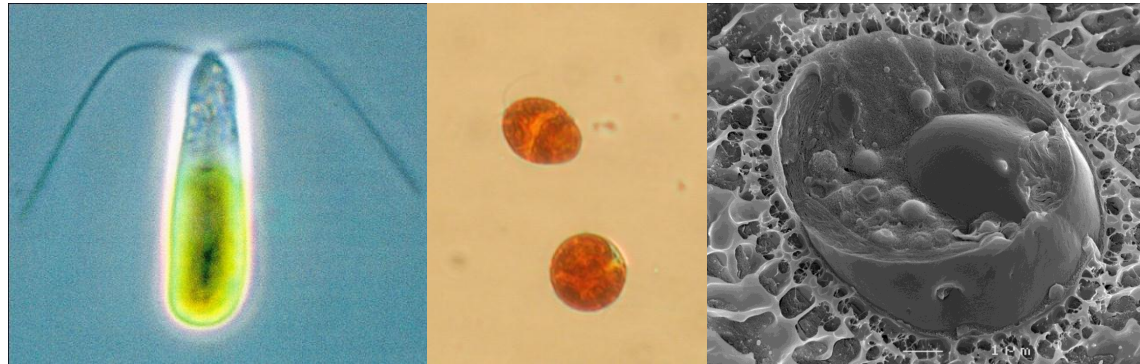


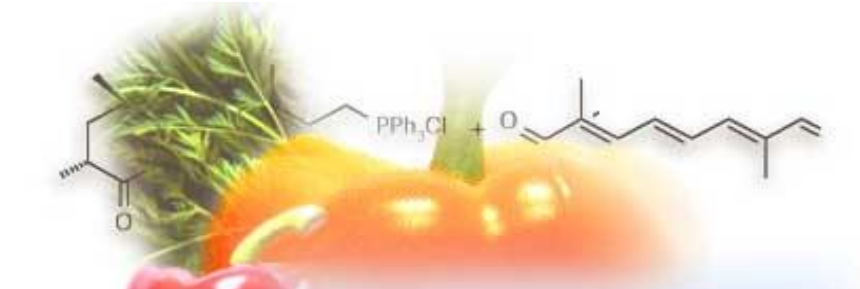
Metabolomics of carotenoid accumulation in *Dunaliella salina*



Packo Lamers

Bioprocess Engineering, Wageningen UR, the Netherlands

Carotenoids



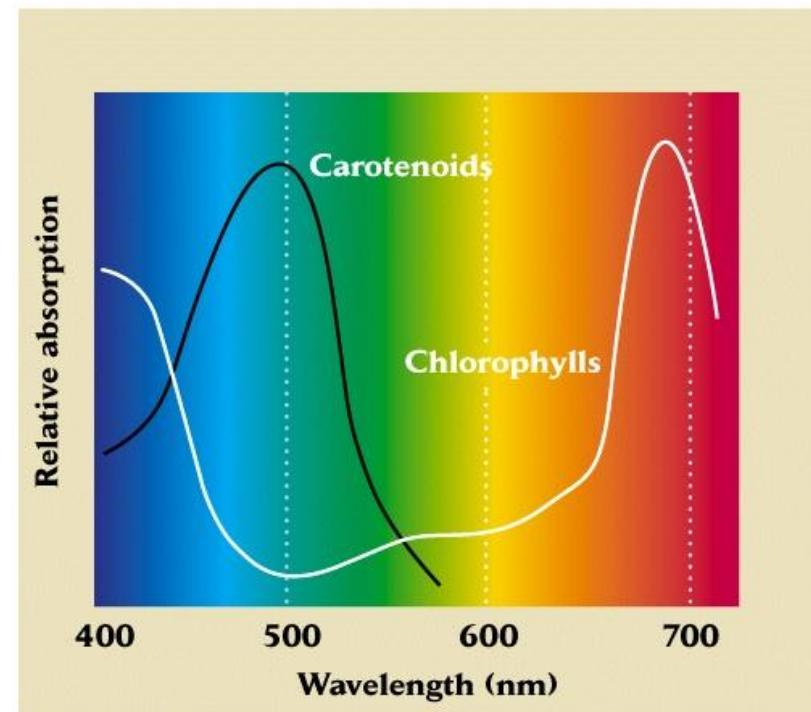
CAROTENOIDS ARE PIGMENTS



Antioxidant activity

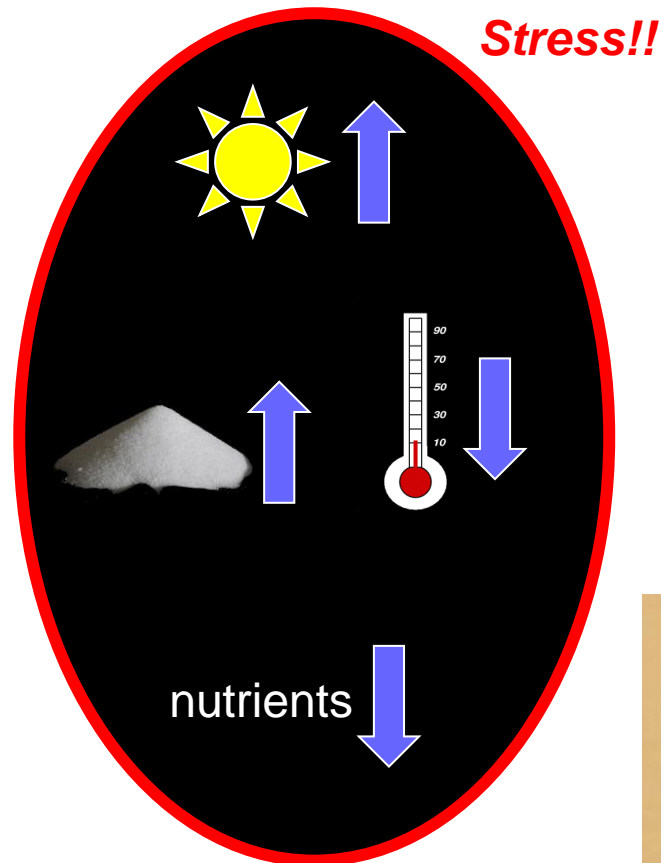
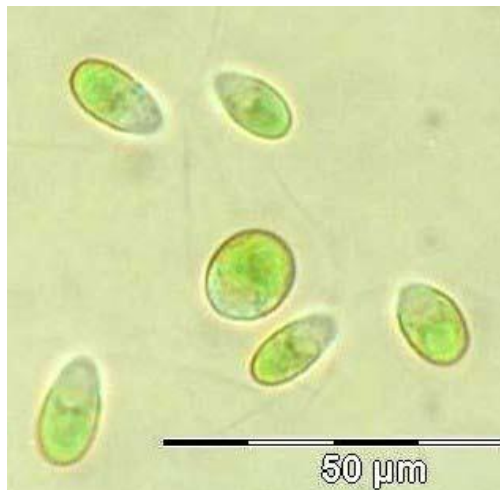


