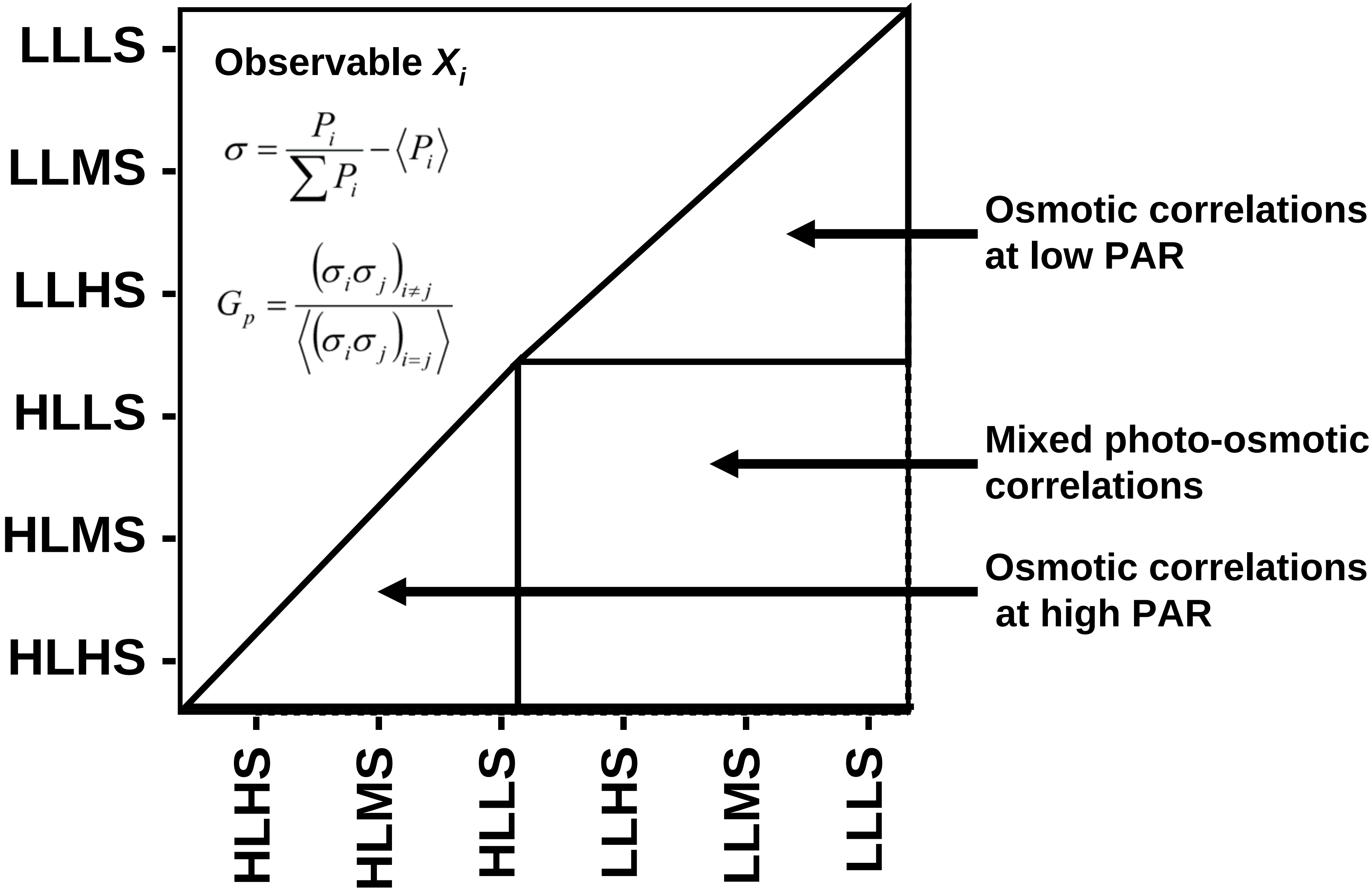
[1,6]: SGR • [Lipid]
[8,7]: motility • complexity
[9,6]: mechanical stability • [Lipid]
[11,6]: pH_{ext} • [Lipid]
[8,6]: motility • [Lipid]
[14,8]: Δ[NAD(P)H] • motility
[15,7]: F_m/F_o • complexity
[16,6]: Y(II) 50 • [Lipid]



physiological biomarker discovery
+ correlation v. causation
= simplified detection of preferred traits

Optimization Method 2: Correlating Culture Conditions

(pair-wise for each observable at steady-state)



