Understanding precision nitrogen stress to optimize the growth and lipid content tradeoff in green algae

Presented at the 2012 Algae Biomass Organization (ABO) Summit in Denver Colorado

Curtis Adams & Bruce Bugbee

BioEnergy Center
Utah State University

Acknowledgements

I appreciate the financial support of:

The BioEnergy Center at Utah State University

US Department of Energy

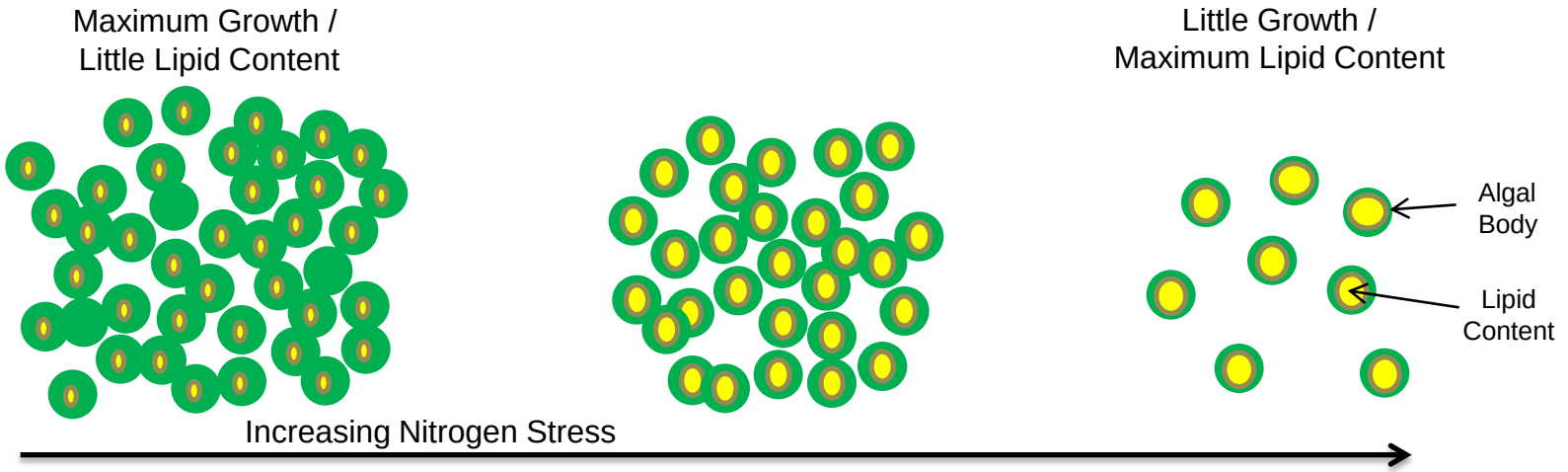
Lachat Instruments (The Hach Company)

Special thanks to:

Lance Seefeldt, Brad Wahlen and Valerie Godfrey, Biochemists

Is nitrogen deprivation a viable method for production of lipid feedstock algae?

- It's well-established that stress—especially N deficiency—promotes lipid formation in some green algae
  - But N deficiency limits growth and thus lipid productivity
- Viability depends on proper tradeoff—not extremes
  - High growth increases yield per unit culture volume
  - High lipid content decreases processing costs per unit biomass
  - Precision stress is needed



An example of precision stress in agriculture

- The tradeoff between vegetative and reproductive growth is managed by precision N stress in tomatoes
  - Too much N = big plants, few fruit
  - Too little N = loss of yield

- Tomato production is a big, profitable industry that has faced a similar challenge

Koch, B and Khosla, R. (2008). The role of precision agriculture in cropping systems. Journal of Crop Production 9:361-381.

