Multi-Scale Characterization of Improved Algae Strains

U.S. Department of Energy (DOE) Bioenergy Technologies Office (BETO) 2019 Project Peer Review

Advanced Algal Systems

Taraka Dale

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Goal Statement

Goal

The goal of this project is to develop tools for generating algae strains improved for productivity and robustness, and to verify that strains generated using these tools demonstrate improved performance at multiple scales, from the bench to outdoors.

Outcomes & Relevance

- Accelerate the advancement of the algae biofuels industry through
  - Delivering tools for generating improved algae strains
  - Informing strategies for indoor-outdoor-indoor experiments
- Enable BETO in reaching their biofuel and cost targets

Quad Chart Overview

Timeline

- **Start Date:** 10/1/12
- **Current Merit Review Period:** 10/1/18 - 9/30/21
- **Percent complete** = 67% since Oct 2012

	Total Costs Pre-FY17	FY17 Costs	FY18 Costs	Total Planned Funding (FY19- End of Project)
DOE Funded	1,325k	600k	600k	1,800k
Cost Share	None			

Partners: Arizona Center for Algae Technology and Innovation (AzCATI, 5%); New Mexico Consortium (NMC, 5%)

Barriers Addressed

- *Aft-C. Biomass Genetics & Development*
- *Aft-E. Algal Biomass Characterization, Quality, and Monitoring*

Objective

Develop and verify tools for generating algae strains improved for productivity and robustness

End of MR Cycle Goal

Deliver at least two microalgae strains with an outdoor biomass productivity (AFDW) of 30% increase over baseline, in a non-freshwater growth medium under a semi-continuous harvesting regime.

1- Project Overview

Strain improvement tool development and testing at multiple scales

Challenge

Productivity needs to be improved to reduce the cost of algal biofuels and bioproducts: Growth and carbon storage

Question

Can we generate improved phenotypes (non-GM or GM) that have sufficient improvements and stability that they can be translated outdoors?

Opportunity

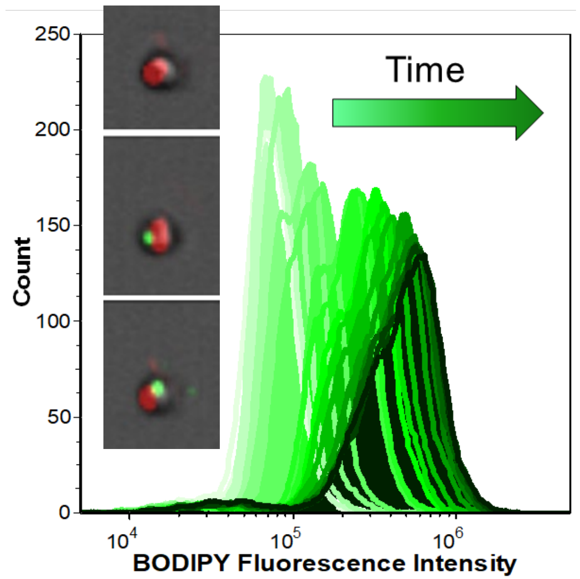
Establish methods for generating improved algae strains and evaluating their performance at multiple scales, from the bench to outdoors, by integrating national lab capabilities in algal biology, flow cytometry, and environmental simulation experiments, and by leveraging BETO's investment in algae testbed facilities.

Technical Objectives

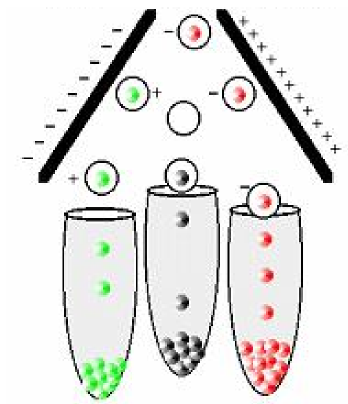
1. Strain Characterization
2. Strain Improvement
3. Outdoor Testbeds

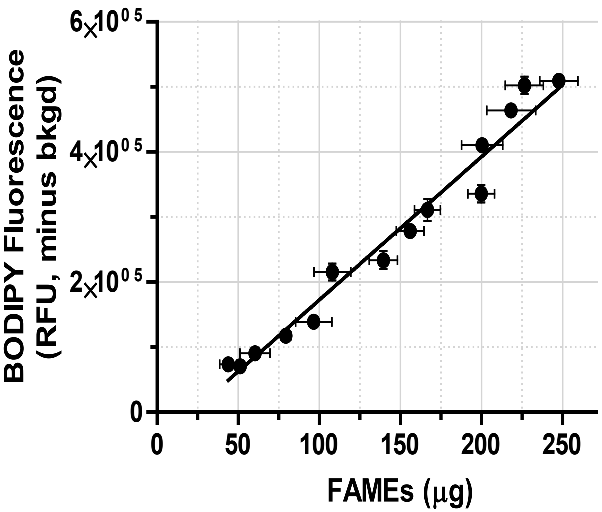
Project Overview: History and Context

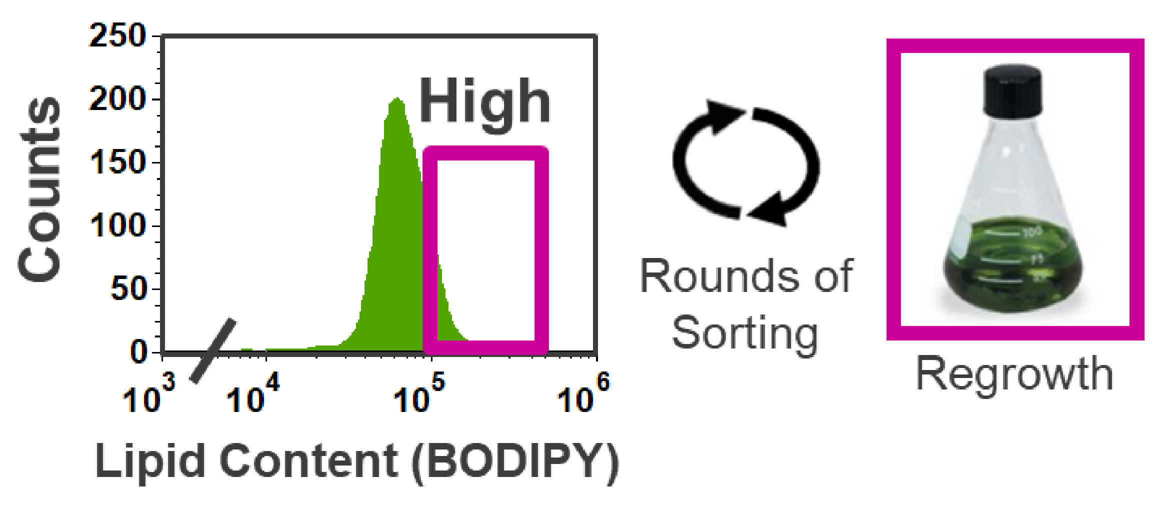
Flow cytometry can be used to track and improve productivity



- BODIPY fluorescence can be tied to neutral lipids (FAMEs)
- Flow cytometry -- 1000s of single cells interrogated per second in a flowing sample stream – 35+ yr history at LANL
- BODIPY staining permits sorting on neutral lipids → FAMEs → Fuel
- Cell morphology can affect dye uptake





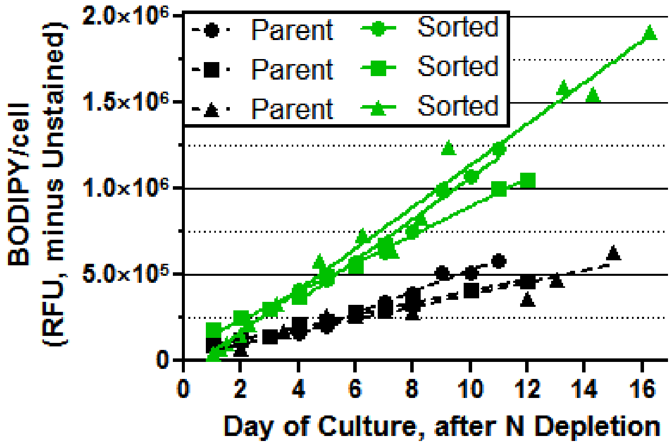


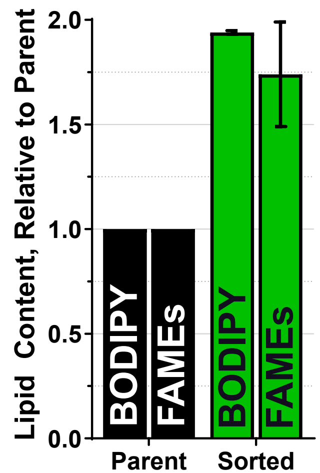
Project Overview: History and Context

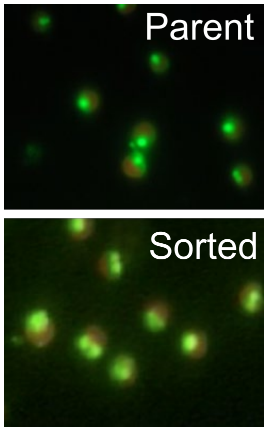
Cell sorting can produce subpopulations with phenotypes lasting years

- Generated new populations for 4 different strains
- 3 separate cultivars for Cs1228
- Verify with FAMEs
- Strains are stable for 2+ years on plates

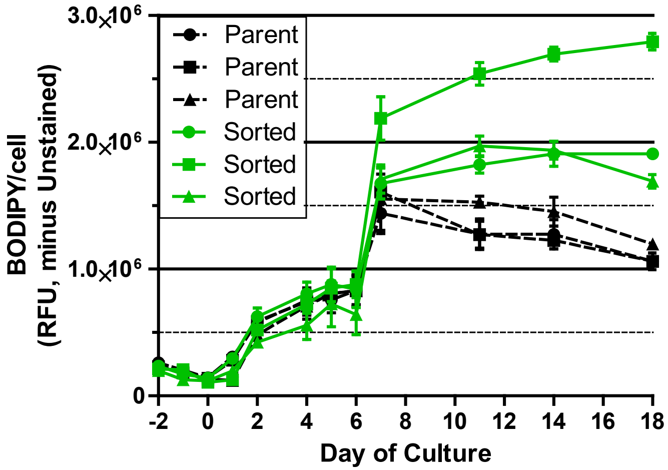
Picochlorum soloecismus

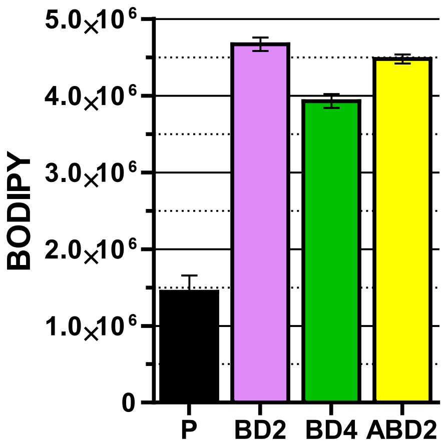


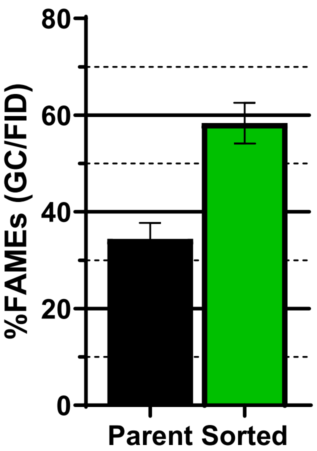




Chlorella sorokiniana 1228







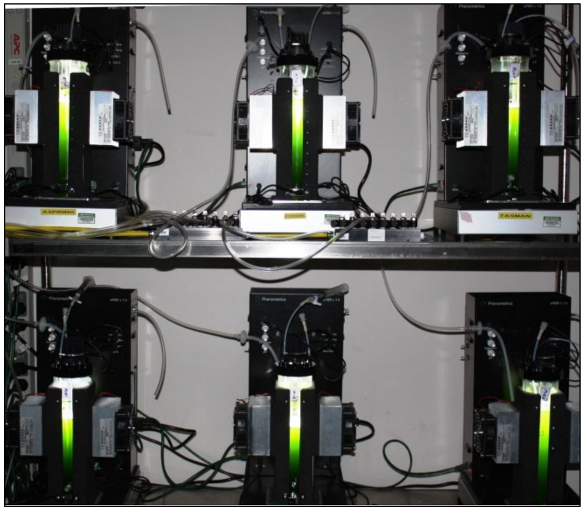
Developing

- Alternate strategies and protocols for recalcitrant strains
- Droplet-based sorting variation
- Mutagenesis