Provitamin A

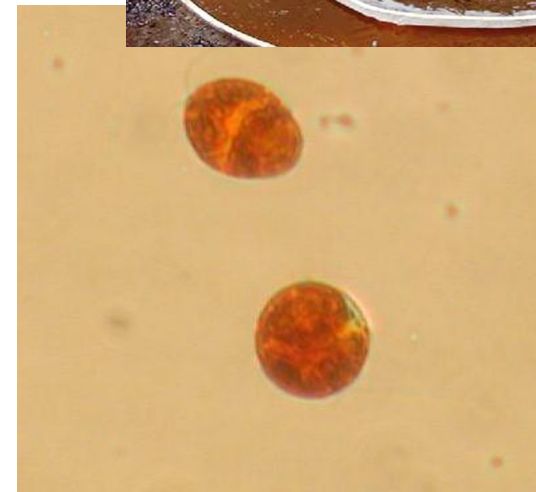



Dunaliella salina

Optimal growth conditions



Carotenoid production



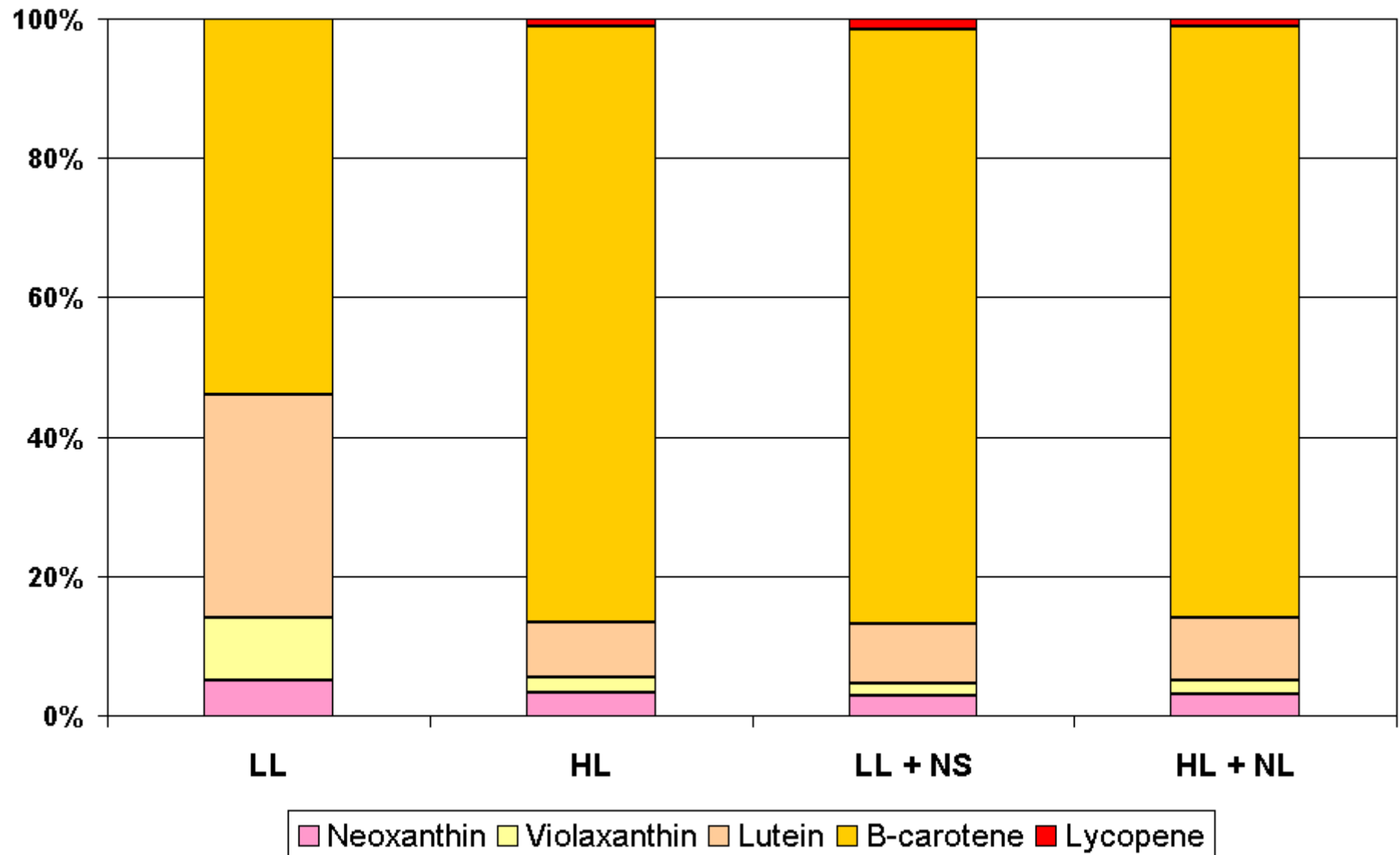


Mechanisms involved in carotenogenesis

Research questions

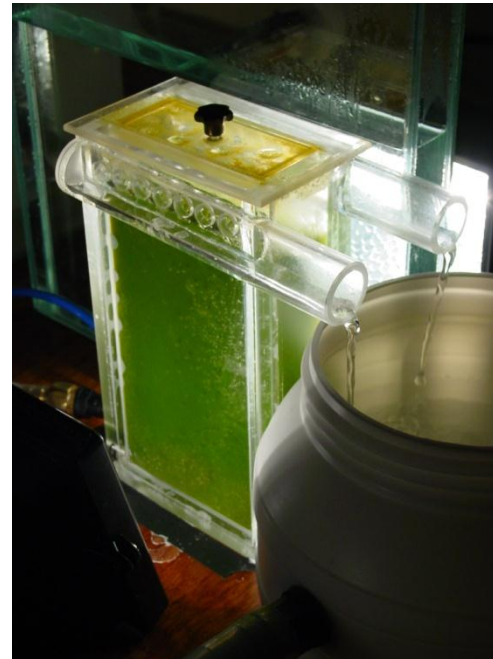
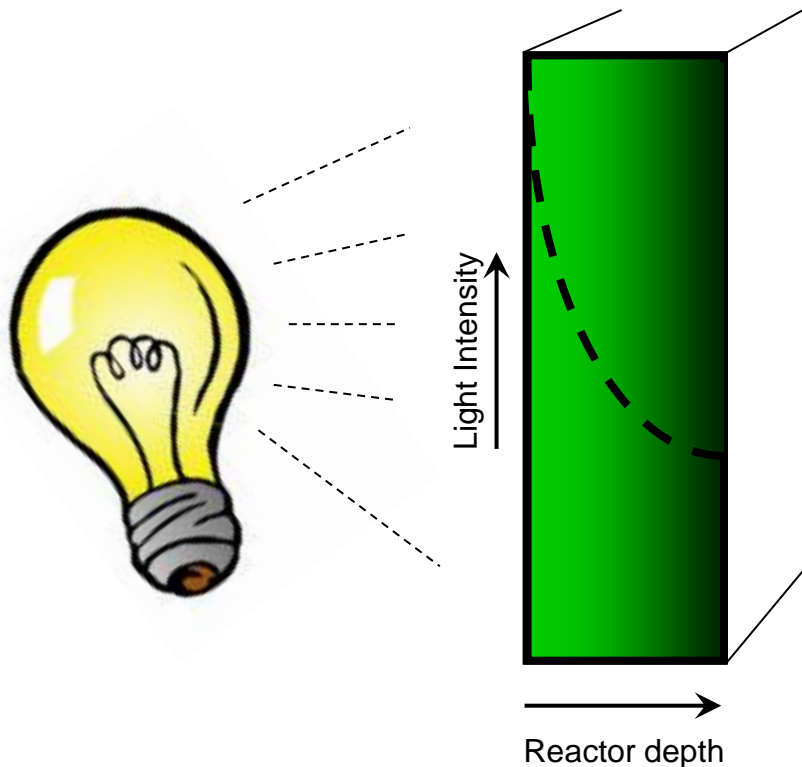