Example: condition optimization for maximum carotenoid, chlorophyll, and lipid

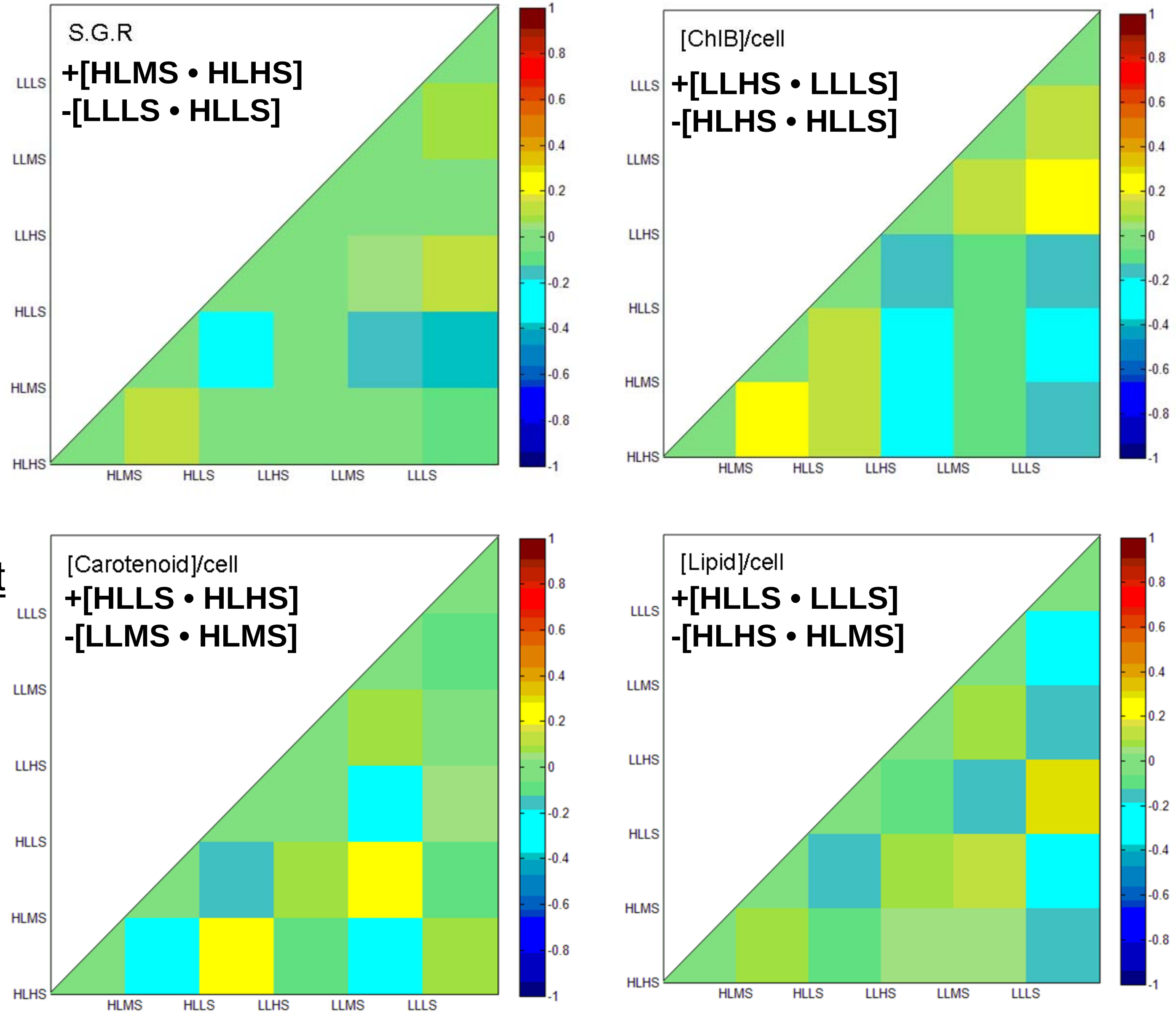
Method isolates dominant factors for each observable:

<u>Optimal for growth</u>
275μE, 2M NaCl

<u>Optimal for carotenoid</u>
275μE, 2.5M NaCl

<u>Optimal for Chl-pigment</u>
50μE, 1.5M NaCl

<u>Optimal for lipid</u>
112.5μE, 0.5M NaCl

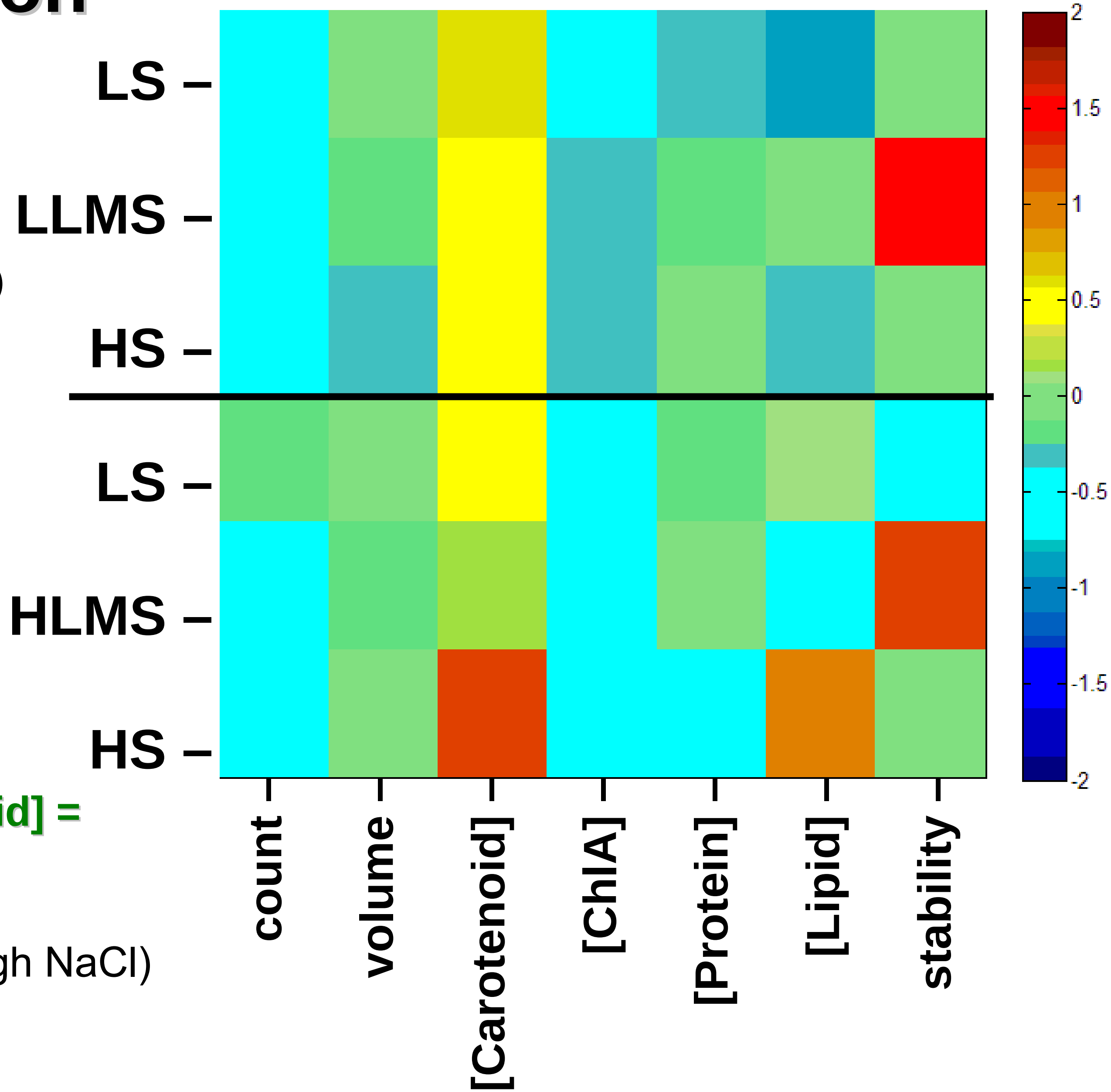


Dynamic cultures 1: nitrate limitation

- lipid "'trigger" only in high PAR, high NaCl (+100% increase in TAG)

- carotenoid enrichment in all conditions

- cell count diminished in all conditions (up to -50%)

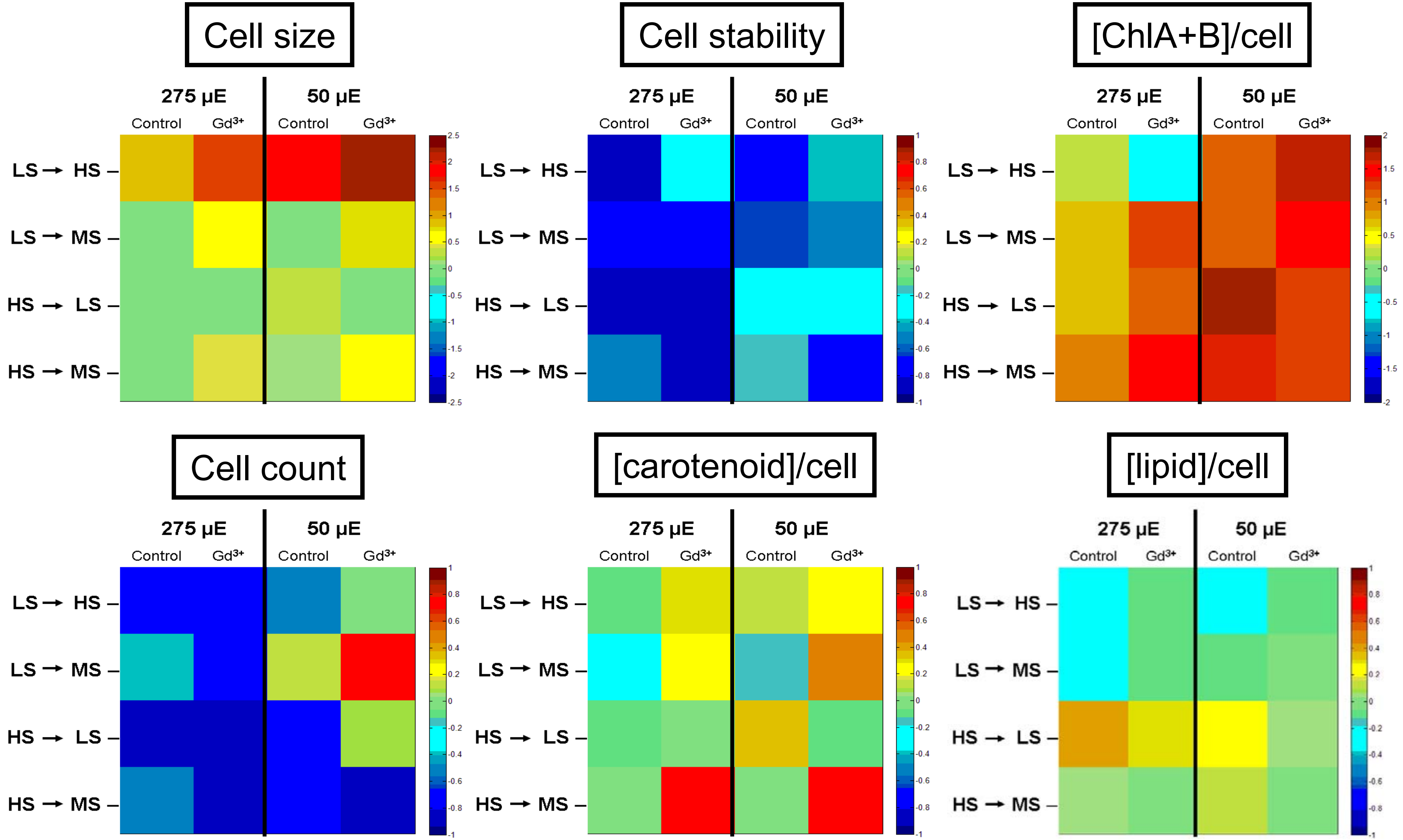


[count] x [volume] x [lipid] = 1.5x control

(but only for high PAR, high NaCl)

Dynamic cultures 2: osmo-stress

(+ block of stretch-activated Ca^{2+} channels)



Challenge:

Find conditions for maximum total lipid (lipid x biomass) using a 2-stage growth model.

