

Limits to Algal Growth Rates and their Alleviation

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Outline

- Introduction
- Intrinsic limitations on the rate of algal growth
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- Bioremediation?
- Conclusions
- Acknowledgements

Introduction

- Algae in carbon mitigation and biofuel production
- Point sources of industrial CO₂ production: sequester CO₂, generate biofuels
- Manipulate algae and culture conditions
- Bioremediation or Bioengineering
- Add a nutrient to specific parts of the ocean where that nutrient is restricting algal growth
- Self-selection of the algae that grow and (?) sequester C

Introduction

- How fast can an organism grow?
- How efficient is the organism in using resources in growth?
- What size is the organism?
- Three questions that cannot be answered directly by genomics, though genomics can help to answer these and related questions, e.g. from rRNA gene copy number

Introduction

- Consider rate and efficiency of growth in for photolithotrophic organisms at the small end of the size range
- Focus on cells, but mention populations

Intrinsic limits on algal growth: comparison with “similar” organisms using other trophic modes

- Chemo-organotrophic micro-organisms have higher maximum specific growth rates (μ_{\max}) than do photo-lithotrophic or chemolithotrophic micro-organisms.
- Comparison involves measurements under optimal growth conditions, with conversion to growth at 20° assuming $Q_{10} = 2$
- Based on Raven (1988) In *Microalgal Biotechnology* (eds. M A and L J Borowitzka) pp. 331 – 356. CUP, Cambridge and Raven (1994) In *Chrysophyte Algae: Ecology, Phylogeny and Development* (eds C D Sandgren et al.) pp. 95-118. CUP, Cambridge

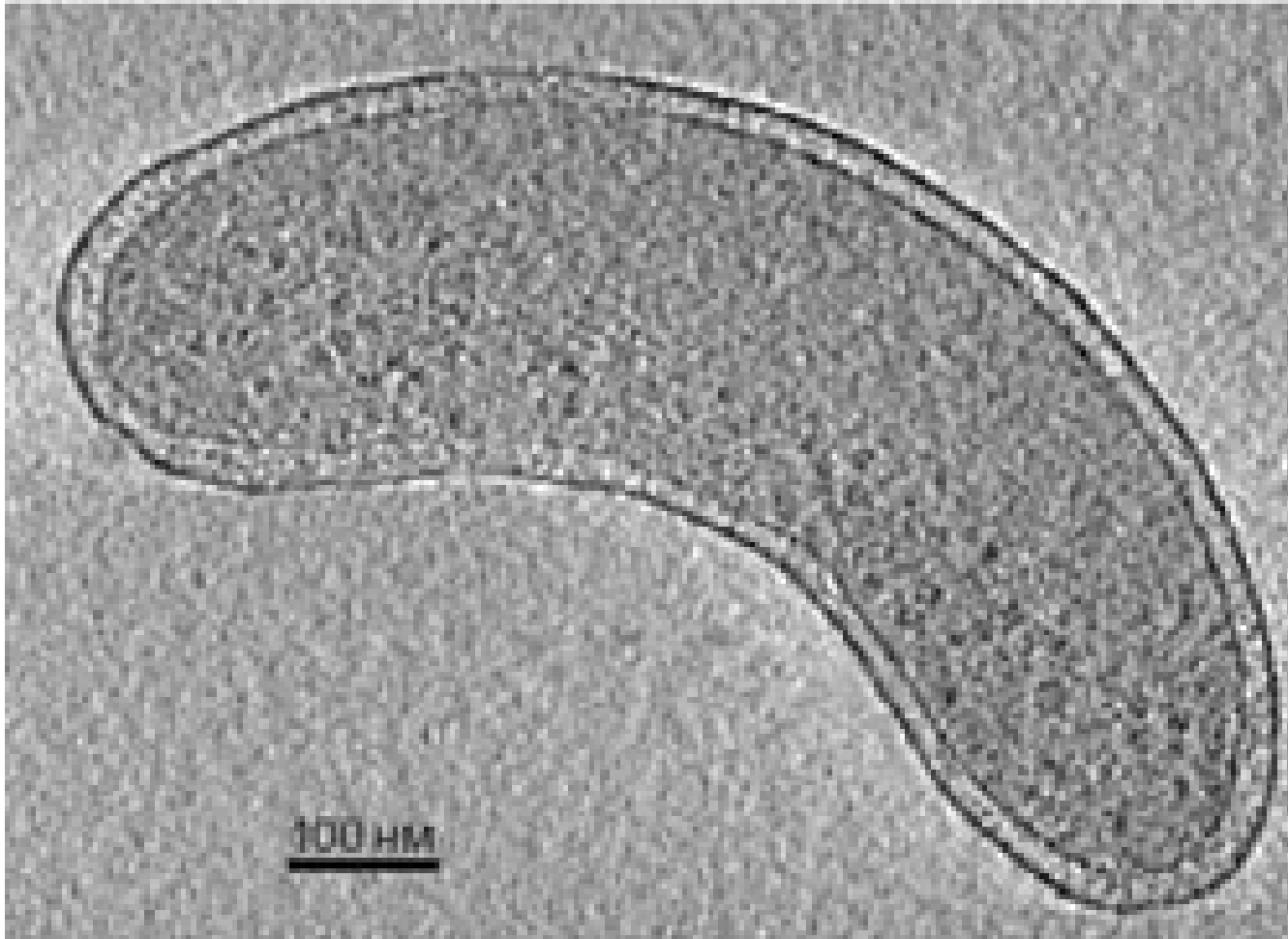
μ_{\max} of micro-organisms with different trophic modes

Organism	Nutrition	μ_{\max} , S^{-1}
Bacteria	Chemo-organo-saprotrophy	$\leq 280 \cdot 10^{-6}$
Bacteria	Chemo-lithotrophy	$\leq 40 \cdot 10^{-6}$
Bacteria	Photo-organo-saprotrophy	$\leq 50 \cdot 10^{-6}$
Bacteria	Anoxygenic photo-lithotrophy	$\leq 27 \cdot 10^{-6}$
Bacteria	Oxygenic photo-lithotrophy	$\leq 24 \cdot 10^{-6}$
Eukarya	Chemo-organo-saprotrophy	$\leq 170 \cdot 10^{-6}$
Eukarya	Chemo-organo-phagotrophy	$\leq 70 \cdot 10^{-6}$
Eukarya	Photo-organo-saprotrophy	$\leq 28 \cdot 10^{-6}$
Eukarya	Oxygenic photo-lithotrophy	$\leq 26 \cdot 10^{-6}$