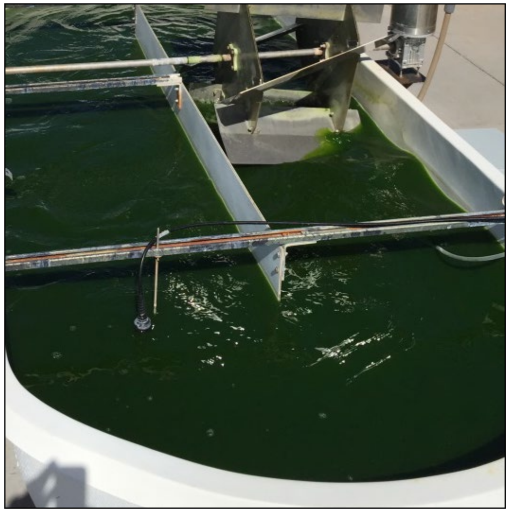
Project Overview: History and Context

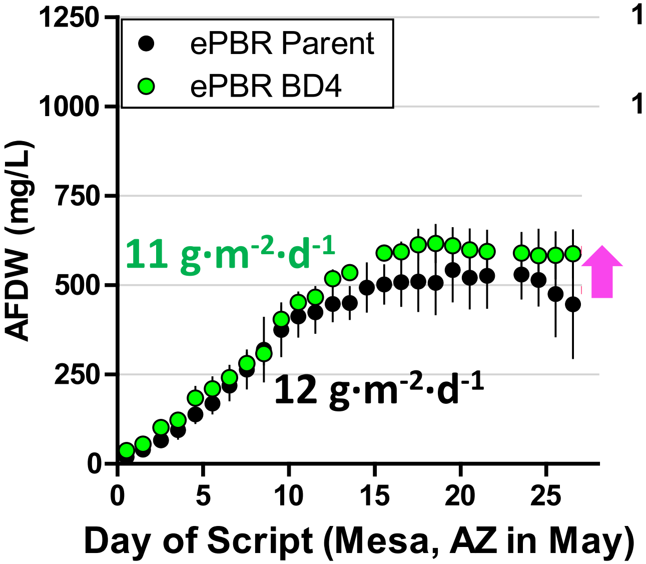
Sorted cultures are at least as fit as parents up to 1000 L outdoors

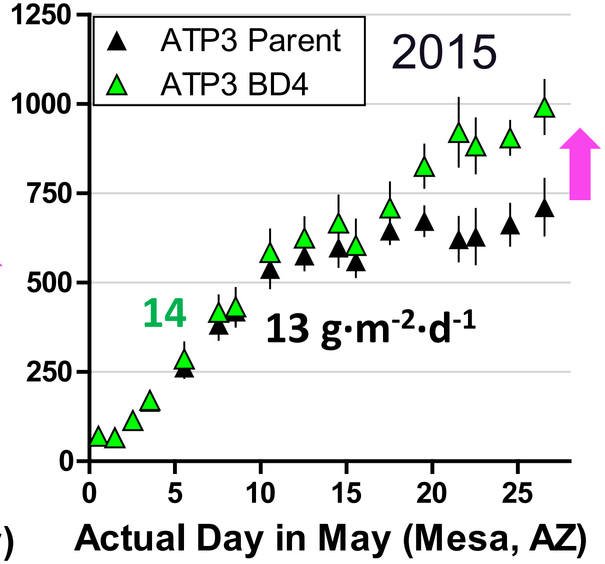
LANL ePBRs



AzCATI Outdoor Raceways







- Environmental simulation in ePBRs and AzCATI ponds
- Relative phenotypes (productivities in g/m^2-d) are similar across these two systems.
- An **improvement** in **total biomass** was observed for BD4
- Productivities for 2 seasons

May/June 2016	
P	**14 g/m^2-d**
BD4	**16 g/m^2-d**

- Carbon storage insufficient to assess lipids outdoors for Cs1228 P/BD4 – but showed lipid phenotype in sorted *N. salina* at 60,000 L in the Cellana ABY1 project ('BR3')

2 – Approach (Management)

- Meetings with the project (LANL) team and individual researchers regarding current experiments, approach, and progress are held weekly
- Meetings with AzCATI and NMC regarding collaborative work held weekly
- Monthly calls between LANL and BETO AAS Team
- Team discusses risks and mitigating strategies early and often
- PI ensures progress and execution of project plan, makes final scientific decisions, meeting milestones, and complies with reporting requirements

Project Interfacing

DISCOVR / State of Technology	Apply tools, share strains and outdoor cultivation lessons learned
ATP^3/AzCATI Testbeds	Cultivation studies and strategies
Functional Characterization of Cellular Metabolism	Share flow cytometry & GM tools
Algal Translational Genomics	‘Omics data
CAP Process Research	Share biomass grown at AzCATI
Algae Biotech Partnership	General sharing of strain info and GM tool development
Genetic Blueprint	
Robust Genome Engineering	
Producing Algae for Coproducts and Energy	

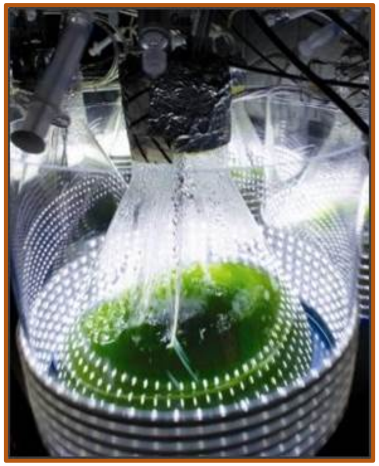
2 – Approach (Technical): Multi-step Process

Characterize strains across multiple cultivation systems

1 Characterization

- Growth – OD, AFDW, Cell Count, [N]
- Single Cell – Size, Shape, Fluor Dyes
- Biochemical – FAMEs, Carbs, Protein

Spin Flask



ePBR

MicroBio Ponds

%Ash

% Protein

% Carbs

% Lipids

2 – Approach (Technical): Multi-step Process

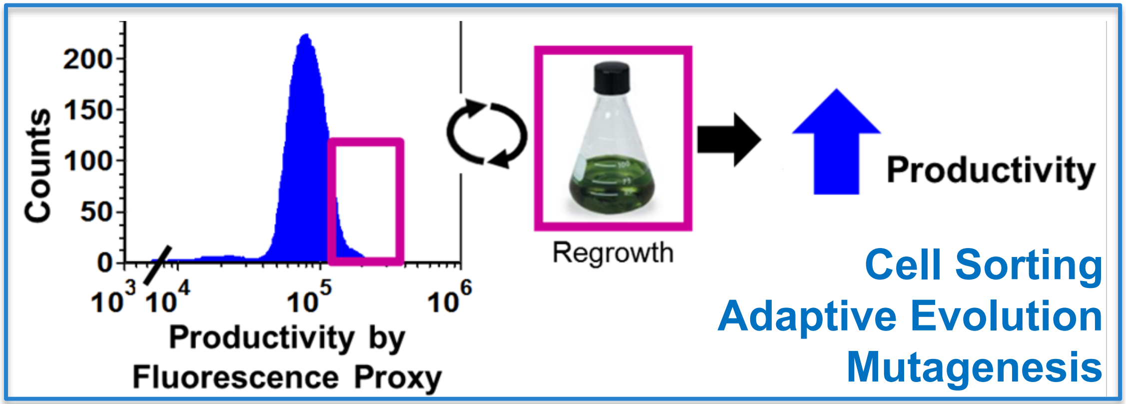
Use non-GM and genetic modification strategies to improve phenotypes

1 **Characterization**

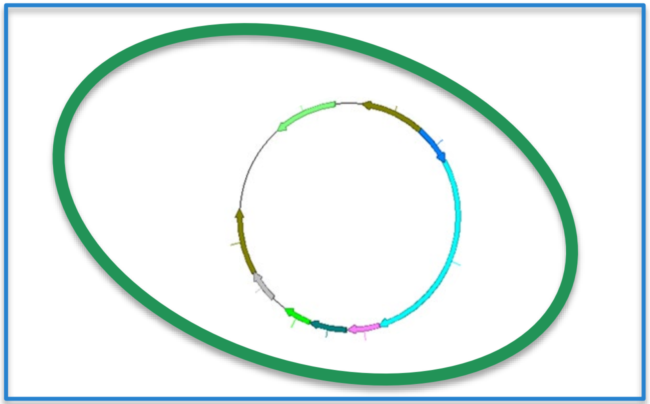
2 **Improvement**

- Carbon Storage – Lipids, Carbs
- Biomass Growth – Early, Linear
- Salinity Tolerance – Non-freshwater

Non-GM



Genetic Modification



2 – Approach (Technical): Multi-step Process

Partner with outdoor testbed for outdoor cultivation

1 **Characterization**

2 **Improvement**

3 **Outdoor Testbed**

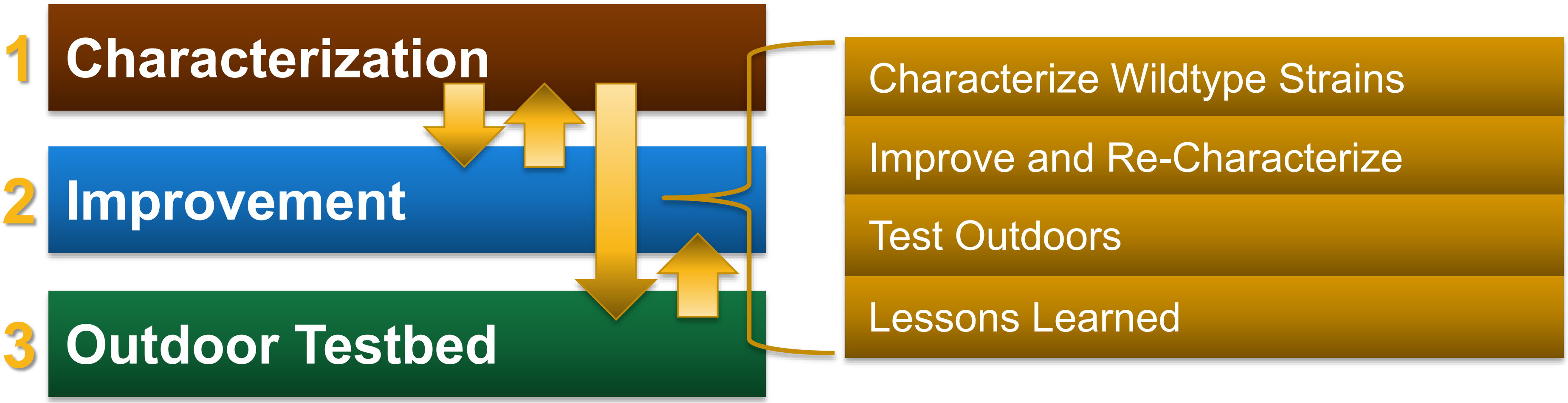
- AzCATI Testbed at ASU, Mesa AZ
- Batch, Semi-Continuous Runs

1000L Open Raceways

Mini-Mobile Flat Panel PBRs

2 – Approach (Technical): Multi-step Process

Integrated team and expertise permits iteration for increased success



Potential Challenges

- Improved strains do not grow well outdoors (are not fit)
- Increases in productivity do not translate to outdoor systems

Critical Success Factors

- Relative indoor performance translates to outdoor systems
- Outdoor testbed facilities remain available

3 – Accomplishments: Overview

Three highlights using our approach will be shared

Improved Salinity Tolerance in *Chlorella*

- *Milestone.* One improved strain from FY17 (+parent) sent to ATP^3 for a final outdoor growth trial.

Picochlorum soloecismus

- *Milestone.* Submit publication on *Picochlorum* strain characterization, including biochemical composition, salt tolerance, and outdoor growth potential.

