GM algae; a Risk-Benefit Assessment

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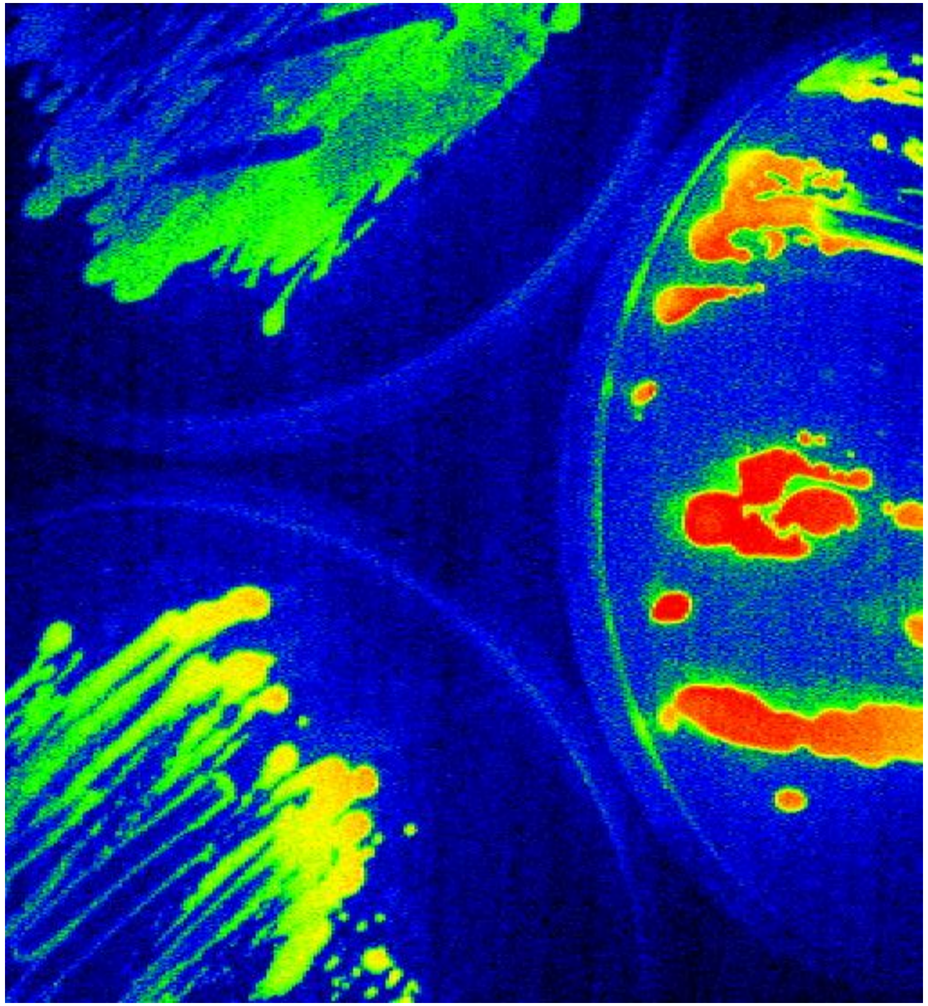
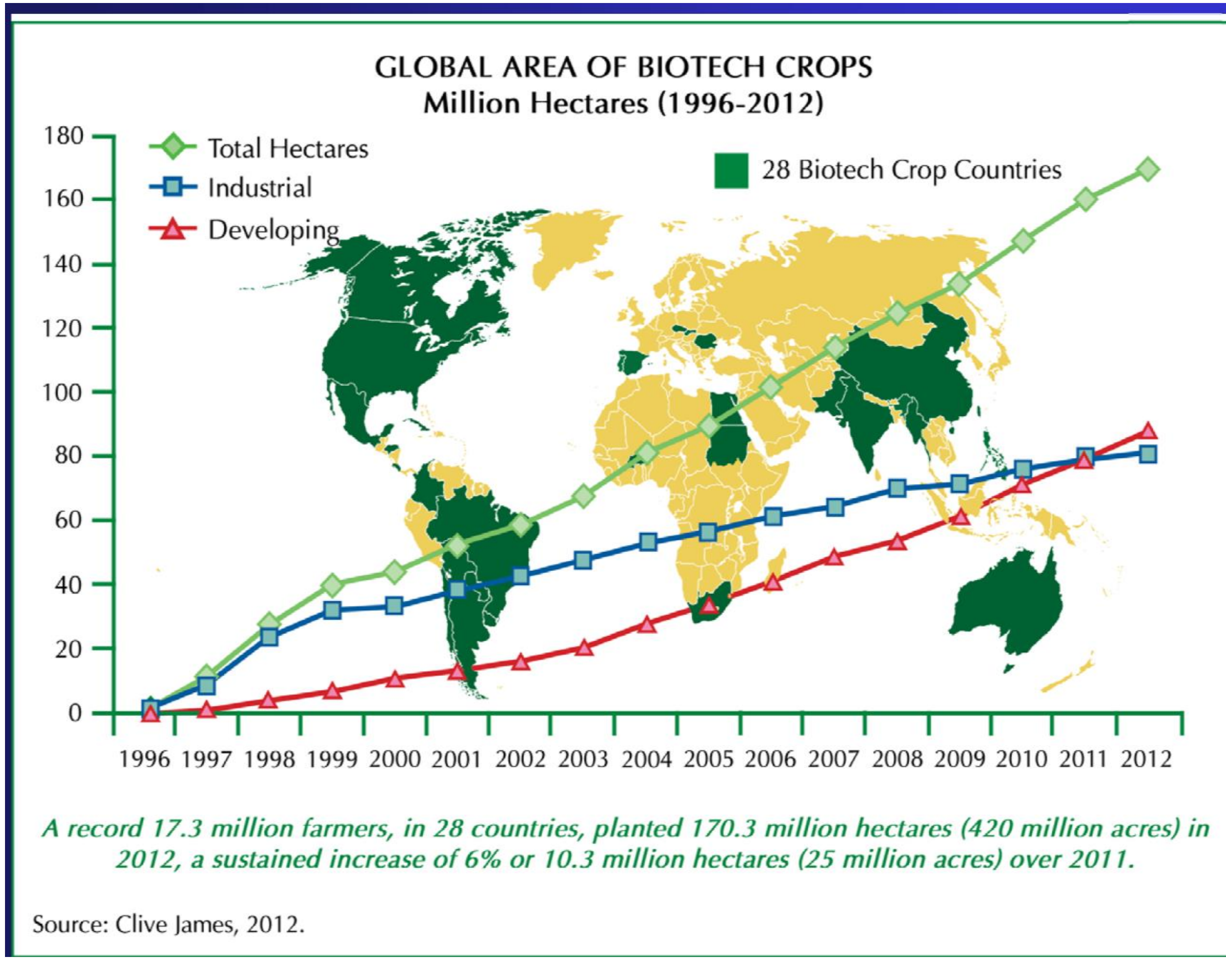
Outline

- Historical perspective; what has been the impact of GM traits on agriculture?
- What are the some of the known potential benefits and risks of GM traits in crops?
- How are GM agricultural traits managed?
- What is the potential for algae in meeting renewable fuel requirements?
- Is their a role for GM traits in algal biomass production?
- What are the perceived risks for GM algae?
- How can risks be mitigated?
- What agencies regulate GM algae?
- Examples of emerging algal GM traits for crop improvement
- Summary



Why GM Technology for Agriculture?

- **Increased income generation:** Increase in crop value from GM traits; $98 billion (1996-2011)
- **GM technology is part of the sustainable solution for agriculture:** 23 billion kg in reduced CO_2 emissions since 1996. 473 million kg reduction in pesticide use. No-till agriculture.
- **Safety record.** More than 1 trillion meals served (1996-2011). No known illnesses from GM foods.
- **International adoption of GM crops**: Developing countries now have greater acreage in GM crops than developed countries. Growing at 6% acreage/year
- **Addressing global challenges with GM technology:**
    - **C4 Rice** (BMGF): The natural genetic diversity available for improvements in rice yield will be exhausted by 2050. There is a need for a quantum leap in crop production to feed the next generation. (Achim Dobermann, DDG, International Rice Research Institute).
    - **BioCassava Plus** (BMGF): There is insufficient genetic variation in cassava to breed for minimal iron requirements in a cassava-based diet; the only option is through transgenics. (Howard Bouis, Harvest Plus)



What are some of the potential benefits derived from transgenic crops?

- Lower costs (major driver in US)
- Reduced energy (no-till) and acreage (greater productivity and sustainability) demands.
- Reduced use of broad-spectrum, synthetic pesticides
- Reduced soil erosion; no till agriculture
- Better nutritional composition of foods - biofortification
- Longer food shelf life
- Increased stress tolerance; drought tolerance
- Renewable production of green chemical feed stocks – e.g., biofuels
- Pharmaceutical production in pathogen free organisms

No till farming: Reduced fuel use, erosion, and CO_2 emissions

What are some of the potential risks associated with transgenic crops?