Top 2 Strategies:

1) **steady-state growth at high PAR, 2.0M NaCl followed by short-term environmental stress with low nitrate: +44% lipid**

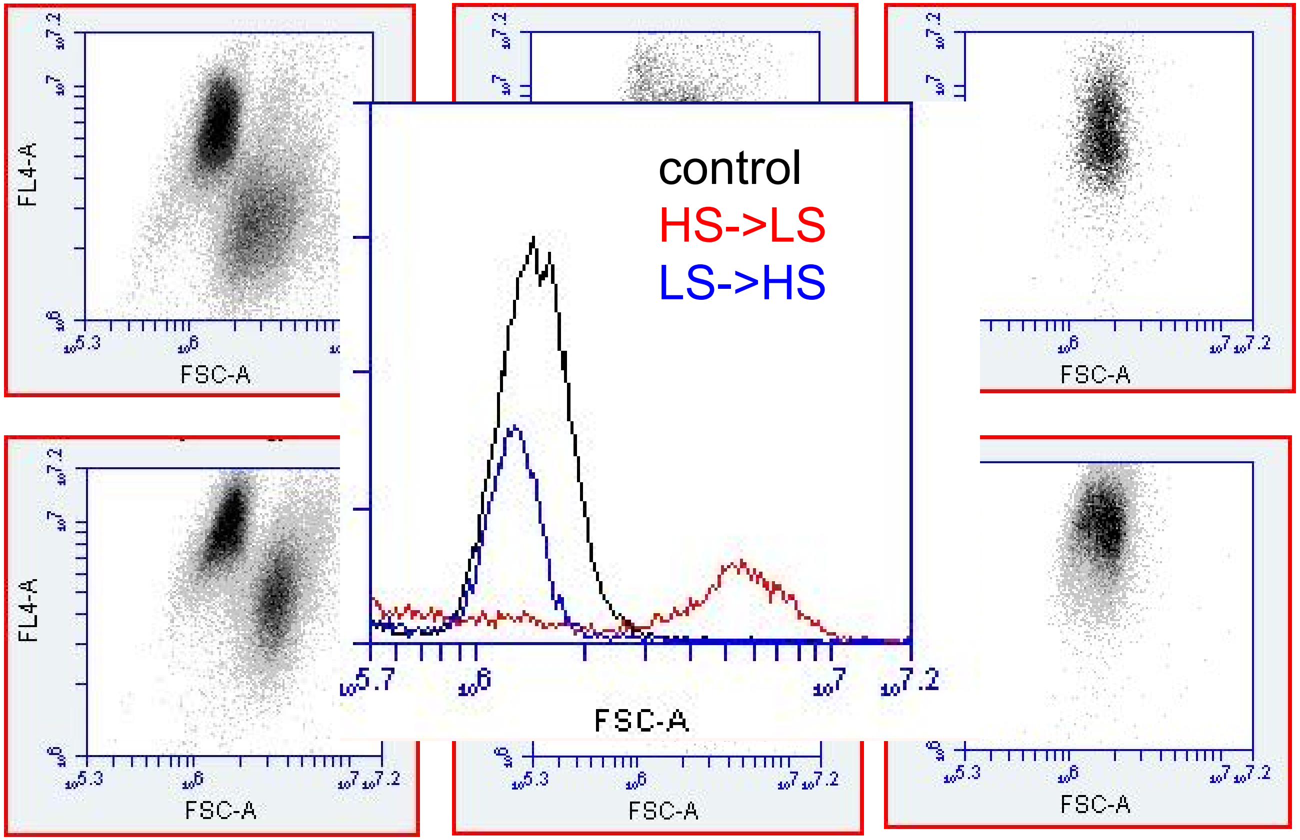
2) **steady-state growth at high PAR, 2.0M NaCl followed by short-term environmental stress with 0.5M NaCl: +31% lipid**

Photo-Osmotic Duality: is there a mechanism for *direct* involvement of carotenoids in the osmotic shock response?

