

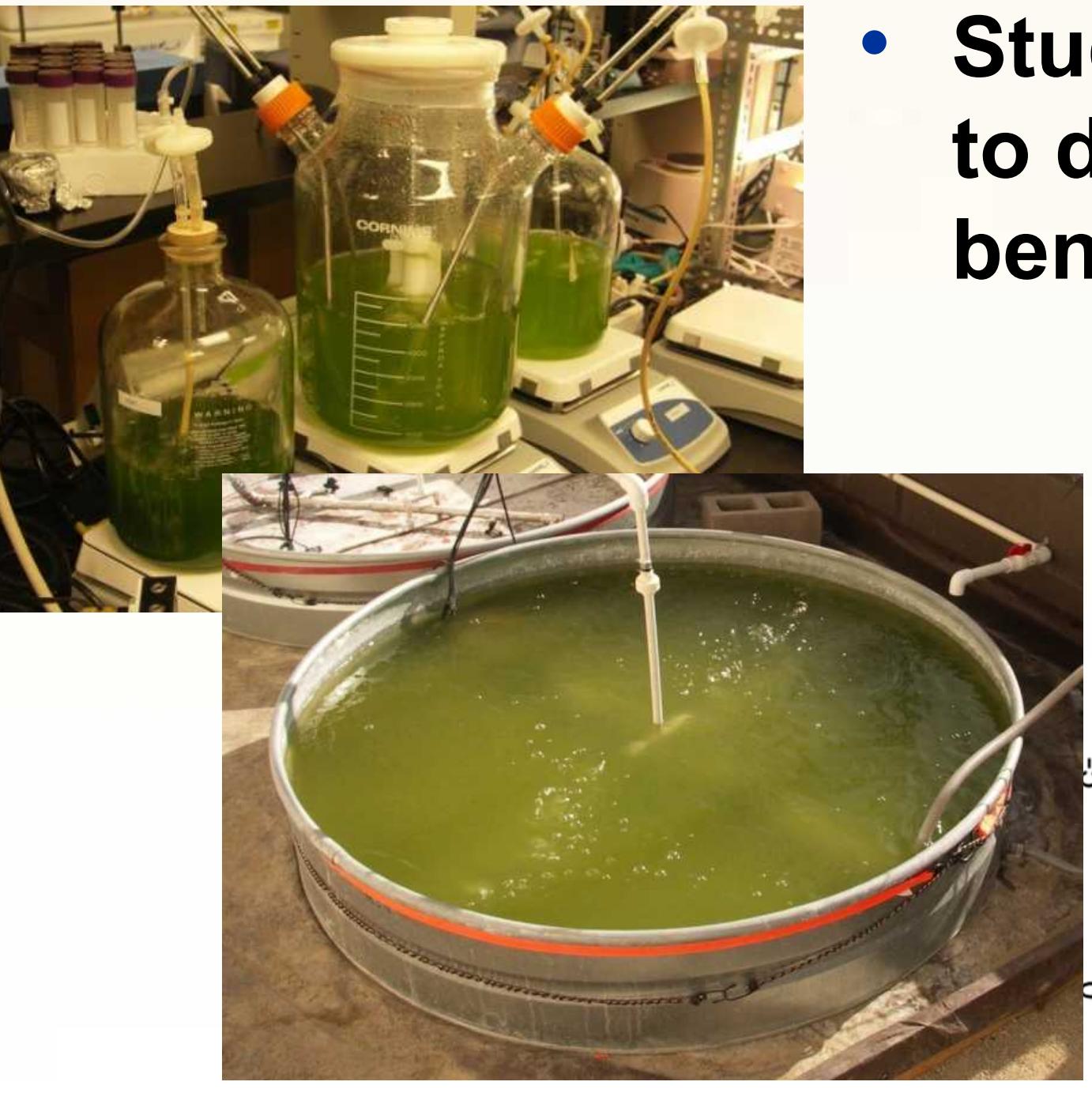
# Optimizing Algal Cultivation & Productivity: An Innovative, Multidiscipline, and Multiscale Approach