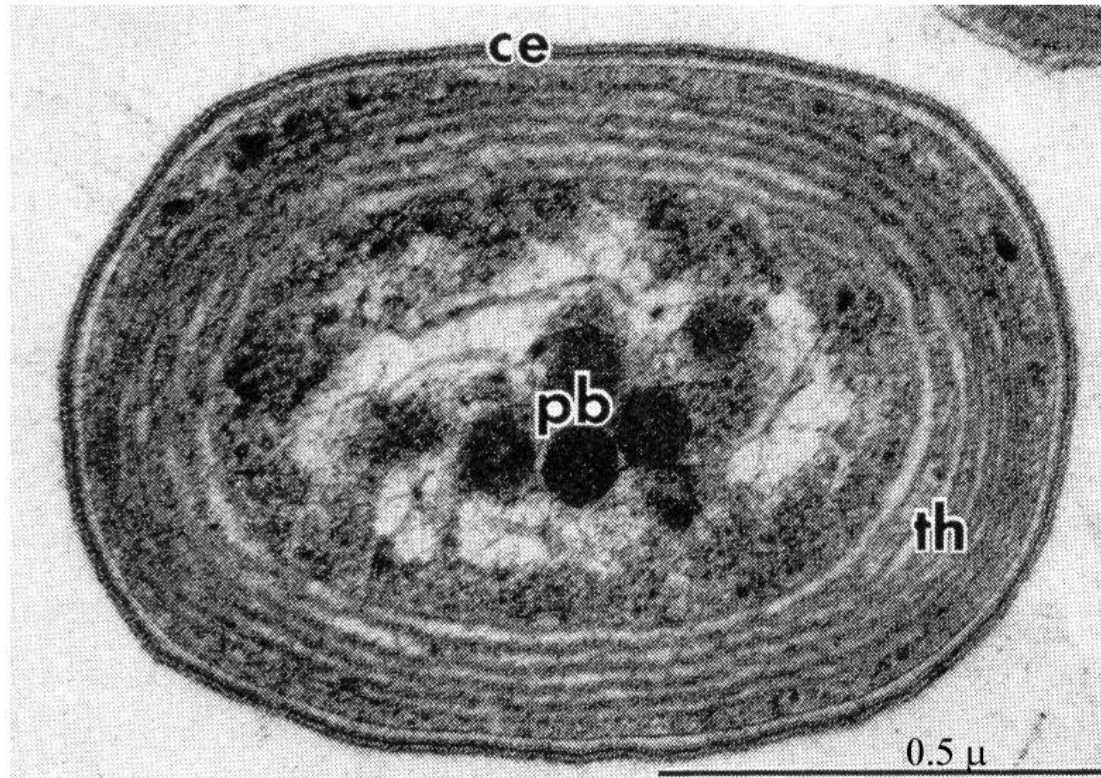
- Bacteria generally have higher μ_{\max} than eukarya with the same trophic mode
- Smallest difference for oxygenic photo-lithotrophy
- Differences not predominantly related to different cell sizes and decreasing μ_{\max} with increasing cell size
- Differences partly related to the fraction of resources in the cell that are allocated to acquisition of organic carbon from the environment (organotrophy) or production of organic carbon from inorganic substrates (lithotrophy)
- Larger fraction of resources allocated to carbohydrate production in lithotrophs than to carbohydrate acquisition in organotrophs, at least in micro-organisms

- Greater range of catalysts needed for photolithophy than for organotrophy
- Fewer genes needed for organotrophy than photolithotrophy
- Smallest known genome of a free-living chemo-organosaprotrophic bacterium is that of a member of the SAR 11 clade, the marine ***Pelagibacter ubique*** HTCC1062: 1354 genes in a 1.31 Mbp genome
- Smallest known genome of a photo-lithotrophic cyanobacterium is that of the marine cyanobacterium ***Prochlorococcus marina*** MED 4: 1716 genes in a 1.66 Mbp genome

Pelagibacter



Prochlorococcus



ce: Cell Envelope
pb: Polyhedral Bodies

th: Thylakoids

- Large number of copies of some proteins per cell volume for some proteins in photolithotrophs
- These proteins account for a large fraction of the total cell proteins in photolithotrophs
- **Rubisco** up to 10% of total protein
- **Apoproteins** of light-harvesting pigment-protein complexes up to 20% of total protein

- Optimal allocation of total cell protein among different proteins to give maximum growth rate under the optimal growth conditions
- About half as much protein per unit cell volume in catalysts processing organic carbon delivered from photosynthesis in photolithotrophs as in the proteins processing the organic carbon delivered from external organic substrates in chemo-organotrophs.

- Other things being equal and optimal, photolithotroph can only grow half as fast as chemo-organotrophs.
- Only half as much machinery for making the monomers needed for DNA, RNA, protein, etc. synthesis, and for polymerising them, in photolithotrophic cells as in chemoorganotrophic cells of the same size

- This partly explains the **differences** in μ_{\max} between a photo-lithotroph and a comparable chemo-organotroph.
- What explains the **absolute values** of μ_{\max} in cells of a given size and a certain trophic mode?