Genetically Modified *Picochlorum*

- *Milestone:* Characterization of at least one genetically modified strain of *Picochlorum* sp., including growth and biochemical composition.

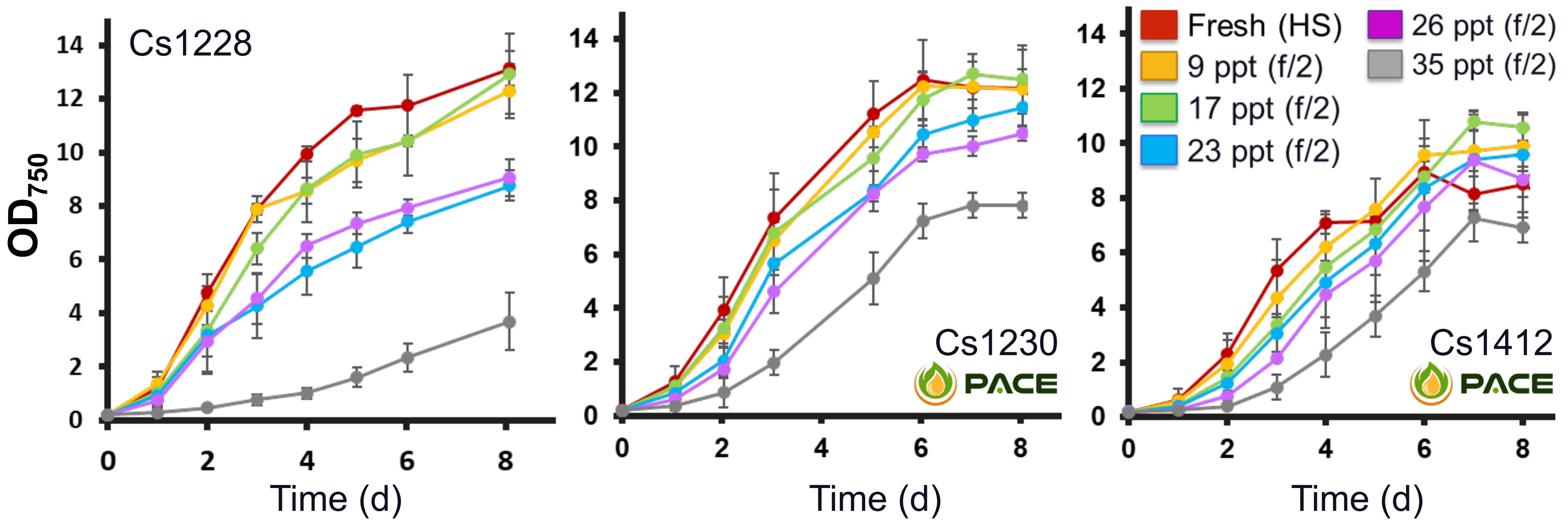
A note on strain choice:
We work with microalgae strains that we understand relatively well in the lab: have varied growth rates in and outdoors, varied carbon storage profiles, and a range of salinity tolerance

3 – Accomplishments: Salinity Tolerance

Shifting away from cultivation in potable water

- Freshwater for drinking and agricultural use comprises a small fraction of available water globally.
- Some of the best growing algae strains are “freshwater”
- Not all algae is grown on the coast, seawater ponds can swing in salinity
- SOT data suggests higher salinity cultures may be more crash resistant

Average seawater: 35 ppt
Drinking water: 0.1 ppt
Limit on agriculture irrigation: 2 ppt

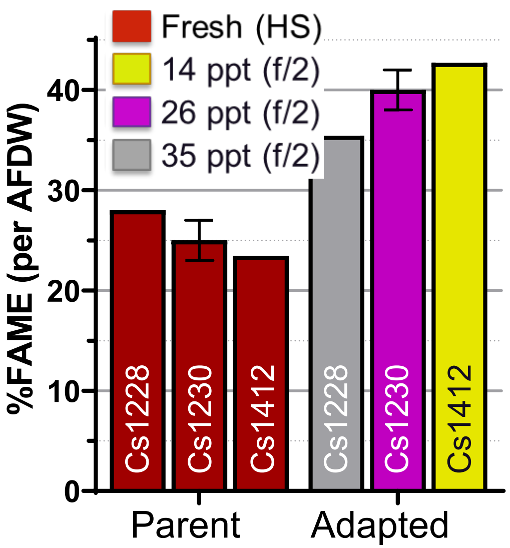


- **First**: Test natural salinity tolerance “shock” experiments
- **Result:** Freshwater strains can be broadly tolerant to salinity shifts, 3 *C. sorokiniana* strains

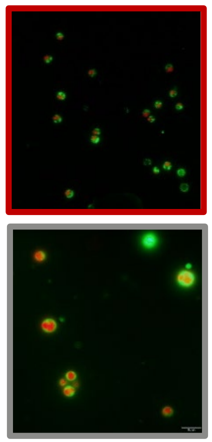
3 – Accomplishments: Salinity Tolerance

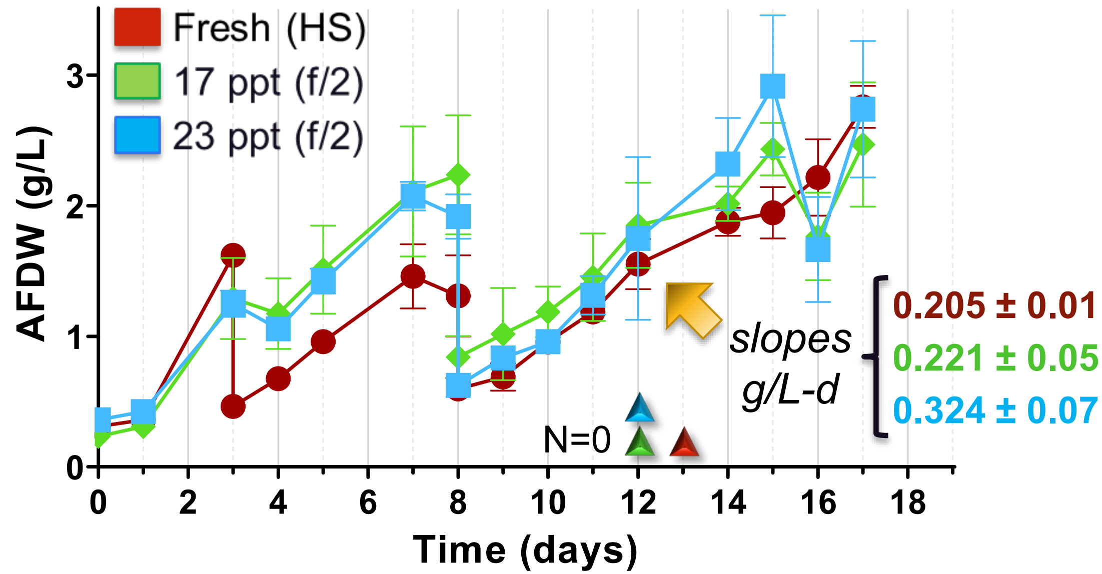
Cultures in higher salinities are fit for outdoor growth

- Noted higher FAMEs (late deplete, by BODIPY and GC-FID) and larger cells by microscopy and flow cytometry

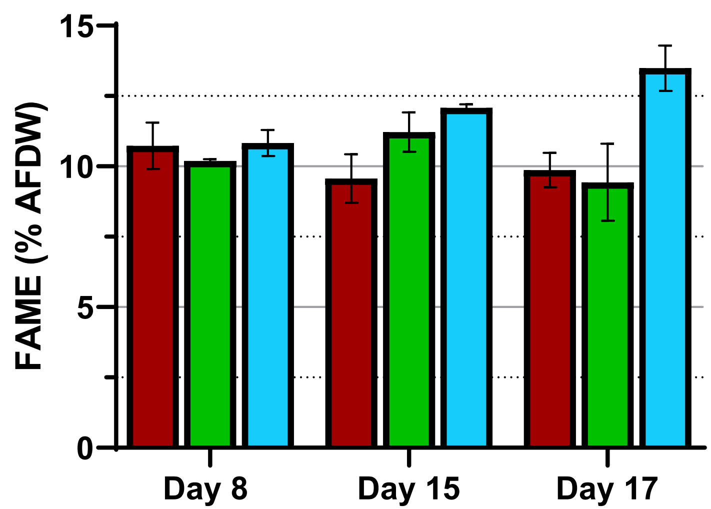


Cs1228





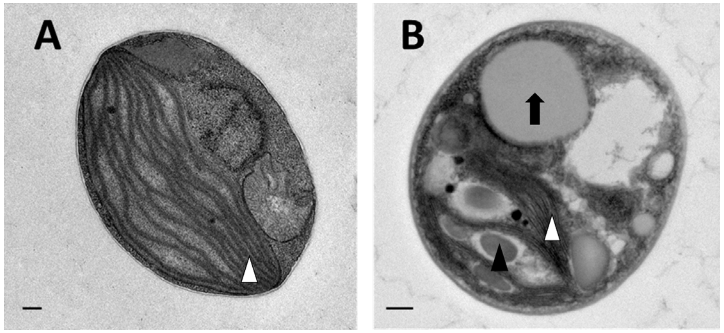
- **Second**: Test Cs1228 outdoors at higher salinty
- **Outcome:** Cs1228 can be adapted to grow to 23 ppt seawater salinity outdoors at AzCATI in July, FAMEs may be increased in early deplete
- *Ongoing*: Manuscript preparation on salinity tolerance and adaptation of *C. sorokiniana x3*

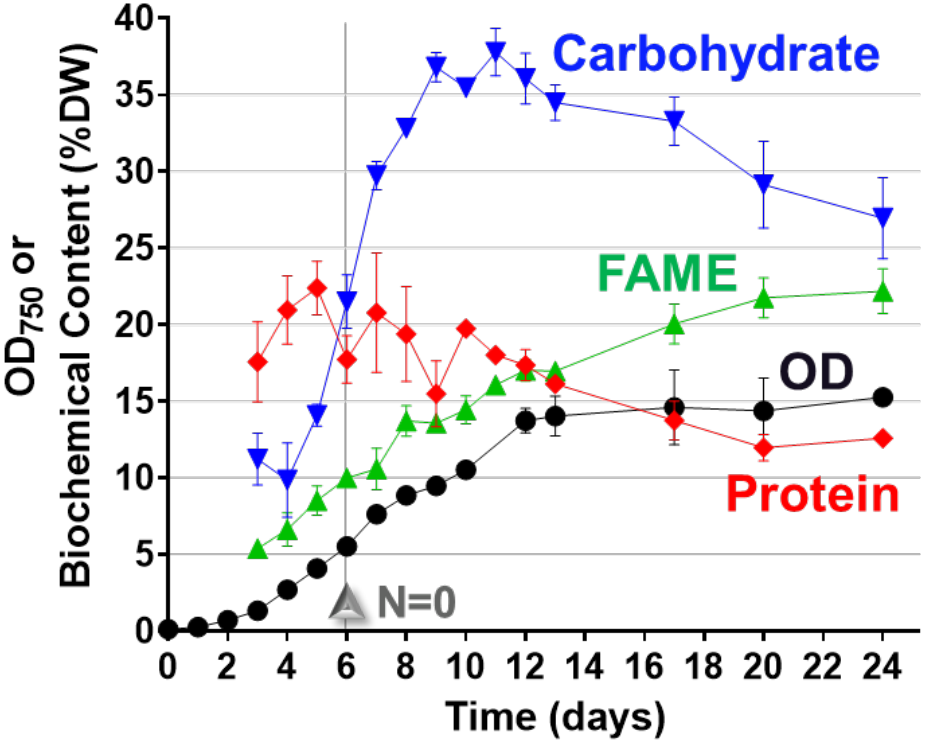


3 – Accomplishments: *Picochlorum soloecismus*

Completed P.s. characterization with growth and biochemical analysis

- *P. soloecismus was* isolated in NAABB, growth & FAMEs evaluated
- Full biochemical profile, outdoor growth and stability unknown
- Genome comparison not conducted
- Salinity tolerance not examined