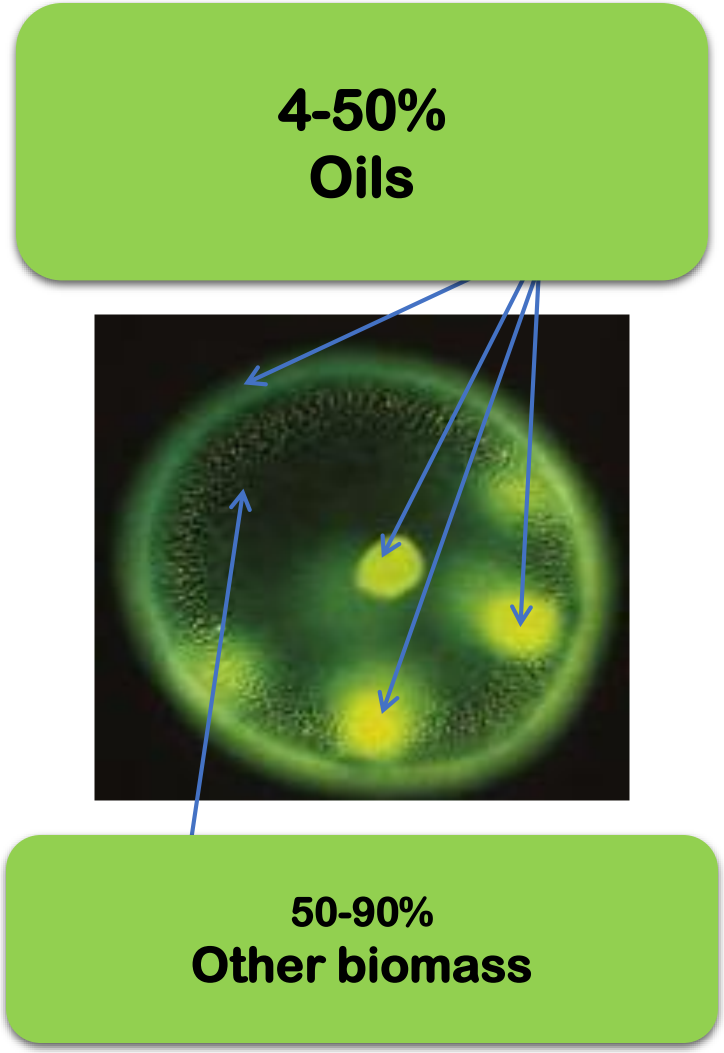
- Transgene introduction may cause an unintended mutation in the plant genome that is undesirable
- Transgene may escape to related plants; pollination of nearby relatives
- Increased use of herbicides to control weeds in herbicide resistant crops
- Development of herbicide resistance in weedy plants
- Widespread planting of genetically uniform strains
- Unanticipated alterations in food composition
- Expression of new allergens
- Problems segregating transgenic from non-transgenic crops
- Greater market control by fewer producers due to high costs of commercializing transgenic crops

Managing risks and benefits:

Example of one regulatory approval strategy for crops (BioCassava Plus)

- Transgenics must meet restrictions of Plant Protection Act (no gene sequences from pathogens or humans)
- Transgene products must be non-toxic and non-allergenic (bioinformatics screen)
- Non-essential DNA sequences should not be included in transgenic plants
- Transgene integration site in plant genome should have no off-target affects
- Yield and nutritional composition (unless enhanced) of transgenic plants should have no substantive alterations relative to wild-type plants
- Confined field trials are conducted under nationally and internationally (Cartegna protocols) recognized standards
    - Fencing, surveillance, fields lie fallow for one year after trial
    - Flowering controlled so no pollen or seed dispersal
    - Potential animal dissemination controlled
    - All plant material must be destroyed at end of trial
- Field trials show no consequential or unintended impacts on yield or environment
- Animal feeding trials show no adverse affects on animal nutrition or health
- Demonstration of complete digestion of transgenic protein in artificial human stomach
- Human feeding trials show no adverse effects on human health or nutrition
- Regulatory review and approval

Why algae now?
Renewable fuels and green chemical feedstocks



Rapid growth rate

(2-10 X faster than terrestrial plants)

- Unlike plants, all cells are photosynthetic
- High photosynthetic efficiency (CCM)
- Double biomass in 6-12 hours

High oil content

- 4-50% non-polar lipids

All biomass harvested

- 100%

Harvest interval

- 24/7; not seasonally, so reduces risk

Sustainable

- Capture CO_2 in ponds as bicarbonate
- Use waste water nutrients
- No direct competition with food

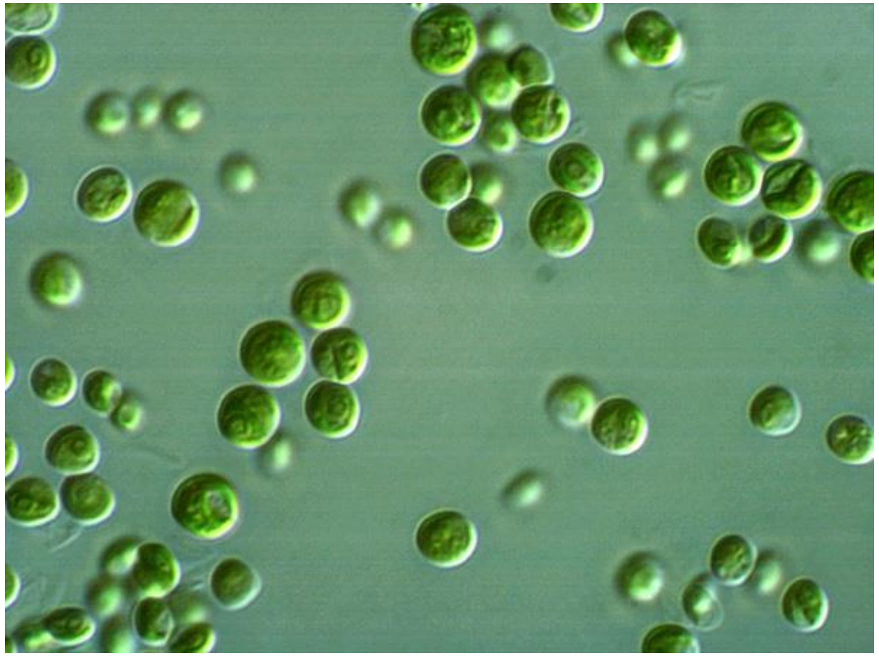
Why is GM technology being considered as part of the solution for algal crop improvement

- In contrast to crop plants, breeding systems have not been developed yet for commercial algal strains

- Other than bioprospecting for better strains and using mutagenesis strategies, introduction of GM traits is currently the most feasible option for strain improvement

- GM traits can be introduced into algae to produce high-value co-products in high volumes

- Unlike crops, GM algae can be grown in contained fermentation systems to reduce the chance of escape



Potential risks associated with the cultivation of GM algae

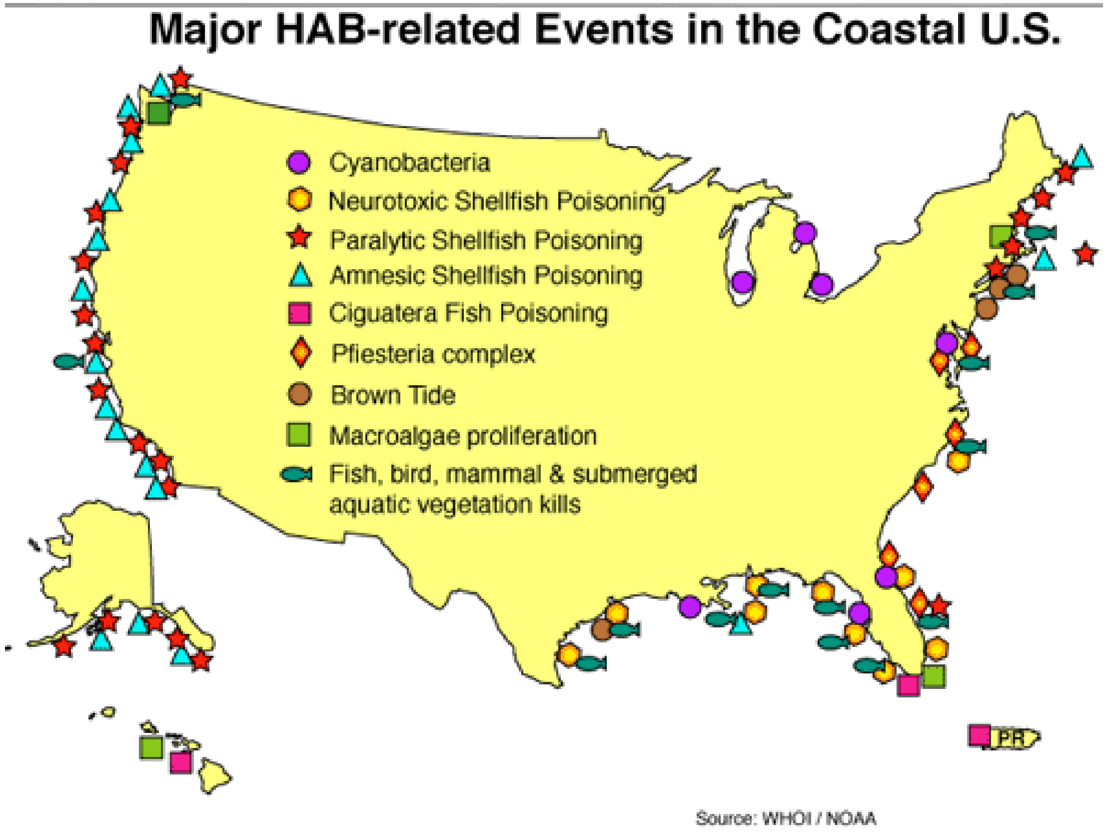
Potential for global dissemination
- Aerosolization and global spread of algae

Persistence in the environment
- Many algae can survive long-term desiccation in soils