Relation of specific growth rate μ to content and reaction rate of catalysts

$$\mu = B_i \cdot C_i \cdot R_i \cdot F_i$$

where

μ = specific growth rate (mol C assimilated \cdot mol C in cell⁻¹ \cdot s⁻¹)

B_i = mol of catalyst of essential reaction i \cdot mol C in catalyst

C_i = mol C in catalyst \cdot mol C in cell⁻¹

R_i = maximum specific reaction rate of the catalyst of reaction i with the reaction product scaled to units of mol C from mol C of product per mol cell C (mol C transformed \cdot mol catalyst⁻¹ \cdot s⁻¹)

F_i = fraction of potential R_i in cell needed to account for observed μ

- Summed over all reactions, the equation would account for all reactions contributing to cell growth (and, with extension, maintenance)
- Consider separately the role of the variables B , C , R and F

$$\mu = B_i \cdot C_i \cdot R_i \cdot F_i$$

- Can B_i be increased by using smaller versions of the catalyst?
- Bacterial and organelle ribosomes smaller than eukaryote cytosol ribosomes
- *Prochlorococcus* has the smallest known urease
- Bacteria have fewer subunits in redox complexes than occur in eukaryotic mitochondria and plastids

- Form II Rubiscos are L_2 , Form I has L_8S_8 ; only LSU has catalytic site
- Downside in present atmosphere if no CCM: Form II Rubiscos maximize specific reaction rate at the expense of affinity for CO_2 and selectivity for CO_2 over O_2
- Cannot maximise specific reaction rate, CO_2 affinity and CO_2/O_2 selectivity of Rubisco simultaneously
- Tcherkez et al. 2006, PNAS 103: 7246-7251

- Light-harvesting complexes – about a 5-fold variation in apoprotein mass per chromophore molecule
- Lowest for chlorophyll- and carotenoid-protein complexes, highest for phycobilins
- Need to consider wavelengths of maximum absorption and specific absorption coefficients in comparing pigments
- Fewer regulatory components in smaller catalysts

- However.....
- μ_{\max} for cyanobacteria no higher than for eukaryotic photolithotrophs of a similar size
- Comparative data are for similar-sized cyanobacteria with phycobilins and eukaryotes without phycobilins: do these protein-costly light-harvesting complexes offset the smaller size of some other catalysts?

$$\mu = B_i \cdot C_i \cdot R_i \cdot F_i$$

- This term is needed to give the important terms the correct units
- Even though this term is lower for smaller versions of catalysts, when summed over all catalysts the mol C in the cell is significantly decreased

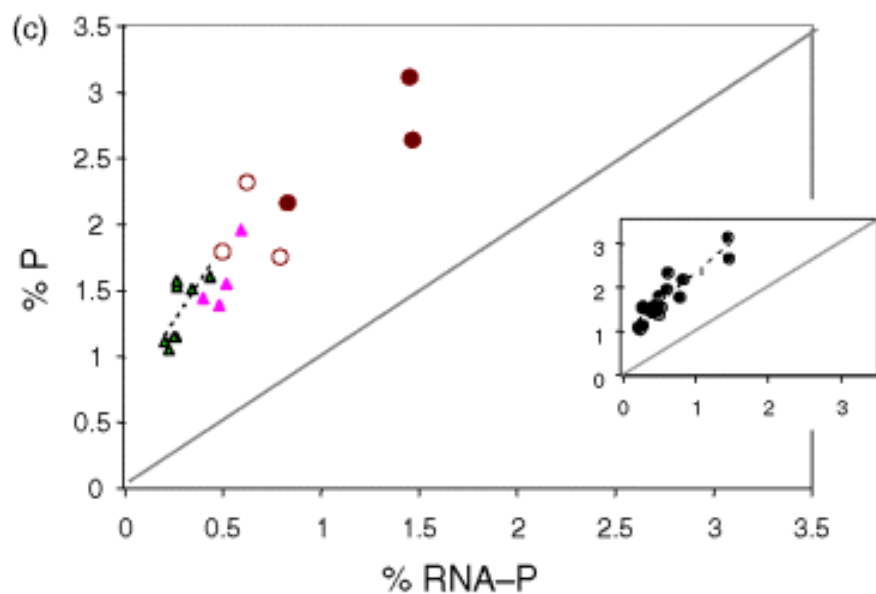
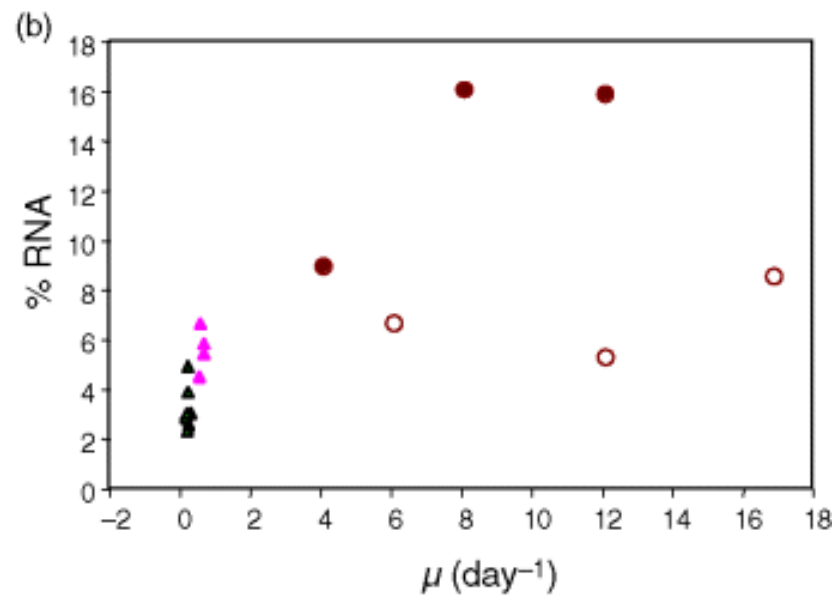
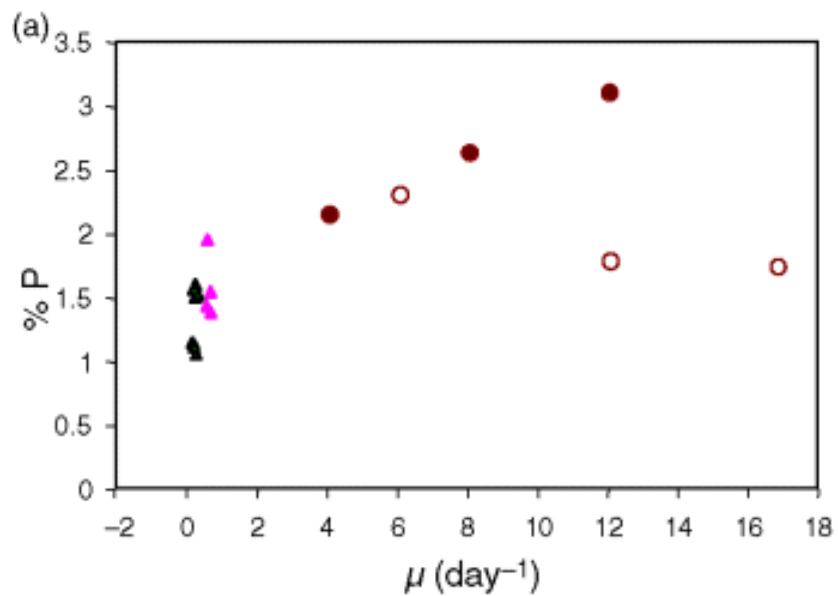
$$\mu = B_i \cdot C_i \cdot R_i \cdot F_i$$