JA Timlin<sup>1</sup>, HDT Jones<sup>1</sup>, A Collins<sup>1</sup>, A Ruffing<sup>1</sup>, TA Reichardt<sup>2</sup>, SC James<sup>2</sup>, JB Ricken<sup>1</sup>, S Scholle<sup>3</sup>, T Turner<sup>3</sup>, DT Hanson<sup>3</sup>, OF Garcia<sup>1</sup>, B Dwyer<sup>1</sup>, C. Trahan<sup>1</sup>, AJ Powell<sup>1</sup>

<sup>1</sup>Sandia National Laboratories, Albuquerque, NM; <sup>2</sup>Sandia National Laboratories, Livermore, CA; <sup>3</sup>University of New Mexico, Albuquerque, NM

## Problem Statement

- Culture sustainability is critical for large scale algae production
- Open raceways: economical operation, uncontrollable environmental variables
- Culture dynamics are accelerated compared to traditional farm crops
- Lack of fundamental understanding of relationship of abiotic and biotic stressors on algal physiology, biology
- Lack of tools to detect and measure early indicators of culture fluctuations



## Technical Approach

- Studying fundamental algal biology in response to dynamic environmental conditions at the benchtop, greenhouse, and raceway scale
  - Improved understanding of algal physiological response
  - Discover spectroscopic signature at the single cell and bulk level that are predictive of culture health
  - Create and validate a predictive model for algal growth and productivity

## Effect of Biotic and Abiotic Stressors on Growth and Productivity

Answering fundamental questions about the dynamic interaction of algae and its environment through novel combination of:

- What is the relationship of CCM function to lipid production?
- What are the mechanisms of algal cell death? How do they relate to lipid production?
- Who are the natural pathogens, predators, competitors in arid ecosystem? What are their potential effects on raceways?

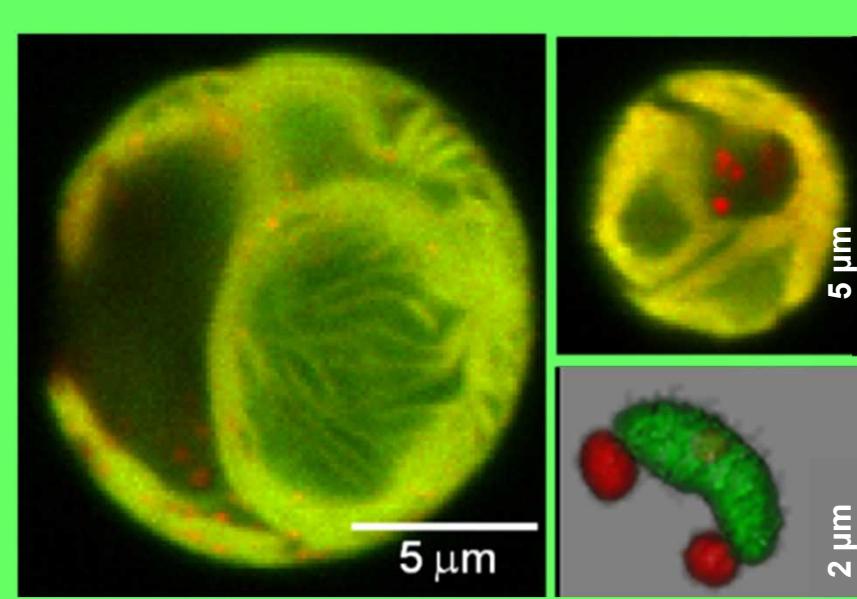


Fig 1. This project compares and contrasts two production strains: *Nannochloropsis salina* and *Dunaliella salina* and the model organism: *Chlamydomonas reinhardtii*. Spectral images of these shown clockwise from lower right. Chloroplast is marked by Chl a (green) while the lipid bodies are identified by lipid-soluble carotenoid (red).