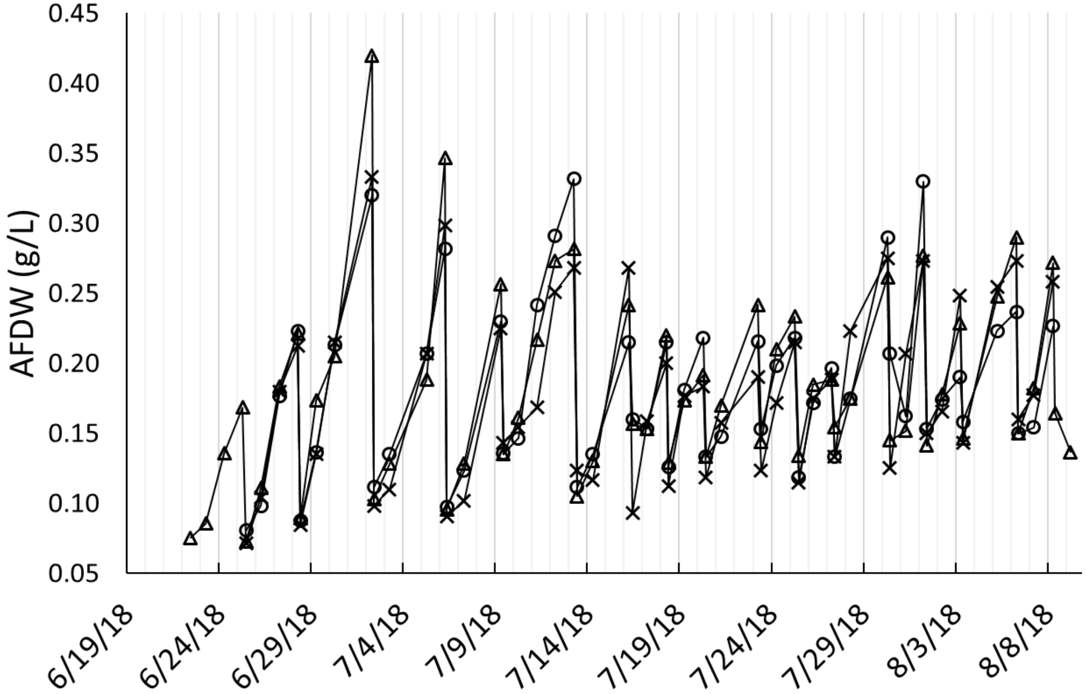


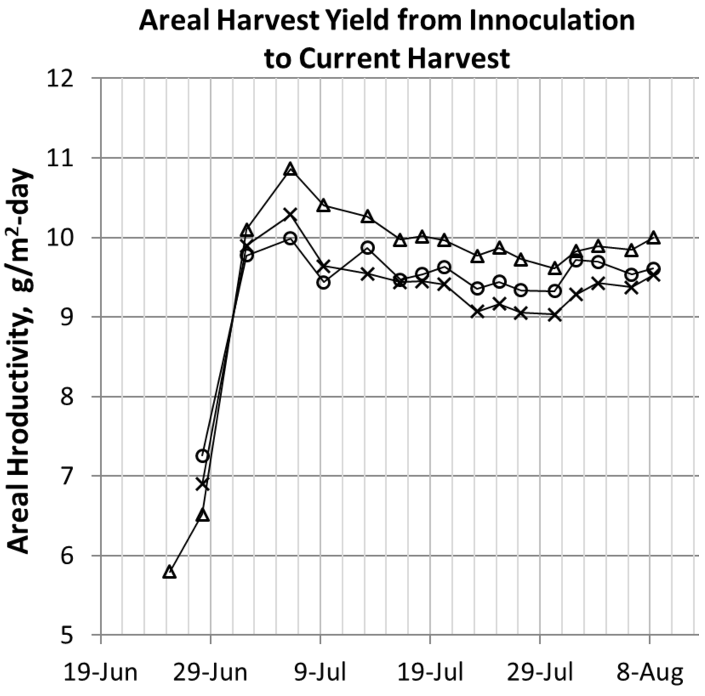
- **First**: Characterize *P.s.* growth/biochem indoors; (genome analysis, salinity not shown)
- **Result:** *P.s.* accumulates both lipids and carbohydrates; 50% DW total carbon storage
- *Ongoing*: Manuscript under review on characterization, genome, transformation method

3 – Accomplishments: *Picochlorum soloecismus*

Completed P.s. cultivation at ASU summers of 2017 and 2018

- *P. soloecismus* cultivated in triplicate, 1000L open ponds for 40+ days in f/2 in summer



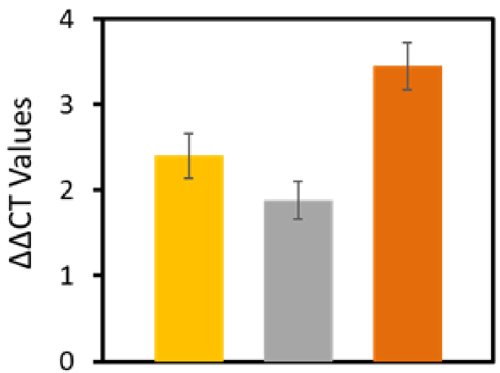


- **Second**: Cultivate *P.s.* outdoors under semi-continuous harvesting conditions
- **Outcome:** *P.s.* grows slow and steady, >9 g/m^2-day, 17 harvests, ***no crashes***
- *Ongoing*: Want to understand what makes *P.s.* so stable relative to other SOT strains

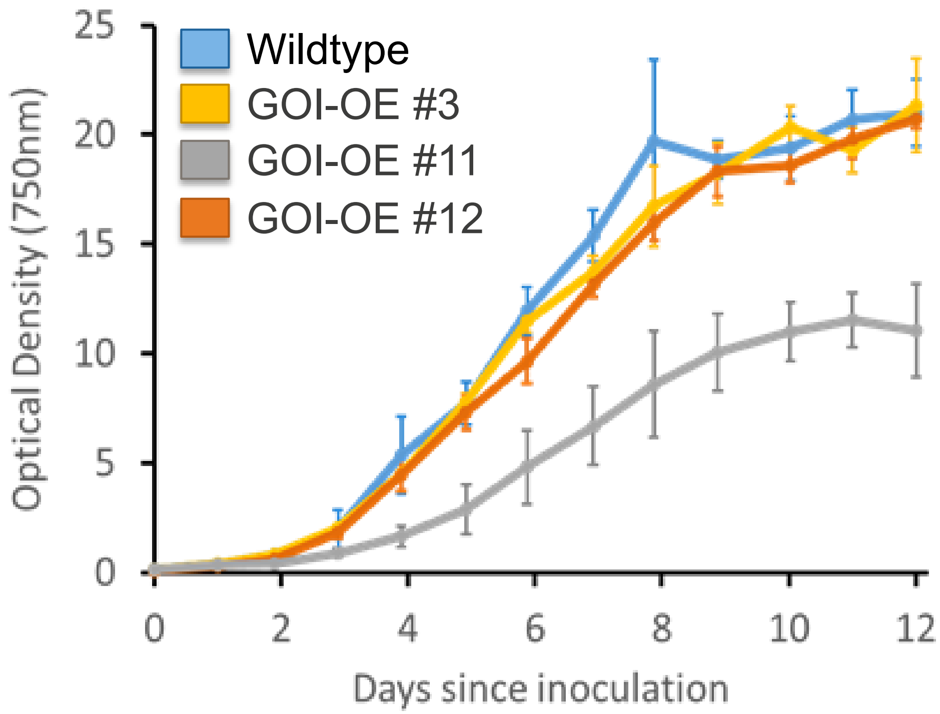
3 – Accomplishments: GM *P. soloecismus*

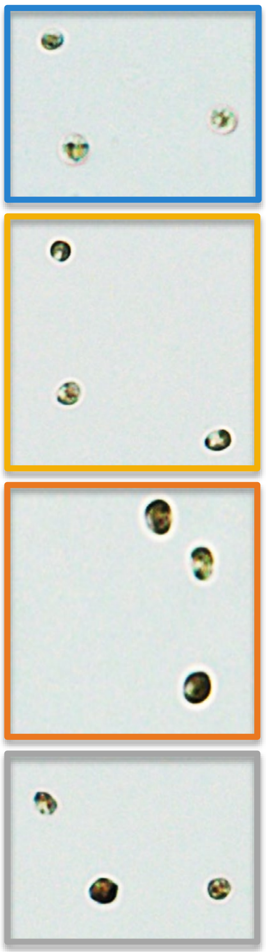
Gene of interest (GOI) shown in plants to boost biomass and stress tolerance

- First *P. soloecismus* transformation method was cumbersome, lines transient
- Developed an electroporation method
- 5 genes of interest PCR (+) confirmed, expression confirmed by RT-PCR and/or Western for 4



Hypothesis: ↑ **Biomass** ↑ **Carbon Storage**

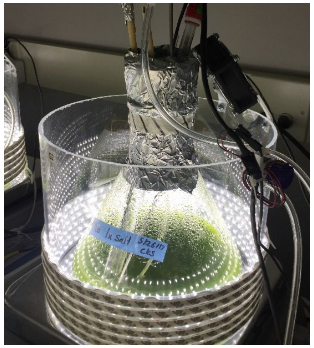




- **First**: Overexpress this gene of interest in *P.s.*
- **Result:** Initial screen of 3 lines → GOI over- expression (OE) lines can grow as well as wildtype, Lugol’s stain suggests higher starch, select #12

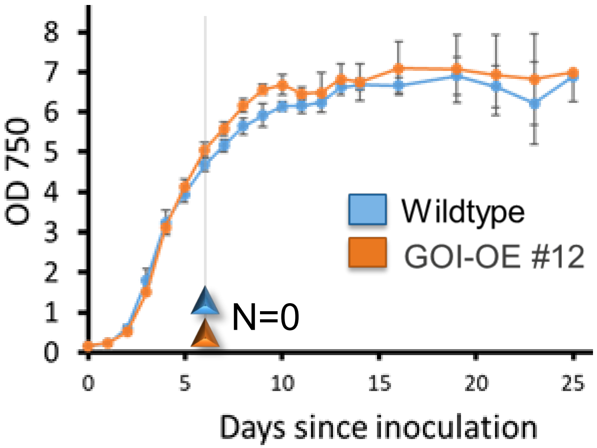
3 – Accomplishments: GM *P. soloecismus*

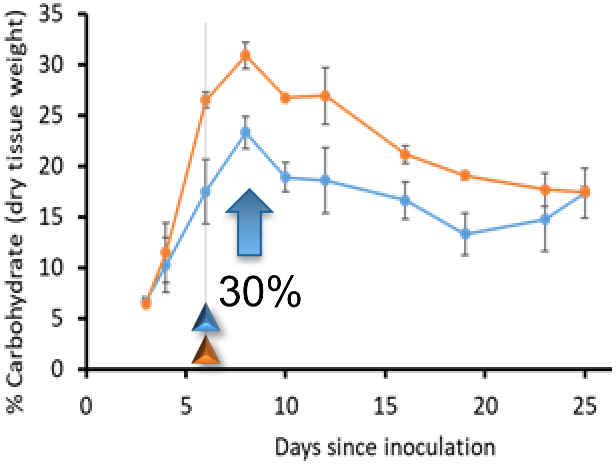
Gene of interest (GOI) boosts carbon storage in P.s.