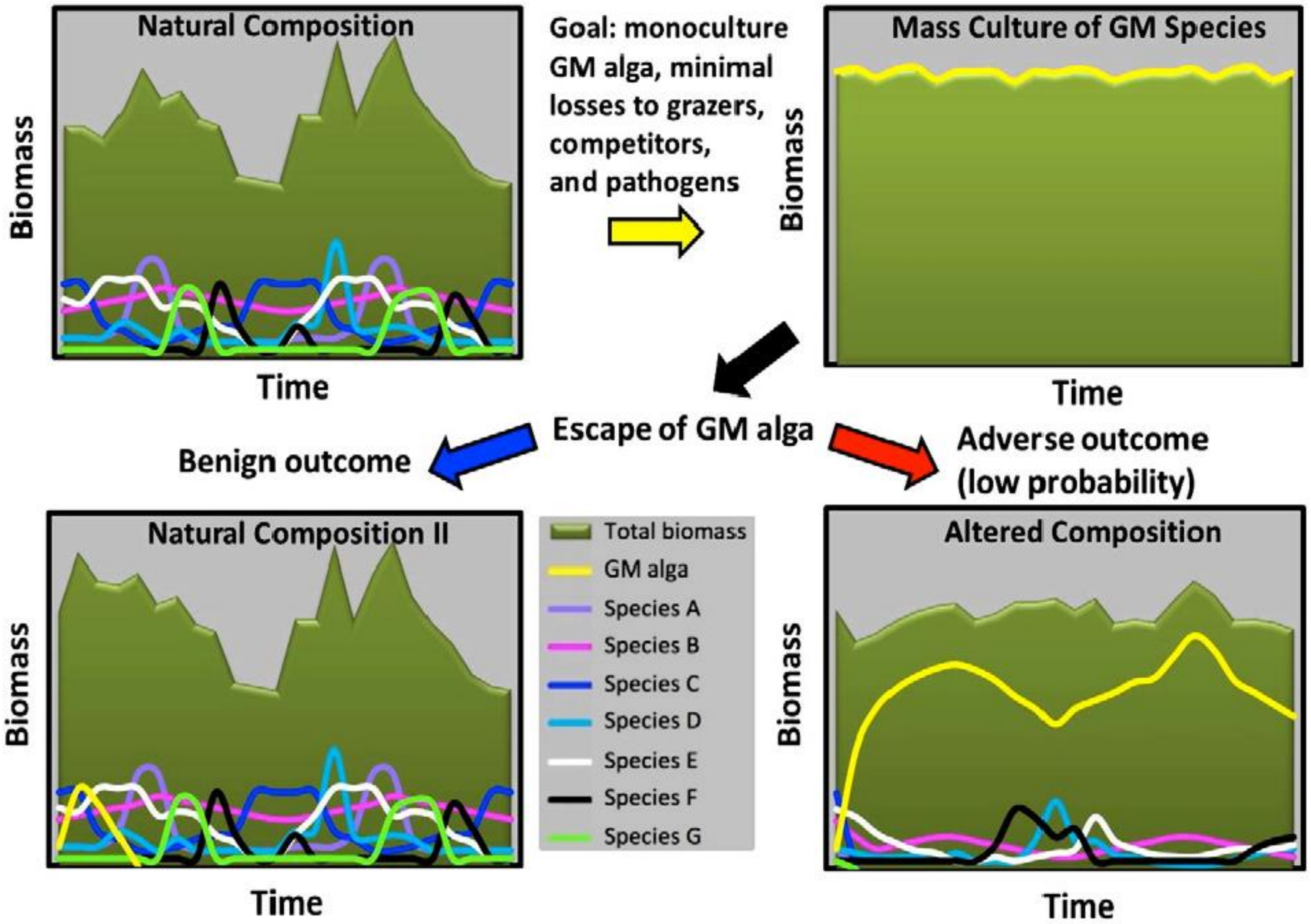
Weedy/Invasive traits
- Enhanced growth in the wild
- Enhanced nutrient utilization
- Toxic to competing algae
- Gene escape

Adverse health impacts needs to be prevented
- No antigen/toxin production

Mitigating the potential for horizontal and/or sexual transfer of transgenes



What are some of the environmental risks?

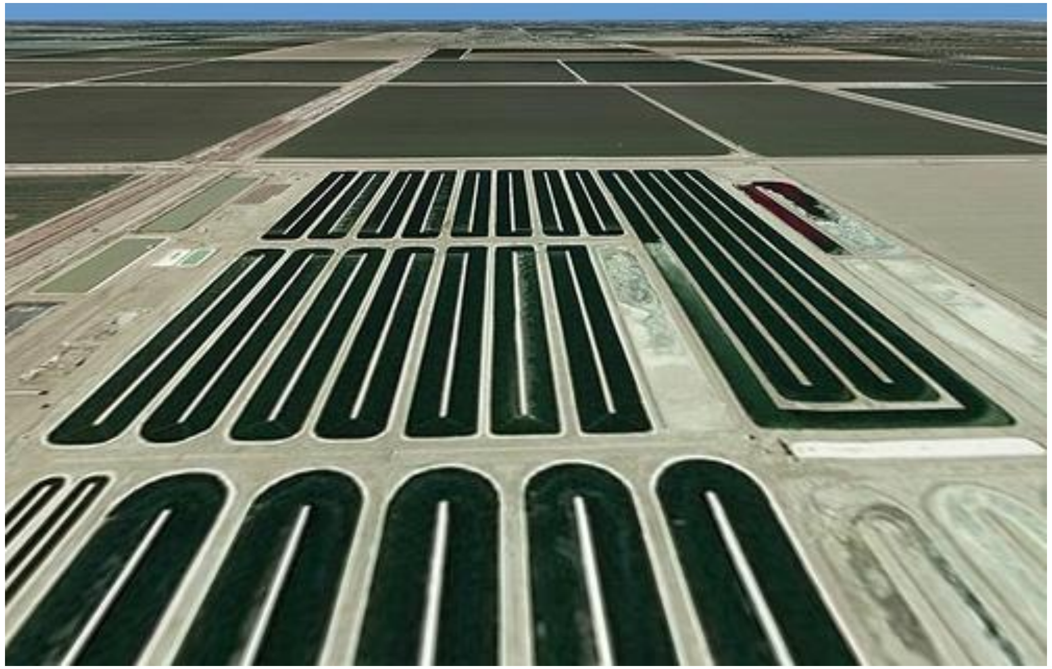


Henley W, Litaker W, Novoveská L, Duke C, Quemada H, **Sayre RT** (2013) Initial risk assessment of genetically modified (GM) algae for commodity-scale cultivation. *Algal Research* 2:66-77.

Risk mitigation for GM algae

Recommendations:

- **GM traits should have minimal impact on the environment**
- **GM traits should ideally reduce evolutionary fitness in the wild**
- **Algae that produce toxins or algae expressing GM traits (toxins, antigens, pathogens, weedy) that are potentially harmful to living organisms and/or disrupt ecosystem health should not be permitted**
- **To evaluate risk potential, controlled field trials should be carried out to evaluate potential or unknown risks**
- **Biocontainment traits can be used to reduce the potential for gene transfer:**
    - **Stacking conditional lethality traits**
    - **Inactivation of genes controlling sexual transmission to reduce gene transfer**
    - **Expression of terminator genes upon escape**

