DOE Bioenergy Technologies Office (BETO) 2021 Project Peer Review

High-Throughput Directed Evolution of Microalgae and Phototrophic Consortia for Improved Biomass Yields EE0008245

March 2021
Advanced Algal Systems

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Colorado School of Mines

Collaboration with Pacific Northwest National Laboratory, Colorado State University and Global Algae Innovations

Project Overview

- *GAI has isolated a diatom (Nitzschia sp.) that is among their superior outdoor strains at the Kauai growth facility. Further improvements in biomass and lipid yield will benefit biofuel applications.*
- *Yields are putatively diminished due to high O_2 levels during periods of high productivity, increases in pH as bicarbonate is assimilated and high temperatures during peak heat of the day.*
- *Both PNNL and Mines have established expertise in building photobioreactors that can mimic the solar day in terms of light intensities and temperatures, including custom turbidostat technology.*
- *Cells can be cultured with increased redox/pH/temperature pressures to select more robust strains under "domesticated conditions".*
- *Starting with an already promising strain, the goal is to further improve yields ~20%.*
- *Additional strains (e.g. cyanobacteria, algae) that grow well in GAI media are also being grown with selective pressures to mitigate risk.*
- *Establish organism consortia.*

1 - Management

Investigator	Roles
Matthew Posewitz Colorado School of Mines	Overall responsibility for ensuring that project obligations are realized. Coordinates routine (weekly or biweekly) project meetings. Responsible for assembly and submission of DOE reports. Responsible for integrating peer-review feedback into the project. Responsible for communication of all research results among team members and enabling all project participants to contribute towards project objectives. Responsible for integrating peer-review adjustments
Jesse Traller Global Algae Innovations (GAI)	Responsible for managing project activities at GAI facilities. Leverages collaborative synergies with other GAI projects when possible. Shapes research thrusts and objectives to focus project on areas that are most likely to improve farm yields. Critically evaluates datasets and formulates experimental design.
Alexander Beliaev Pacific Northwest National Laboratory (PNNL)	Responsible for the overall coordination of ‘omics’ data collection and ensuring that targets are responsive to BETO goals. PNNL is also leading several aspects of strain evolution. Responsible for helping to shape project goals and directions and ensuring open communication across the project.
Jason Quinn Colorado State University (CSU)	Responsible for geographically resolved TEA and LCA and focusing the project on solutions that are scalable and tractable. Integrates advances from across a portfolio of projects where possible to facilitate progress.

Goal Statement

- *Use directed evolution approaches as a tool for the improvement of photoautotrophic microorganism biomass yields.*
- *Leverage strains grown by Global Algae Innovations (GAI) to further improve productivities by enabling improved tolerance to higher pH, O_2 concentrations and temperature.*
- *Directed evolution has a long and successful history in industrial microbiology but has not been leveraged extensively with microalgae.*
- *Ultimately select strains/consortia that attain 24 g/m^2/day in the Kauai testbed.*

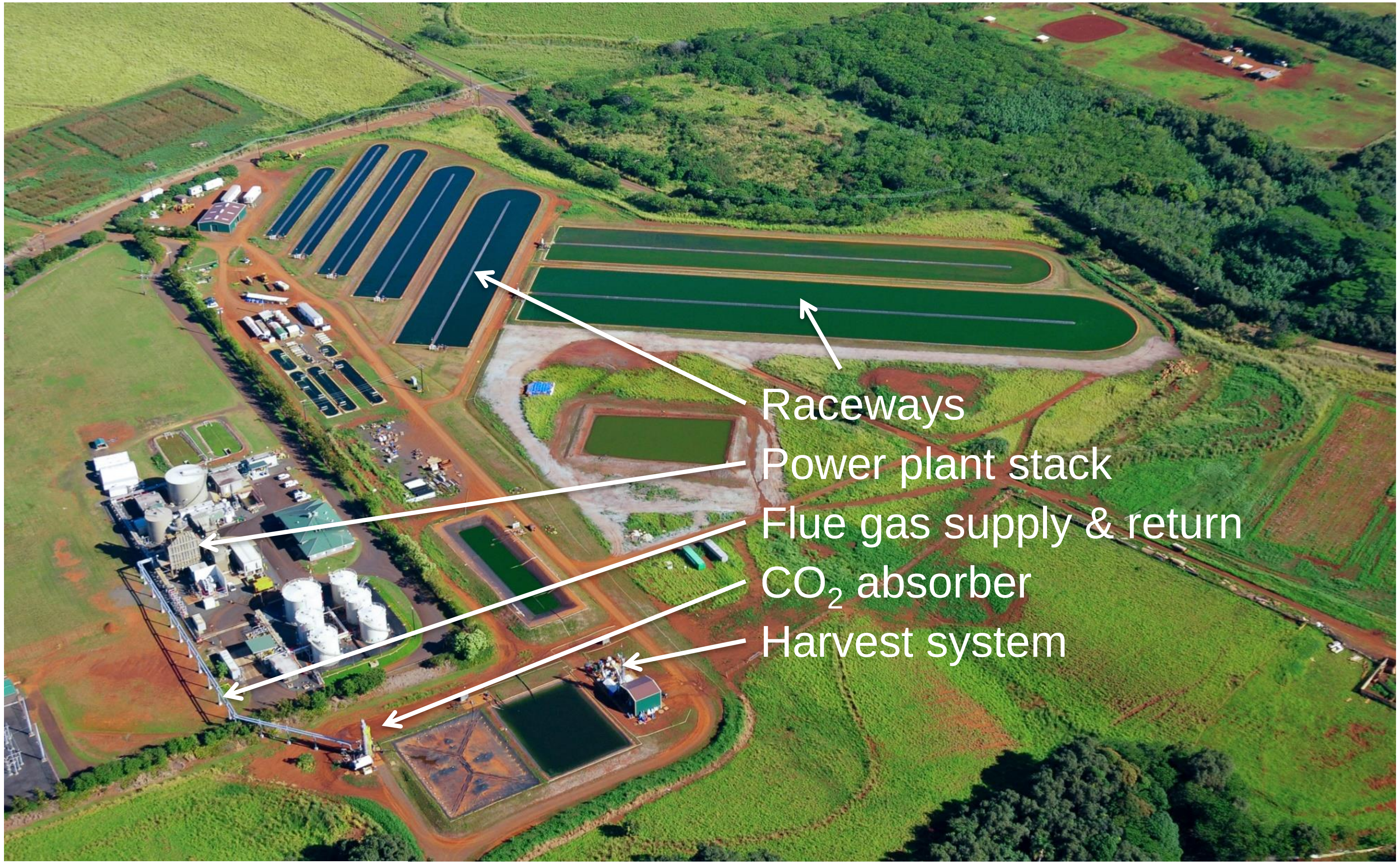
2 – Approach

- *Strains of GAI Nitzschia and species of Nannochloropsis are systematically cultured under escalating pressures in directed evolution experiments to adapt strains that are more tolerant to high pH, high temperature and/or high $O_{2.}$ These are common stressors in algal biomass ponds and can limit productivity. Using a suite of custom built bioreactors, we are applying constant pressure at each of these stresses individually and together to select for faster growing strains under pond conditions.*
- *The largest challenges include the ability of a strain to adapt to the selected stress, as well as the ability to maintain the adapted line, especially if an extended period of low stress culturing in a pond would occur.*
- *The recent Go/No-Go included the ability to reach >30 g/m^2/d in laboratory photobioreactors in an attempt to exceed 24 g/m^2/d in the pond at GAI. Exceeding the outdoor target putatively requires a lab-to-field performance buffer.*
- *The primary technical metric is algal productivity (g/m^2/d) and quality (gallons of gasoline equivalent).*

3 – Impact

- *The overarching goal is to use directed evolution to improve algal biomass yields at the GAI algal growth facility. Specifically, we are targeting productivities of 24 g/m^2/day.*
- *Algal cultivation in domesticated ponds promotes stressful conditions in the forms of redox stress, high pH and high temperature.*
- *These stress parameters are hypothesized to limit yields of a top GAI producer (Nitzschia sp. GAI-229) – as well as many other stains of biotechnological interest.*
- *We hypothesis that directed evolution will improve GAI-229 ponds productivities by enabling cells to be more productive during periods of peak stress.*
- *Methodology is likely applicable to many other strains.*
- *GAI is already an algal biomass provider – advances in productivity will benefit their existing technologies.*
- *Development of 'omics' datasets that correlate and predict enhanced productivity and identify environmentally robust algal isolates are anticipated.*