- Can R_i be increased?
- For Rubisco, trade-off between R and selectivity between CO_2 and O_2 and affinity for CO_2
- Difficult or impossible to break this correlation, granted the reaction mechanism
- Scope for increasing R_i for other high-expression catalysts

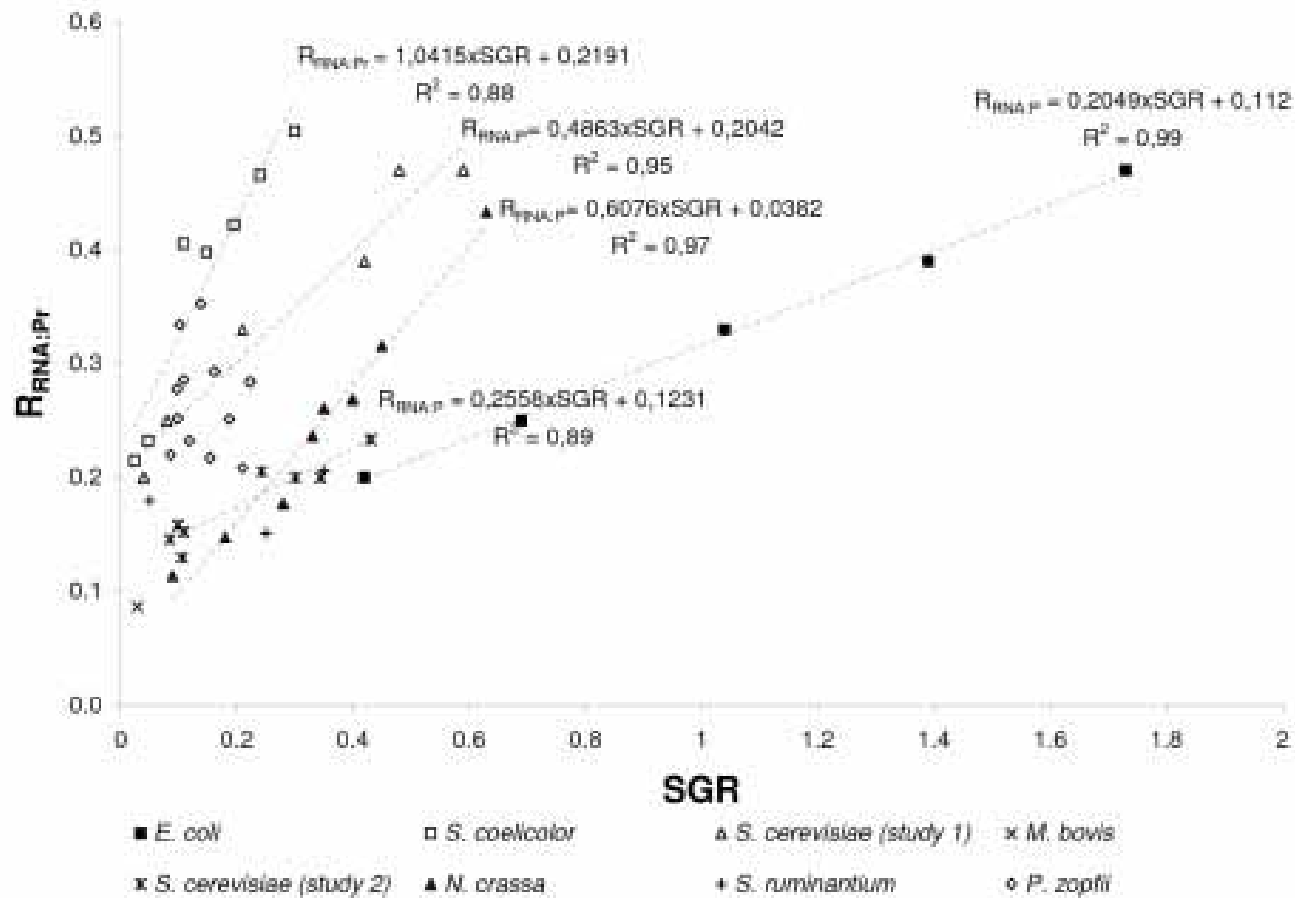
$$\mu = B_i \cdot C_i \cdot R_i \cdot F_i$$

- Are all the enzymes, transporters, components of the light-harvesting machinery, etc., operating at their maximum specific reaction rate in growing cells?
- **No, not even at μ_{\max}**
- rRNA only function in peptide chain elongation in microalgae at less than 70% of the maximum possible rate at μ_{\max} : in some cases as little as 8%
- (Flynn, Raven, Finkel, Quigg, Rees & Beardall, *J. Phycol.*, in press)

- Growth Rate Hypothesis (GRH) predicts that μ is a linear function of rRNA content, with a constant specific reaction rate of rRNA at all rRNA contents
- Related to the geochemical prediction of phosphorus as limiting resource for global primary productivity
- Growth Rate Hypothesis does not seem to apply universally to phytoplankton, though it applies to some chemo-organotrophs



- ▲ *Daphnia pulicaria*
- ▲ *Daphnia pulex*
- *Escherichia coli*
- Lake bacteria



- Rubisco is not usually completely activated
- Rare to find all of the cellular Rubisco of cyanobacteria in the carboxysomes where it is active in inorganic carbon concentrating mechanisms (CCMs)
- Rare to find all the cellular Rubisco in the pyrenoids of those eukaryotes have CCMs based on pyrenoids
- Fraction of Rubisco in pyrenoids varies through the cell cycle
- Marty Spalding's talk

- Metabolic control analysis: estimate control coefficients – in simple terms, increment in flux through a pathway per unit increment in activity (content) of enzyme, transporter, light harvesting complex, ribosome, etc. (Burns, Heinrich, Kacser, Rappoport).
- Shows that there is significant variation in the extent to which individual catalysed reactions in a sequence control the overall rate.