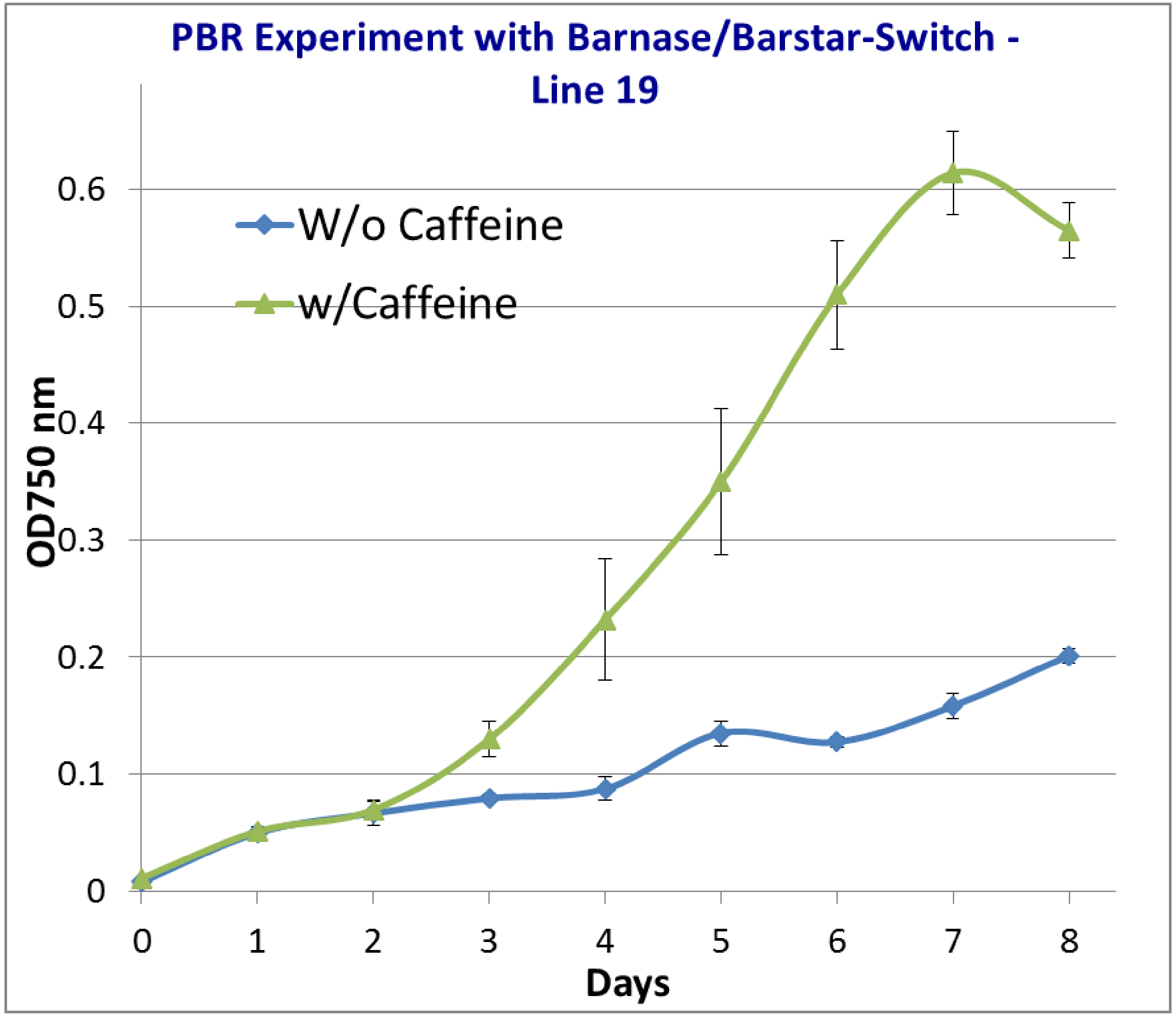


Snow A and Smith VH (2012) Genetically engineered algae for biofuels: a key role for ecologists. *Bioscience* 62: 765-768.

Henley W, Litaker W, Novoveská L, Duke C, Quemada H and Sayre RT (2013) Initial risk assessment of genetically modified (GM) algae for commodity-scale cultivation. *Algal Research* 2:66-77

Gressel J, van der Vlugt CJB, and Bergmans HEN (2013) Environmental risks of large scale cultivation of microalgae: Mitigation of spills. *Algal Research* 2:286-298**.**

Using inducible gene switch technology to express terminator genes upon escape



Growth takes place in the presence of caffeine

Algae have impaired or no growth in the absence of caffeine (in the wild)

Sathish Rajamani et al. in preparation

Regulation of GM algae

- Approval for the release of GM algae is regulated by the EPA under the Toxic Substances Control Act (TSCA) for engineered microorganisms
  - A TSCA Experimental Release Application (TERA) for GM algae requires at least 60 days advance notice for EPA review and approval

- A Microbial Commercial Activity Notice (MCAN) requires 90 days advance notice for EPA review and approval prior to commencing commercial activities

- Additional regulatory agencies (USDA and FDA) may control the release and commercialization of GM algae depending on the products produced and their use.

For further information see:
http://www.slideshare.net/djglass99/david-glass-regulatory-presentation-and-case-study-bio-pac-rim-conference-december-2013?next_slideshow=1

How Do We Make Algal Biofuels Work?

Based on NAABB LCA/TEA analyses, substantive increases in biomass yield and large reductions in harvesting costs are required to make algal biofuels feasible

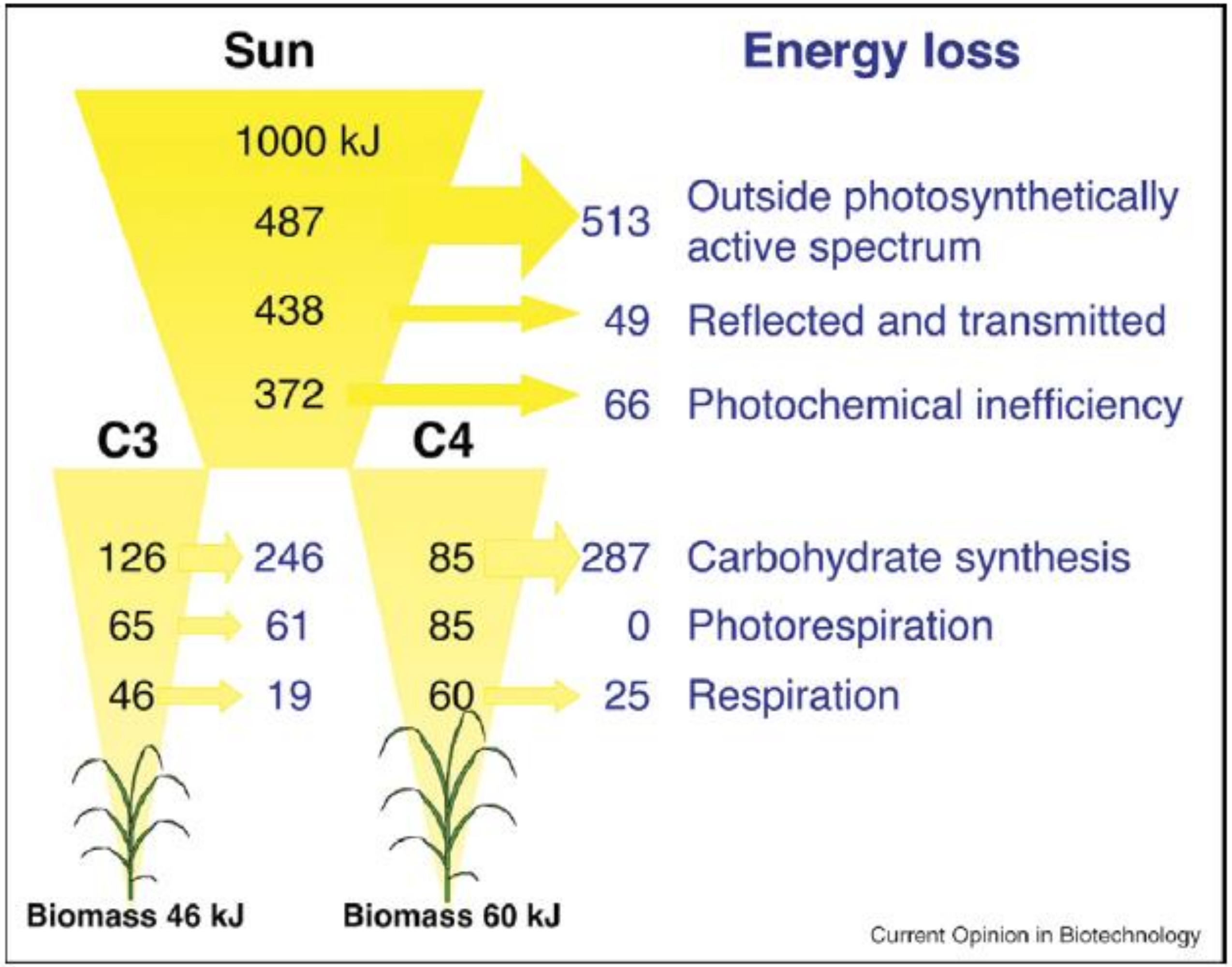
Scenario	Base	Best Case
Biology	Generic algae	GMO
Cultivation	Open Pond	Arid Raceway
Harvesting	Centrifuge	Electrocoagulation
Extraction/Fuel Conversion	Wet Solvent	HTL-CHG
Nutrient Recycling	No	Yes
Biomass Production (Tons/yr)	120,000	380,000
Crude Oil Production (gallons/yr)	4,700,000	52,000,000
Products	Oil and delipidated algae	Oil and methane
Location	Pecos, TX	Tucson, AZ
Total cost/gallon	$230 - 16	~$ 8.00

3X yield increase

< 5% energy content

NAABB LCA/TEA team
James Richardson
Meghan Downes
Eric Dunlop
Mark Wigmosta

An example of an GM trait
Improving biomass production efficiency through optimization of photosynthetic light harvesting and conversion efficiency
Is it risky?