Fig 2. Airlift photobioreactor analysis to assess how photosynthetic adaptation to CO<sub>2</sub> affects lipid production. Low CO<sub>2</sub> levels in a culture induces carbon concentrating mechanisms (CCMs). It is unknown if investment in cellular machinery to run the CCM consumes lipid stores or if there is a net increase in storage from greater efficiency.



Fig 3. Sevilleta LTER experiment to understand dynamic food web including algae predators.

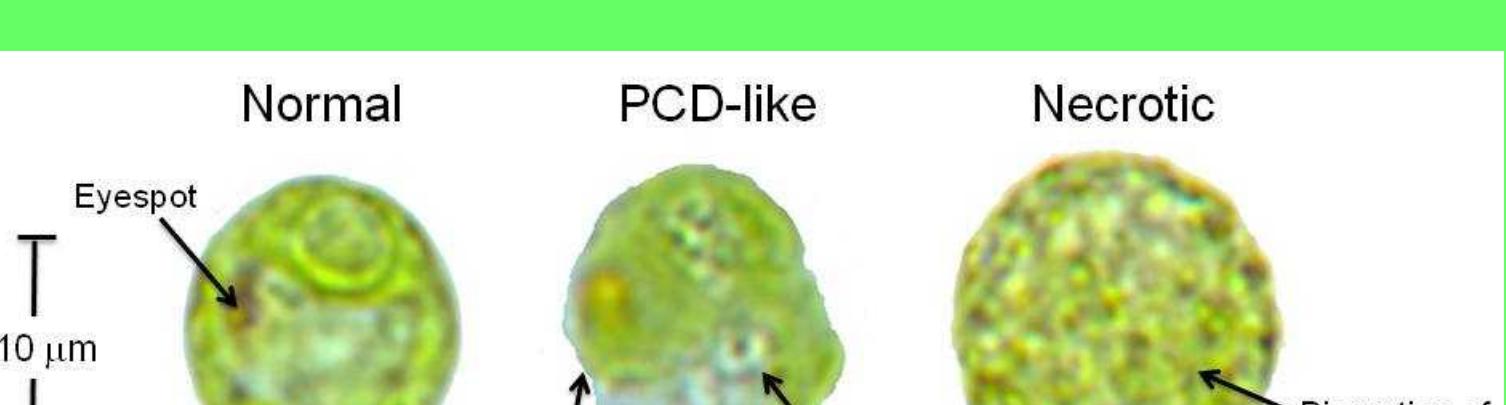


Fig 4. Optical microscopy captures the morphological effect of heat stress on *C. reinhardtii*. Exposure to 55°C for 10 min results in programmed cell death, while 85°C for 2 min induces a necrotic response.

Condition	Increased Lipid Production	PCD Inducer
High light	X	X
Nitrogen starvation	X	X
High temperature	X	X
Osmotic stress	X	X
Senescence	X	X
pH stress	X	?
Phosphate limitation	X	?
Sulfate limitation	X	?