Algae cultivated on CO_2 supplied from power plant flue gas since June 2014



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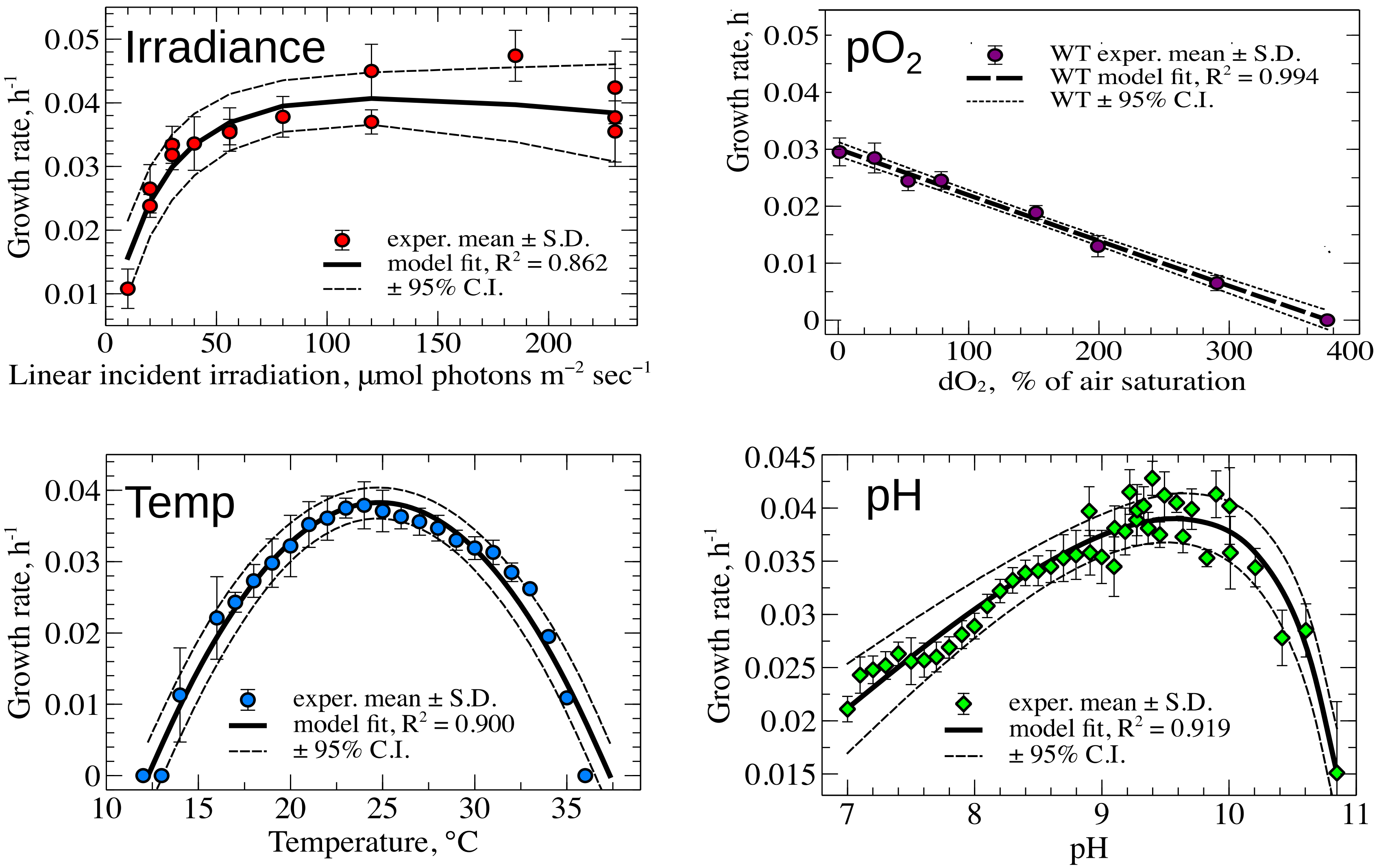
4 – Progress and Outcomes

- *Temperature, pH, O_2 optima mapped*
- *Two runs of stress adaptation finished*
- *Temperature adaptation finishing*
- *Bioreactor growth in laboratory exceeding 30 $g/m^2/d$*
- *Transcript network being constructed*

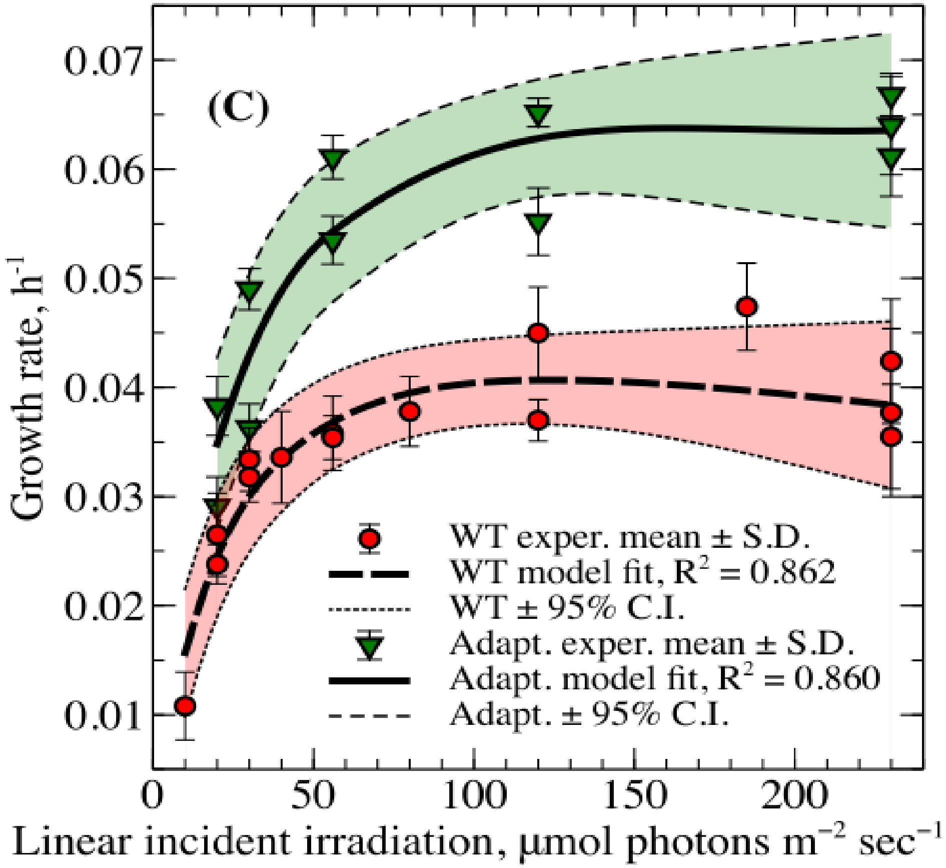
4 – Progress and Outcomes
Mapping Growth Boundaries and Physiological Stress

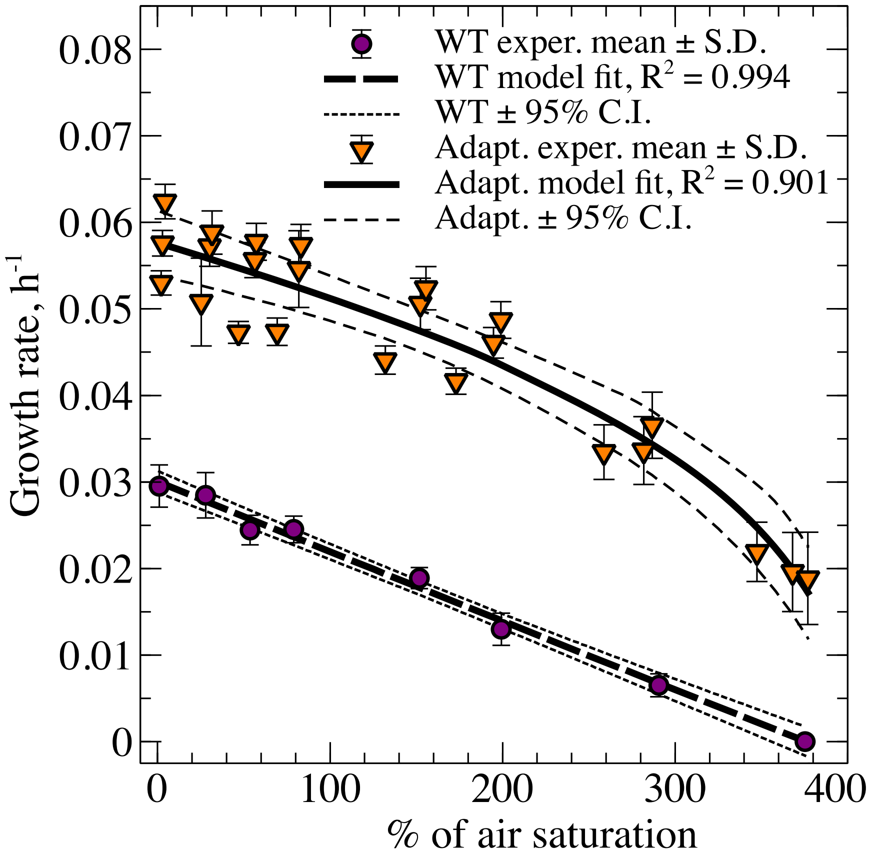


- **Irradiance: no inhibition**
- **pO_2: 50% inhibition at 2x saturation**
- **pH: 9-10 optimum**
- **Temp: 22 - 28^{o}C optimum**

Bohutskyi, McClure, Hill, Burch, Traller, Posewitz, Beliaev (*in preparation*)