Light capture
55% losses

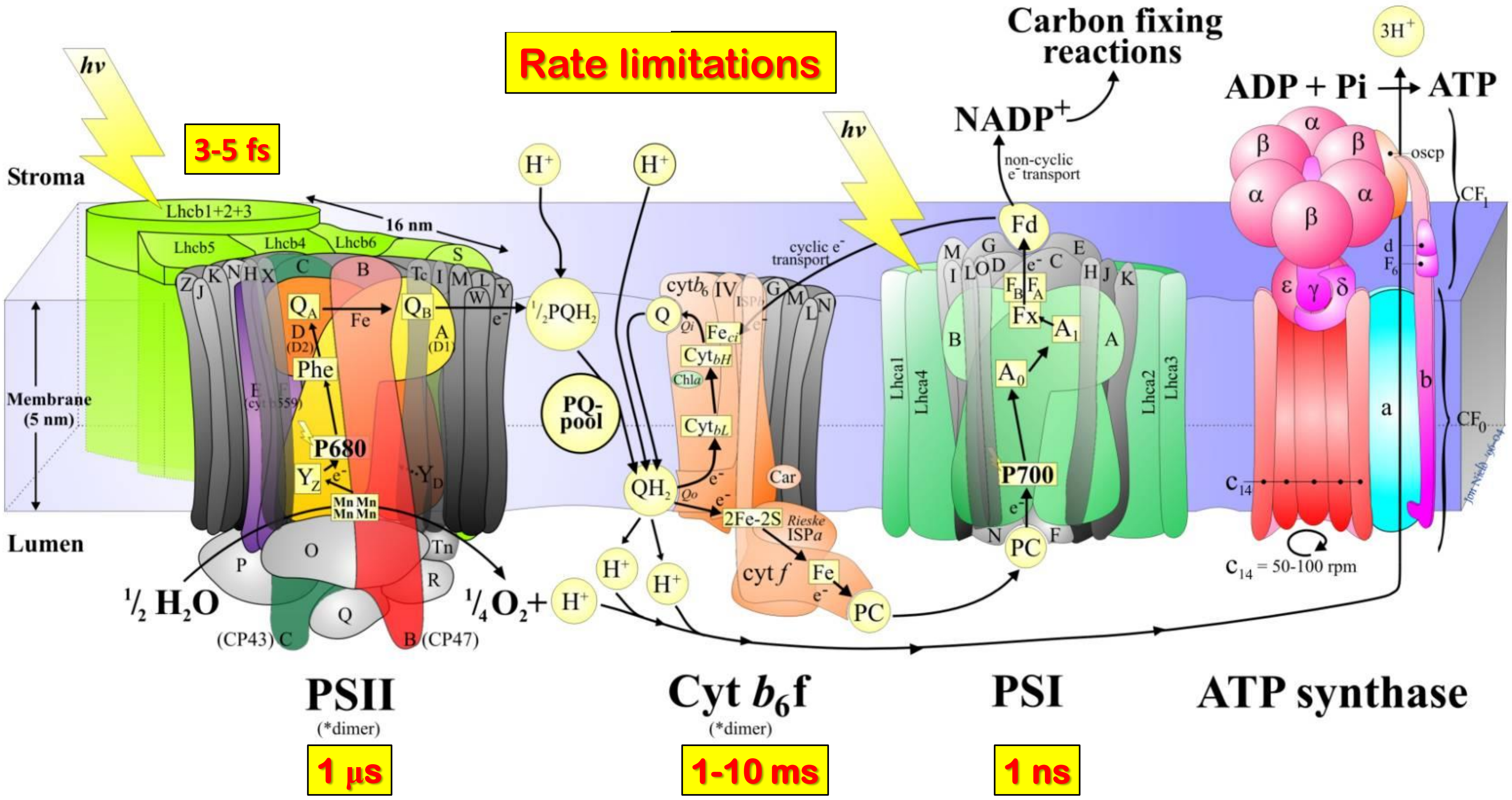
Energy conversion
30-40% losses

Energy accumulation (sink)
4-6% gain

Zhu et al., (2010) *Ann. Rev. Plant Biol.*, 61: 235-261; **Subramanian S, Barry A, Pieris S and Sayre RT (2013)** ***Biotechnol. Biofuels*** **6:150-162**

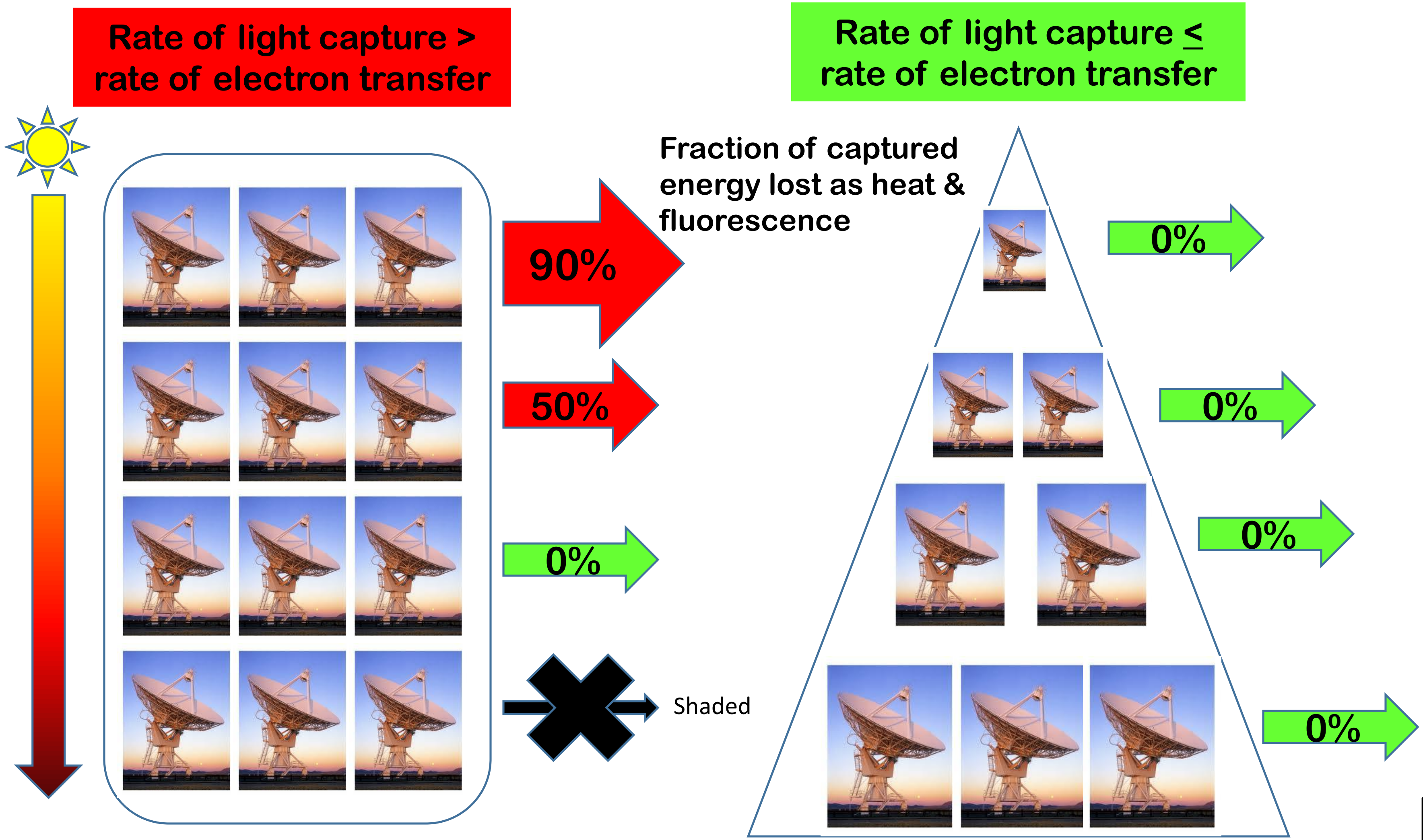
Kinetic bottlenecks in electron transfer impede the conversion of light into chemical energy

Maximum rates of photon capture at full sunlight intensities are 10 times faster than maximum electron transfer rates



Stoichiometry of PSII/Cyt b_6f/PSI = 1:1:1

Optimizing light-harvesting antenna designs for greater fitness in mixed and single species systems

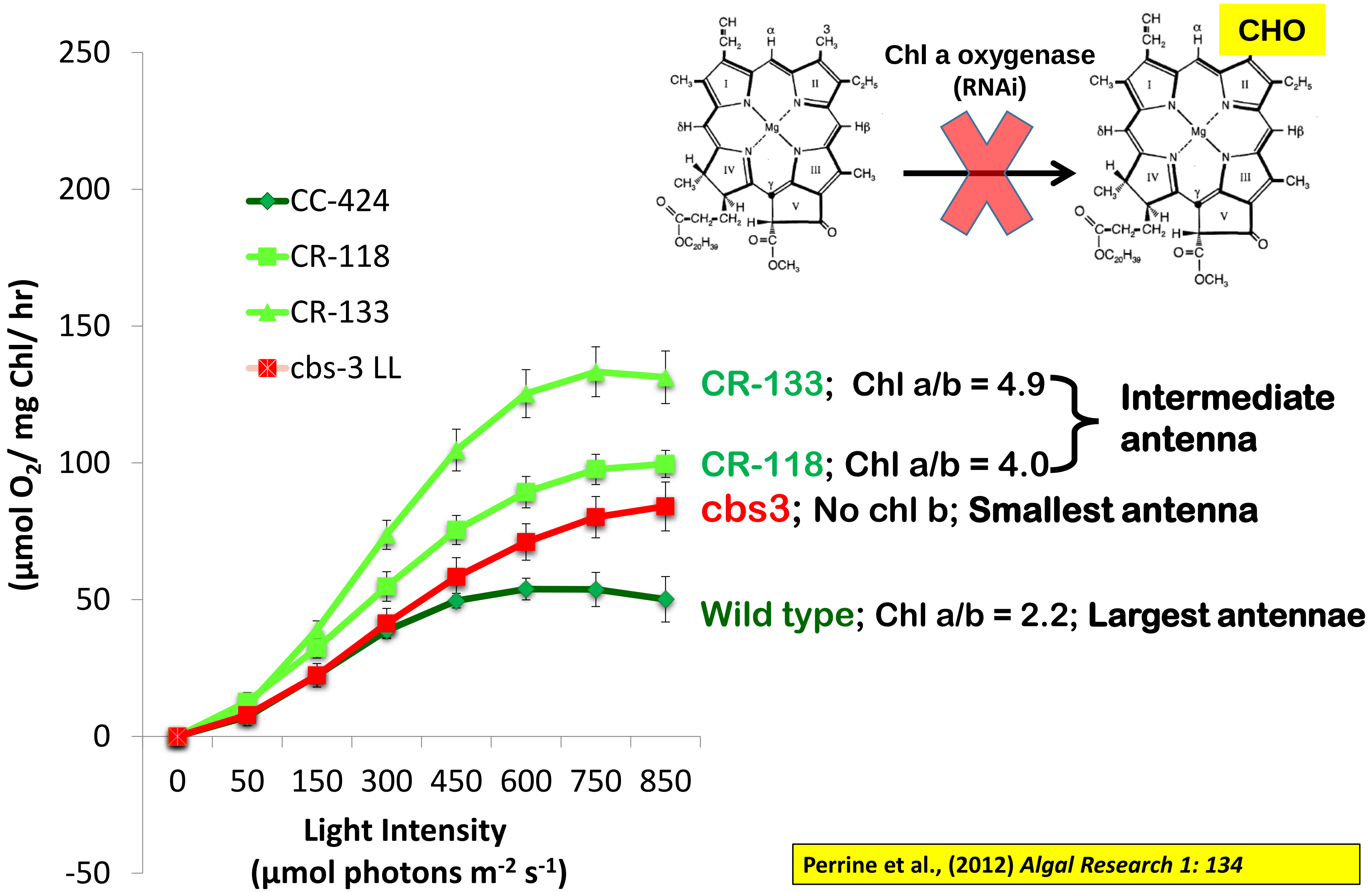


Optimal light capture strategy to compete in polycultures

Optimal light harvesting strategy in monocultures

Perrine et al., (2012) Optimization of photosynthetic light energy utilization by microalgae. *Algal Research* 1:134-142.