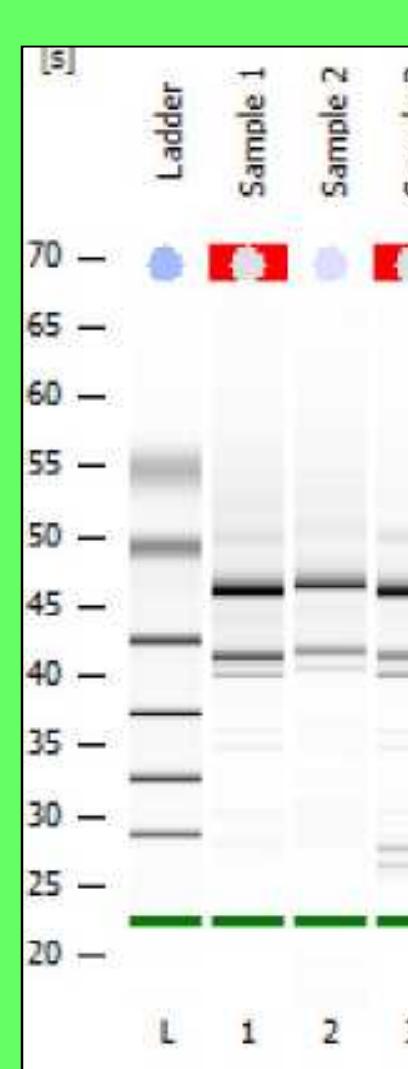


Fig 5: Transcriptomic Analysis of Programmed Cell Death (PCD) in *Chlamydomonas reinhardtii*. Stressors that induce PCD are also implicated in increasing lipid production. Genes & metabolic pathways involved in PCD will be identified via genome-wide expression studies in the model species *C. reinhardtii*. A few genes implicated in PCD in higher plants have been identified in the *C. reinhardtii* genome, but full complement of genes is essentially unknown. These investigations will be integrated with advanced imaging.

Can we identify spectral signatures that correlate with algal culture health:

- Within single cells?
- At benchtop level?
- At standoff distances?

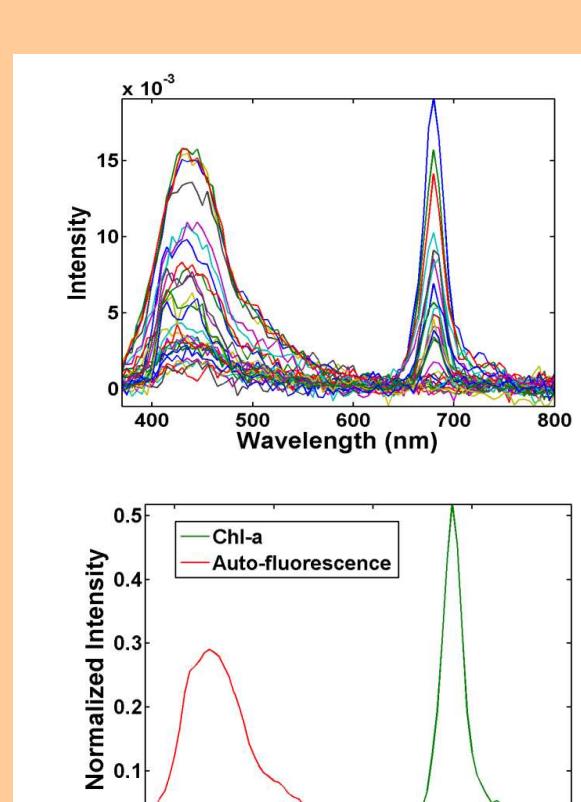


Fig 6. Multivariate Curve Resolution was used to extract the pure spectral signatures and the associated intensities from fluorescence spectra obtained from a sealed 1 cm cuvette. Sealing the cuvette caused a temporal change in cell health.