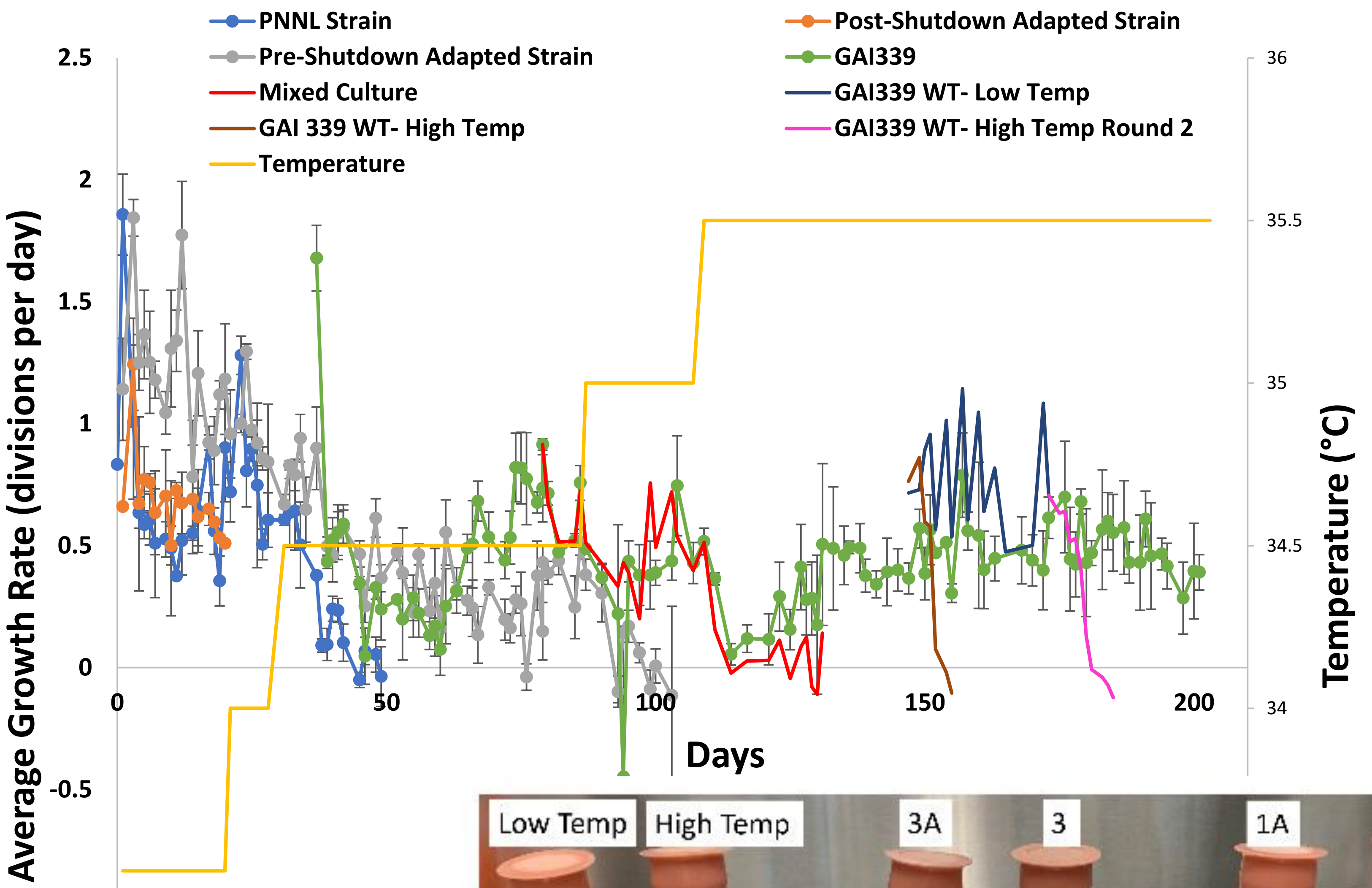
4 – Progress and Outcomes
Adapting to High Light/High Redox

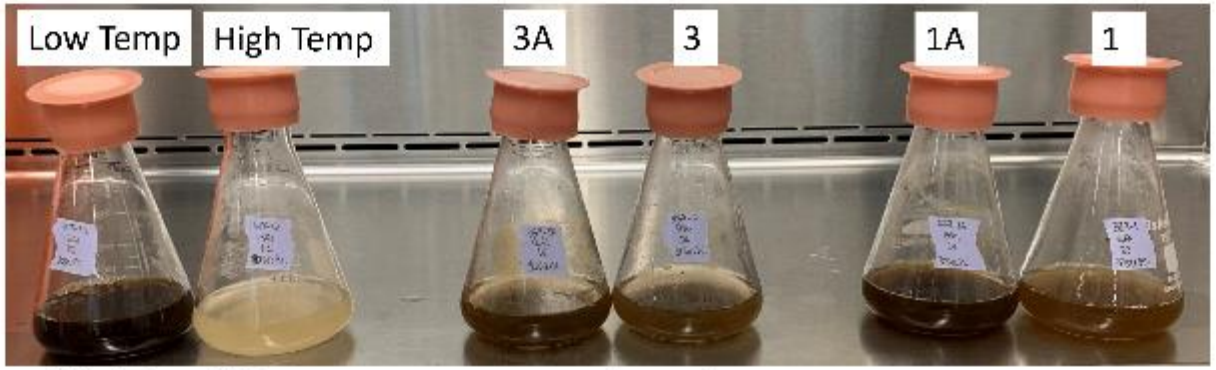




- Turbidostat cultivation enables directed evolution through tight control of growth rates
- Prolonged exposure to redox stress results in adaptation
- The evolved strain demonstrates improved redox tolerance and >60% increase in growth rate