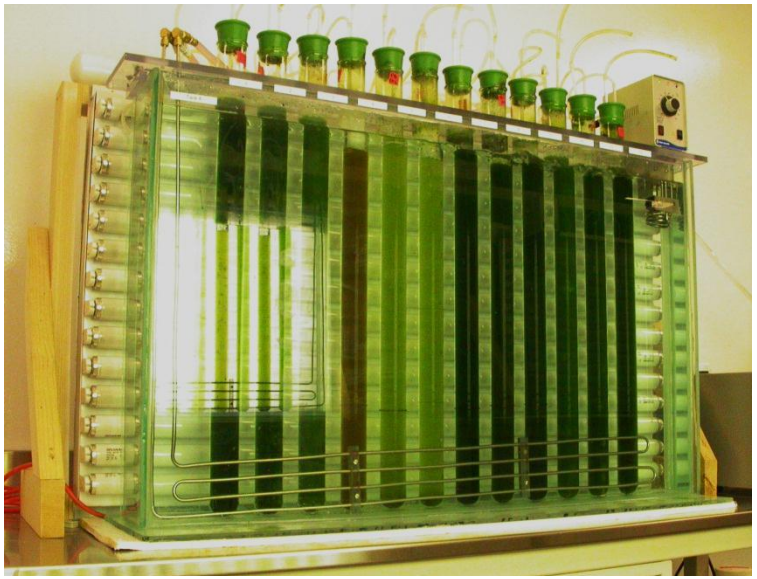
A perspective on nitrogen deprivation for algae lipid production

- The Aquatic Species Program suggested mutual exclusivity of nutrition favoring growth and lipids (Sheehan *et al*., 1998):
  - Increased lipid content in N stress led to cessation of cell division
  - As a result, despite increased oil content, lipid productivity was equal or lower with N deficiency
- This conclusion was generally based on extreme nutritional conditions
- The perspective in the field has changed little since 1998

Where is research needed?

- We have a poor quantitative understanding of the effects of N deprivation on lipid production in algal cultures
  - Growth and lipid content tradeoffs
    - In particular with intermediate levels of stress
  - Timing of lipid accumulation
  - Magnitude of stress required
  - Species differences
    - What characteristics make one species better than another as a lipid feedstock?