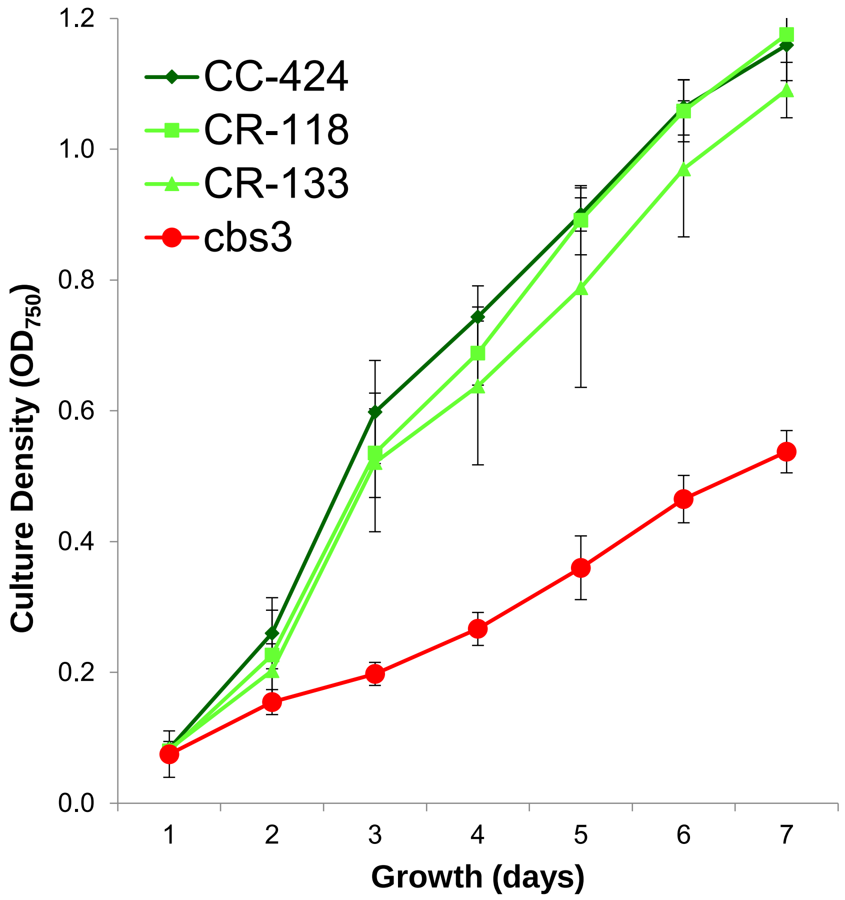
Algae with intermediate Chl b levels have intermediate light-harvesting antennae sizes and 2.5-fold higher photosynthetic rates



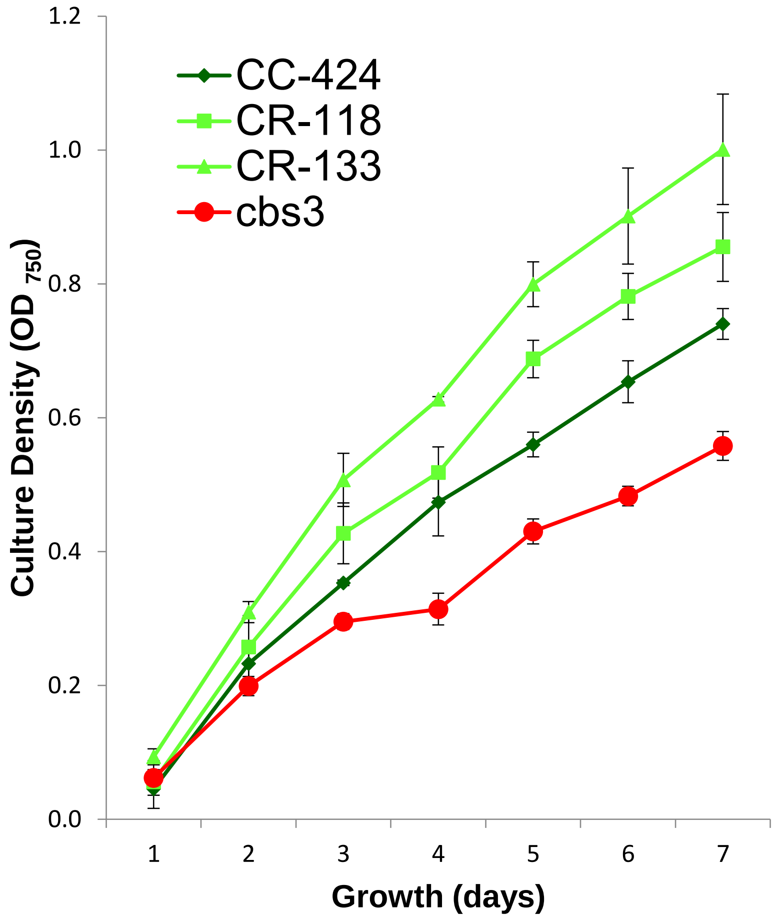
Perrine et al., (2012) *Algal Research 1: 134*

Algae with intermediate antenna size (CR) have 40% higher biomass productivities than WT (CC-424) algae at saturating light intensities

Growth under 50 µmol photons $m^{-2}s^{-1}$
(LOW LIGHT)



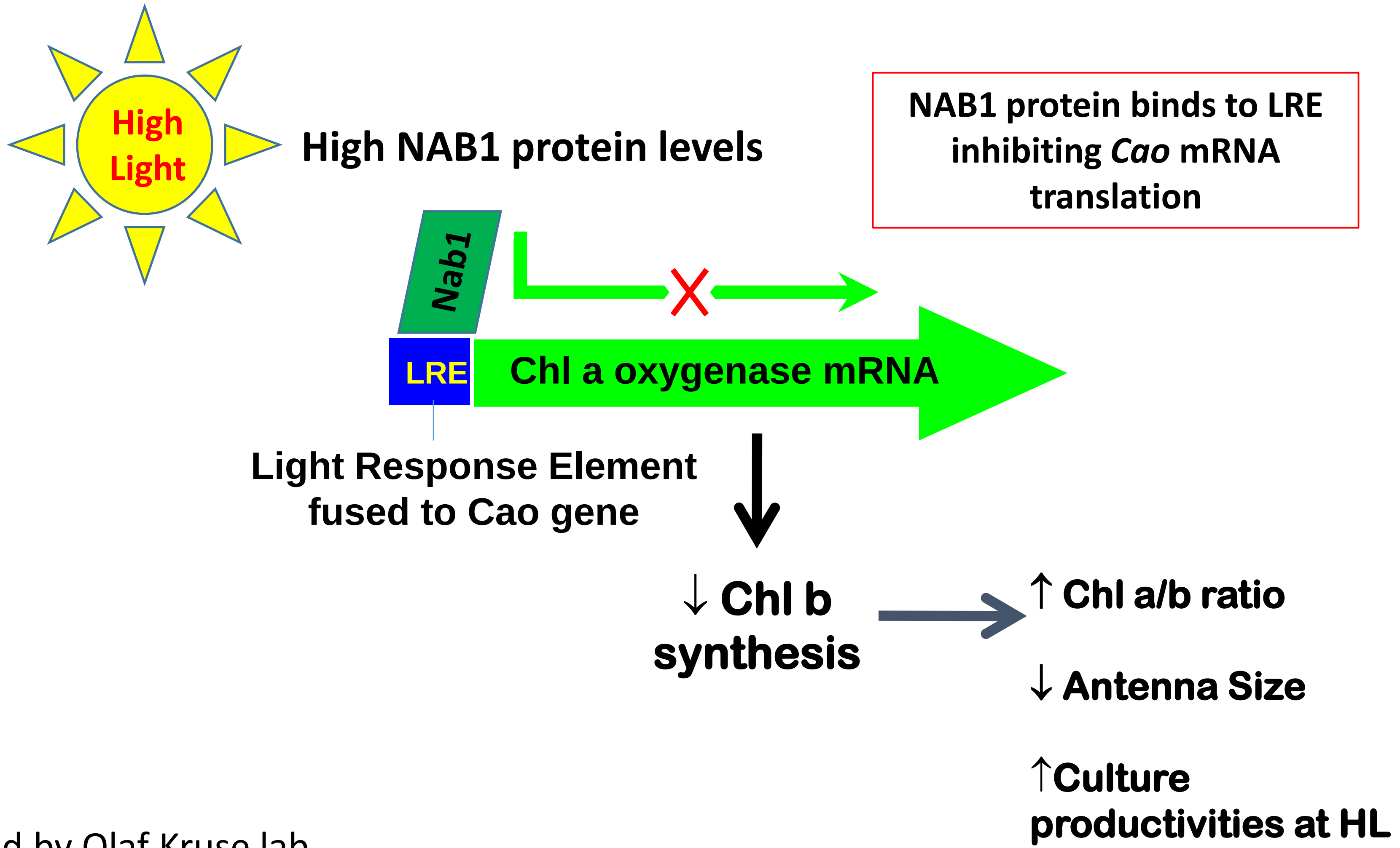
Growth under 500 µmol photons $m^{-2}s^{-1}$
(SATURATING LIGHT)