4 – Progress and Outcomes
Adapting to High Temperature





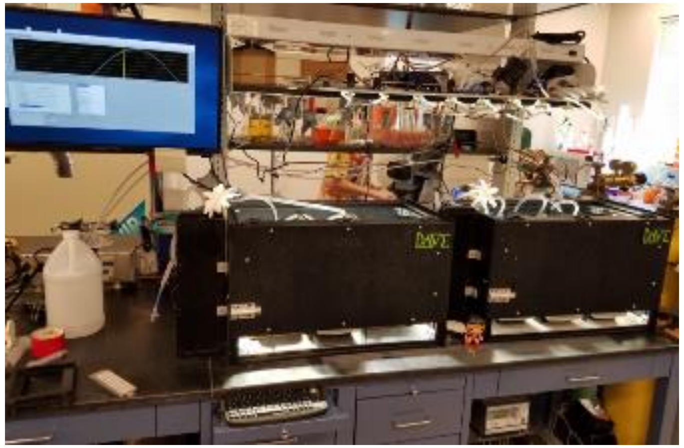
Wild Type 339

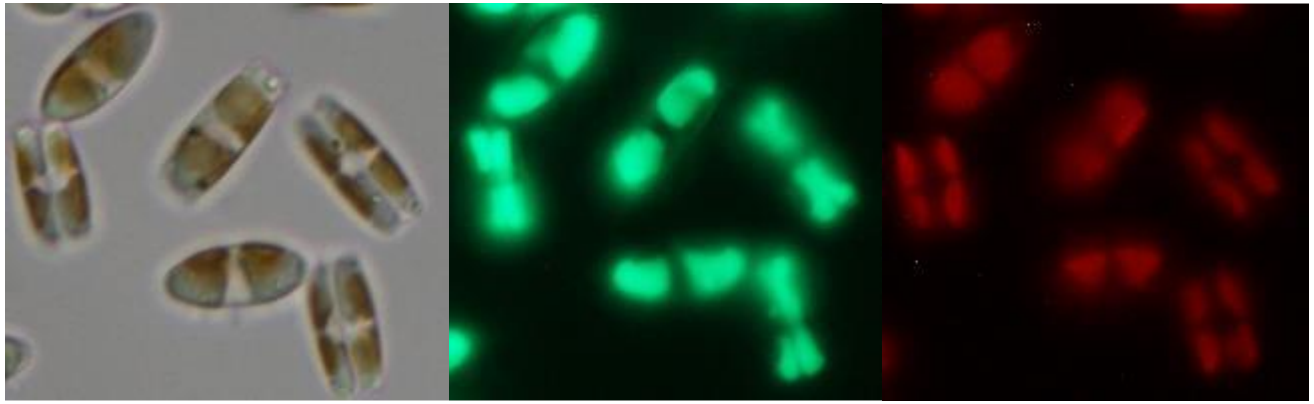
339 Adapted Replicate 3

339 Adapted Replicate 1

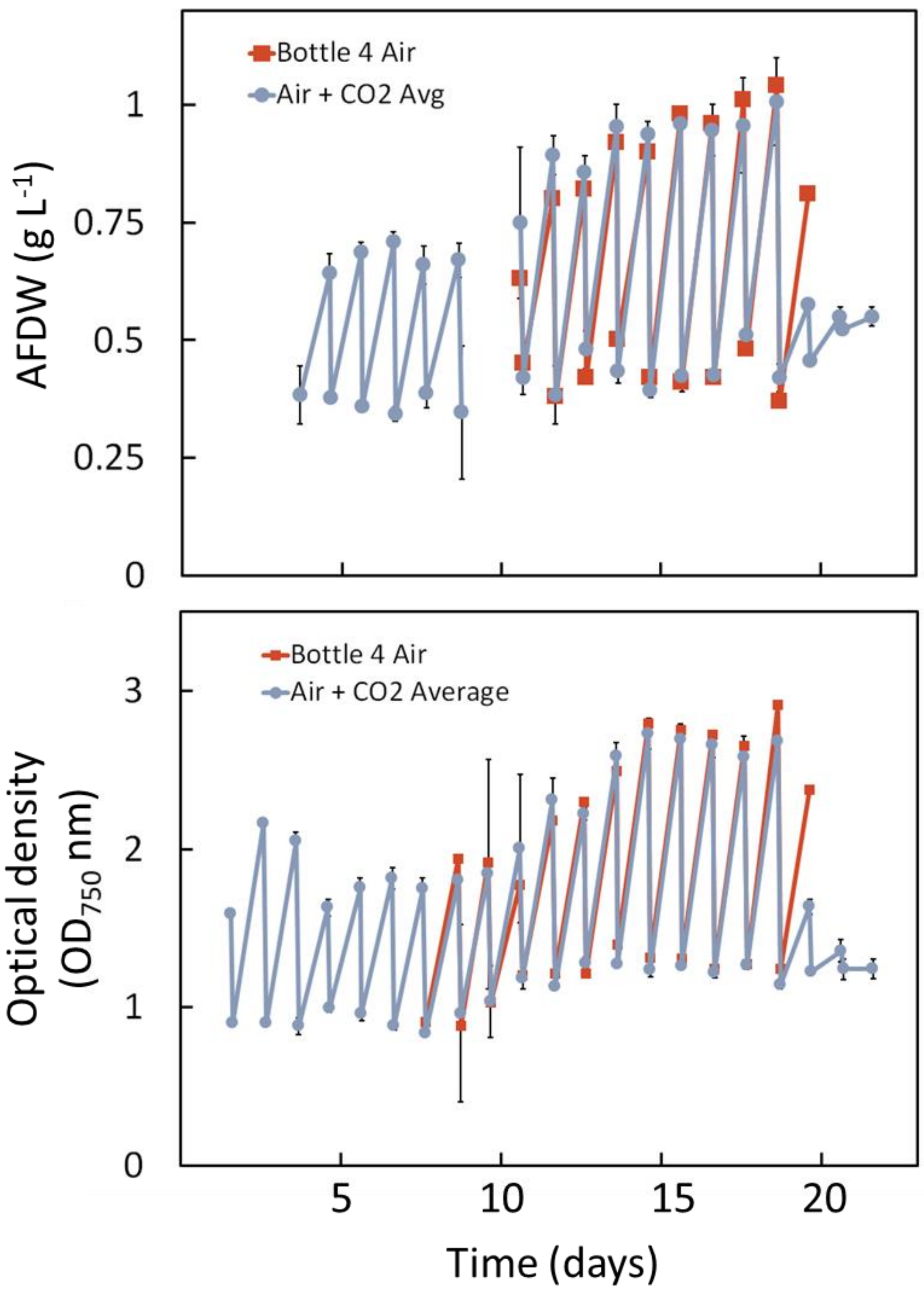
- Eight-month temperature adaptation
- Samples at 37°C
- Control strain has been placed in incubator 3 successive runs and is unable to survive
  - Thermal adaptation succeeded and will terminate soon
- Diel testing underway
- Low temp performance being vetted for “trade-off”
- Clonal isolates being tested

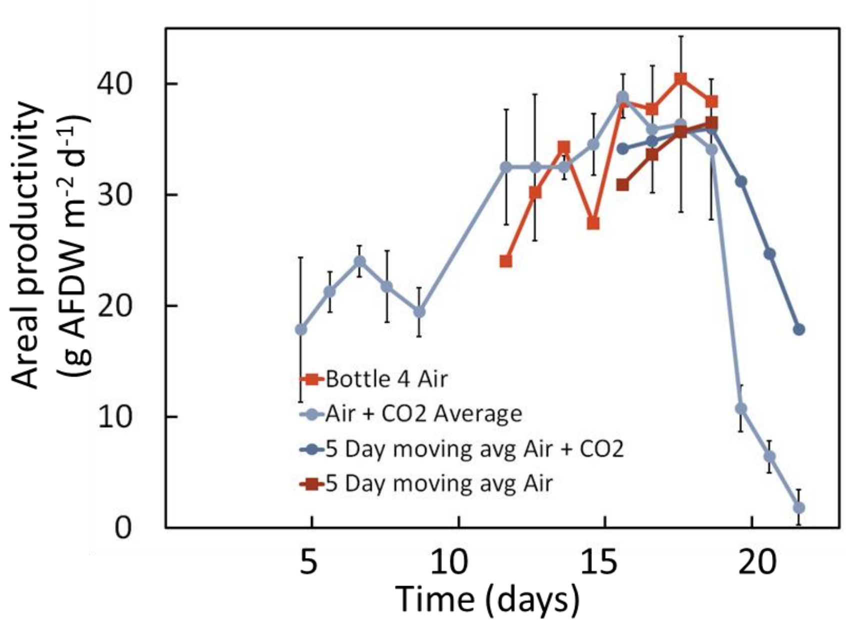
Optimization of diel cycle growth parameters for high productivity in the high-stress evolved PNNL strain of GAI 229

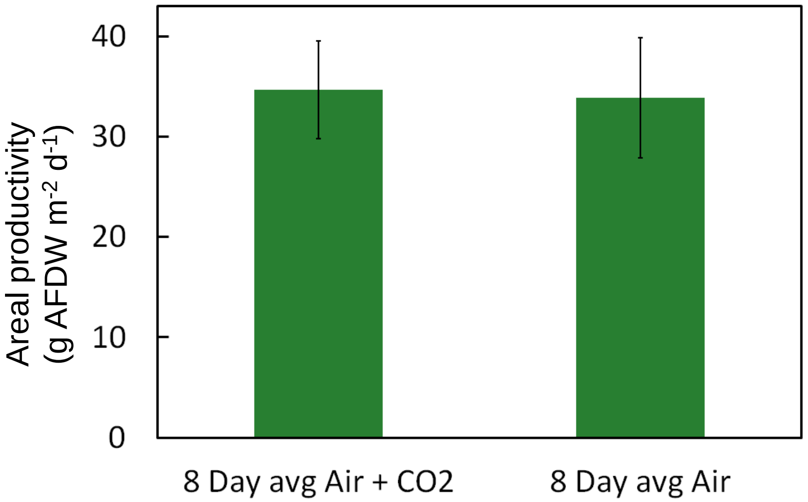




4 – Progress and Outcomes
Bioreactor productivities

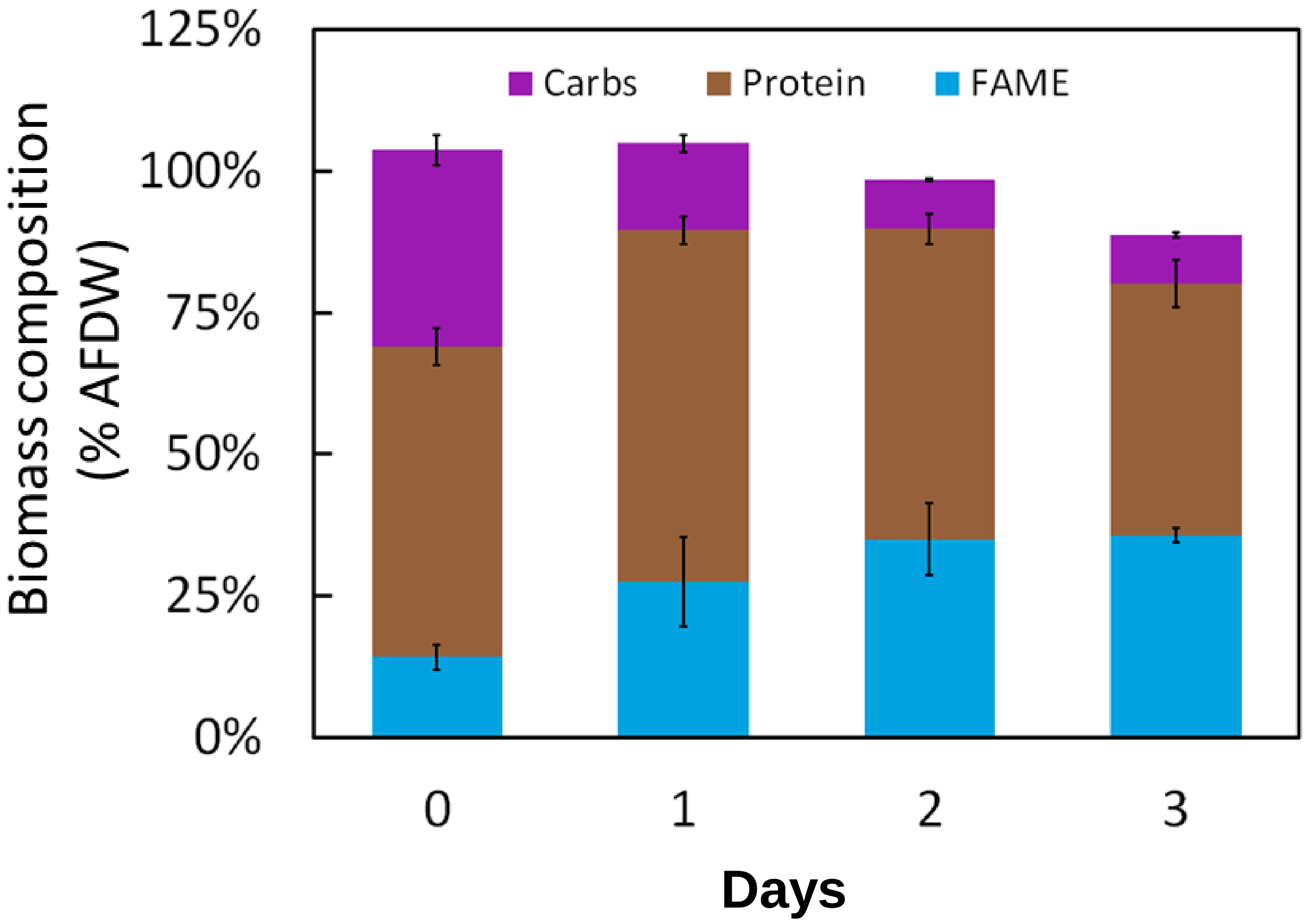






Bioreactor diel productivities are >35 g/m²/d exceeding project milestones prior to outdoor runs