- Similar effects on stress response by light intensity and nitrogen starvation?
- Relation between fatty acid and carotenoid metabolism?
- Global stress-sensing mechanism?

Carotenoid composition in batch



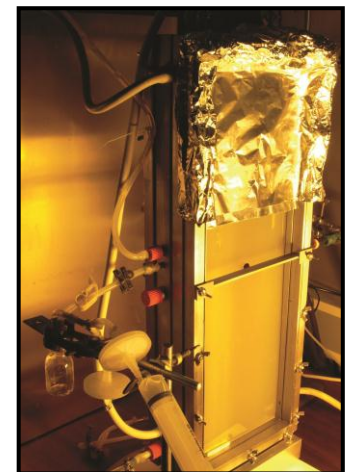
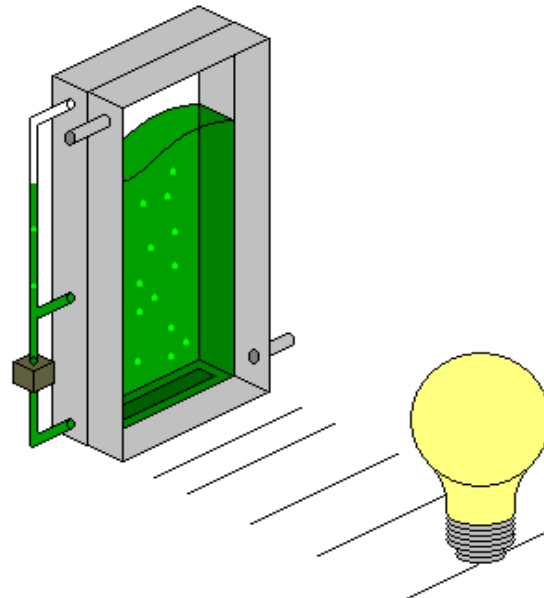
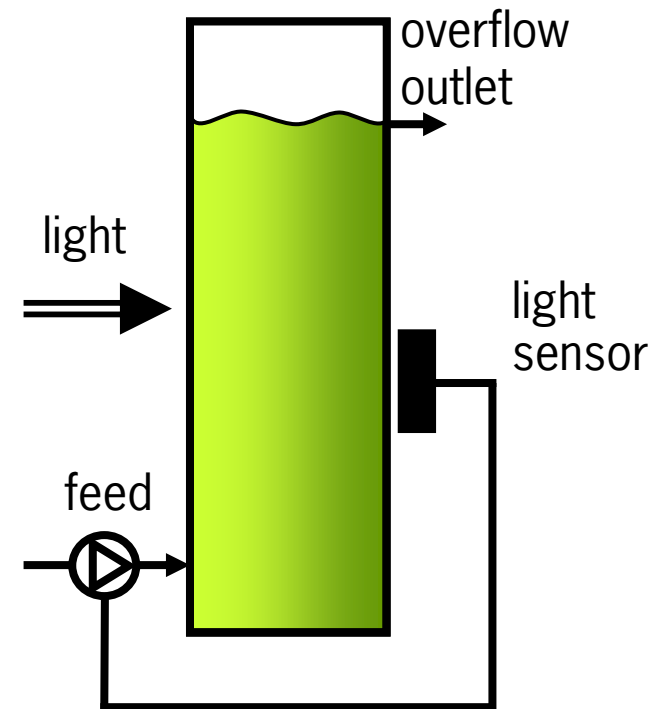
Stress assessment