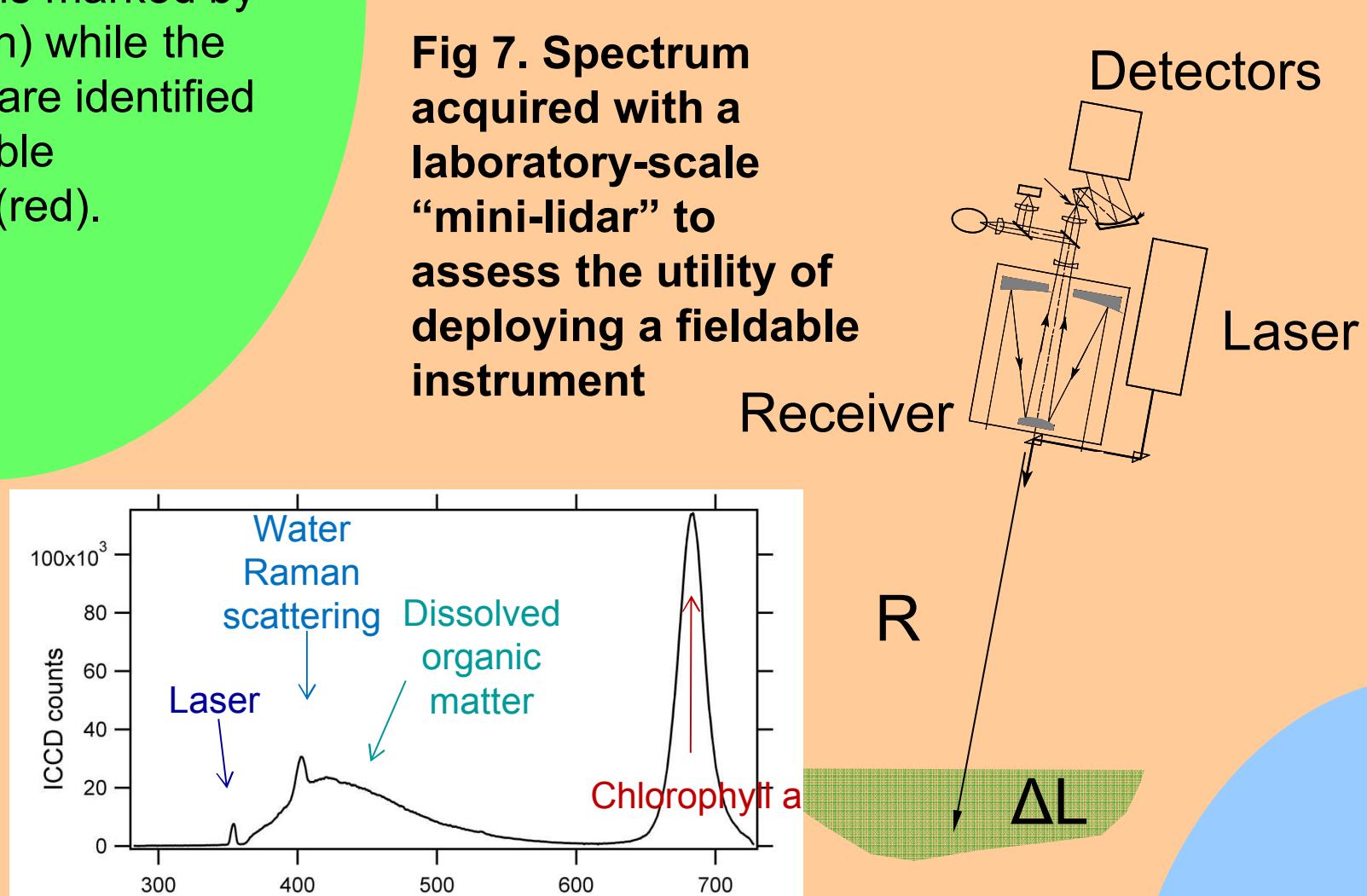


Fig 7. Spectrum acquired with a laboratory-scale "mini-lidar" to assess the utility of deploying a fieldable instrument

Fig 7. Spectrum acquired with a laboratory-scale "mini-lidar" to assess the utility of deploying a fieldable instrument

Fig 8. Parameter sensitivity study reveals key experiments to conduct.