4 – Progress and Outcomes
Bioreactor productivities

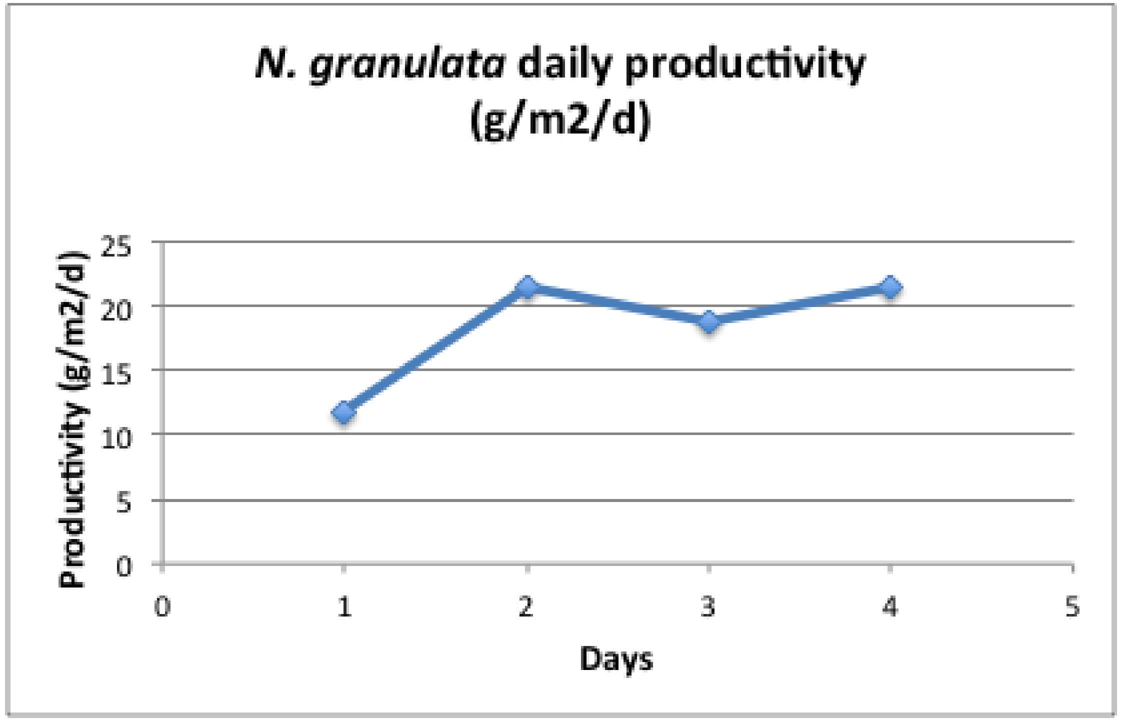


Proximate analysis at the end of bioreactor diel run (Day 0) and after nutrient limitation (Days 1-3).

Lipid yields ~30%

4 – Progress and Outcomes

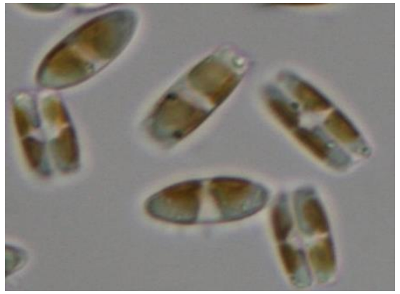
Strain Mitigation



Strains of Nannochloropsis (left) and Picochlorum (right) adapted to GAI media for risk mitigation – both exceed 20 $g/m^2/d$ running Kauai summer scripts. Picochlorum is in the 30 $g/m^2/d$ range

4 – Progress and Outcomes
'Omics toolkit'