- Light intensity one of the most important inducers of carotenogenesis

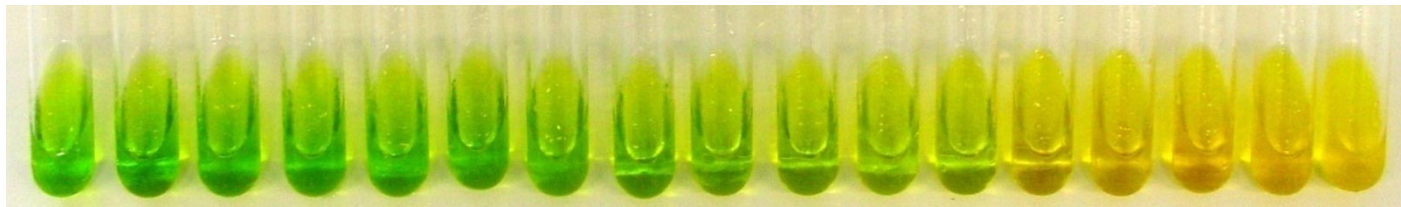
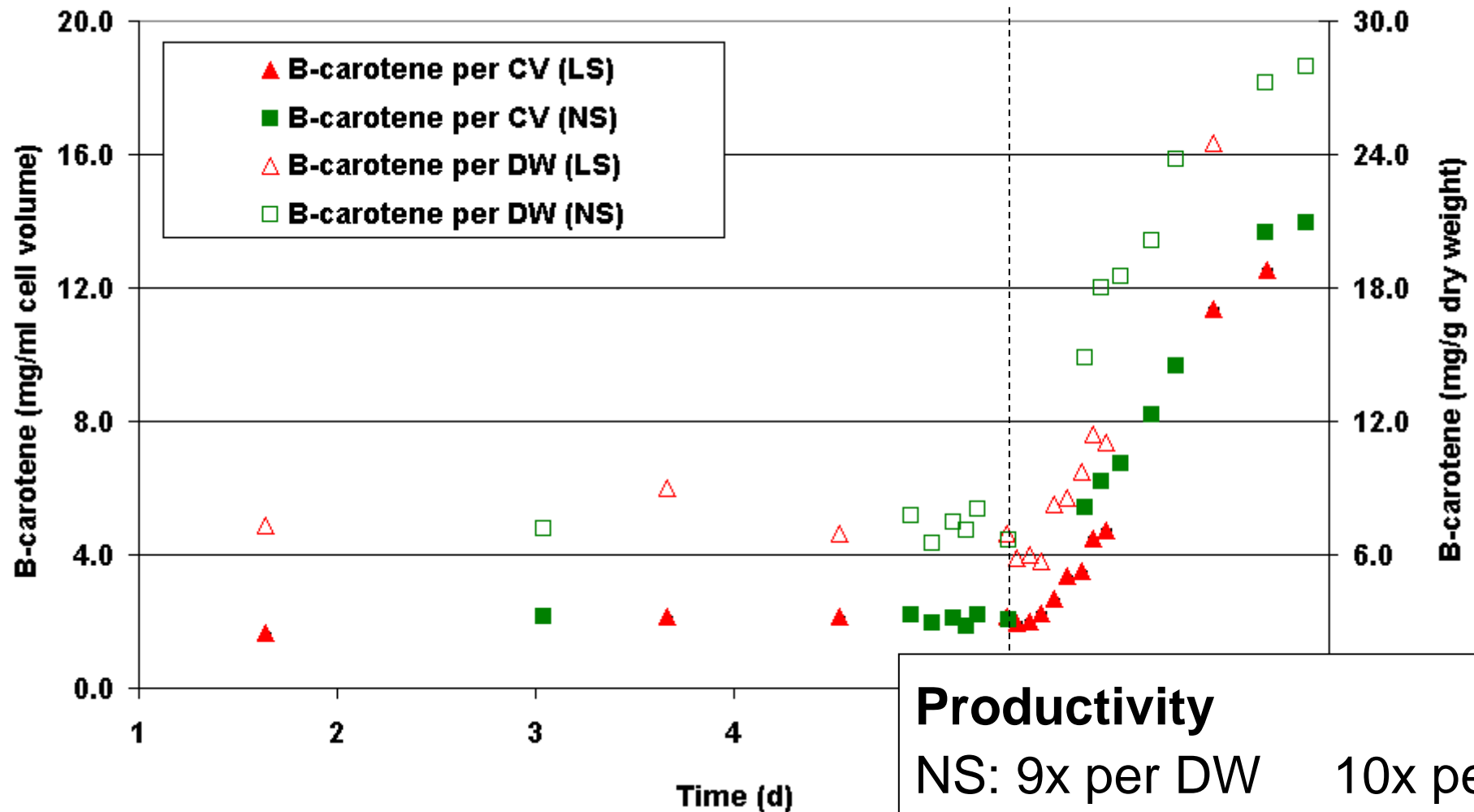


Turbidostat

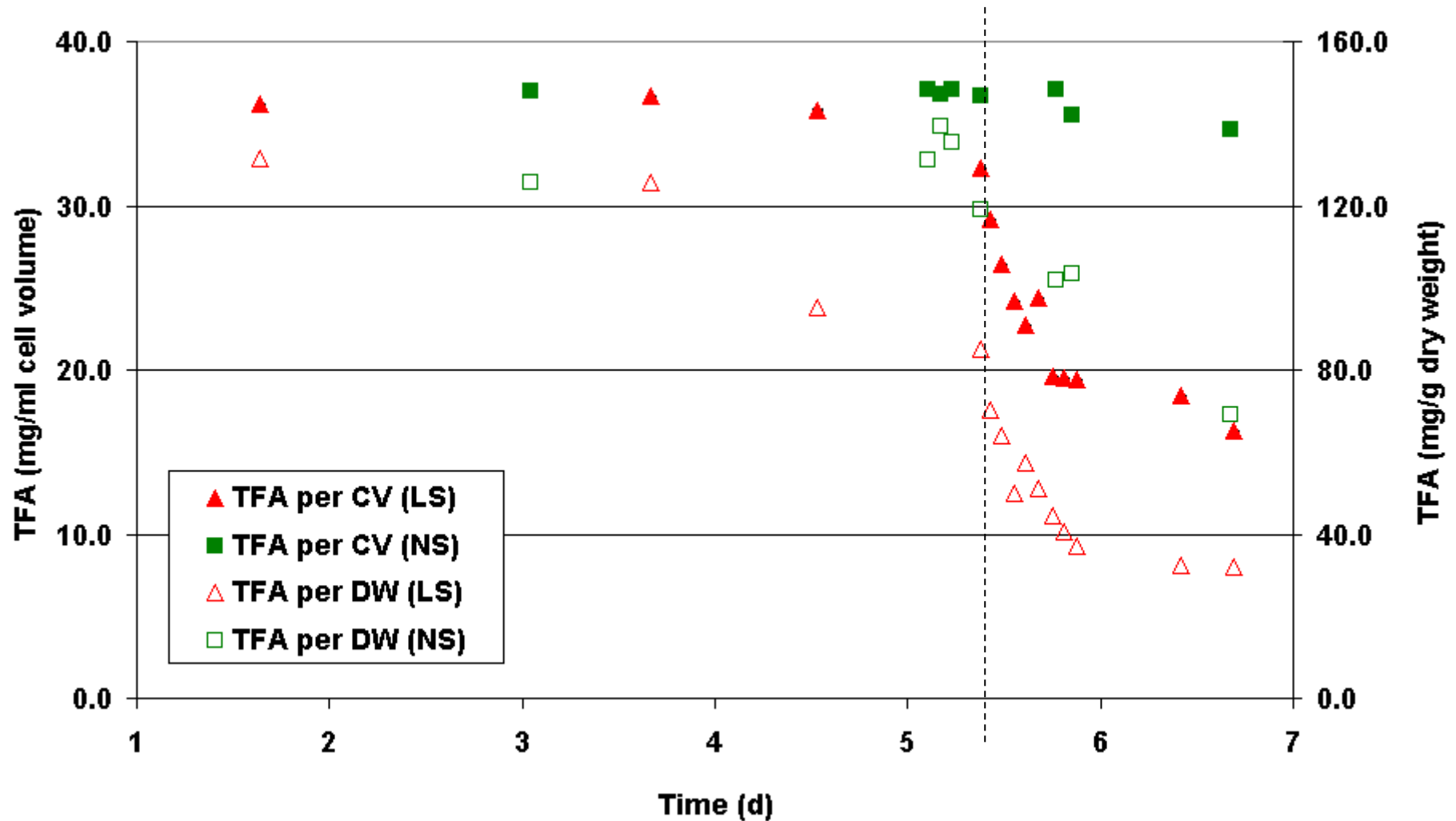
- Turbidostat
- Defined and stable light regime
- Stepwise increase of light
 - $200 \rightarrow 1400 \mu\text{mol}/\text{m}^2\text{s}$
- Nitrogen run-out