2.5M NaCl | 1.5M NaCl | 0.5 M NaCl

275 µE

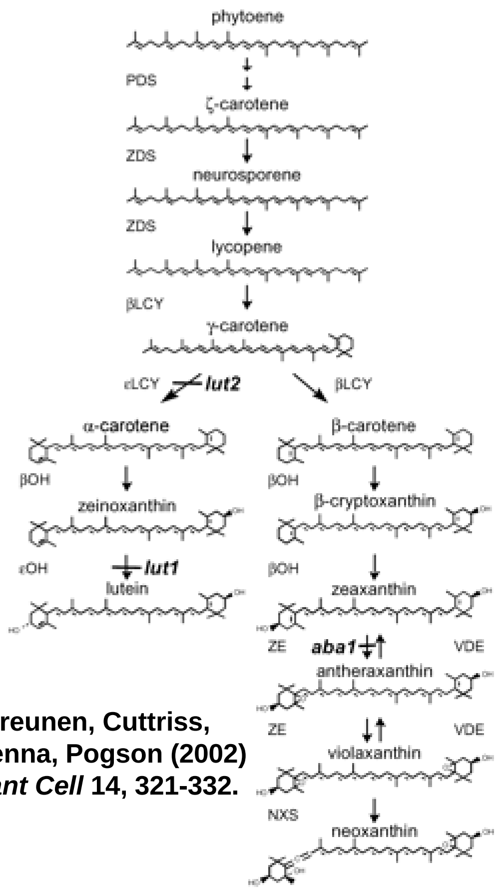
50 µE



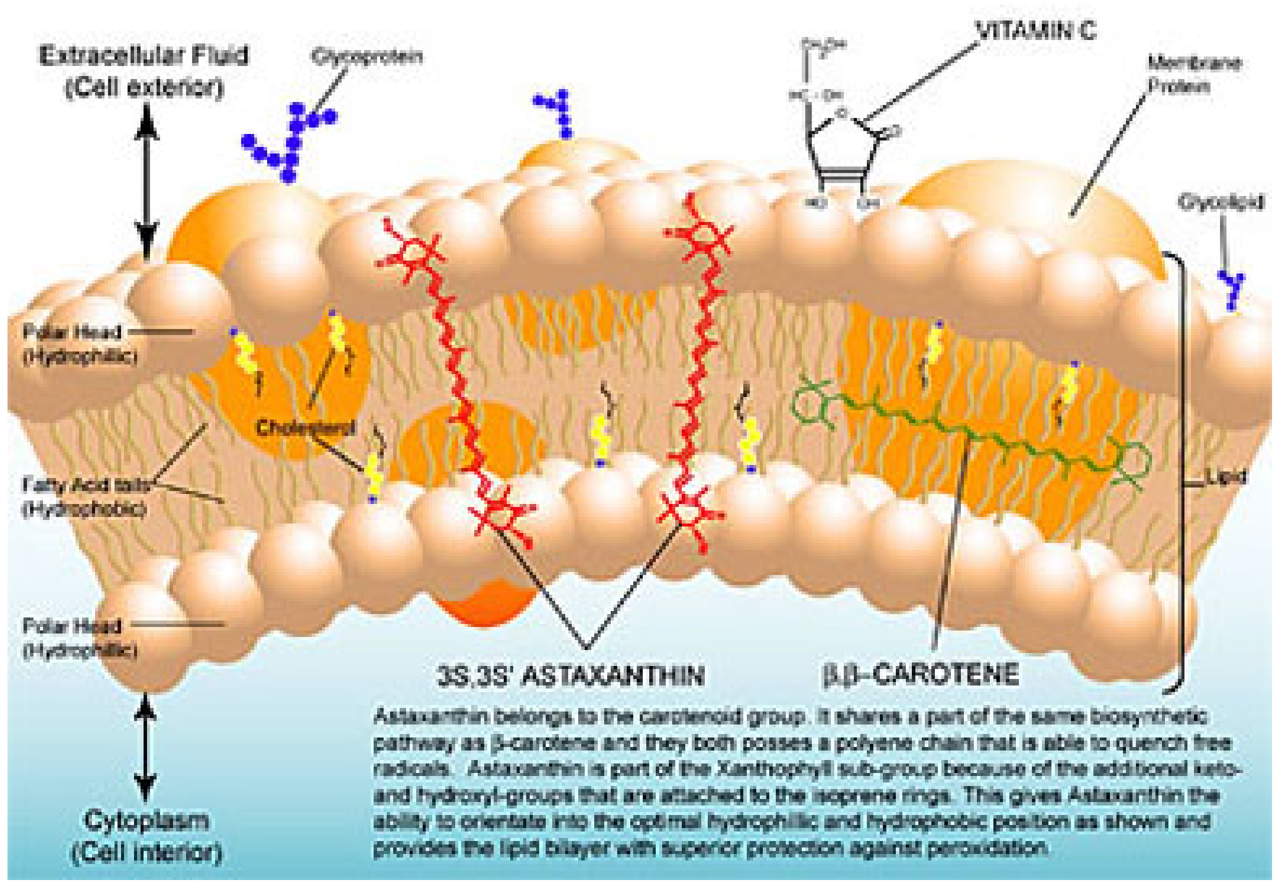
DPA block of carotenoid biosynthesis induces morphological symptom of osmotic shock in elevated salt conditions

Polyakov NE, Focsan AL, Bowman MK, Kispert LD (2010) Free radical formation in novel carotenoid metal ion complexes of astaxanthin *J. Phys. Chem. B.* 114(50):16968-77.

Carotenoids: hydroxylated?



Park, Kreunen, Cuttriss, DellaPenna, Pogson (2002) *The Plant Cell* 14, 321-332.



Lignell & Wood (2004) *Innovations in Food Technology*

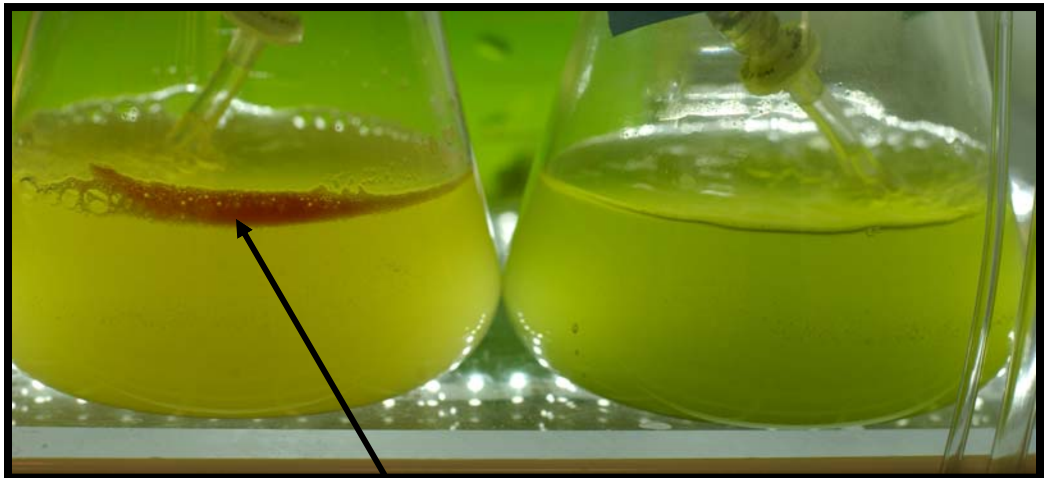
Protection from:

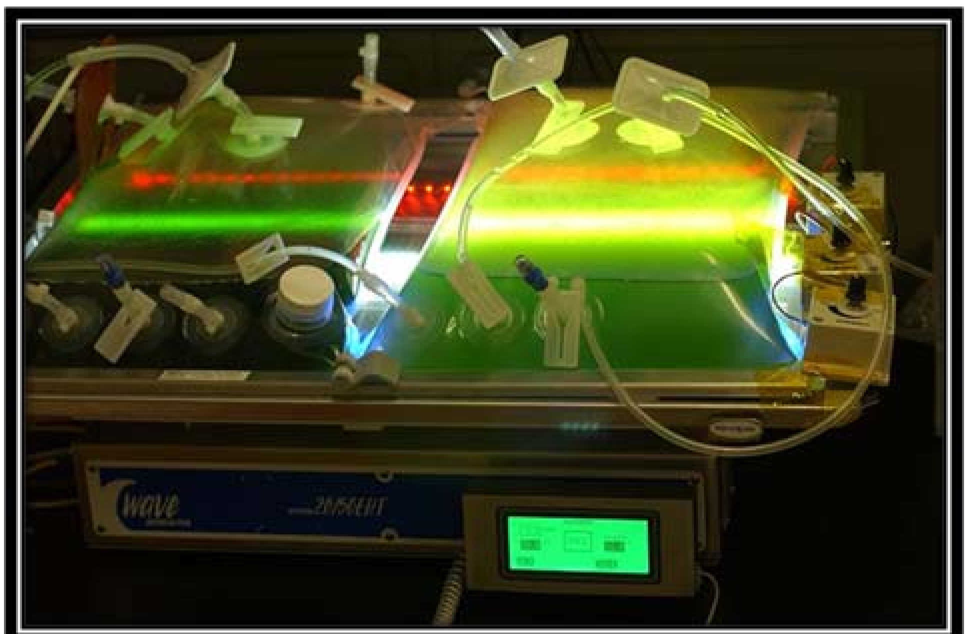
- Excessive light (PAR, UV)
- Free-radicals
- elevated $[Ca^{2+}]_{int}$?

Jin E, Ferth B, Melis A (2003) A mutant of the green alga *Dunaliella salina* constitutively accumulates xeaxanthin under all growth conditions. *Biotechnol. Bioeng.* 81, 115-24.

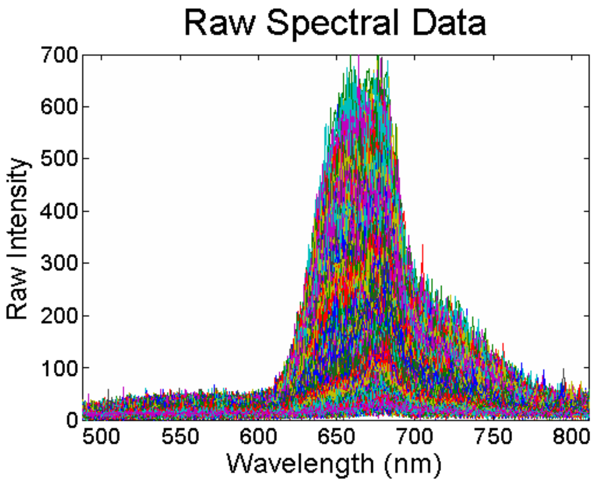
Conclusions

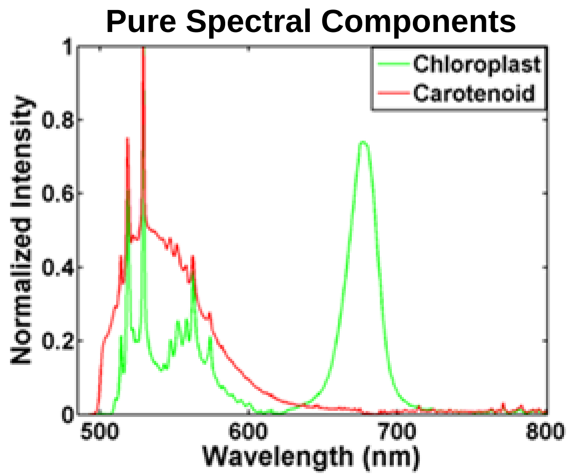
- Rigorous methods and visualization tools for comparing and optimizing multiple parameters in steady-state and dynamic cultures
- Isolation of biomarkers for physiological responses and corresponding yields of algal bio-products
- Predict multi-stage growth condition for maximum lipid production in *Dunaliella salina*
- Show evidence for active role of (hydroxy?) carotenoids in calcium-mediated osmo-regulation