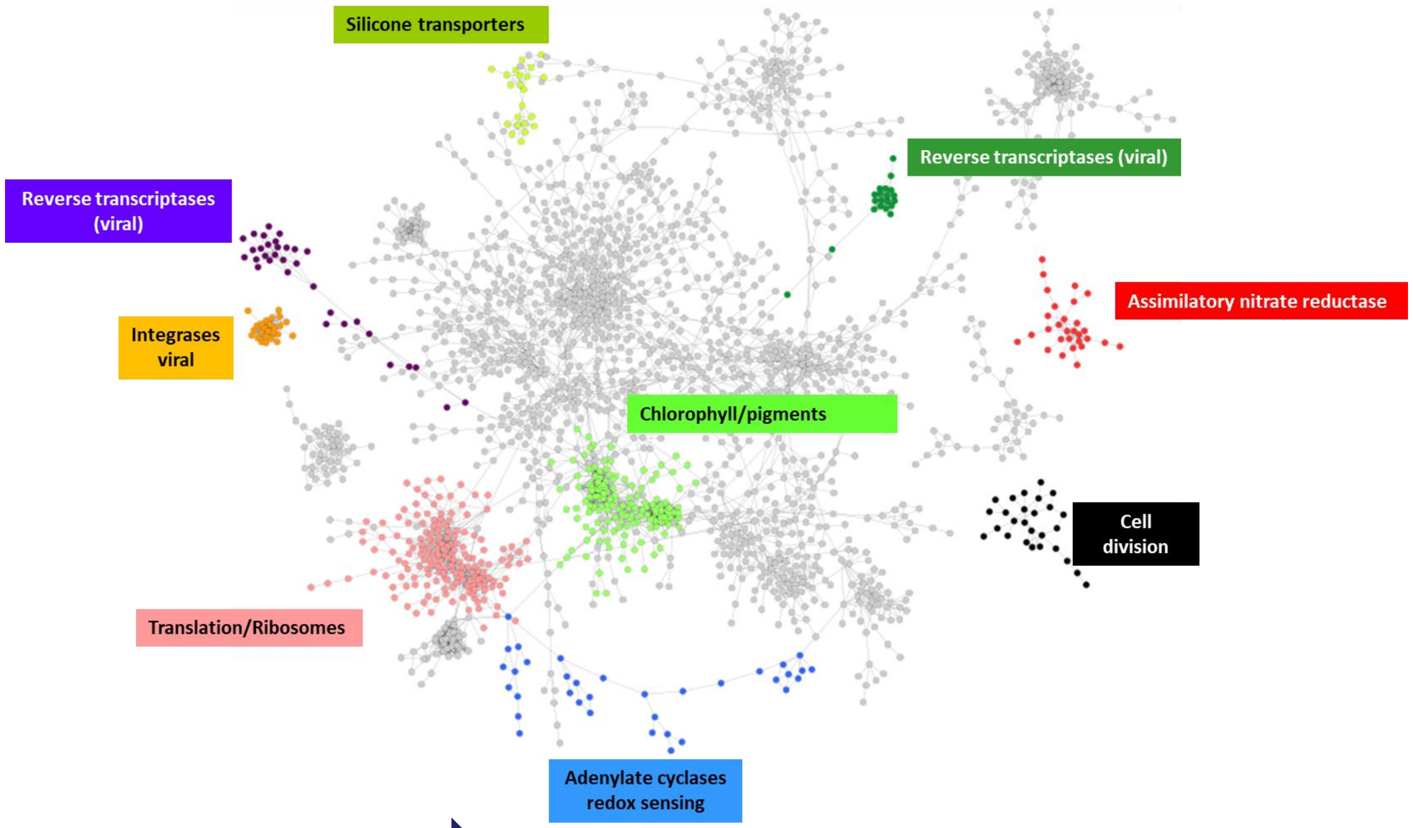
48 pond-relevant conditions



RNAseq

Wild-type & adapted strains

JGI-EMSL FICUS



Resequencing, proteomics metabolomics

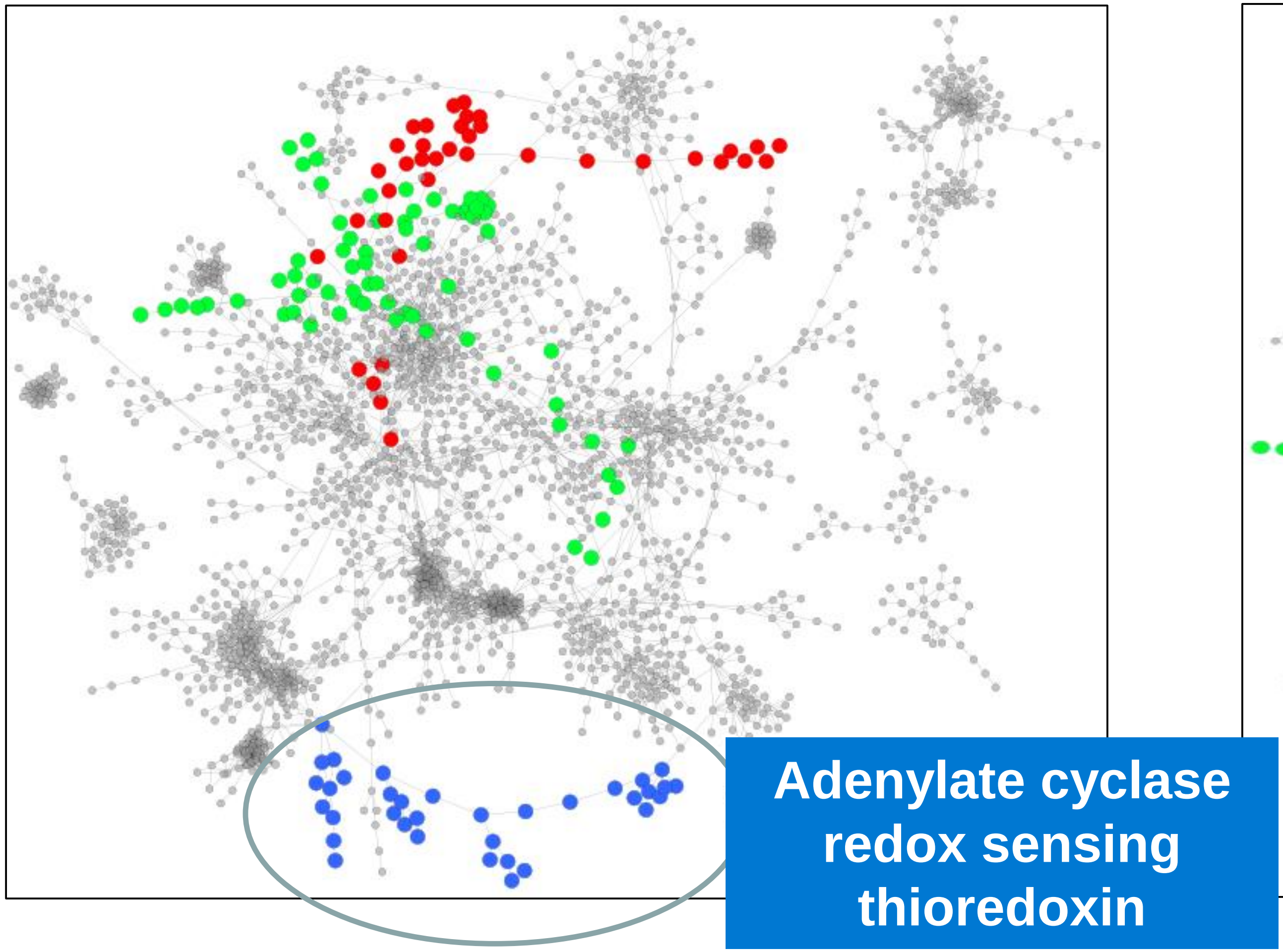
Genetic and metabolic markers in adapted high-productivity strains

Transcriptomic network-based approach details key gene clusters

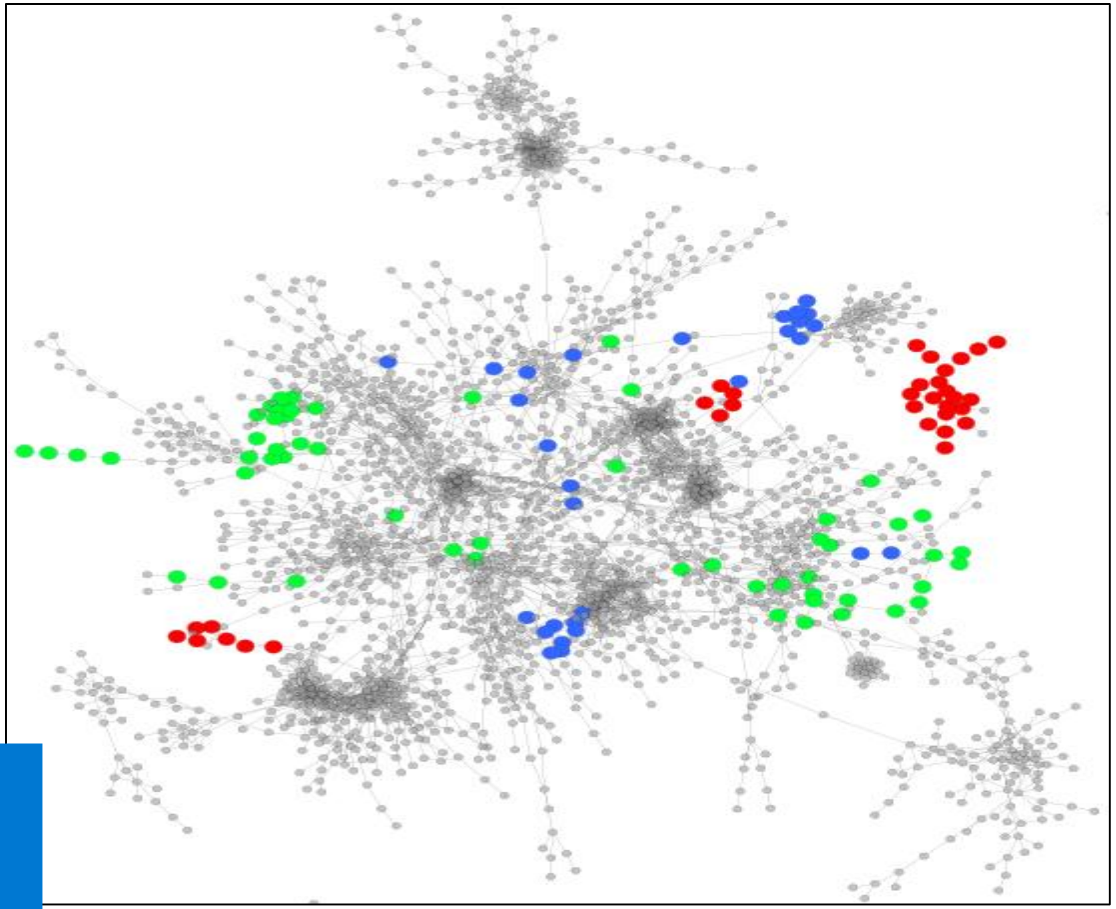
4 – Progress and Outcomes

'Omics toolkit'