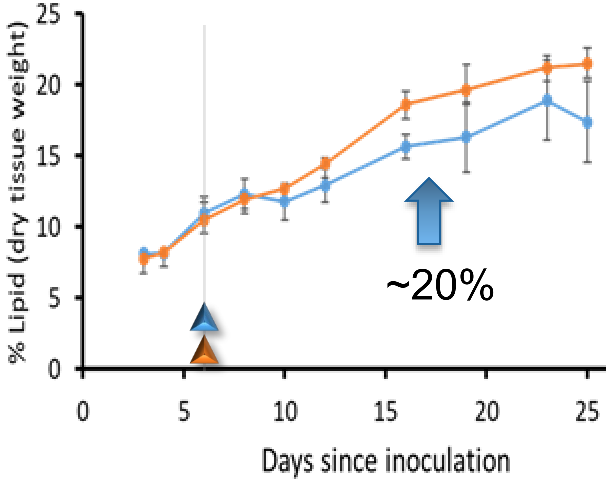


- Spin flask on 16:8 light dark, 25C, pH 8
- Transformants are more stable than before, have had since Oct 2017
- Plan to test in ePBRs

Result: Biomass in ***P.s.*** Carbon Storage







- **Second**: Compare growth and biochemical composition of wt and GOI-OE *Picochlorum*
- **Outcome:** GOI-OE #12 grows as well as WT and accumulates more FAMEs and carbs
- *Ongoing*: Final biochemical and photosynthesis measurements, manuscript in prep

4 – Relevance to R&D and Industry

Delivering production-relevant strains and informing in-out-in approaches

Goal

- Develop tools for strain improvement, test/verify that tools generate strains that are fit outdoors, learn from outdoor experiments to improve tools, deliver strains and tools to algae community

Industry Relevance

- Aim to
  - Work with **relevant strains under outdoor-relevant conditions** indoors -- media, light cycle/intensity, productivities in g/m^2-d where possible
  - Move **outdoors relatively quickly** – non-GM improvement methods do not require regulatory approval, 8 outdoor runs since May 2015
  - **Adapt laboratory experiments** to better reflect outdoor performance

Technology Transfer

- Share knowledge gained at conferences and publications

4 – Relevance to BETO Mission

Delivering production-relevant strains and informing in-out-in approaches

- Strategies for **strain improvement and relating laboratory to outdoor data (and back again) are needed** to increase productivity and reach BETO cost targets
- **Work closely with AzCATI, DISCOVR, and the SOT** Team to share lessons learned & accelerate implementation of the best strains & practices
- Using production-relevant strains:
  - *P. soloecismus* was selected to be part of the summer SOT trials -- high performer in LEAPs and stable in summer at AzCATI
  - *C. sorokiniana* crashes at AzCATI in summer but showed 13-14 g/m^2-d in May 2015, 2016
  - Salinity adaptation results helped inform DISCOVR salinity results

Season (Months)	**2018 SOT g/m^2-d**
Spring (MAM)	15.2
Summer (JJA)	15.4
Fall (SON)	8.5
Winter (DJF)	7.7
Annual Average	11.7

- Contributes to **BETO Milestones**:
  - 2021: Develop strain improvement toolkits and technologies that enable algae biomass compositions in environmental simulation cultivation conditions that represent an energy content and convertibility of 80 GGE of advanced biofuel per ash-free dry weight ton of algae biomass.
  - 2025: Increase the summer seasonal areal productivity to 25 grams per square meter per day ($g/m^2/d$) from the 2016 baseline of 13.3 $g/m^2/d$.

5 – Future Work

New tasks work towards quarterly milestones (M) and 18mo Go-NoGo (GNG)

Characterization

- Continue to move towards more cost effective **media**
- Examine interplay between **abiotic stressors**: salinity, temperature, light (M)
- **Semi-continuous harvesting** experiments in ePBRs and mini-ponds (M)
- ‘**Omics** of improved algae strains

Improvement

- Continue to develop **mutagenesis** strategies to add genetic diversity (M, GNG)
- Characterize the other **mutant *P. soloecismus* lines** (M)
- Expand **molecular toolbox** for *Picochlorum*, add Cs1228
- Modify **gel microdroplet-based sorting** strategy to improve linear growth rates

Outdoor Testbed

- Continue to **partner with AzCATI** to conduct outdoor runs (M)
- Continue to strengthen our **indoor-outdoor-indoor** approach
- Examine if we can isolate cultures that blow in/crash outdoor ponds “**work with the land**”
- Evaluate **operational strategies** for further productivity improvements

Summary

1. Overview

We are **establishing methods for generating improved algae strains and evaluating their performance at multiple scales**, from the bench to outdoors, using national lab core expertise

2. Approach

We are using **an iterative process between: Strain Characterization, Strain Improvement**, and transitioning to our **Outdoor Testbed** partner

3. Progress

- We are establishing **methods for increasing productivity & robustness** using cell sorting, adaptive evolution, and genetic modification approaches
- ***C. sorokiniana*** can be adapted to **higher salinities with possible boost to carbon storage**
- ***P. soloecismus*** has been **further characterized**, and **stable GM lines generated with increased carbon storage phenotype**

4. Relevance

We are **developing approaches for strain improvement and strain downselection that can be leveraged in other projects** (DISCOVR, competitive projects) and handed off to **external stakeholders**

5. Future Work

We will continue to **develop non-GM and GM-based strain improvement methods**, **evaluate strains** across scale, and **use the outdoor data to inform the next indoor experiment**

Acknowledgements

- C. Raul Gonzalez Esquer
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- Babetta Marrone

- Sangeeta Negi
- Taylor Britton

New Mexico CONSORTIUM

ATP3 Algae Testbed Public-Private Partnership

- J. McGowen
- J. Forrester
- S. Chin
- J. Izzett

AzCATI Arizona Center for Algae Technology and Innovation

U.S. DEPARTMENT OF ENERGY | Energy Efficiency & Renewable Energy

BIOENERGY TECHNOLOGIES OFFICE

Additional Slides

Presentations/Publications Since Last Peer Review

Publications

- Gonzalez-Esquer CR, Wright K, Sudasinghe N, Carr CK, Sanders CK, Turmo A, Kerfeld CA, Twary SN, Dale T* (Under Review). Demonstration of the potential of *Picochlorum soloecismus* as a microalgal platform for the production of renewable fuels. *Algal Research*.

- Tyler CRS, Sanders CK, Erikson RS, Dale T, Twary SN, Marrone BL (Under Review). Functional and phenotypic flow cytometry characterization of *Picochlorum soloecismus* DOE101 isolates. *Algal Research*.

- Gonzalez-Esquer CR, Twary SN, Hovde BT, Starkenburg SR (2018). Nuclear, chloroplast, and mitochondrial genome sequences of the prospective microalgal biofuel strain Picochlorum soloecismus. *Genome Announcements*, 6(4), 1-2.

Presentations & Session Chairs

2019 Dale T. Symposium on Biotechnology for Fuels and Chemicals. Invited co-chair/convener of the session, *Lipid production and processing*

2019 Dale T. 13th Annual Algae Biomass Summit Biology Track Chair. Invited co-organizer of Biology sessions.

2018 Dale T. University of Georgia. *Optimizing metabolic flux for improving the productivity of biofuels and bioproducts.* Invited seminar.

2018 Dale T. 12th Annual Algae Biomass Summit Biology Track Chair. Invited co-organizer of Biology sessions.

2018 Sudasinghe N, Sanders C, Kwon I, Cirigliano E., Wright K, McGowen J, Dale T, 12th Annual Algae Biomass Summit. Poster. *Saline adaptation of freshwater microalga* Chlorella sorokiniana *for sustainable biofuel production.*

Presentations/Publications Since Last Peer Review

Presentations, continued

2018 Wright K, Gonzalez-Esquer CR, Sudasinghe N, Sanders CK, Paez J, Starkenburg S, Dale T, 12th Annual Algae Biomass Summit. Poster. *Overexpression of [gene of interest] increases starch accumulation in* Picocholorum soloecismus.

2018 Dale T. Society for Industrial Microbiology and Biotechnology. Invited co-chair/convener of the session, *Metabolic engineering of photosynthetic organisms*.

2018 Dale. T. BIO World Congress on Industrial Biotechnology. Invited speaker and panelist. Panel topic: *Test in the lab, field, or somewhere in between? Are there strategies to fast track algae biofuels and products research?*

2018 Gonzalez-Esquer, CR. 8th International Conference on Algal Biomass, Biofuels and Bioproducts. Oral presentation. *A modular approach for the metabolic redesign of microalgal production platforms*

2018 Gonzalez-Esquer, CR. New Mexico Consortium Seminar Series. Oral presentation. *A modular approach for the metabolic redesign of microalgal production platforms.*

2017 Dale T. 11th Annual Algae Biomass Summit. Oral presentation. *Leveraging natural genetic variation in* chlorella sorokiniana *to increase biomass productivity.*

2017 Dale T. 11th Annual Algae Biomass Summit Session Chair. Invited session chair.

2017 Sanders CK and Dale T. 7th International Conference on Algal Biomass, Biofuels, and Bioproducts. Poster. *Growth of microalgae in conditions of increased salinity*.

2017 Dale T, Lindsey-Carr C, McGowen J, Pelle B, Dichosa A, Starkenburg S. 7th International Conference on Algal Biomass, Biofuels, and Bioproducts. Poster. *Leveraging natural genetic variation in* chlorella sorokiniana *to increase biomass productivity.*

Presentations/Publications Since Last Peer Review

Presentations, continued

2017 Gonzalez-Esquer, CR. 7th International Conference on Algal Biomass, Biofuels and Bioproducts. Poster. *Picochlorum soloecismus DOE101 as a novel microalgae platform for the production of biofuels.*

2017 Dale T. Visit by the European Union Deputy Director General for Energy and EU delegation, Bradbury Science Museum. Invited briefing. *LANL's biofuels and bioproducts program.*

2017 Algae Testbed Public-Private Partnership Spring 2017 Workshop. Invited speaker and lab module instructor. *Algal flow cytometry.*

2017 New Mexico State Convention for the American Association of University Women. Invited banquet speaker. *LANL's biofuels and bioproducts program.*

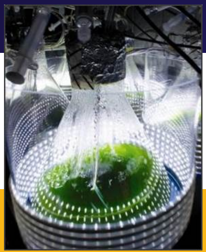
Previous Peer Review Comments/Responses

- This project has shown significant ability in being able to sort for lipid concentration and separately for productivity. The work they are doing to down-select strains from in-door experiments and have the results translate to outdoors performance addresses issues that the industry has experienced. However, I think that having a goal of biomass accumulation instead of growth rate can be very misleading due to potential growth lag times.
- The project is focused on important questions of strain improvement and “flask-to-farm” issues. Some of the stated accomplishments require considerably more validation. Future plans are quite ambitious and may require a narrower focus.
- Overall, this is sound project with clear and relevant objectives. Integration of this project with others helps to standardize results relevant to the field. Clear and successful transition of lab-based assessment to field-scale pilot studies show a sound approach and well-thought-out pipeline for bio-feedback and relevant results.
- Very interesting work on productivity improvement, but I would like to see work at a scale larger than 1,000 L ponds to demonstrate that productivity improvements would hold as you move up in scale, as the project has demonstrated in moving from the lab to the field.
- Work conducted in this project is highly relevant for BETO and should be particularly commended for working with production strains and considering applications of project outputs for algal production at scale. It is also effective at interactions with other national facilities and leveraging resources such as national testbeds. The overall technical approach would benefit from improving experimental design to increase confidence in the validity of strain improvements.
- This project addresses an important issue of utilizing advanced cell sorting and genetic tools to develop improved cell lines. If the project is able to overcome the transience of advanced phenotypes, the project has a strong platform to benchmark performance in a pipeline that spans the lab to the outdoor field conditions.

PI Response: Thanks to the reviewers for their thoughtful comments and general support of this work. We agree that ongoing validation is important for project success and aim to continue to do so. We agree that larger-scale data (greater than 1,000 L) would be relevant to collect. Should resources become available for such a task, we may pursue it; meanwhile, we will leverage similar work conducted at the testbed facilities and by our industry collaborators. Regarding validation of the improved strains and transience of phenotype, we have noted multi-year stability of improved phenotypes, in the lab for multiple strains and up to the 1,000 L scale for the one strain tested with that particular question in mind. We could have communicated this more clearly, as well as our onboarding of mutagenesis strategies to isolate even more stable phenotypes, which we did not have time to discuss but is ongoing. We take the reviewer’s point regarding productivity calculations and will be extensively characterizing the new improved-growth phenotypes to better understand the impact on relevant productivity measurements.