- Naïve optimal allocation of resources among catalysts would have them all sharing in control of overall flux to an extent weighted by the cost of synthesising and maintaining the catalyst
- Large catalyst molecules with low specific reaction rates (e.g. ribosomes, Rubisco, light-harvesting complexes) are often over-represented in their effect on overall flux
- Compromise between resource cost of synthesis and fractional limitation of growth rate?

- Theory suggests that kinetic heterogeneity in a pathway is needed for stability and self-correction, so an equal share of the control of flux by all catalysts might not be of selective advantage even if it was possible
- Oscillations may be manifestations of self-correction and of kinetic heterogeneity

- Example might be spontaneous oscillations in the rate of photosynthetic CO₂ uptake and O₂ production in constant conditions
- More common in C₃ than C₄ land plants; only recently found in an alga
- Kühn and Raven 2008 *Photosynthesis Research* 95: 37-44

- Evidence that significant increases in photosynthetic and growth rates are possible by decreasing expression enzymes of the photorespiratory carbon oxidations cycle
- High expression today a 'memory' of earlier atmospheric compositions
- Early = before 1750, including low CO₂ and CO₂/O₂ in the Pleistocene glacial maxima
- Zhu, de Sturler and Long (2007) *Plant Physiology* 145: 513-526
- Zhu, Long and Ort (2008) *Current Opinion in Biotechnology* 19: 153-159

Conclusions on the catalyst by catalyst approach

- Possible trebling in μ_{\max} of oxygenic photo-lithotrophs (and other nutritional categories) by
- Making all catalysts equally growth-limiting or optimising extent to which a catalyst limits relative to resource costs of generating unit catalytic capacity for each catalyst (**optimal allocation**)
- Having every catalyst present as its smallest variant (***B***)
- Having every catalyst present as its highest specific reaction rate variant (***R***)
- Having all copies of the catalysts active (***F***)
- Not achieved in the nature or, yet. experimentally
- Need for self-correction and stability in pathways
- Variations in the environment in the real (and even the biotechnological) world

Larger-scale replacements

Maximise **B and R**

- CO₂ fixation pathways
- The Benson-Calvin Cycle (photosynthetic carbon reduction cycle or PCRC) involves Rubisco, a large, low specific reaction rate carboxylase with a relatively low CO₂ affinity and O₂ as an alternative, competing substrate
- Apparently it is mechanistically impossible to ‘improve’ all kinetic parameters of Rubisco **simultaneously** (Tcherkez et al. 2006 *PNAS* 103: 7246-7251)
- **Bob Blankenship’s talk**

- In the present atmosphere photosynthesis using the Benson-Calvin cycle uses
- A photorespiratory carbon oxidation cycle (PCOC) to remove the phosphoglycolate generated by the oxygenase reaction of Rubisco when CO₂ enters by diffusion, and in some cases,
- A CCM* (inorganic carbon concentrating mechanism) that concentrates CO₂ near Rubisco which increases the rate of the PCRC and decrease the rate of the PCOC
- *Marty Spalding's talk

- Several other autotrophic CO₂ assimilation pathways are known, some with lower energy requirements and/or relatively high CO₂ affinity with no competition from O₂.
- Could large-scale genetic engineering put these pathways into oxygenic photolithotrophs and decrease resource costs of photosynthesis and/or increase the photosynthetic rate and μ_{\max} ?
- Naturally occur only in some chemo-lithotrophic and some non-oxygenic photo-lithotrophic Archaea and Bacteria (rest have PCRC), with very small contributions to global autotrophic CO₂ fixation today

Quantitative significance of autotrophs other than oxygenic photolithotrophs

(Raven, Aquatic Microbial Ecology, in press doi 10.3354/ame01315)

- Present day chemolithotrophs and non-oxygenic photolithotrophs: **~0.4 Pg C year⁻¹**
- Present day aquatic photolithotrophs: **>50 Pg C year⁻¹**
- Present day terrestrial photolithotrophs: **~60 Pg year⁻¹**
- Chemolithotrophs and non-oxygenic photo-lithotrophs before oxygenic photosynthesis (~ 2.7- 3.8 Ga ago): **< 5 Pg C year⁻¹**
- Chemolithotrophs before photosynthesis (~ 4 Ga ago): **~0.001 Pg year⁻¹**

- How do these pathways compare with the Benson-Calvin cycle (PCRC) for
- Energetics?
- Kinetics?

Energetics of the six autotrophic inorganic carbon assimilation pathways with carbohydrate as the product

- Benson-Calvin cycle (PCRC) in the absence of photorespiration or a CCM:
3 ATP and 2 NAD(P)H per CO₂
- Reductive tricarboxylic acid cycle:
1.67 ATP and 2 NAD(P)H per CO₂
- 3-Hydroxypropionate pathway:
2 ATP and 2 NAD(P)H per CO₂
- 3-Hydroxypropionate/4-Hydroxybutyrate pathway:
3 ATP and 2 NAD(P)H per CO₂
- Dicarboxylate/4-Hydroxybutyrate pathway:
2.67 ATP and 2 NAD(P)H/Fd⁻ per CO₂
- Wood-Ljungdahl pathway to sugars
1 ATP and 2 NAD(P)H per CO₂

(Raven, Aquatic Microbial Ecology, in press doi: 10.3354/ame01315)

Kinetics of the six autotrophic inorganic carbon assimilation pathways with carbohydrate as the product