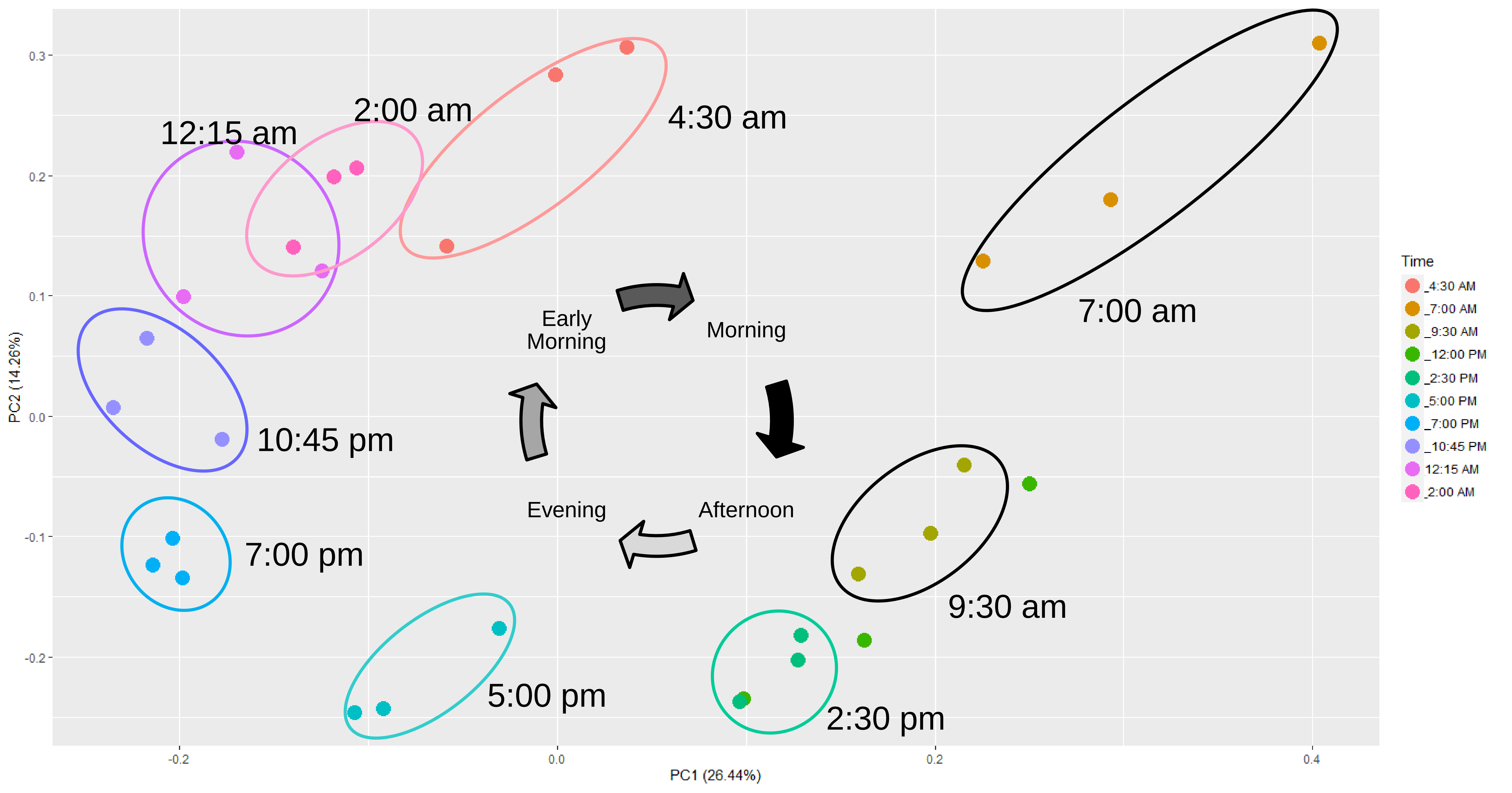
Full Dataset Network



Network without Adapted Strain



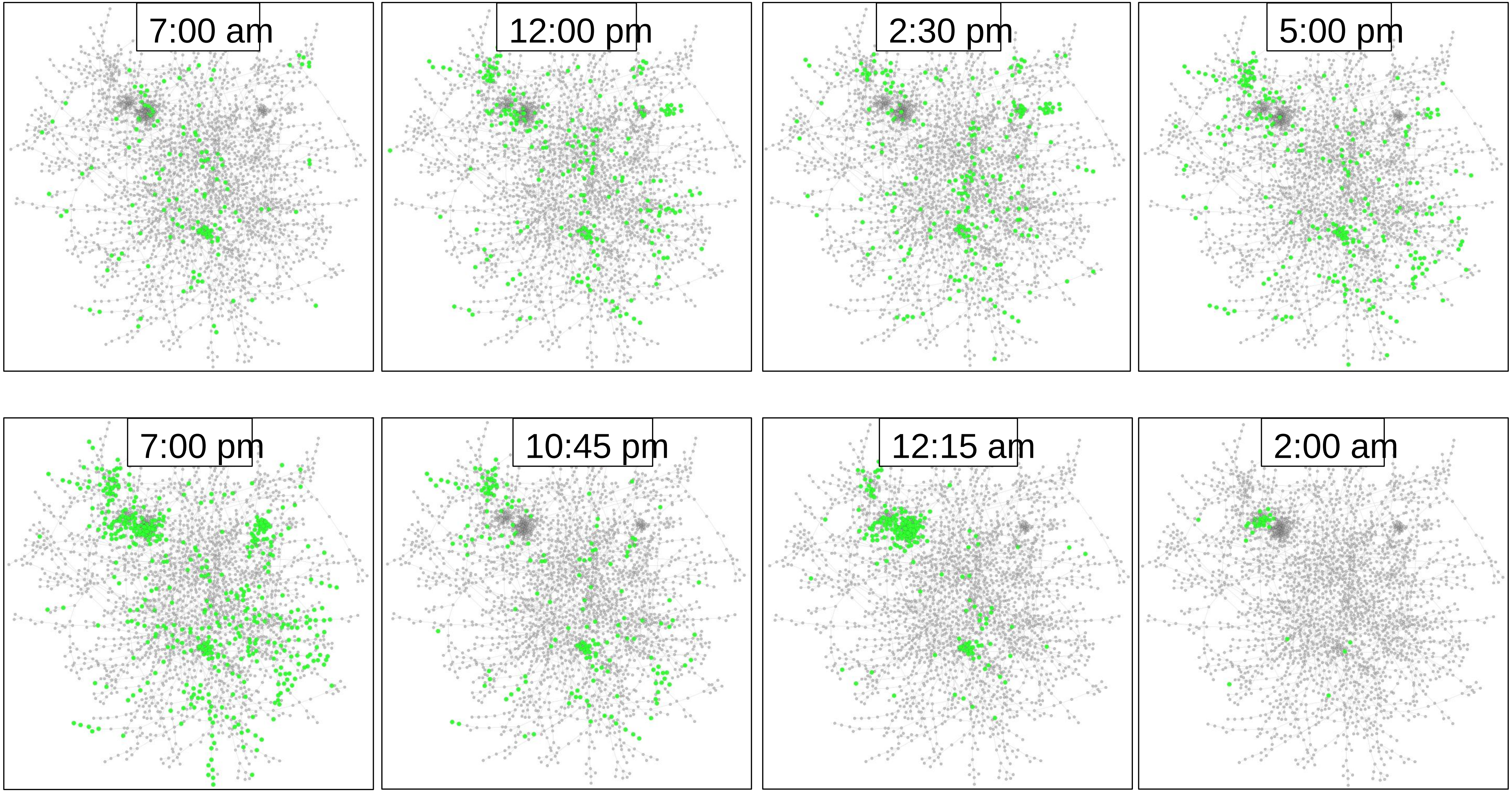
Preliminary transcriptomic network-based analysis suggests altered thioredoxin and redox sensing are important in adapted strain and differentiated from parent strain



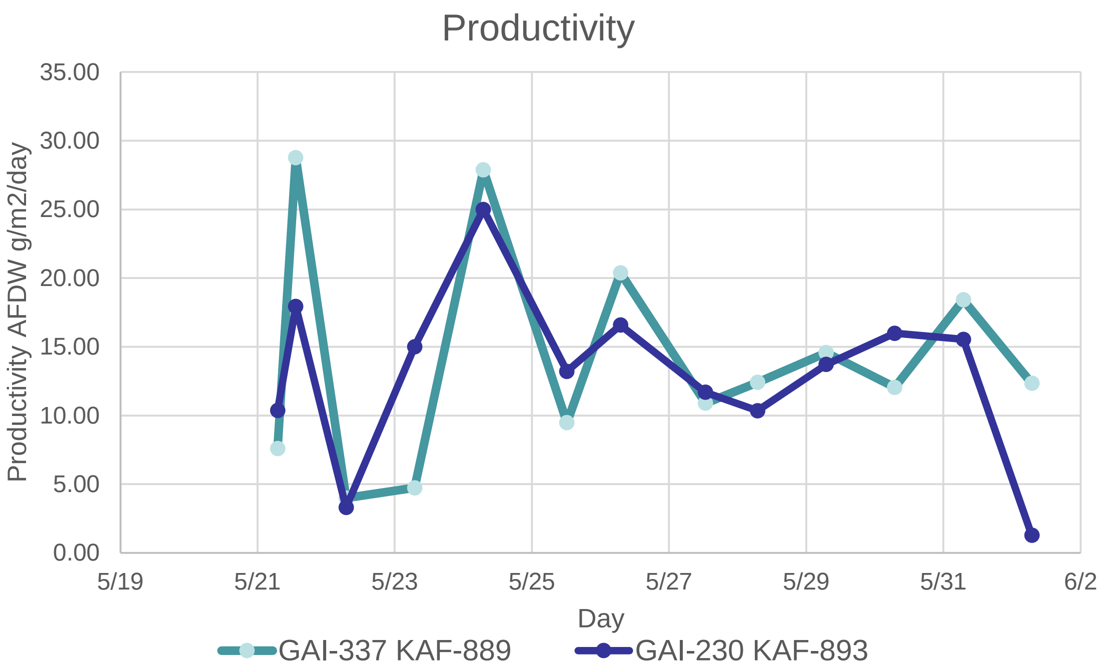
2:00 am
12:15 am
4:30 am
7:00 am
Early Morning
Morning
10:45 pm
Evening
Afternoon
7:00 pm
9:30 am
5:00 pm
2:30 pm
PC1 (26.44%)
PC2 (14.26%)
Time
_4:30 AM
_7:00 AM
_9:30 AM
_12:00 PM
_2:30 PM
_5:00 PM
_7:00 PM
_10:45 PM
12:15 AM
_2:00 AM

Differentially Expression Genes for Each Timepoint vs. 4:30 am.

DEGs are green overlayed on a network of all data showing co-expressed genes



First adaptation trial outdoors: Initial results did not demonstrate significant difference compared to wild type. However, in limited outdoor testing to date, peak productivities were attained by adapted strain on highest productivity days. Additional summer campaigns are necessary.