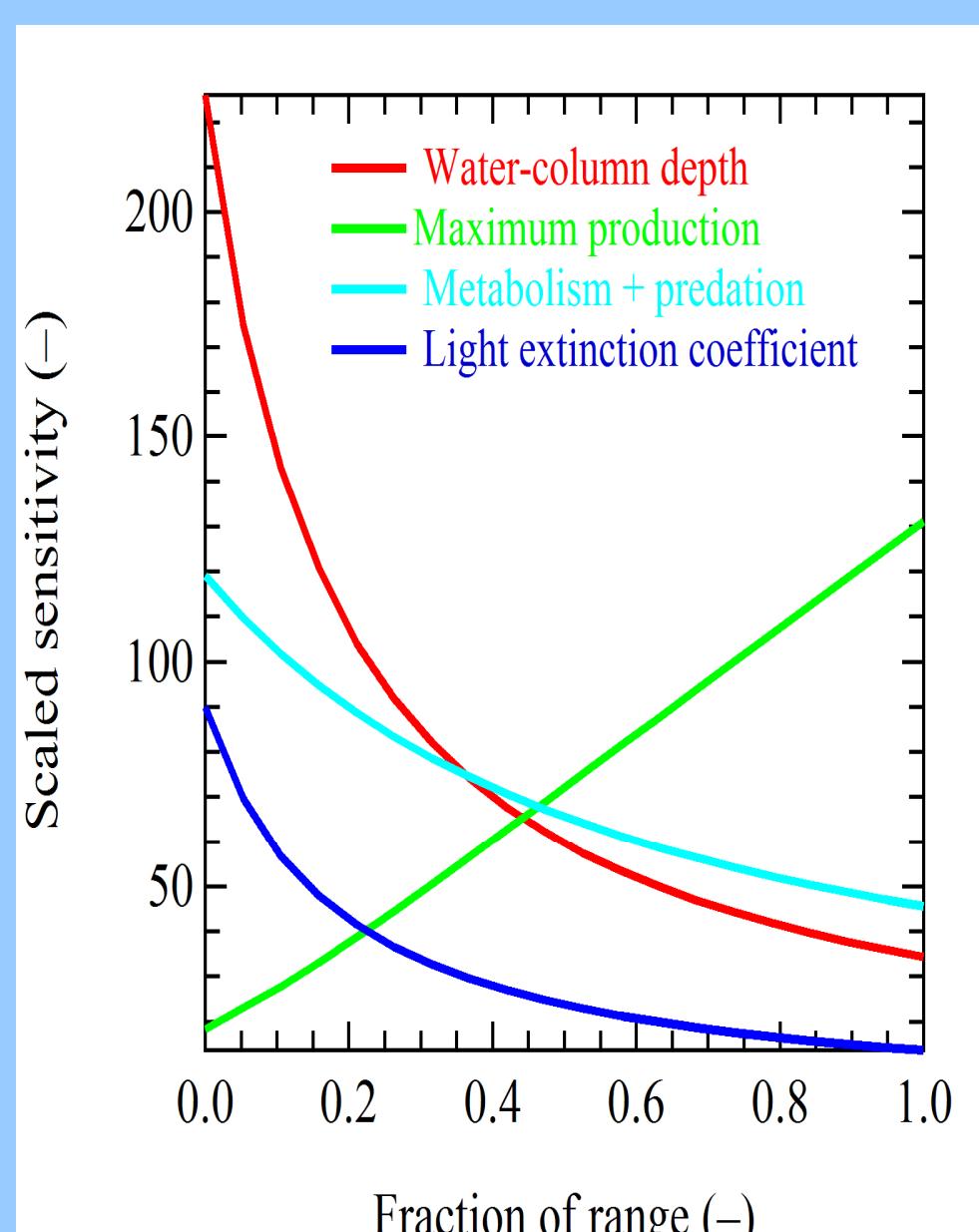


Table 1. Relationship between programmed cell death (PCD) and lipid production in algae

James and Boriah "Modeling Algae Growth in an Open-Channel Raceway", J Computational Biology, 2010, 17:1-11.

$$\frac{\partial}{\partial t} B(\mathbf{x}, t) = (P - B_M - P_R) B(\mathbf{x}, t)$$

$B(\mathbf{x}, t)$  is the spatio-temporal algal biomass ( $\text{gC/m}^3$ )

$P$  is the production rate (1/day)

$B_M$  is the basal metabolism rate (1/day)

$P_R$  is the predation rate (1/day)

Use experimentally determined relationships to populate the model – In turn the model helps reduce the number of experiments to be done and extends results to other configurations.

Lay foundation for early detection of culture "change."

## Spectroscopic Signatures of Culture Health