- Benson-Calvin cycle (PCRC):
 $K_{1/2} \text{CO}_2 > 10 \text{ mmol m}^{-3}$, O_2 competitive inhibitor
- Reductive tricarboxylic acid cycle:
 $K_{1/2} \text{CO}_2 > 1,300 \text{ mmol m}^{-3}$, O_2 does not compete but does inhibit
- 3-Hydroxypropionate pathway
 $K_{1/2} \text{CO}_2 \sim 10 \text{ mmol m}^{-3}$, O_2 does not compete or (apparently) inhibit
- 3-Hydroxypropionate/4-Hydroxybutyrate pathway, Dicarboxylate/4-Hydroxybutyrate pathway:
 $K_{1/2} \text{CO}_2 > 2,000 \text{ mmol m}^{-3}$, O_2 does not compete; does it inhibit?
- Wood-Ljungdahl pathway:
 $K_{1/2} \text{CO}_2 \sim 40,000 \text{ mmol m}^{-3}$, O_2 does not compete but does inhibit

(Raven, Aquatic Microbial Ecology, in press doi: 10.3354/ame01315)

Good News

- All the alternative pathways needs less energy to run them at present atmospheric CO₂, than the Rubisco/PCRC mechanism, especially when energy needed for the PCOC or the CCM are considered.
- Some pathways have higher CO₂ affinity, none have O₂ as a competitive inhibitor

Bad News

- Some of the alternative pathways have some degree of O₂ inhibition, or even destruction of enzymes. They function mainly in anoxic or hypoxic environments.
- Some have very low CO₂ affinity
- Could this be remedied by GM???

Biotechnological and biogeoengineering implications of intrinsic constraints

- Oil-producing algae: lower specific growth rate than same-sized cell with no intracellular oil because less room for catalysts
- Even if oil is extracellular, lower specific growth rate because photosynthetic products are diverted into an end-product rather than producing more catalysts of growth
- Unavoidable outcome
- Less of a problem if whole alga is harvested

Extrinsic Limits on algal growth

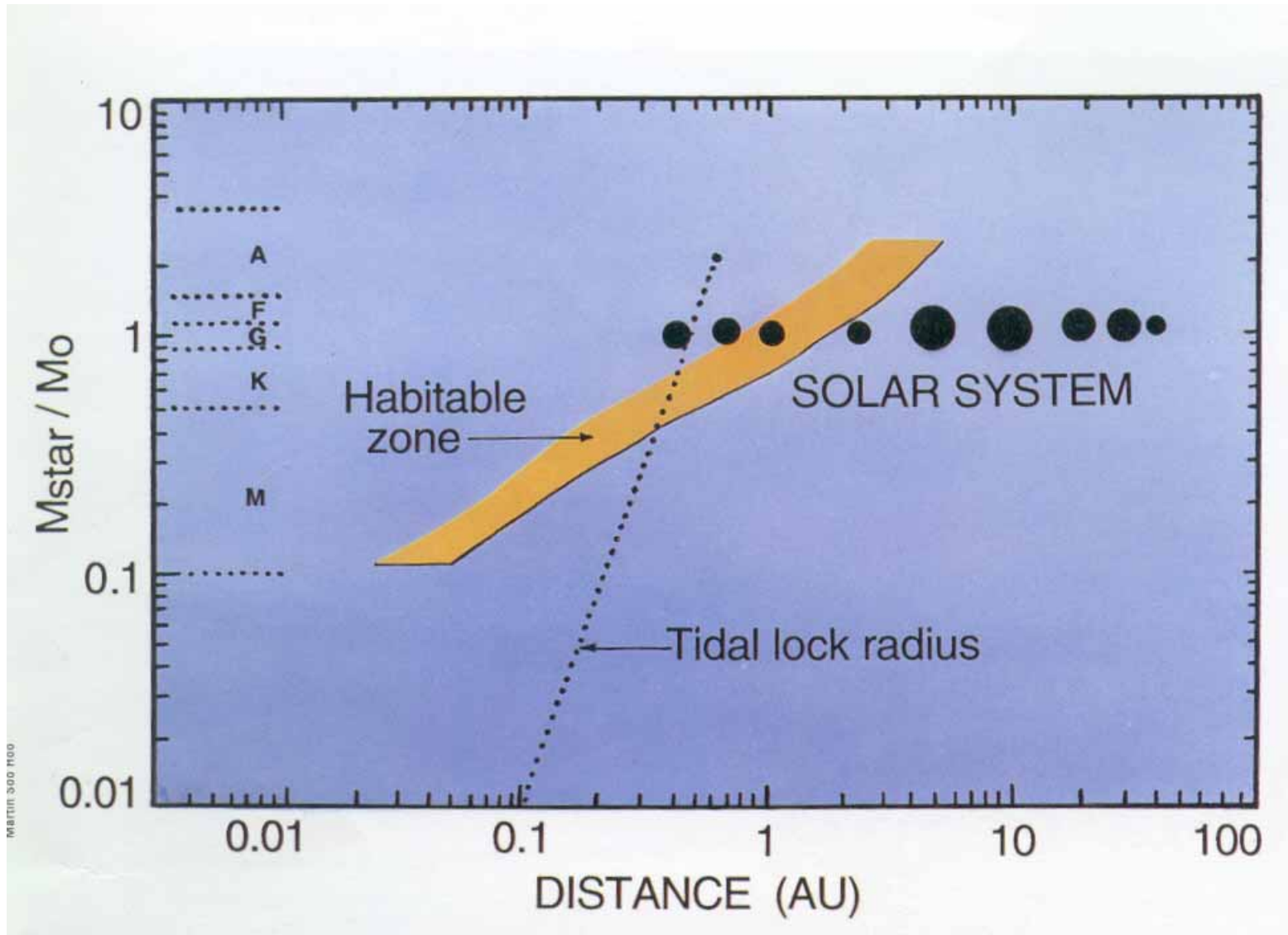
- Photosynthetically Active Radiation
- Full sun at noon $\leq 2 \text{ mmol photon m}^{-2} \text{ s}^{-1}$
(400-700nm)
- If all incident PAR is absorbed, net primary productivity of 1 mol C assimilated into cell material per 12 photons absorbed (both assumptions optimistic!) = net primary productivity of $\leq 170 \text{ } \mu\text{mol C m}^{-2} \text{ s}^{-1}$

- Assumes all cells are strictly light-limited and are acclimated (phenotypic)/adapted (genotypic) to their radiation microclimate, i.e. are part of a microbial mat with problems of nutrient supply and harvesting
- In a stirred suspension culture stirring is never fast for cells to instantaneously 'see' a constant radiation environment in a dense, totally absorbing, culture within the reaction time (a few ms) of photosynthesis
- Ben Hankamer's talk, Marcel Janssen's talk