Engineering self-adjusting, light-harvesting antenna systems for dynamic control of photosynthetic efficiency

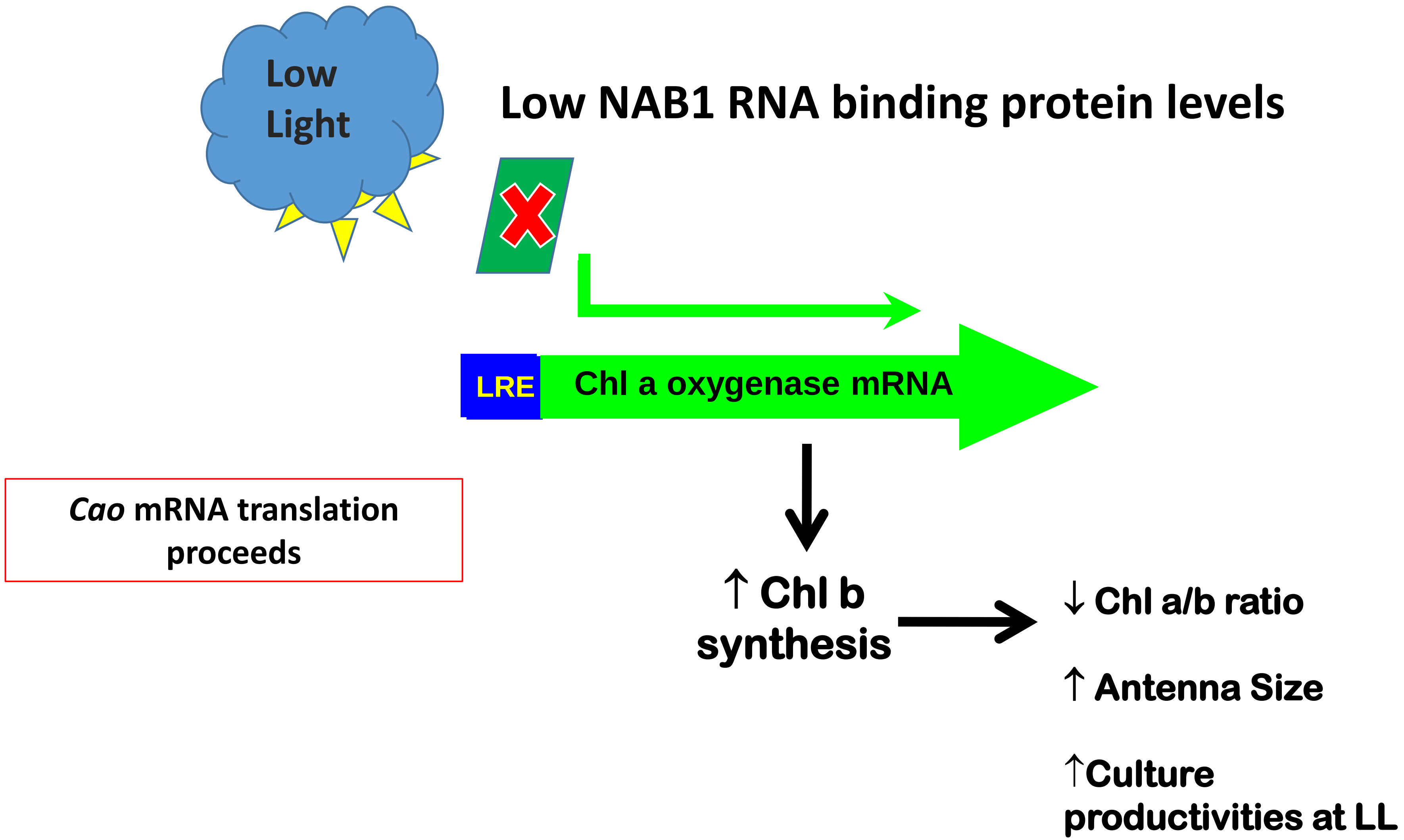
Engineering antenna sizes that self-adjust to changing light intensities: Reducing chlorophyll b accumulation in high light to decrease antenna size

Chlamydomonas Chl a oxygenase (no Chl b) mutant background
transformed with LRE-Cao construct

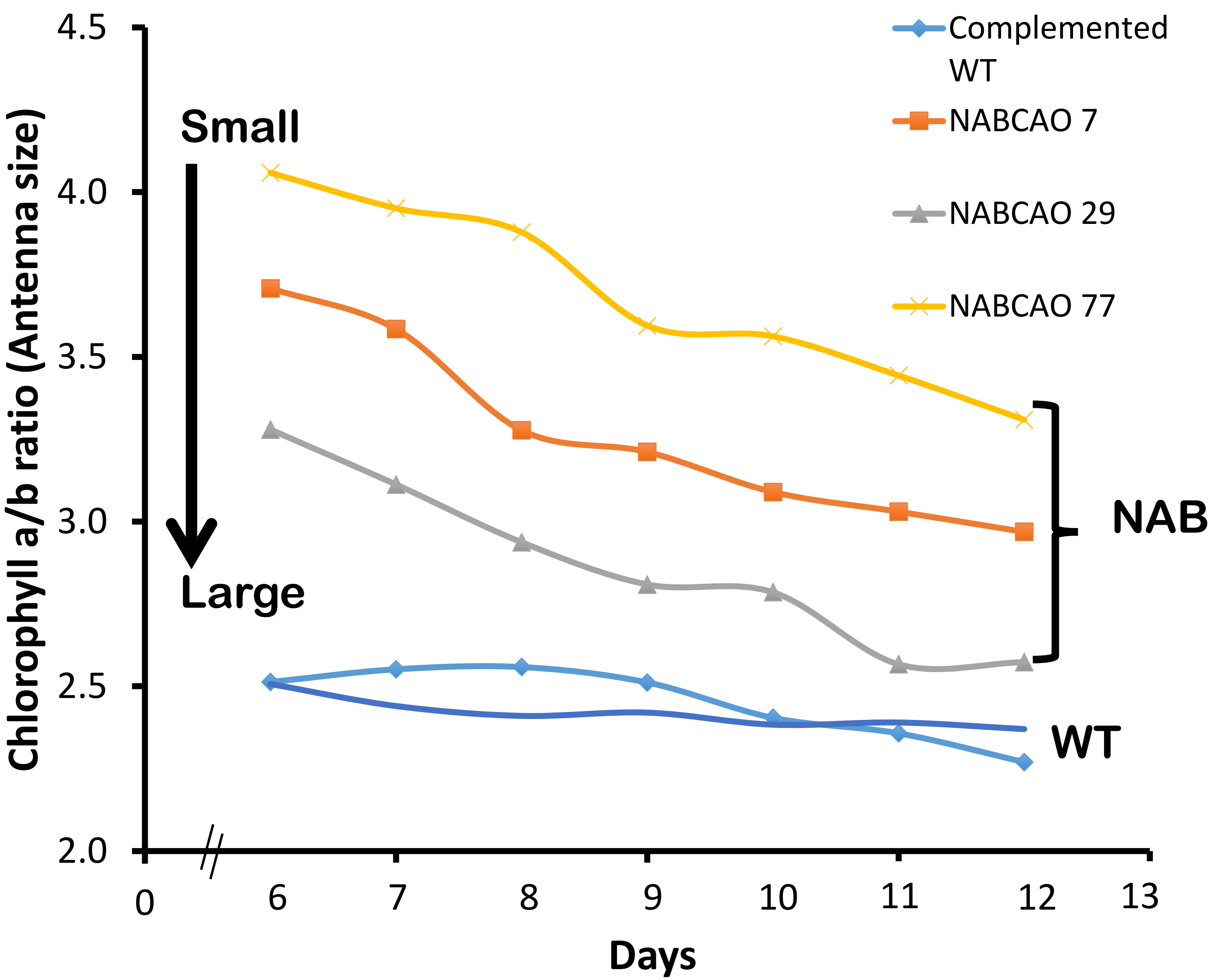


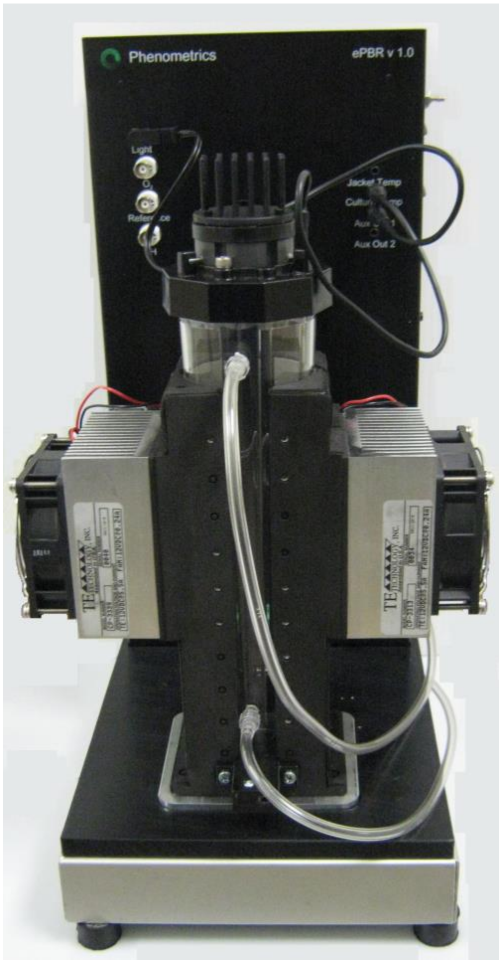
NAB1 protein characterized by Olaf Kruse lab