Methods

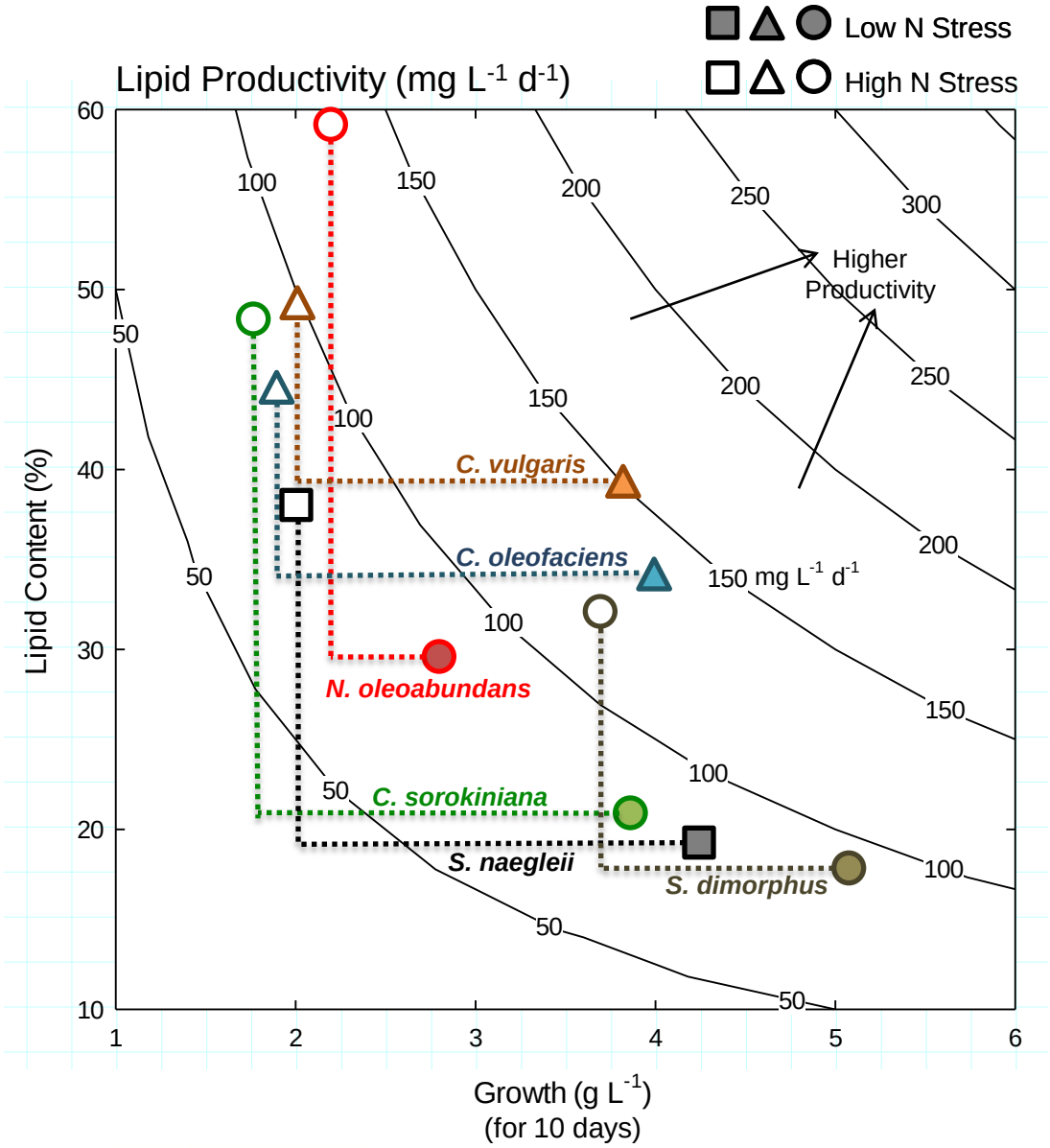


Air-lift, glass bioreactors in plexiglass water tank for temperature control; fluorescent lighting behind

- In 12-day, axenic batch cultures (1% CO_2), we took daily measurements of:
  - Growth – Spectral measurements and filtration
  - Biomass lipid content – Lipids extracted and converted to FAME (biodiesel), quantified by GC
  - Tissue N – Perkin-Elmer CHN Analyzer (Model 2400)
  - Solution N – Lachat QuikChem 8500 Automated Ion Analyzer
- Two N stress treatments:
  - Low N stress (11 mM N) – Not nutrient replete
  - High N stress (4 mM N) – Not severely limiting
- Six species of oleaginous green algae
  - *Chlorella sorokiniana, Chlorella vulgaris, Chlorococcum oleofaciens, Neochloris oleoabundans, Scenedesmus dimorphus, Scenedesmus naegelii*

Growth and Lipid Content Tradeoffs

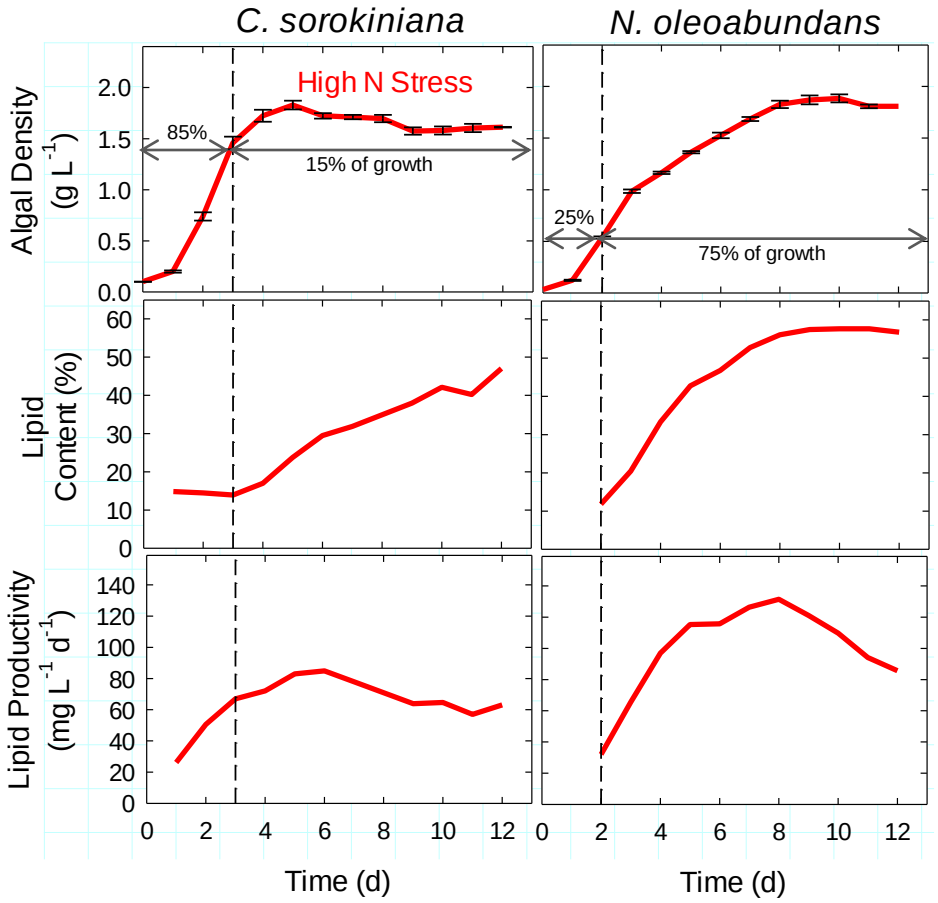
Tremendous differences observed in response to N supply