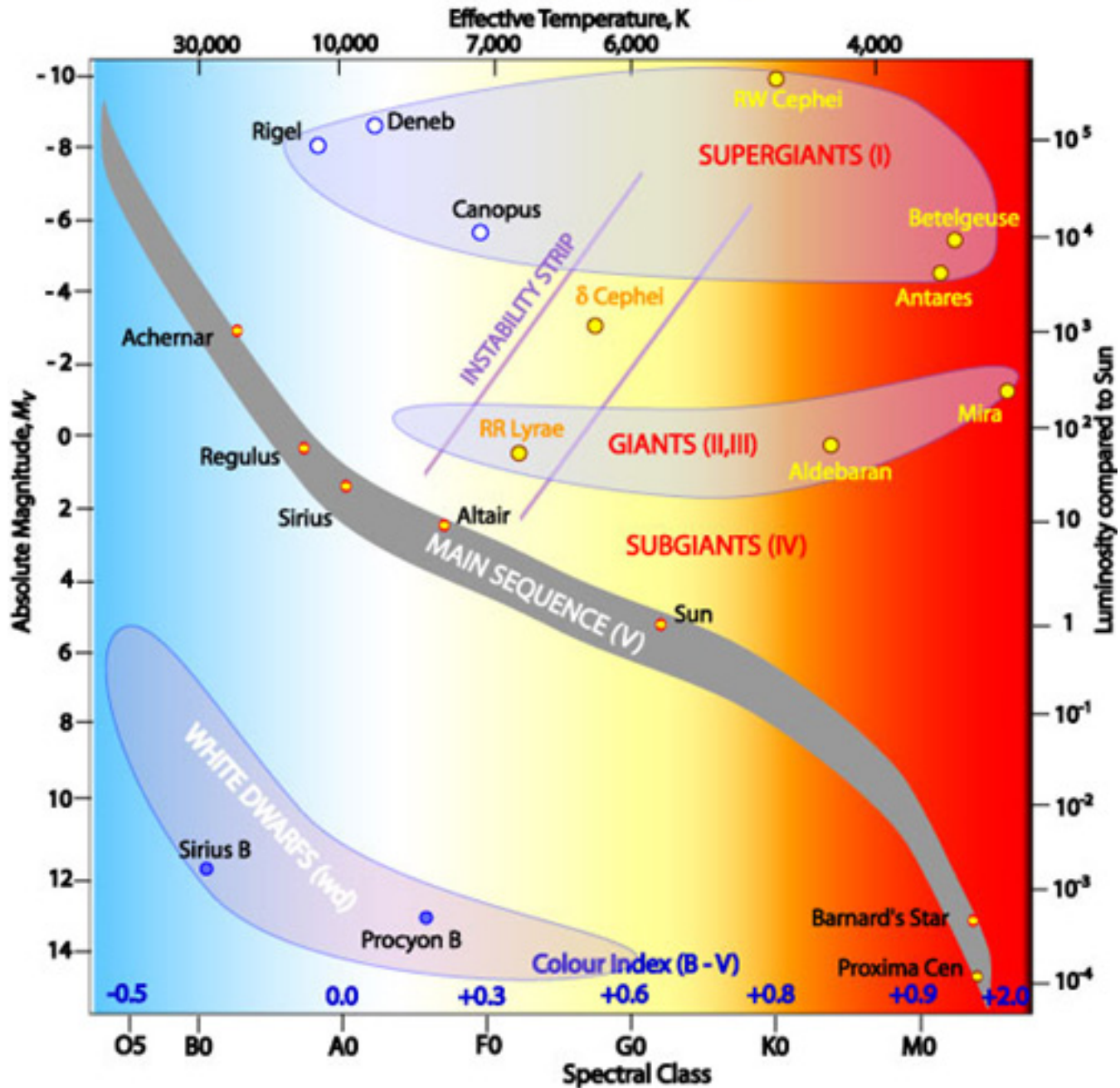
- Quota of light-harvesting machinery which is optimal for one incident photon flux density is not optimal for the other photon flux density
- Spatial variations in PAR are encountered in a mixed, attenuating environment (natural or in biotechnology) at a given water surface PAR
- Also variations in PAR through the day.
- Limits on the extent and rate of acclimation of the light-harvesting apparatus that would be useful in natural selection:
- Dimier, Geider, Raven, Benoit, *Limnology and Oceanography* **53**: 823-836

- Nowhere on Earth has constant natural PAR at a level adequate to support photolithotrophy
- Hydrothermal vents in mid-ocean ridges operate 24/365 but generate only a very low PAR (similar to surface full moonlight: apparently adequate for Chlorobi), and only for a few decades in a given location
- Look to exoplanets: 373 known at 13 August 2009 (<http://exoplanet.eu>) currently known – most are not Earth-like planets (ELPs)
- Continuous light for half of a tidally locked ELP in the (continuously) habitable zone of a M star