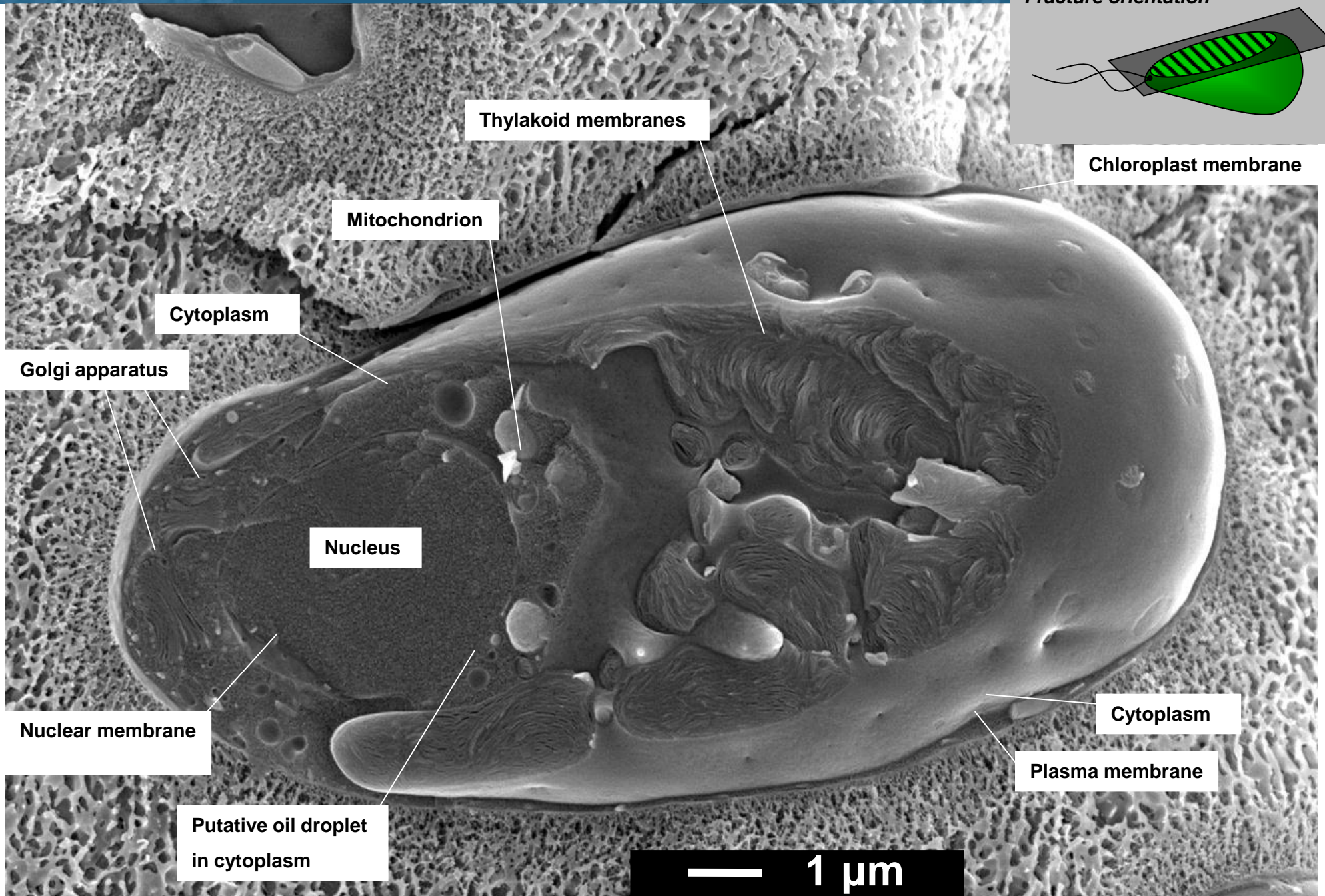
β -carotene



Total fatty acids

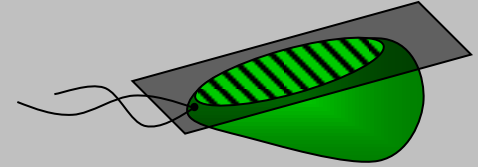


Non-stressed *Dunaliella salina*

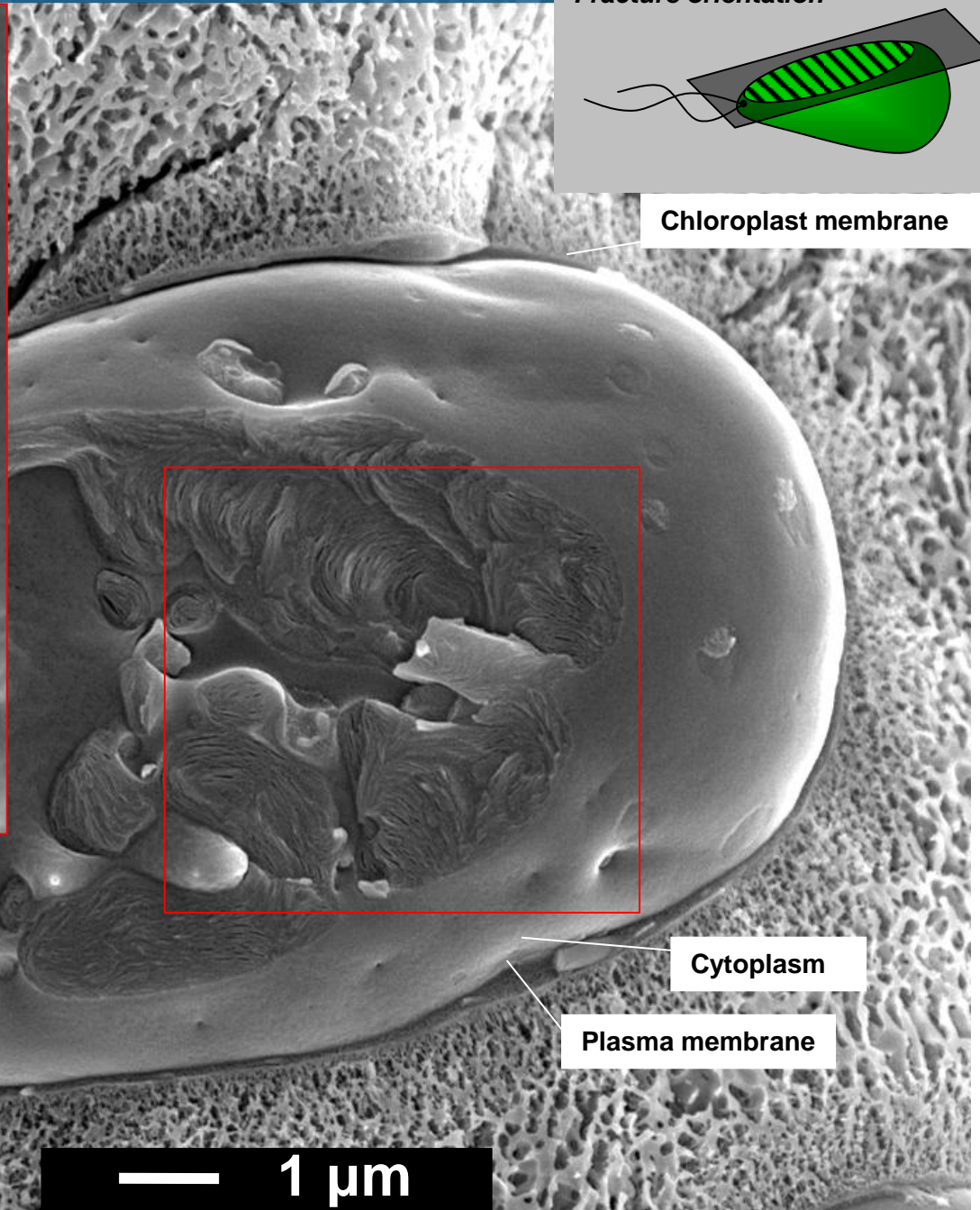
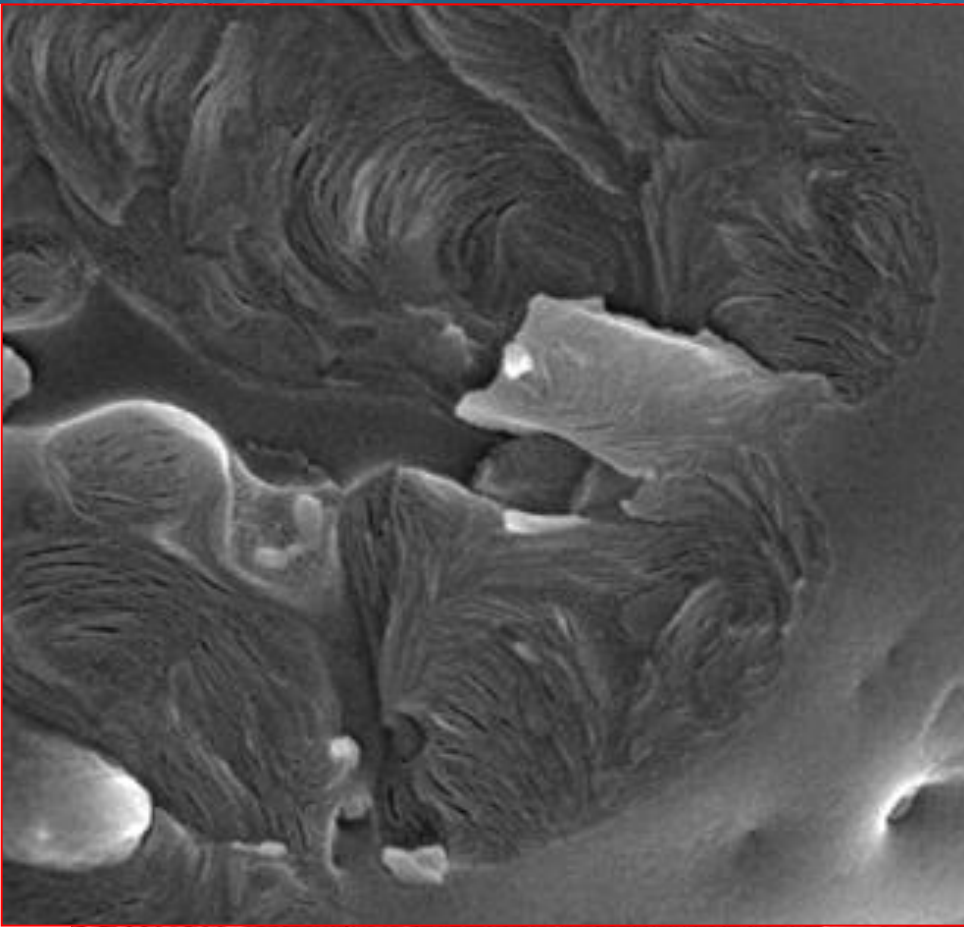