Cultivation Systems Comparison

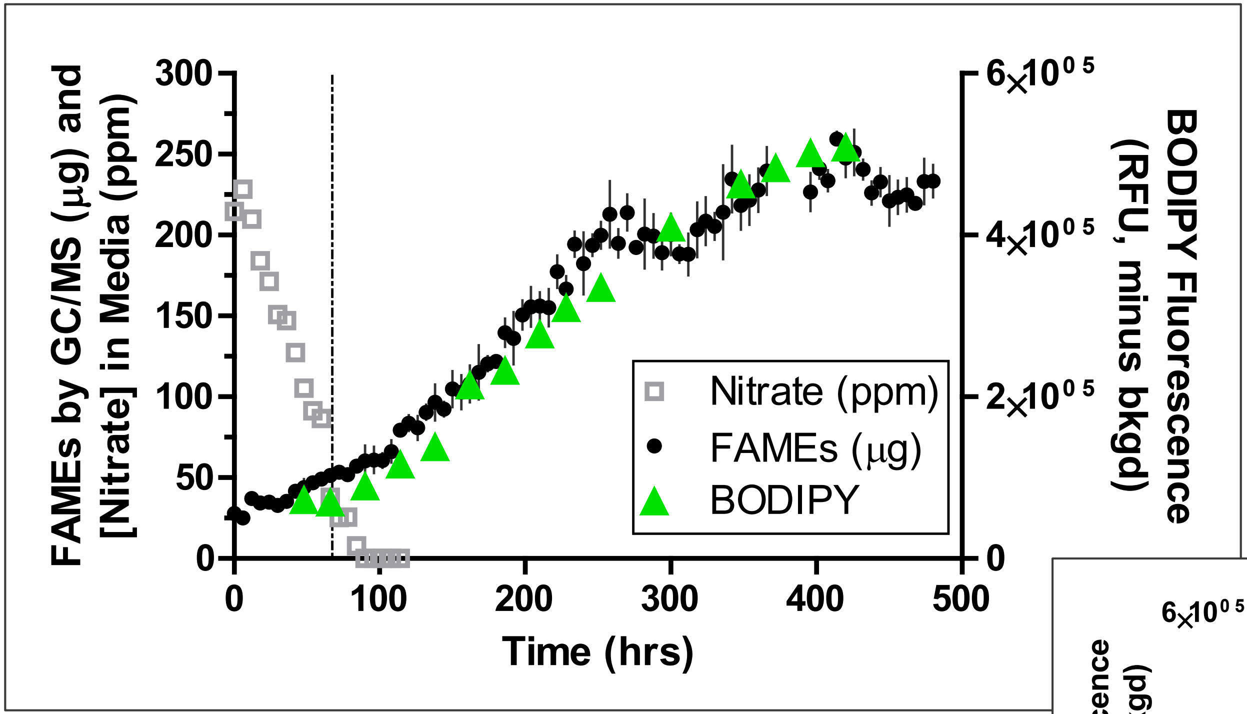
Indoor and Outdoor

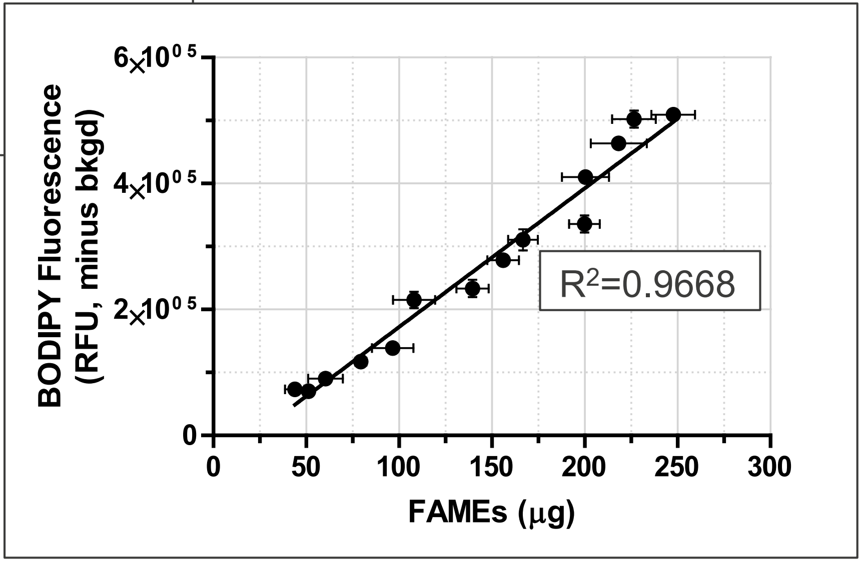


Variable	Lighted CO_2 Incubator	Spin Flask	Environmental Photobioreactors (ePBRs)	MicroBio Ponds	Outdoor Ponds (AzCATI)
System	Flasks (~30 ml)	Flasks (1-1.6L)	Cylinder ~0.6L	Raceway 50-100 L	Raceway 1000 L
Mixing	Shaker	Stir bar	Bubbled air & stir bar	Paddlewheel	Paddlewheel
Temp	Constant (25C)	Constant (25C)	Sinusoidal light/temp script	Greenhouse	Seasonal temps
Lights (μmo/m^2-s)	300, 16:8 on/off	800, 16:8 on/off	2000, script, sinusoidal	1200, sinusoidal with artificial add	Seasonal daylight
Media	HS (fresh), F/2 (marine)	HS (fresh), F/2 (marine)	HS (fresh), F/2 (marine)	HS (fresh), F/2 (marine)	HS or BG11 (fresh), F/2 (marine)
Operational Conditions	Batch, replete/ deplete, high [biomass]	Batch, replete/ deplete, high [biomass]	Batch or semi-continuous, replete/deplete, outdoor [biomass]	Batch or semi-continuous, replete/deplete, outdoor [biomass]	Batch or semi-continuous, replete/depl, outdoor [biomass]
Productivity	OD_{750} & cell counts time course, AFDW end point	OD_{750} & cell count time course, AFDW end point	OD_{750} & cell count time course, AFDW end, calculated slope g/m^2-day	$OD_{750,}$ cell count, AFDW time course, slope or harvested g/m^2-day	$OD_{750,}$ cell count, AFDW time course, slope or harvested g/m^2-day
Biochemical	End point	End point	Time course	Time course	Time course
CO_2 Delivery	Constant 1% CO_2	pH control by CO_2	pH control by CO_2	pH control by CO_2	pH control by CO_2