Average productivity
Wildtype - 12.87 g/m2/day
Adapted - 13.37 g/m2/day

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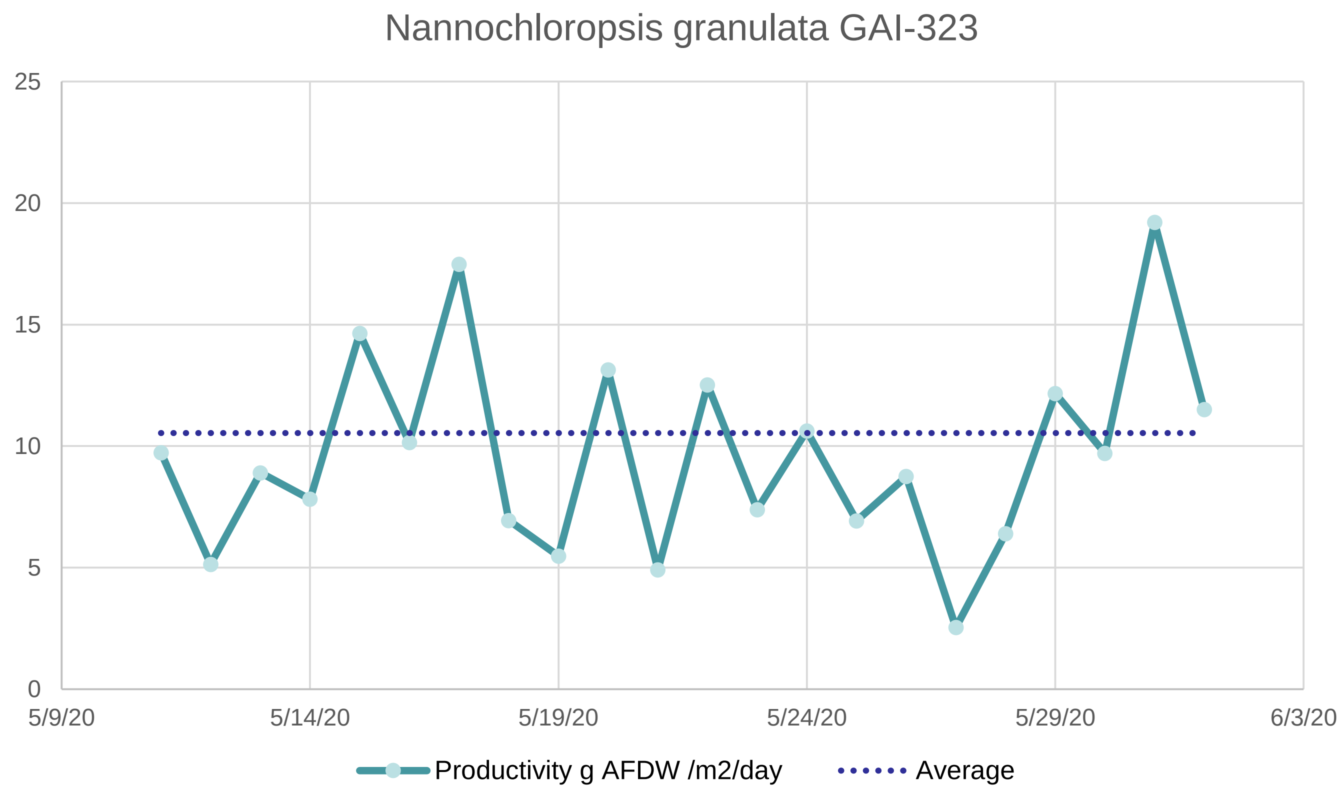
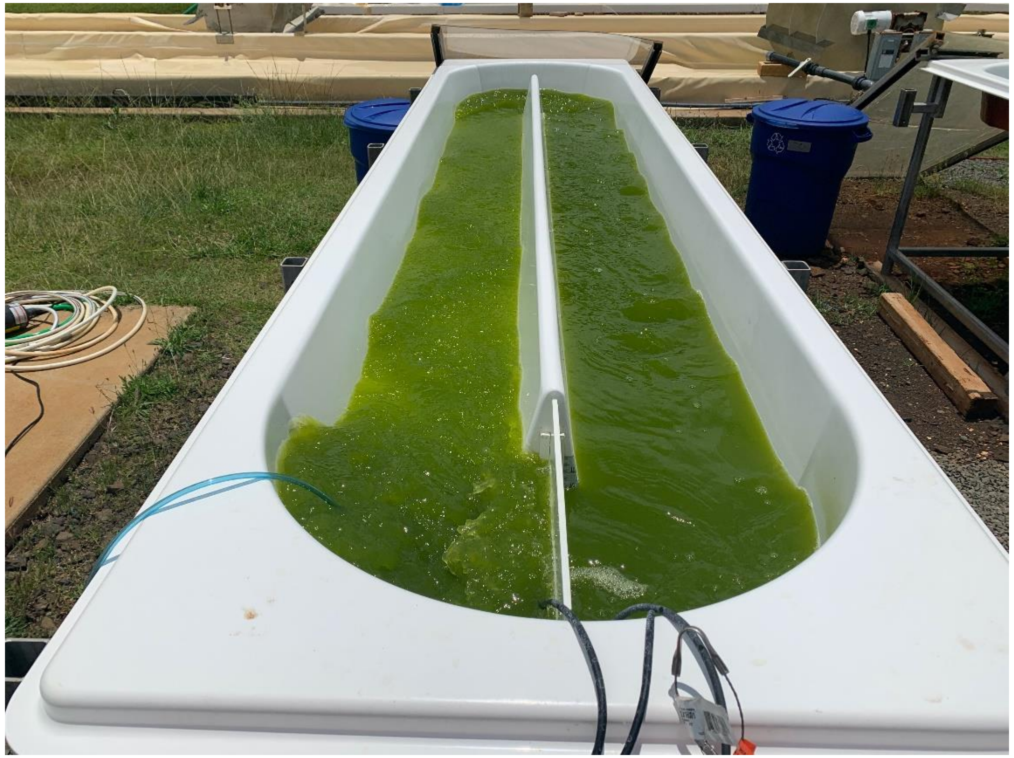
GAI-323 – *Nannochloropsis granulata* outdoor testing





26 day average productivity: 10.53 g/m2/day
Average pH: 8.91

Initial results promising, but needs additional adaptation to higher pH to demonstrate full suitability as GAI production strain

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Summary

- ***Directed evolution is a powerful tool to improve biotechnology phenotypes.***
- ***By using laboratory culturing facilities that better correlate with outdoor environmental conditions, selective pressures can be imposed that improve strain performance.***
- ***GAI-229 has been adapted to higher temperature and redox stress levels. Initial laboratory bioreactor experiments show improved biomass productivities. GAI-229 is inherently stable at higher pHs and adaptation to higher pH was not deemed necessary. Additional high-performance strains (Nannochloropsis and Picochlorum) have been adapted to GAI media and may be used as risk mitigation.***
- ***Thorough productivity tests at the pond are required to determine if laboratory productivity gains are realized outdoors.***

Publications, Patents, Presentations, Awards, and Commercialization

Presentations

- Posewitz M.C., *Characterization of Algal Phototrophs for Potential Biotechnology Applications.* January 2019 Western Photosynthesis Conference. Friday Harbor, WA.

Publications

- LaPanse, A., Krishnan, A., and Posewitz, M.C. Adaptive Laboratory Evolution for algal strain improvement: methodologies and applications. *Algal Research* (2021), **53**, 102122.
- Oliver A, Podell S, Pinowska A, Traller JC, Smith SR, et al. Diploid genomic architecture of Nitzschia hildebrandi str. GAI293, an elite biomass production diatom. Manuscript in prep. – in collaboration with PEAK project DE-EE0008121
- Bohutskyi, McClure, Hill, Burch, Traller, Posewitz, Beliaev et al. Exploring the physiological and genomic underpinnings of high biomass productivities in and industrially relevant diatom, Nitzschia hildebrandi str. GAI293. *In preparation.*

Quad Chart Overview

Timeline
- Project start date: August 15, 2018
- Project end date: December 31, 2021
- Percent complete: 75%

	FY20 Costed	Total Award
DOE Funding	$785,354	$1,919,178 (70%) $540,000.00 PNNL (20%)
Project Cost Share	$83,543 (11%)	$273,243 (10%)

Project Partners*
- PNNL
- Global Algae Innovations
- Colorado State University

Project Goal
This project specifically addresses BETO MYPP challenges in algal **Biomass Availability/Cost** (Aft-A).

End of Project Milestone
Increase biomass yields by ~20% above background strains in GAI high bicarbonate/high pH media. Attain 24 $g/m^2/day$ algal biomass at the GAI facility in Kauai.

Funding Mechanism
DE-FOA- 0001628 (2017)

Responses to Previous Reviewers' Comments

- Maintaining and verifying that evolved strains dominate outdoor ponds.
  - We are using extensive genetic and phenotype resources to determine whether inoculum from adapted strains remains in the ponds. We recognize that this a serious risk and are prepared to collect samples at the start of outdoor campaigns and at the end to determine if the adapted strains dominated.
- Adaptation may lessen fitness at other conditions (e.g. lower temperature)
  - We are testing across diel culturing rapidly to test adapted strain productivities under dynamic conditions.
  - We are also testing whether high-temp adapted strains grow similarly to control strains at lower temperatures.