Non-stressed *Dunaliella salina*

Fracture orientation



Chloroplast membrane



Nuclear membrane

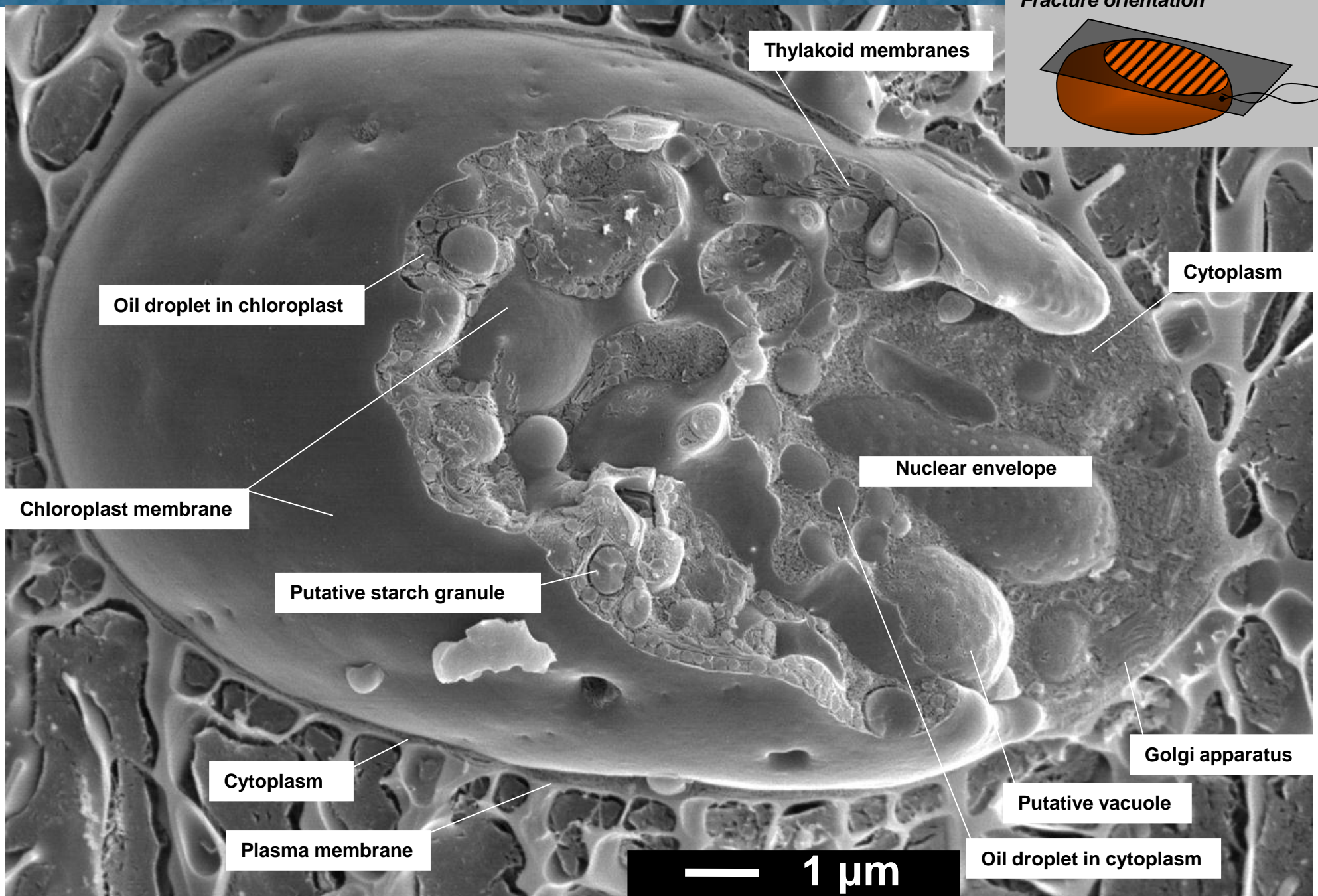
Cytoplasm

Plasma membrane

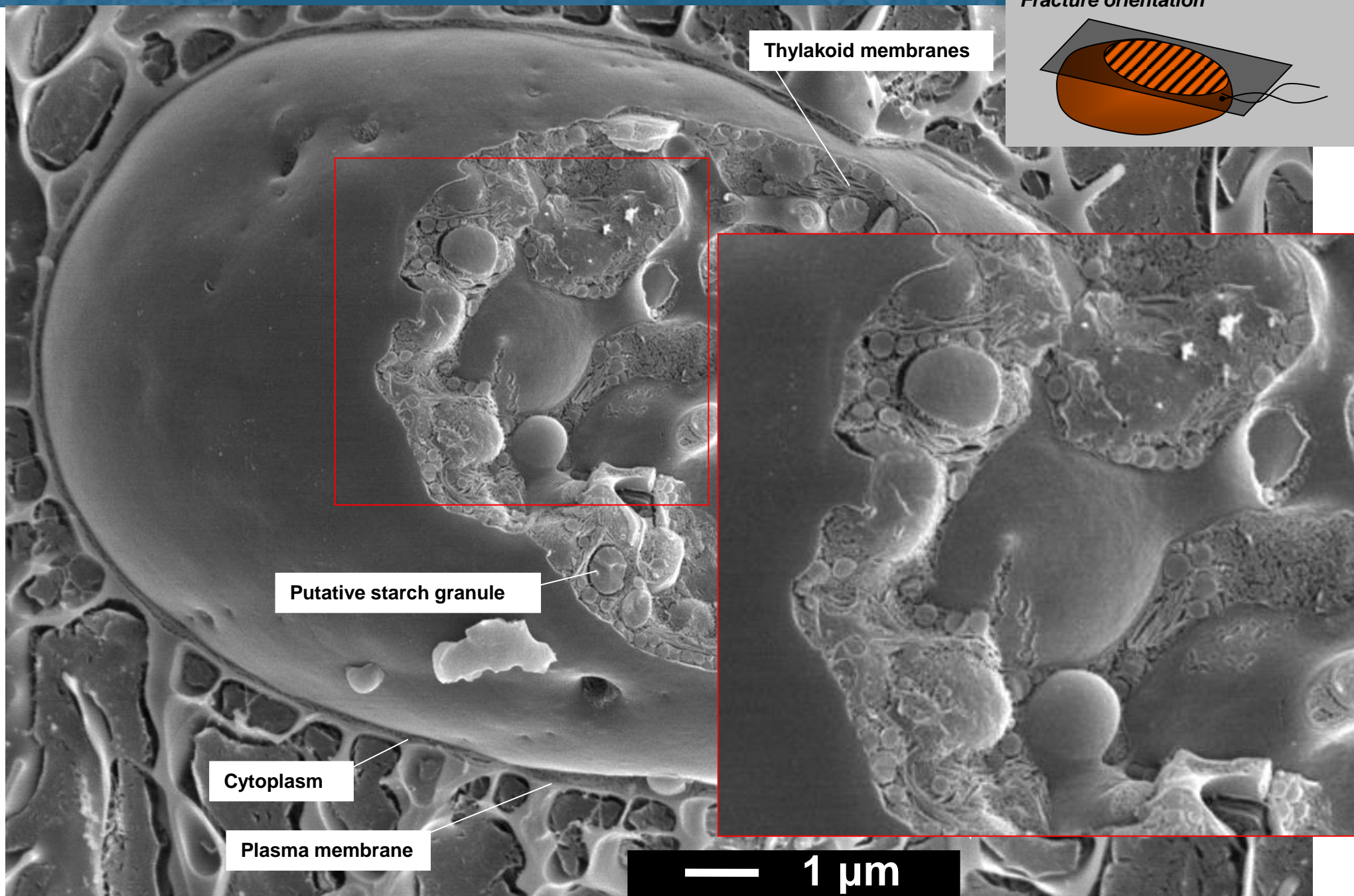
Putative oil droplet
in cytoplasm

— 1 μm

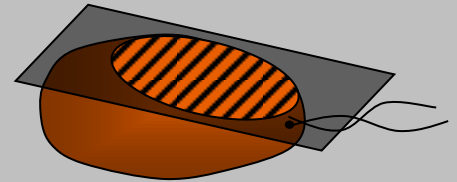
Stressed *Dunaliella salina*



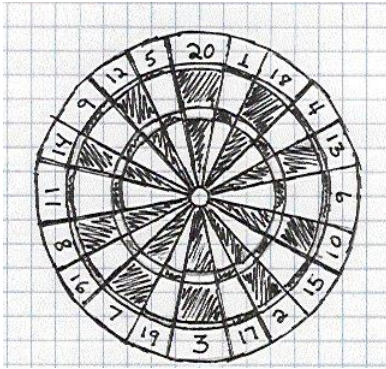
Stressed *Dunaliella salina*



Fracture orientation

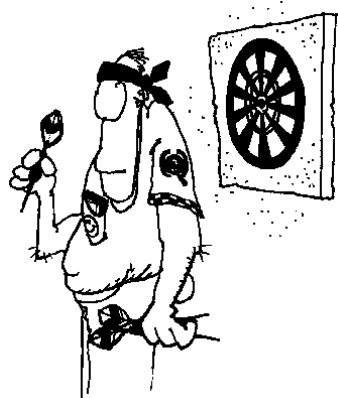


Metabolomics



Targeted

- Aimed at specific metabolites
- Quantitative when reference standards are available

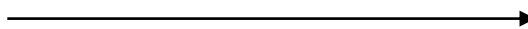


Untargeted

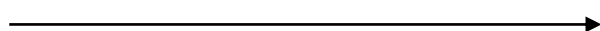
- As many metabolites as possible
- Global impression of metabolome (semi-quantitative)