Fluorescence and Lipid Content Can be Correlated

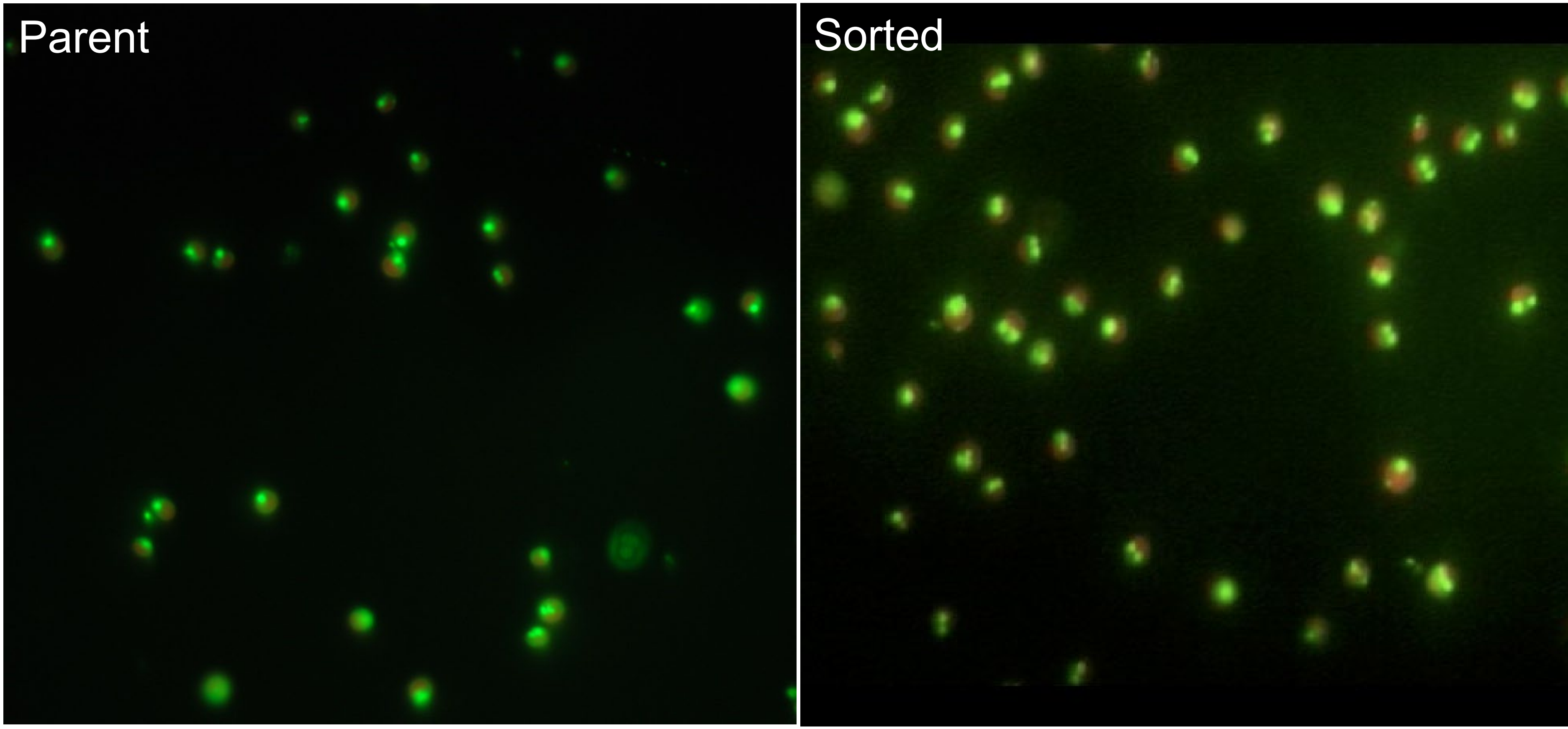
FAME and BODIPY data over time





Fluorescence Microscopy, Parent vs Sorted

100x images, BODIPY staining

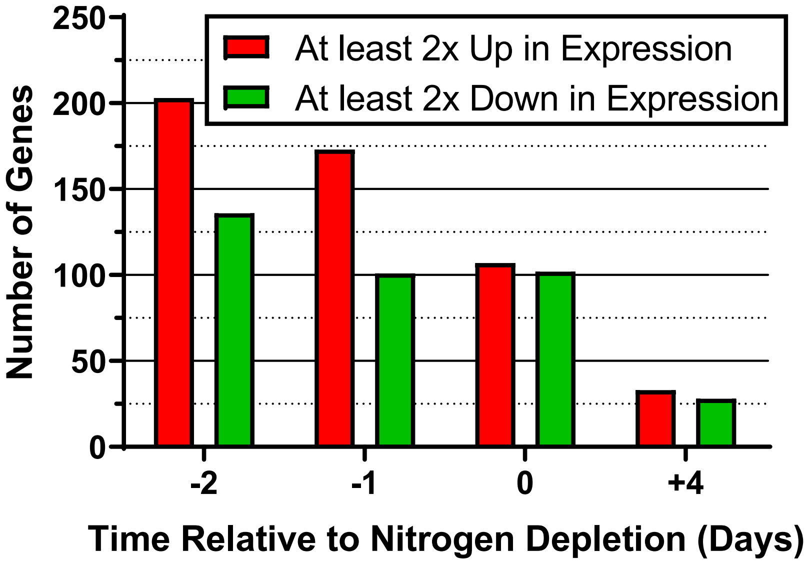


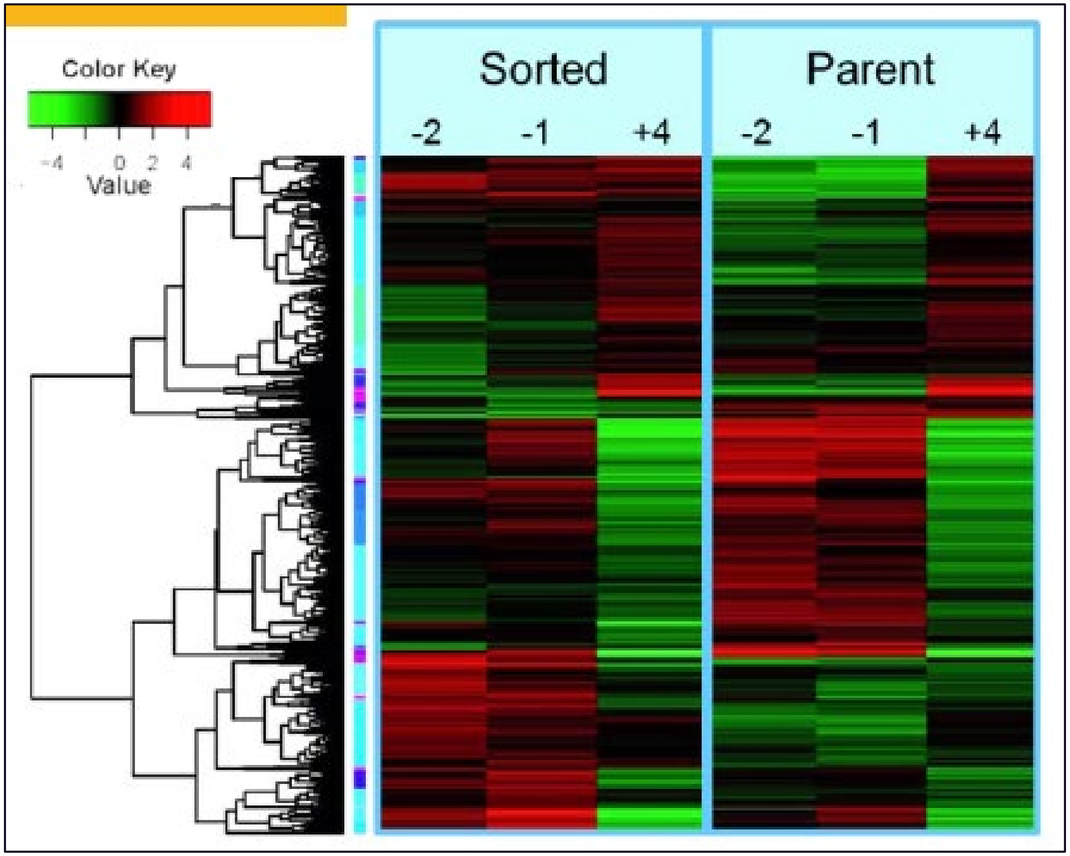
Background

Transcriptomes are different between Parent and Sorted populations

- Genome resequencing showed a few SNPs and InDels
- Transcriptomic analysis indicates changes in gene expression between Sorted and Parent

Maximal Difference in Gene Expression Observed on Day -2

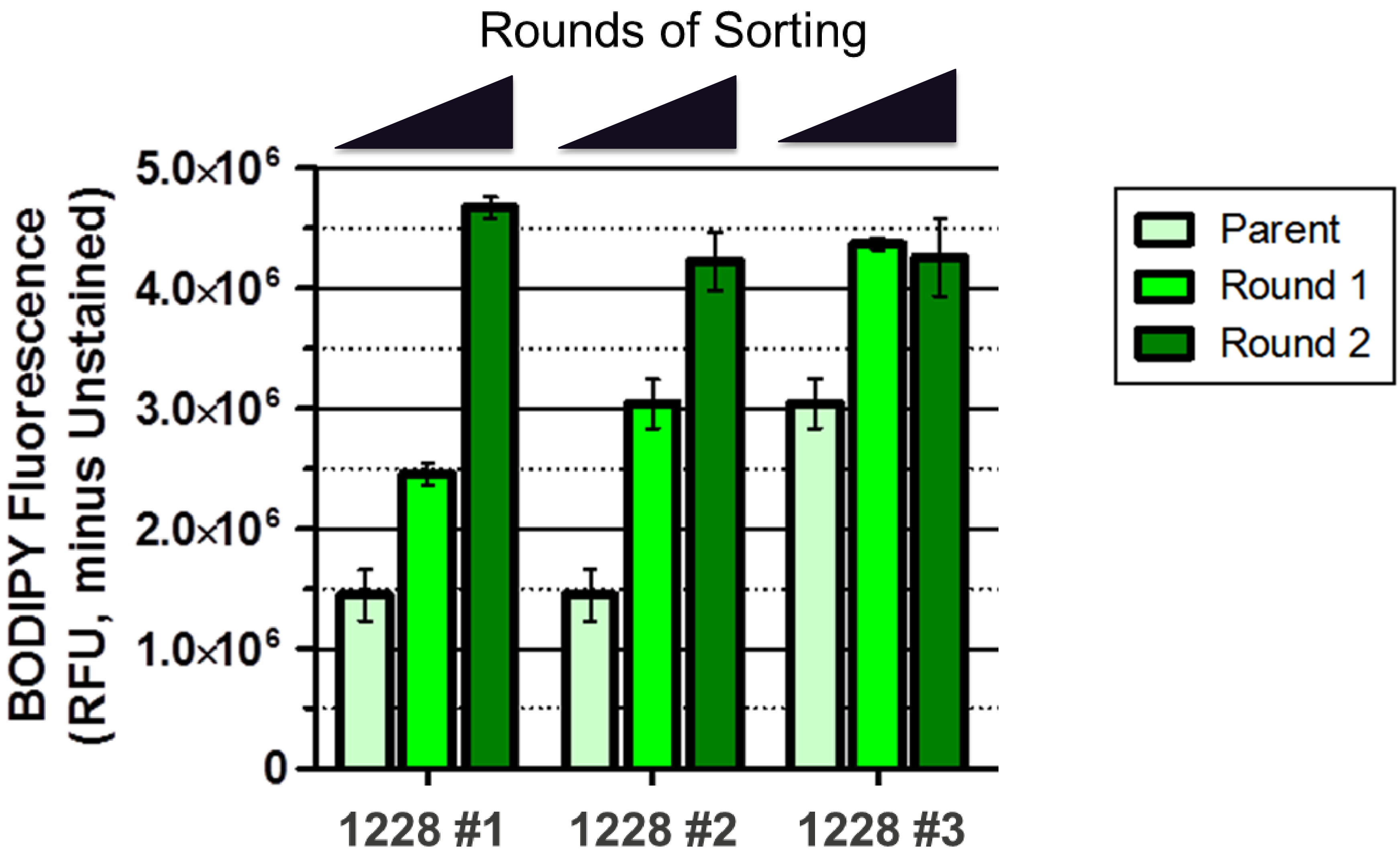




- Affected pathways included fatty acid synthesis, TCA cycle, starch and sucrose metabolism, and others

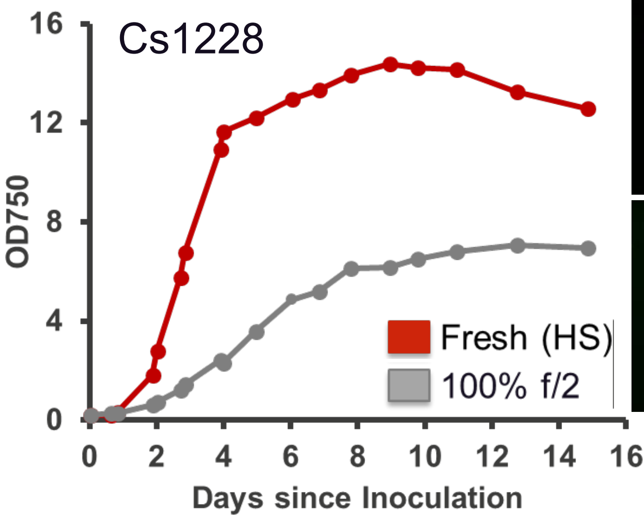
Increases in BODIPY with Rounds of Sorting

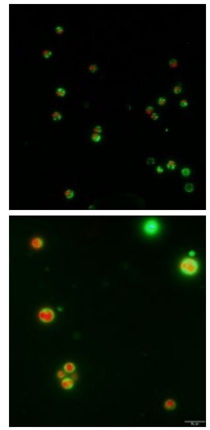
Several different populations achieved for Cs1228

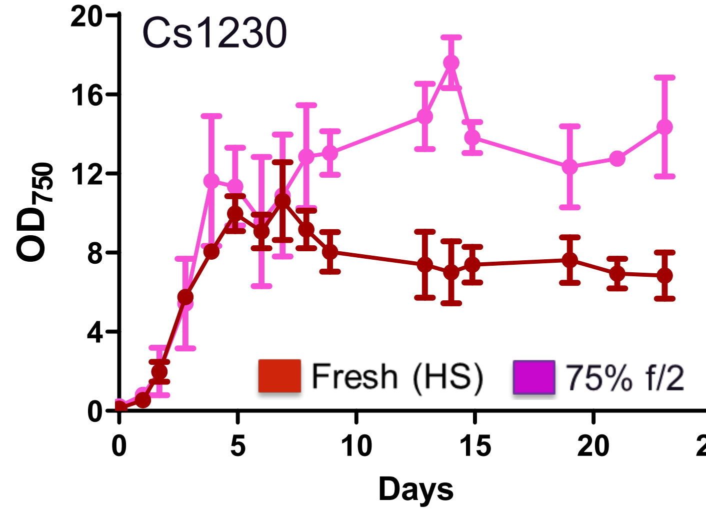


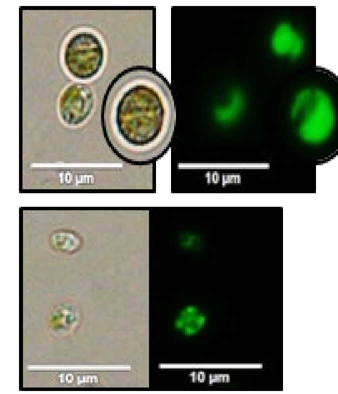
Examples Growth Curves of Salt Adapted *Chlorella*