Continuously Habitable Zone



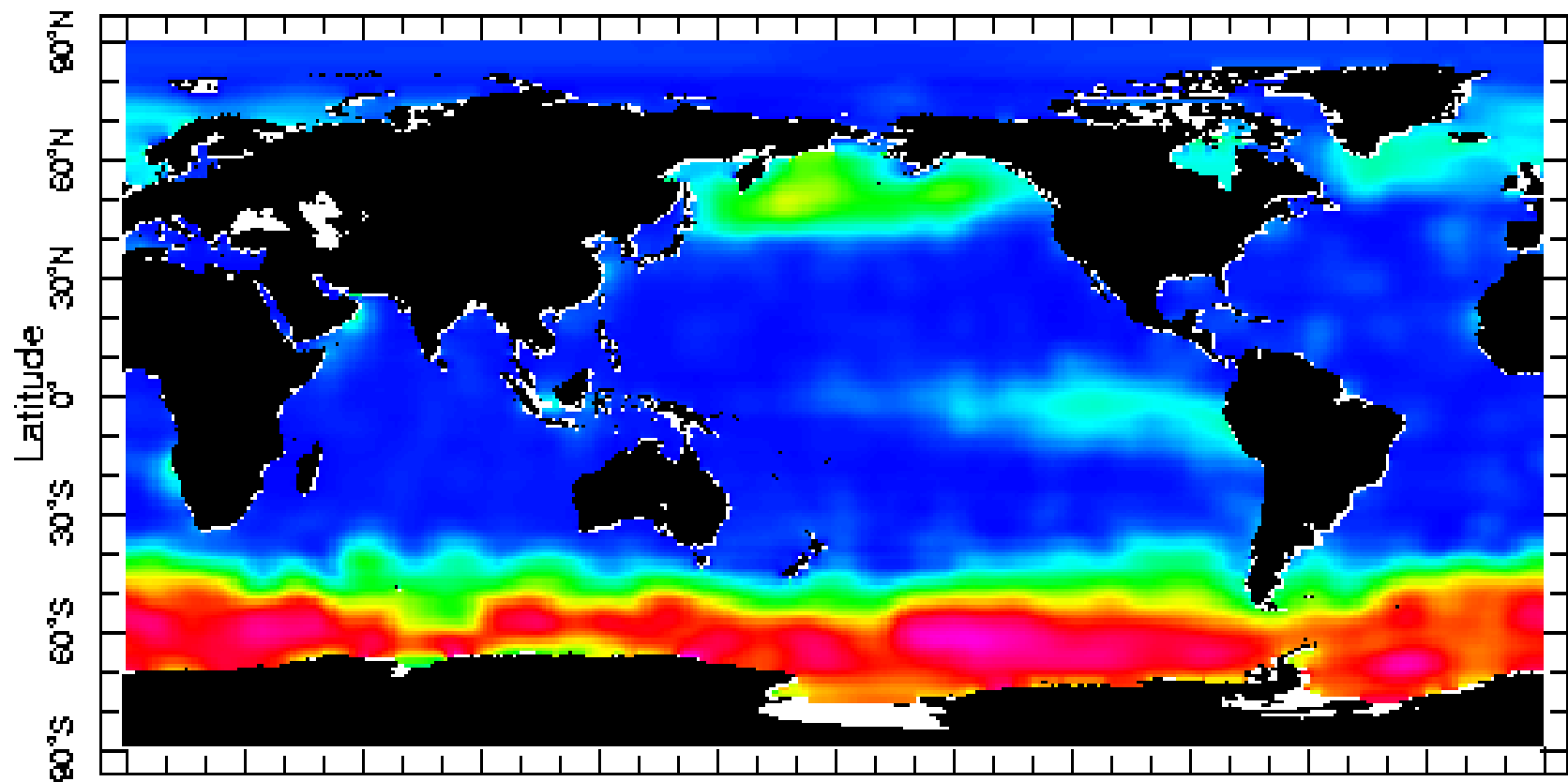
Hertzspung-Russell Diagram



- Supply of nutrients (including CO₂) by bubbling
- CO₂ from industrial effluent – at high concentration, but then problems with stripping out all the CO₂ in the cultures
- Problems with nutrient removal and recycling from some harvested biofuels
- Intrinsic factors – minimize use of non-C nutrients: energy costs for N fertilizer, limited supply of high-grade rock phosphate

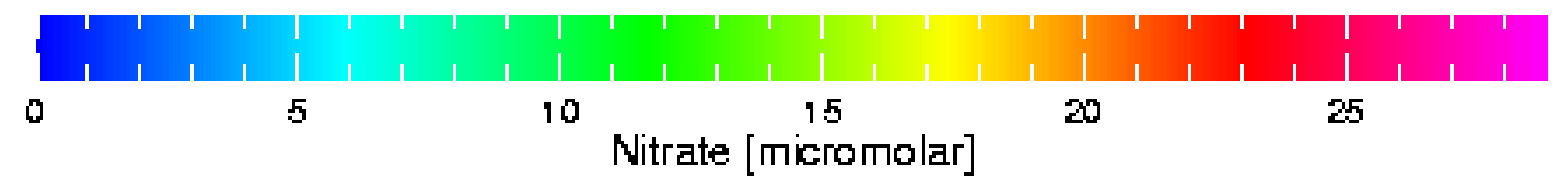
Biogeoremediation (Biogeoengineering)?

- Limitation of phytoplankton growth in the ocean is limited by
- Light: seasonality at higher latitudes, and deep mixing of surface waters
- Nutrients: could grow faster if there was a higher concentration of nutrients
- In order of area in which a given element is limiting growth: $N > Fe > P$



0° 30°E 60°E 90°E 120°E 150°E 180° 150°W 120°W 90°W 60°W 30°W 0°
Longitude

0.0 m



Biogeoremediation (Biogeoengineering)?

- Add Fe^{2+} to Fe-limited areas of the ocean, $\text{CO}(\text{NH}_2)$ to N-limited areas, HPO_4^{2-} to P-limited areas
- Stimulate photosynthesis and growth of (e.g.) larger diatoms ✓
- Short-term drawdown of CO_2 ✓
- Sinking of the additional organic C to deep ocean?
- Woodward et al. 2009, Current Biology **19**: R615-R623

Biogeoremediation (Biogeoengineering)?

- Even with such sinking, effect on surface ocean and atmospheric CO₂ smaller than originally suggested X
- Unintended consequences, e.g. deep ocean anoxia due to added organic C, production of N₂O and CH₄ – much more powerful greenhouse gases than CO₂ X
- Energy/carbon costs of producing and distributing the fertilizers ?

Biogeoremediation (Biogeoengineering)?

- Energy/carbon cost problem might be decreased using tubes to below the upper mixed layer to bring up high-nutrient water
- High nutrient from decomposition of sedimented organisms
- Power by wave action with a non-return valve
- Problem: deep sea water is also enriched in CO₂!
- Lovelock and Rapley (2007) Nature 449: 403

Conclusions

- Significant increases in the growth rate and/or resource use efficiency of photolithotrophs is possible
- For microalgae, should allow a given harvest to be obtained with less biomass and/or less resource input, or a greater harvest with the similar biomass and resource input
- Bioremediation: possibly

Acknowledgements

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- Posterity for giving me retrospective permission to me to muse on astrobiology, ancient carbon cycles and the like instead of devoting my whole time to considering biofuels, the effects of ocean acidification on marine biota, and how rice productivity could be increased