Increasing Chl a oxygenase activity and elevating Chl b levels in low light to increase antenna size



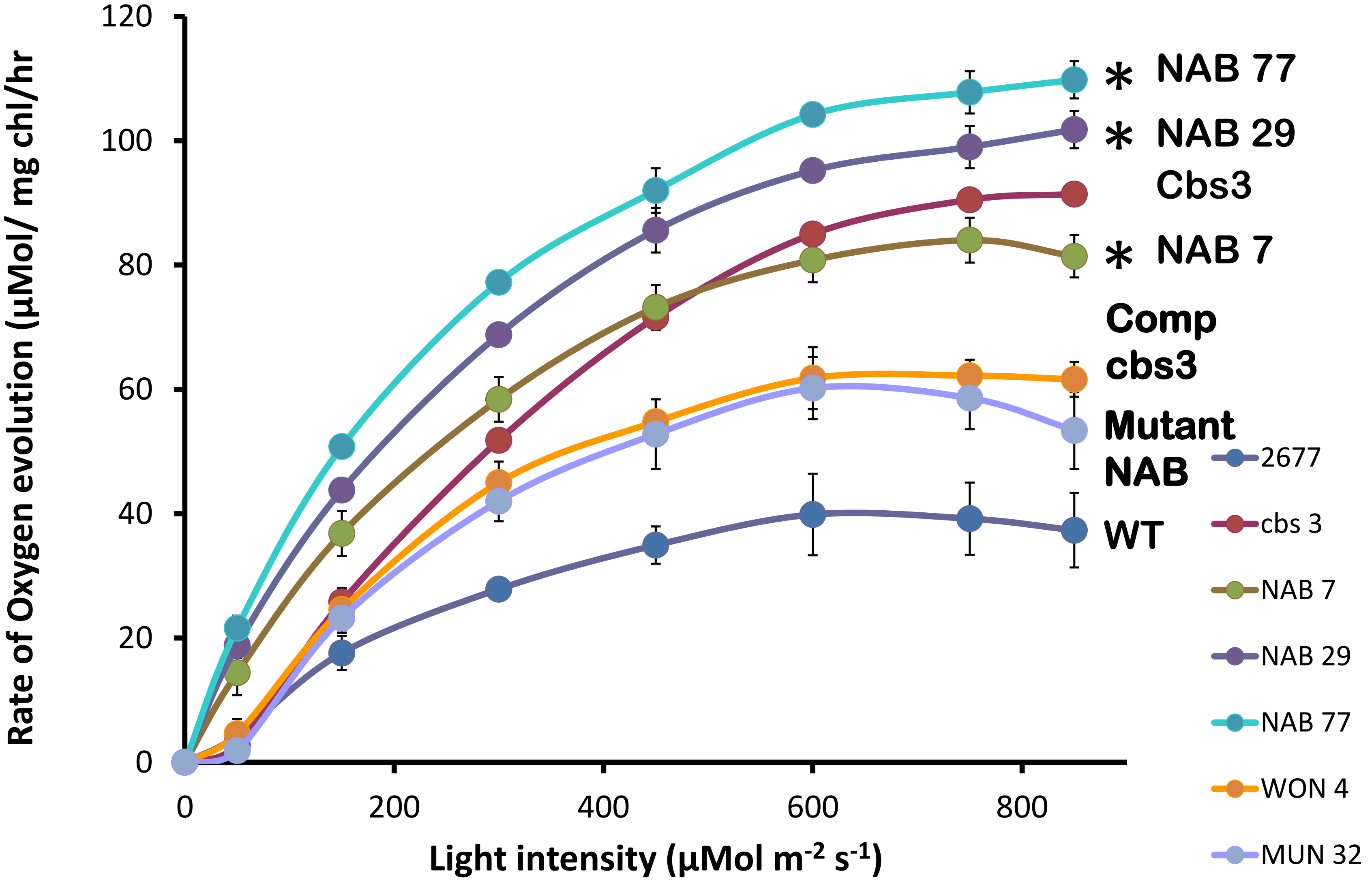
Does antennae size self-adjust?
Antenna get larger as culture (self-shading) grows



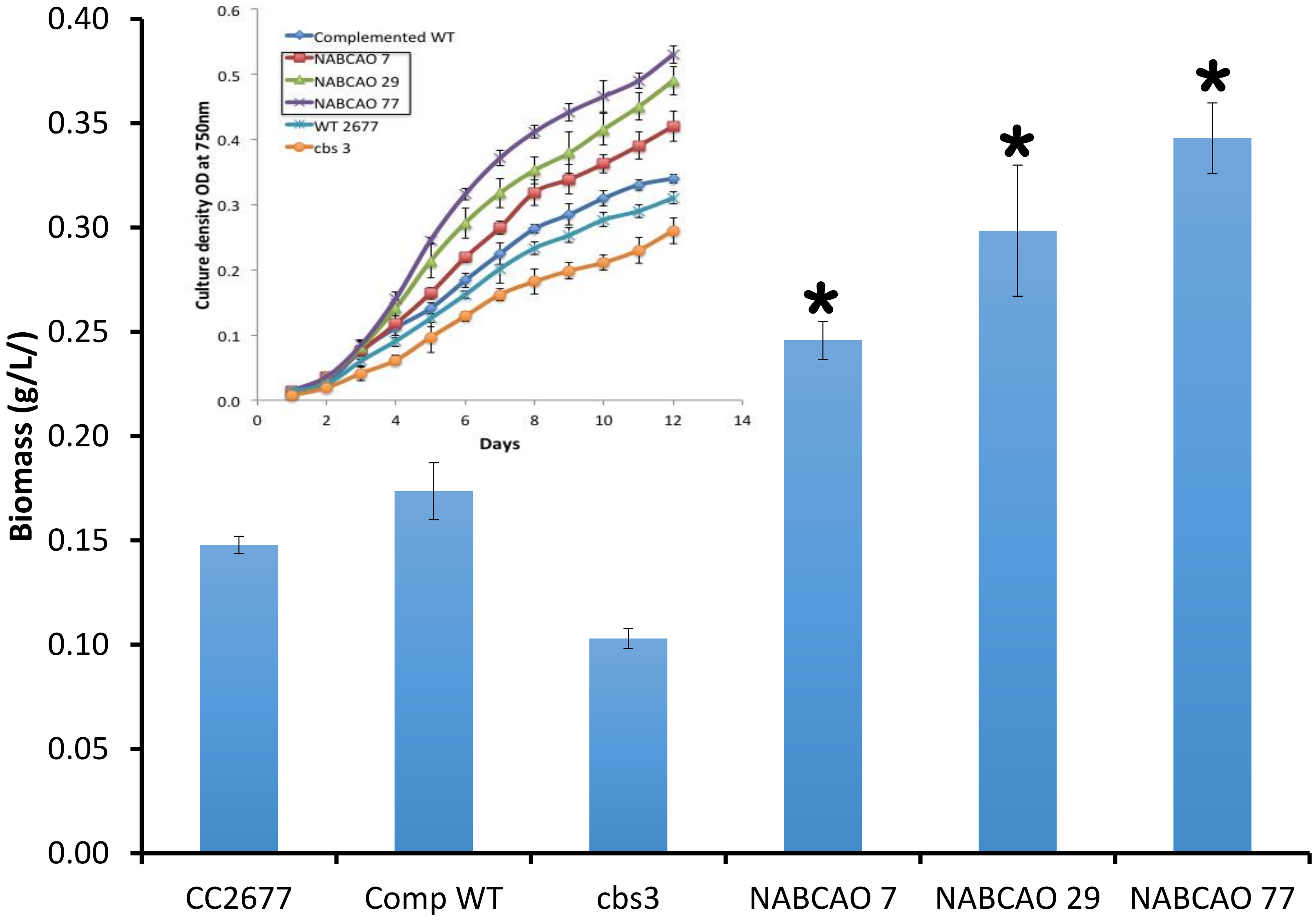


Phenometrics PBR

Photosynthesis in algae with self-adjusting antenna (NAB lines) is 3X greater than wild type in monocultures



Transgenics with self-adjusting antenna produce > 2-fold more biomass than wild-type algae



Could these traits impart weediness?

Competition studies indicate not

Wild-type algae shade out transgenic algae with smaller antenna

Summary

- For algal biofuels to become economically feasible:
  - Yield will need to be increased $\geq$ 3X over current production rates
  - A higher energy return on investment (> 10) will also be required
  - Reductions in carbon emissions and enhanced environmental services (nutrient recycling, reduced water use)
- The greatest risk potential for GM algae is “weediness” leading to ecosystem disruption
- However, many GM traits (higher oil content, reduced competitive abilities) are likely to reduce fitness in wild
- Stacked bio-containment strategies can be employed to reduce the potential for escape
- Regulatory approval should include controlled field trial assessments to predict potential invasiveness and other risk factors
- Federal regulations for release of GM algae are present and being improved