Untargeted metabolomics

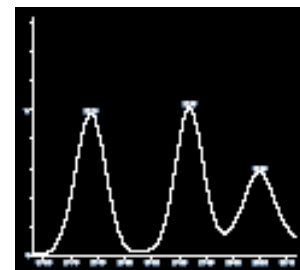
Metabolite extraction



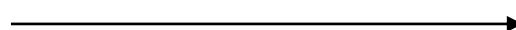
Chromatography



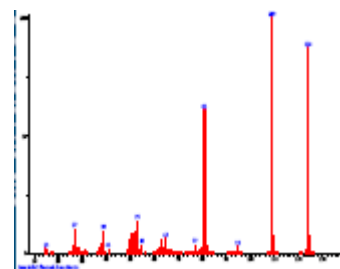
separation based on
chemical properties



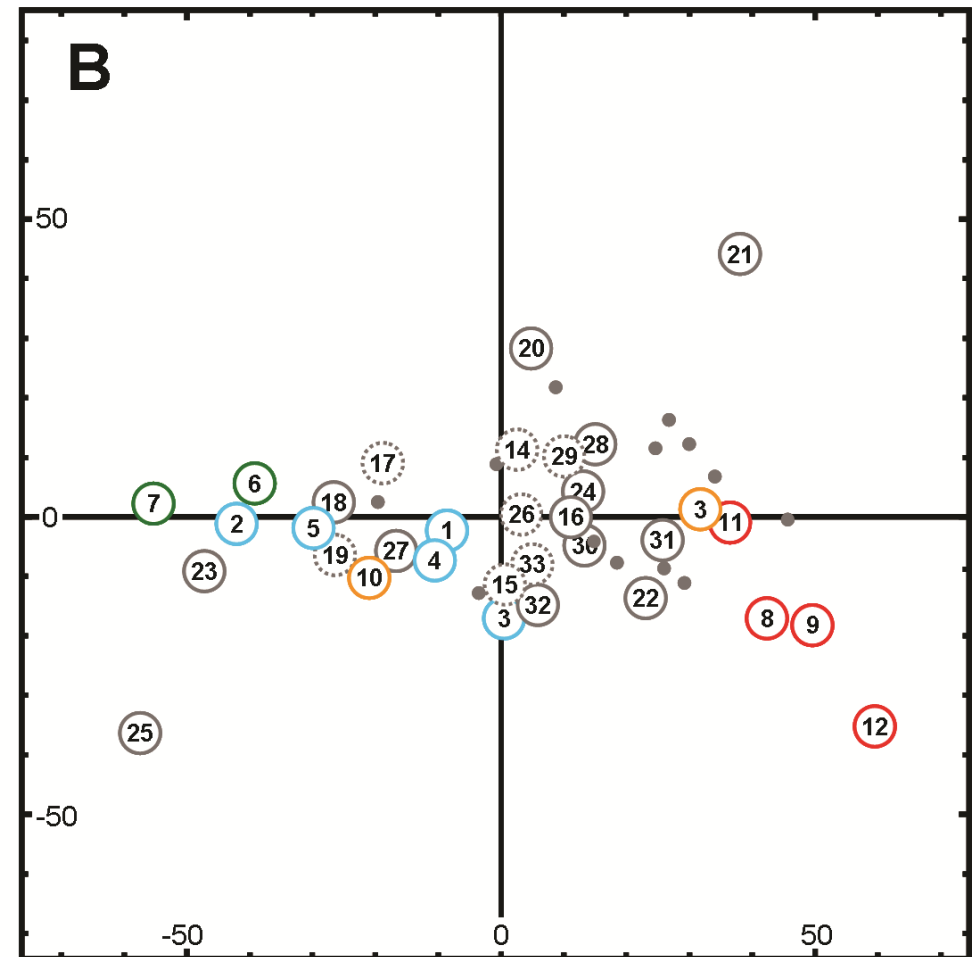
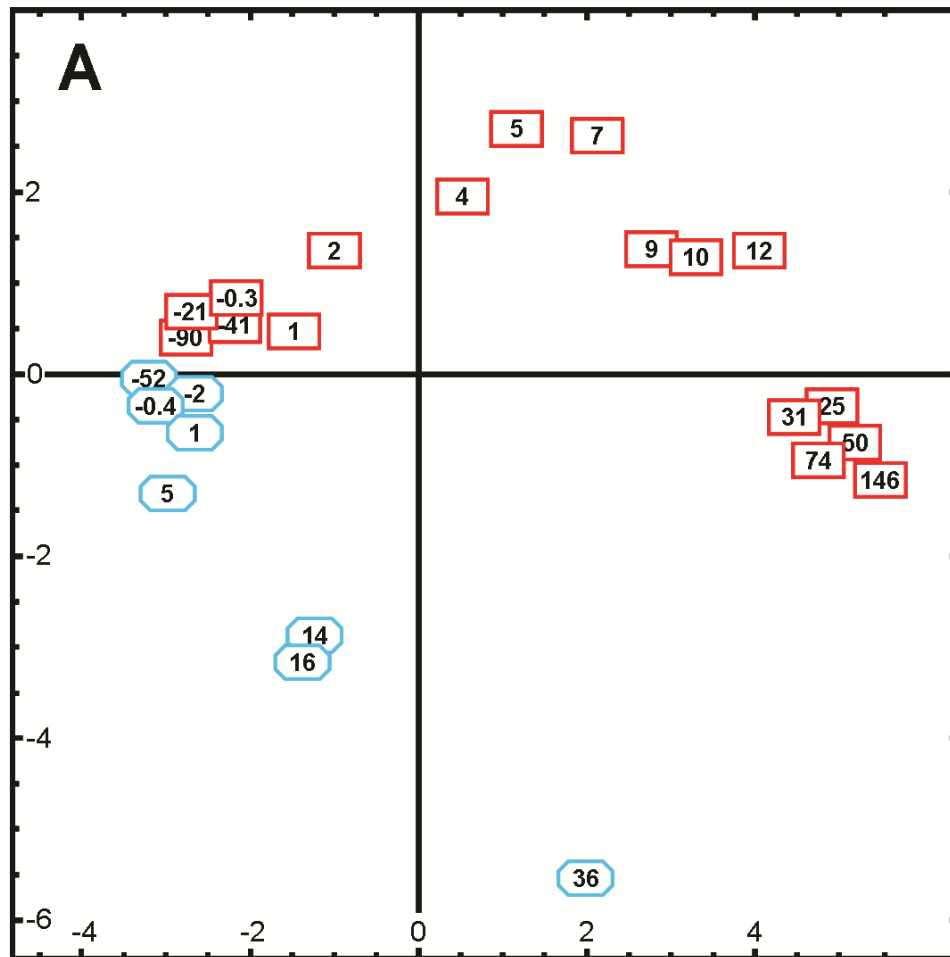
Mass spectrometry



mass determination
of all components



(a)polar metabolites



PC1: 51% (horizontal)
PC2: 25% (vertical)

Stress sensing hypothesis

One mechanism to detect:

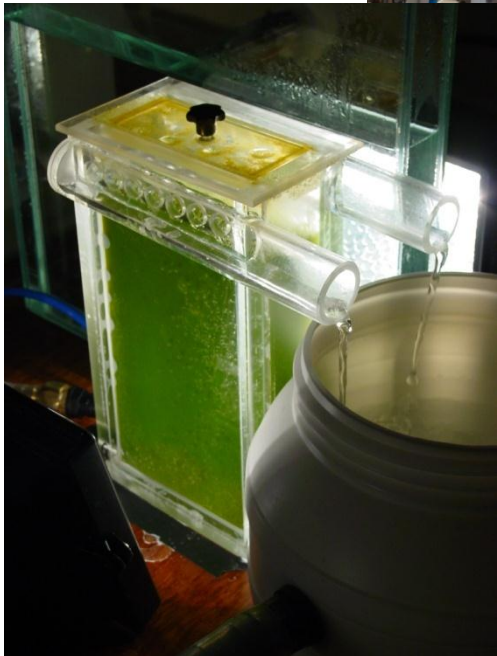
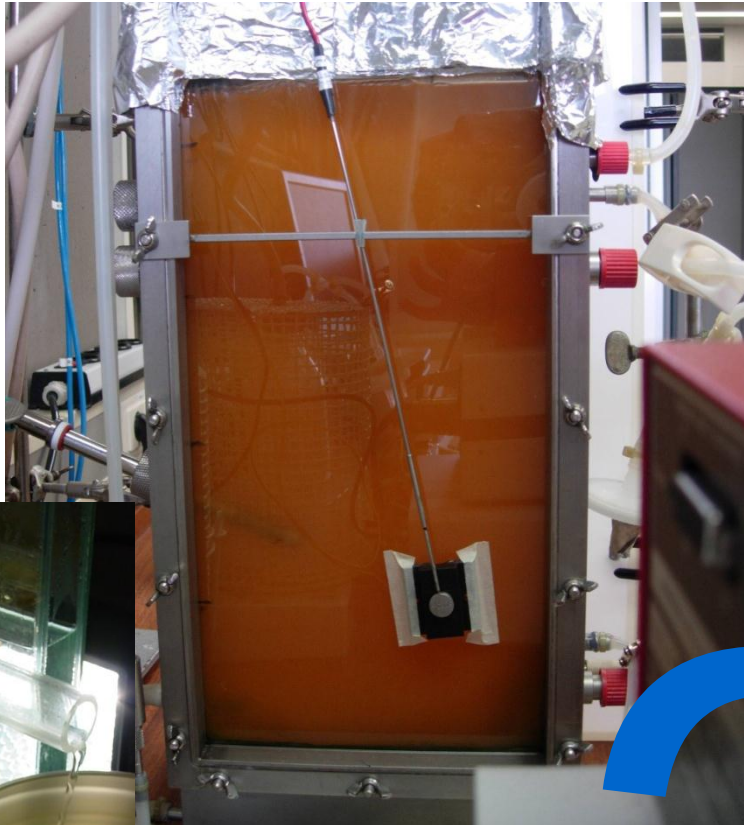
Imbalance between supply and demand of energy



Conclusions

- High light stress and nitrogen starvation cause similar responses
- Total fatty acid and carotenoid metabolism are not correlated
- Possibility for a global stress-sensing mechanism