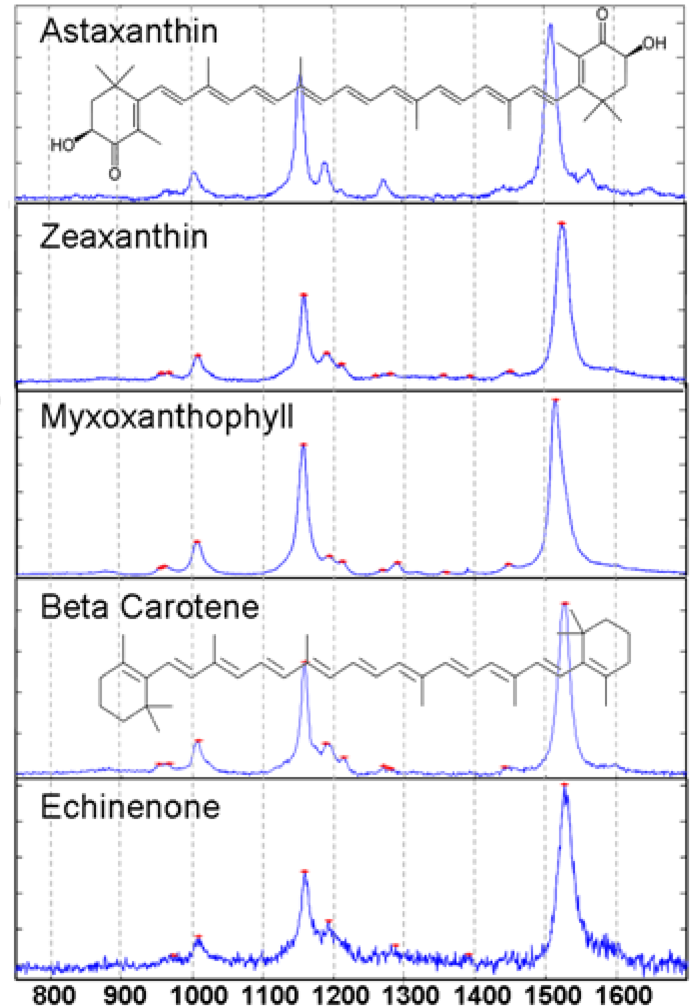

β-carotene

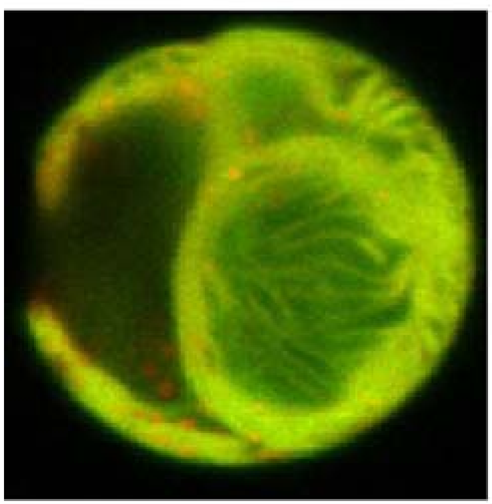


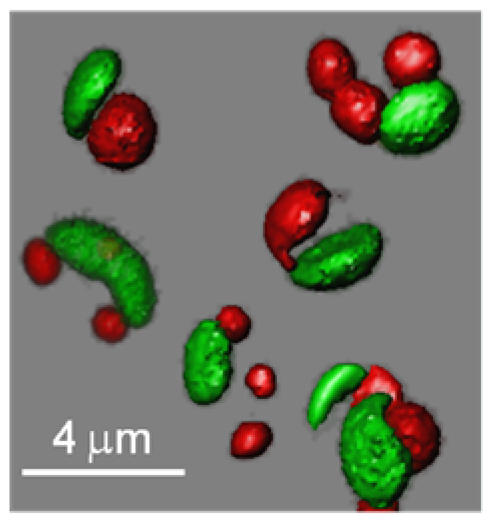
SNL's Spectroscopic Capabilities

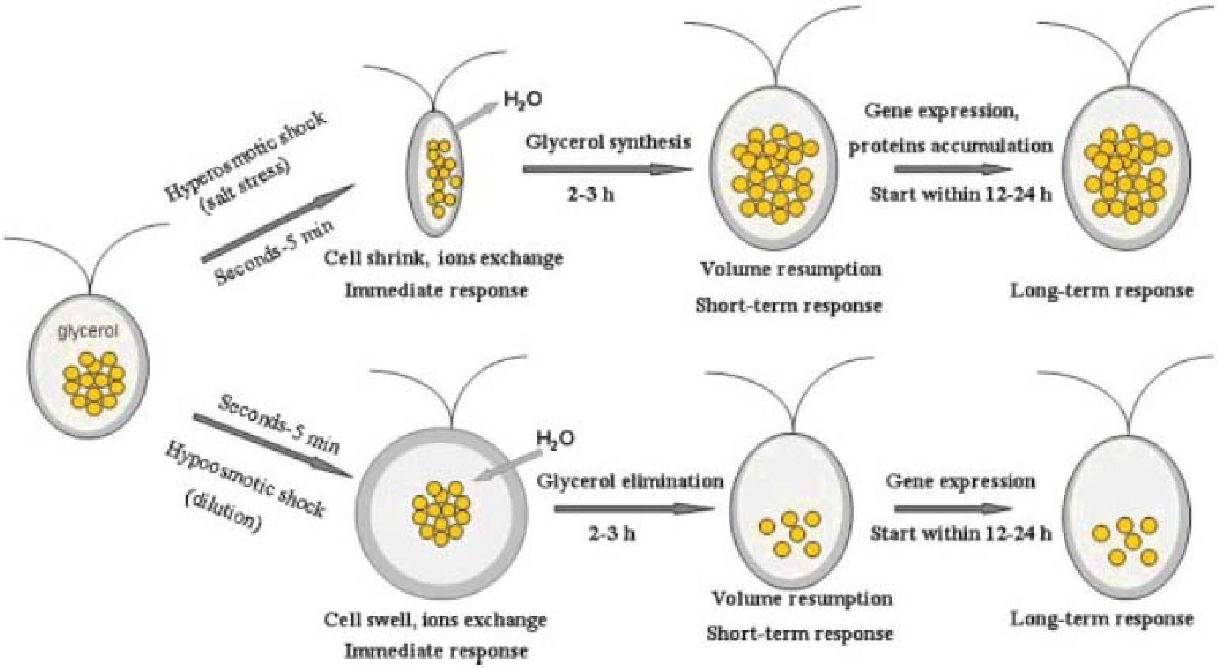


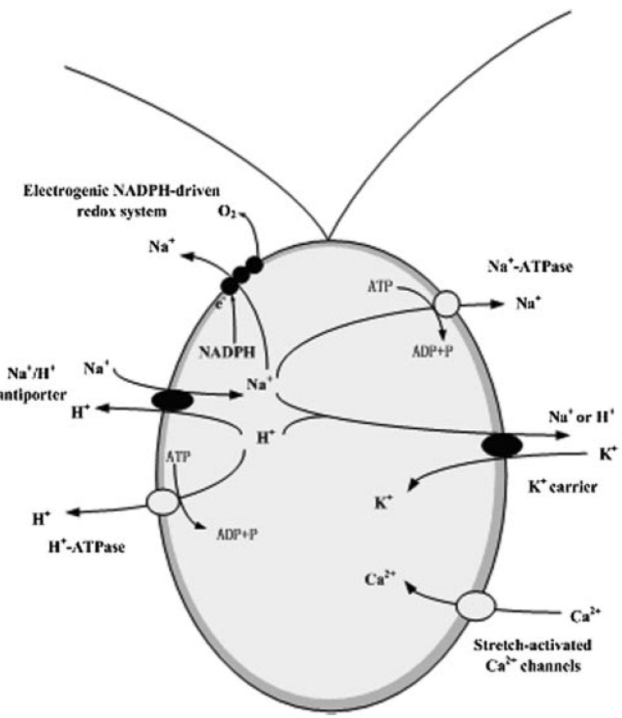




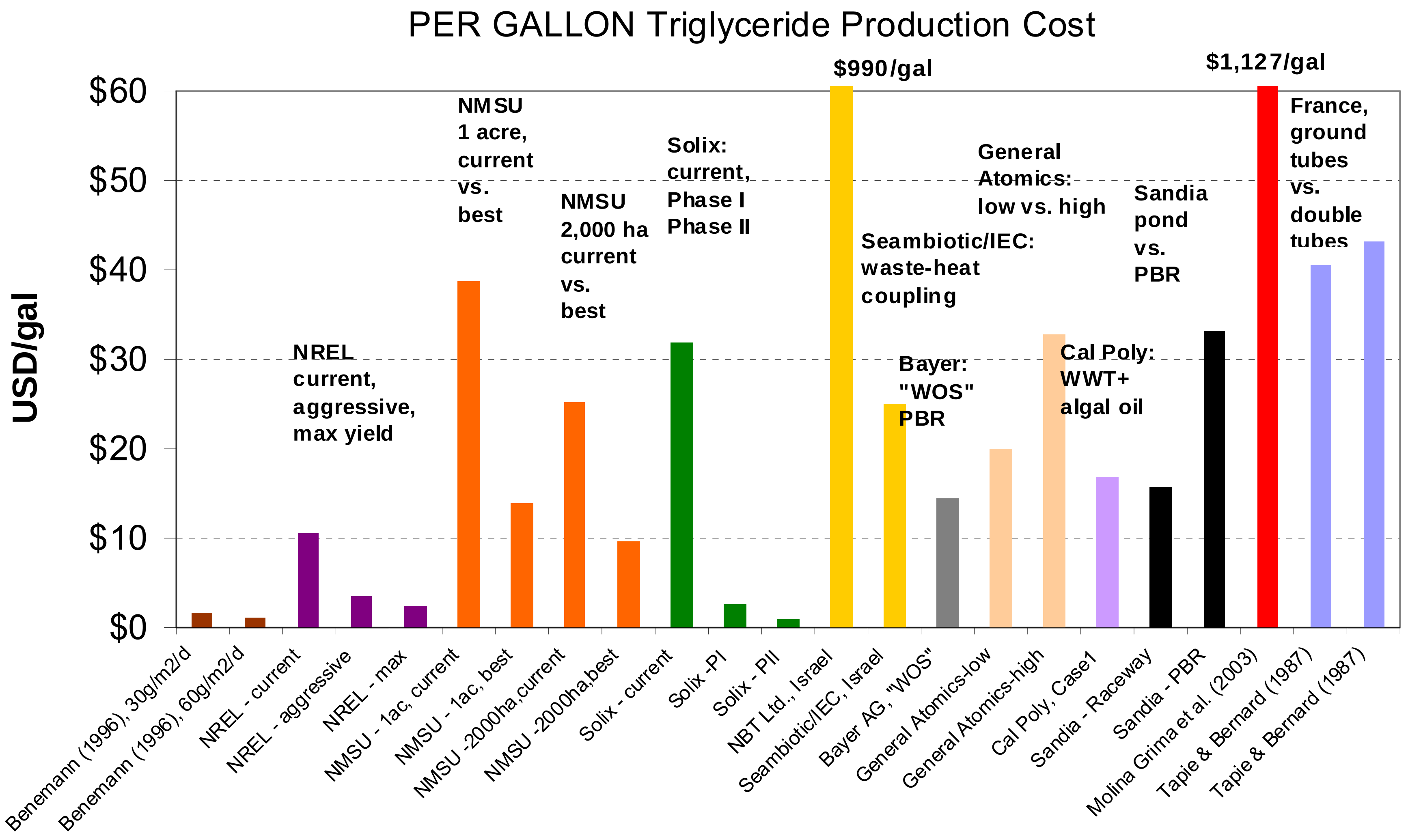





glycerol
Hyperosmotic shock
(salt stress)
Seconds-5 min
H2O
Cell shrink, ions exchange
Immediate response
Glycerol synthesis
2-3 h
Volume resumption
Short-term response
Gene expression,
proteins accumulation
Start within 12-24 h
Long-term response
Seconds-5 min
Hypoosmotic shock
(dilution)
H2O
Cell swell, ions exchange
Immediate response
Glycerol elimination
2-3 h
Volume resumption
Short-term response
Gene expression
Start within 12-24 h
Long-term response


Electrogenic NADPH-driven
redox system
O2
Na+
e-
NADPH
Na+-ATPase
ATP
Na+
ADP+P
Na+/H+
antiporter
Na+
Na+
H+
H+
Na+ or H+
K+
K+ carrier
K+
ATP
H+
H+-ATPase
ADP+P
Ca2+
Ca2+
Stretch-activated
Ca2+ channels

Widely varying analysis indicates there is not consensus on the echno-economic feasibility of algal biofuels.



Average = $109 USD/gal

Variability is wide, Std. Dev. = $301 USD/gal

Algae has many advantages over corn, cellulosic materials, and other crops as an alternative to petroleum-based fuels.

Gallons of Oil per Acre per Year	
Corn	18
Soybeans	48
Safflower	83
Sunflower	102
Rapeseed	127
Oil Palm	635
Micro Algae	5000-15000

- *Will not compete with agricultural lands for food and feed production.*
- *Will reduce deforestation area (Science, 2008).*
- *Avoids fresh water depletion.*
- *Produces higher energy content fuels.*
- *Uses CO_2.*