- Three categories of response (with increased stress):
  1) Increase in lipid content exceeded decrease in growth
  2) Decrease in growth exceeded increase in lipid content
  3) 1:1 tradeoff
- The data highlights need for species-specific nutrition
  - Lower level stress for some, higher level for others

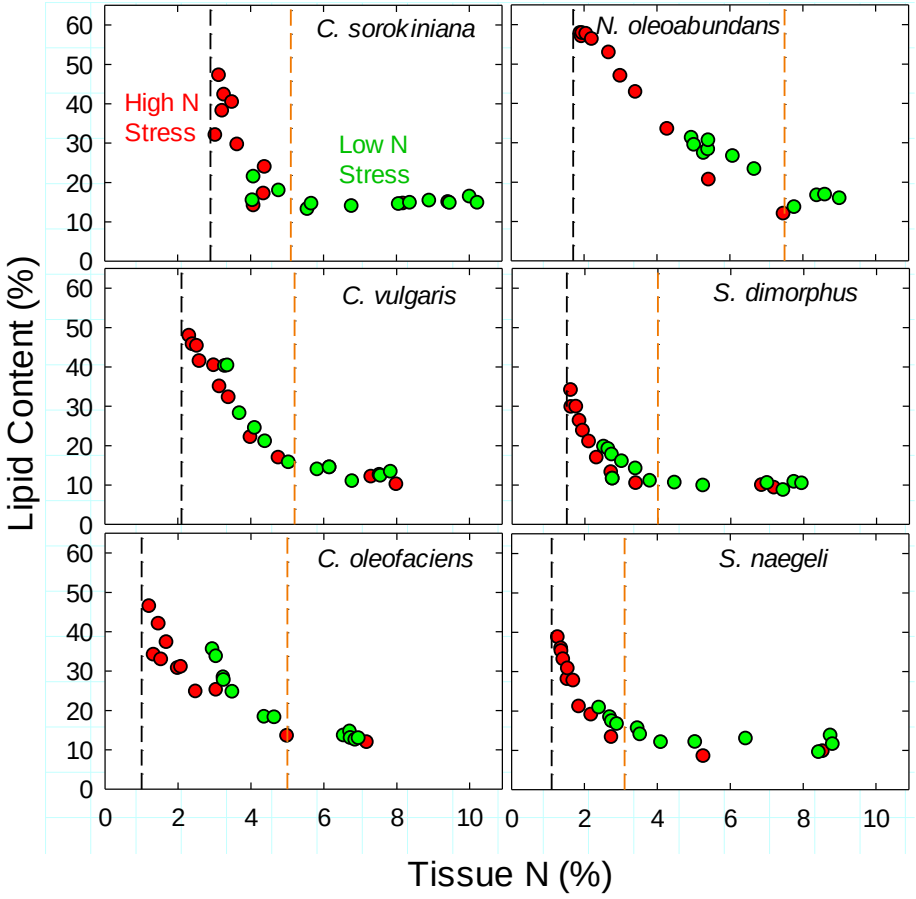
The timing of lipid accumulation

- Some algae grow first, then accumulate lipids; others do both at the same time
- Concurrent growth and lipid accumulation always resulted in higher lipid productivity



The magnitude of nitrogen stress

The timing of lipid accumulation reflects stress responses



- Wide variation in stress response among species
- A larger range in tissue N indicated better lipid productivity
  - This can be used to identify the best lipid producers
- The most promising species will combine concurrent growth and lipid accumulation with high lipid content
- Cultures can be managed by mass balance
  - Tissue N can be estimated with measurements of solution N and growth

Conclusions

- Precision N stress can be used to optimize growth and lipid content tradeoffs
  - Optimization requires low-level stress for some, high-level for others
- Species selection should be broadened to include:
  - Concurrent growth and lipid accumulation
  - Response to minimal N stress
- N deprivation is a viable method for increasing lipid productivity
  - There is great promise among the natural species