Questions/discussion

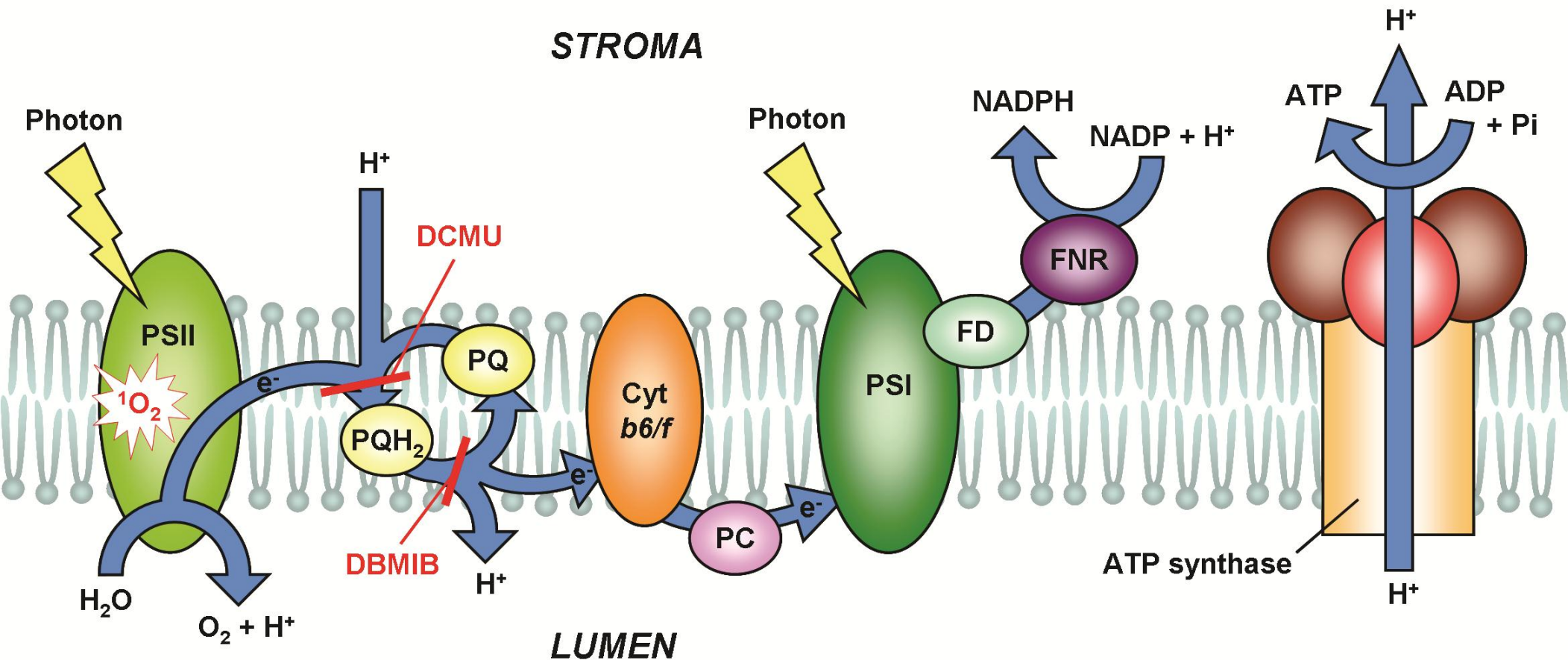


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Harry Jonker
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Yuri Tikunov
Adriaan van Aelst

www.algae.wur.nl

Stress signal & sensor



Plastoquinone pool redox regulation for:

LHCII complex synthesis (*D. salina* & *D. tertiolecta*)

Astaxanthin production (*H. pluvialis*)

Inhibitors

	LL		HL		NS (LL)	
	<i>growth</i>	<i>β-carotene</i>	<i>growth</i>	<i>β-carotene</i>	<i>growth</i>	<i>β-carotene</i>
No inhibitors	100 %	1.7	100 %	6.9	100 %	10.6
+ DCMU	86 %	1.9	65 %	4.6	48 %	5.2
+ DBMIB	69 %	1.9	-	-	66 %	10.0



Carotenoid applications

Pharmaceuticals & personal care



Food



Feed

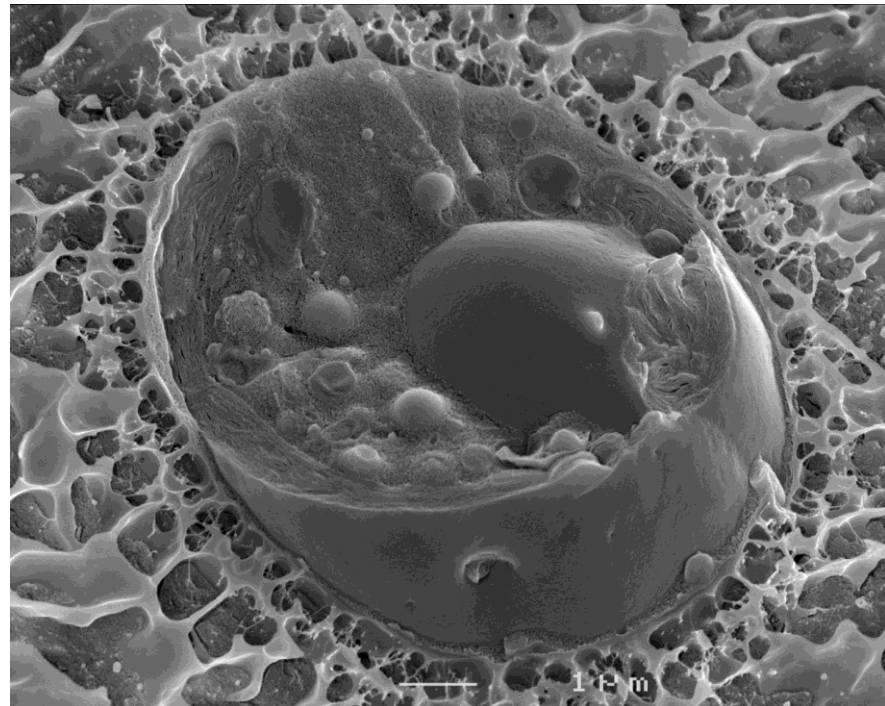
Fertilizers



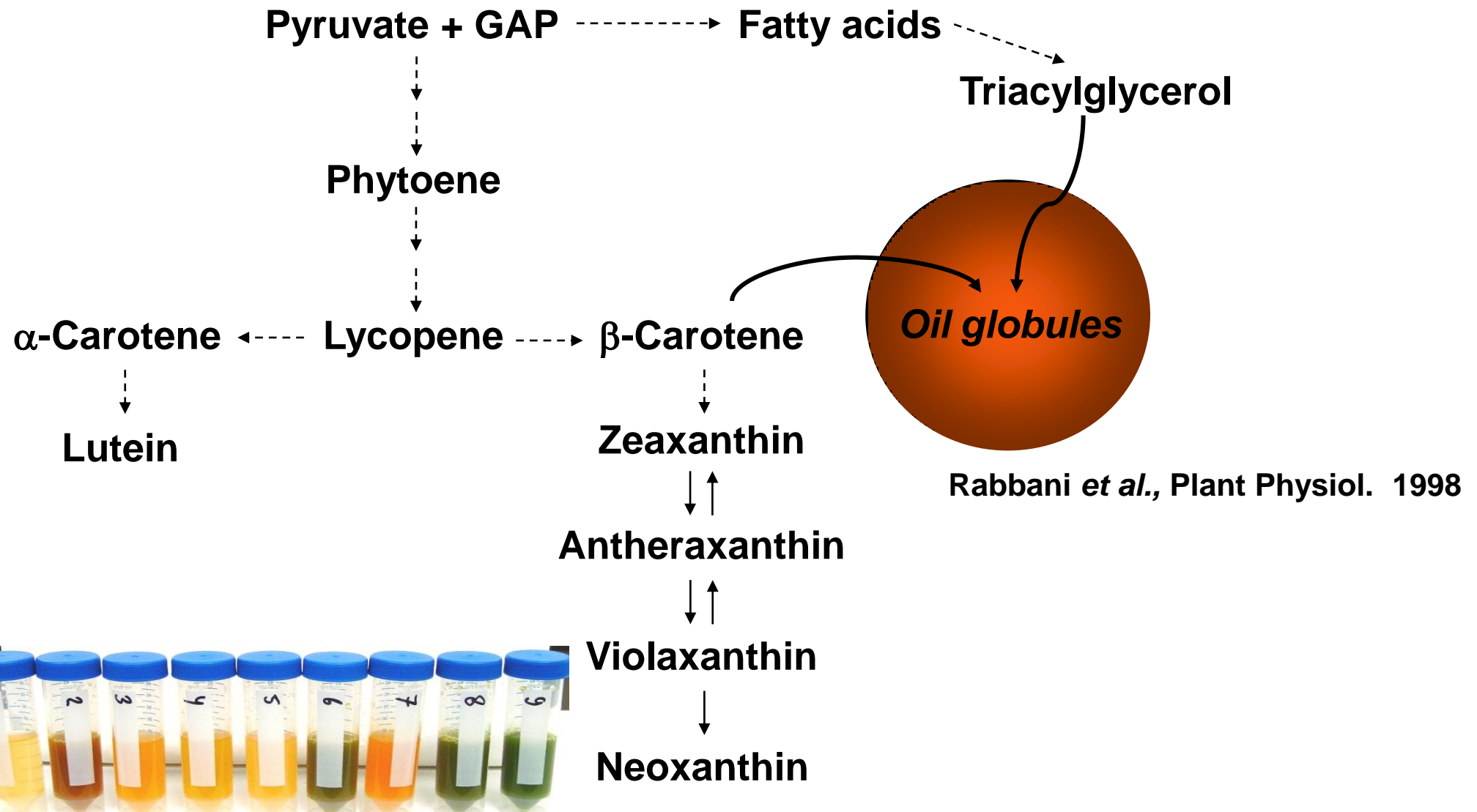
Dunaliella salina



- Green microalga
- Unicellular
- Biflagellate
- Halotolerant (6 - 30% NaCl)



Link with fatty acid synthesis?



Research questions

- Similar effects on stress response by light intensity and nitrogen starvation?
- Relation between fatty acid and carotenoid metabolism?
- Global stress-sensing mechanism?