Adapted cultures show higher FAME and altered cell morphology

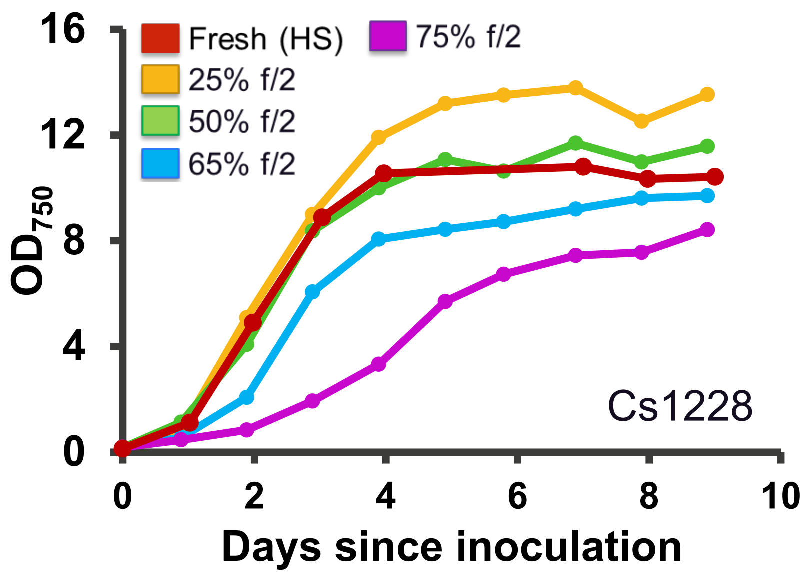


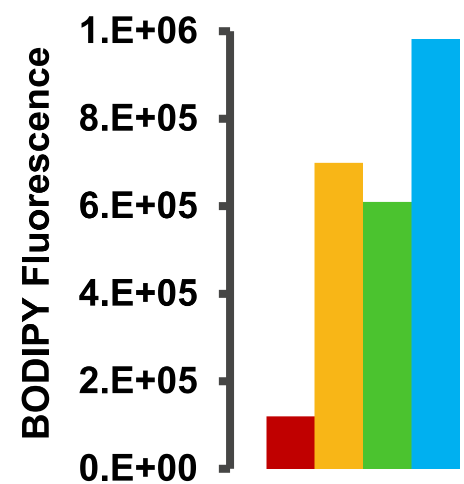






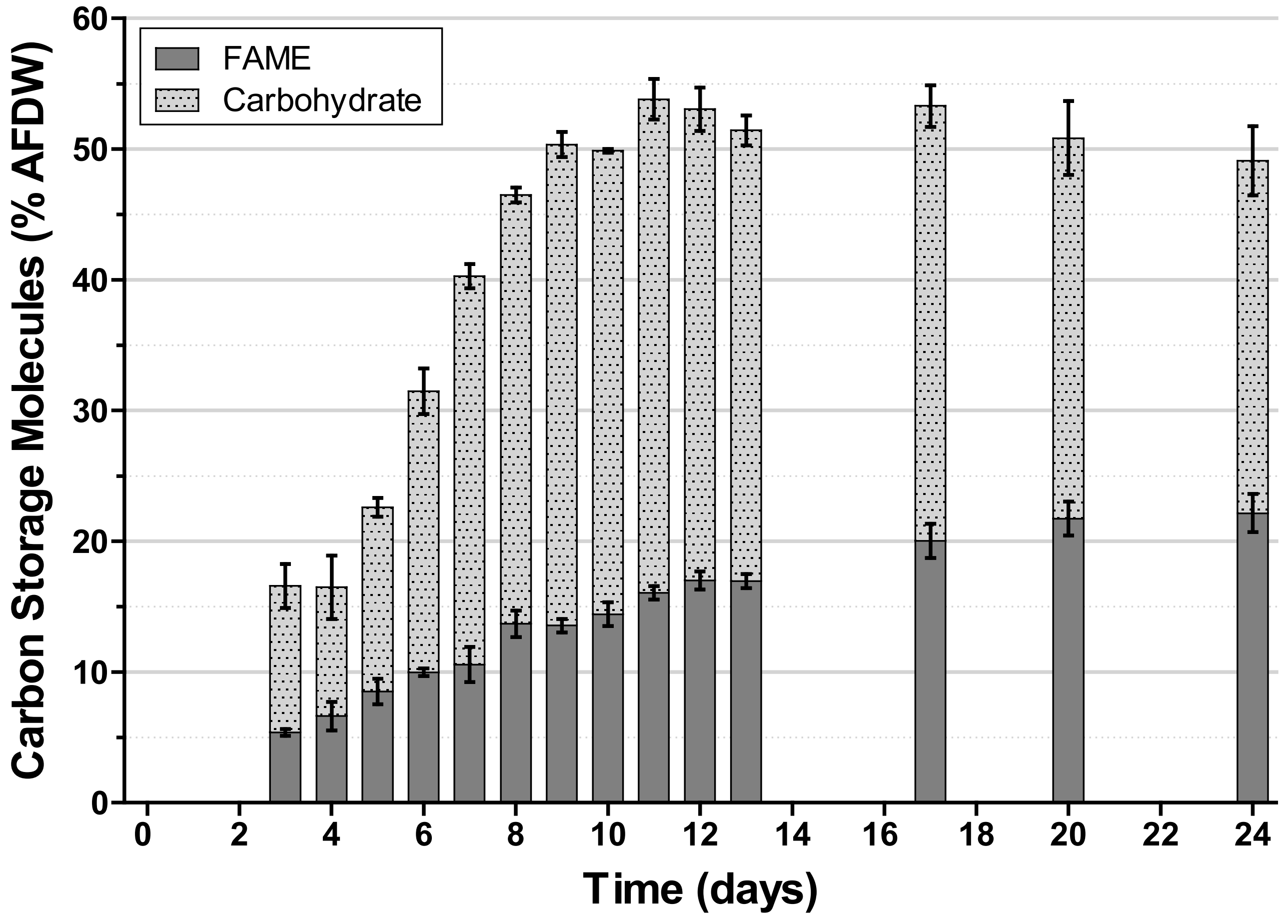
- Adapted cultures show higher FAMEs and larger cells by microscopy and flow cytometry.
- But some adapted cultures still don't grow very well
- For Cs1228 went back to brackish conditions for testing growth outdoors

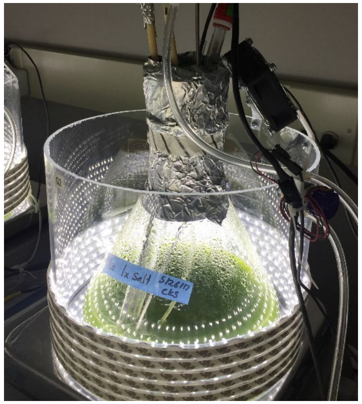




Carbon Storage in *Picochlorum soloecismus*

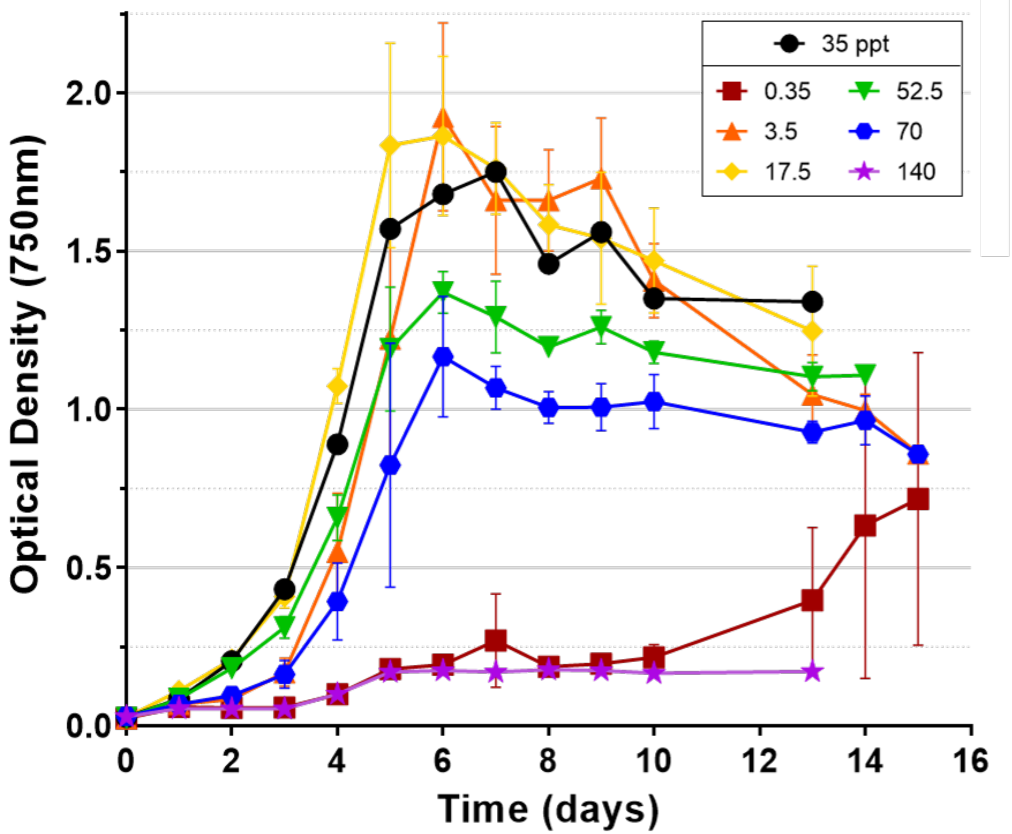
Carbon storage molecules make up 50% of dry biomass under N depletion

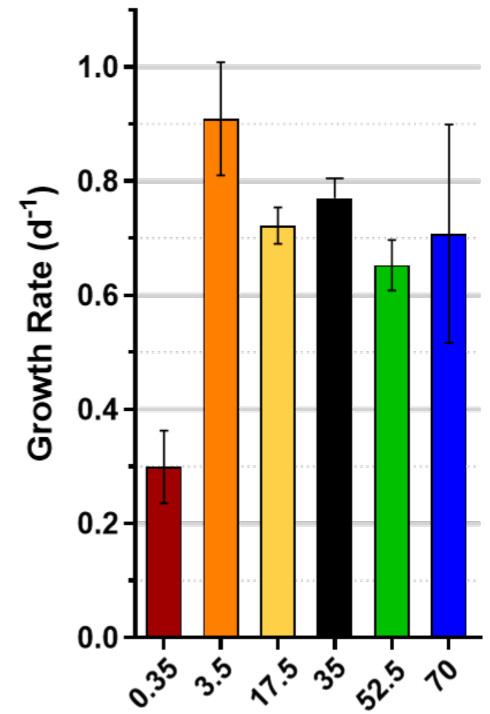




Salinity Tolerance of *Picochlorum soloecismus*

Pico is tolerant to a broad range of salinities

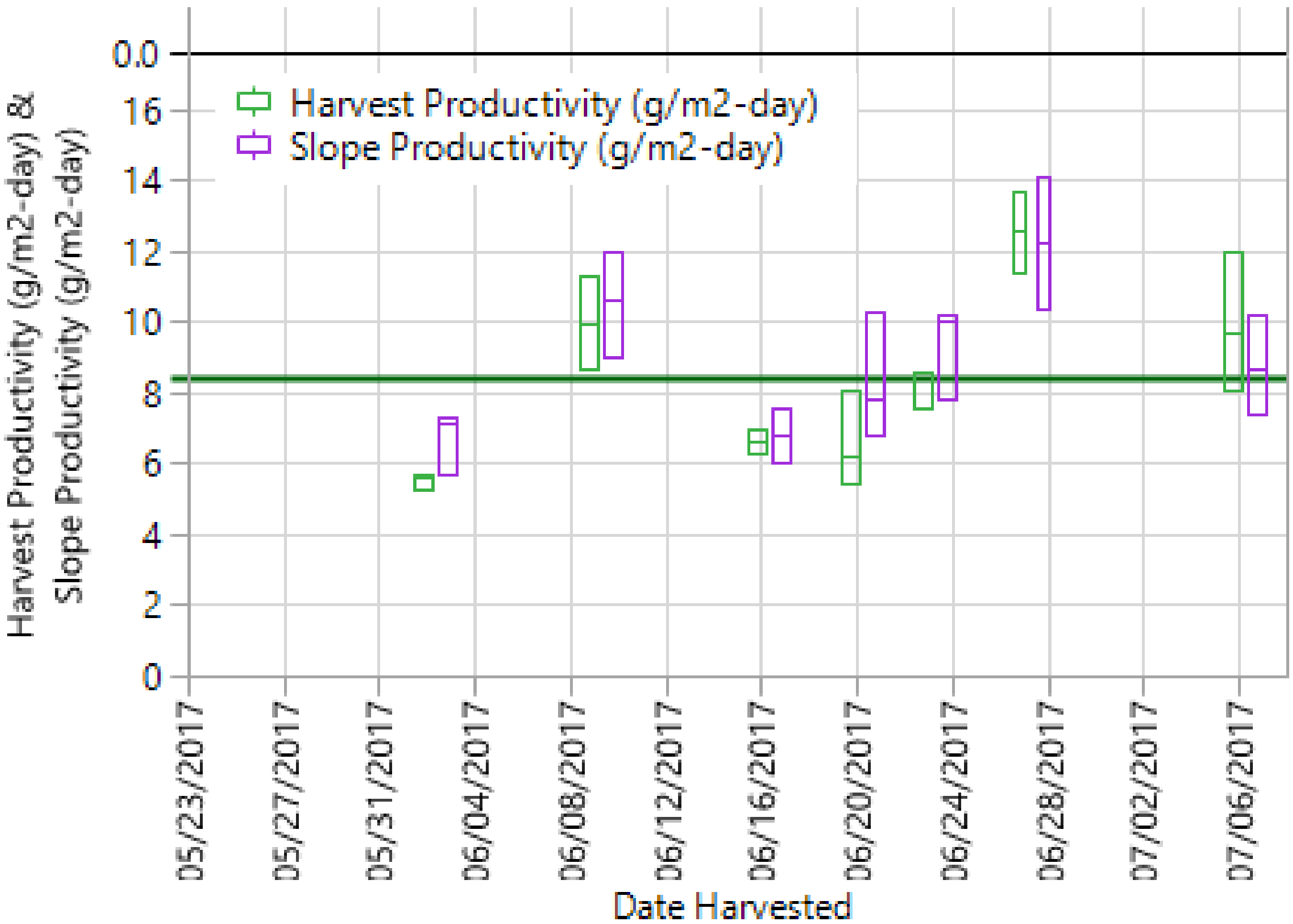


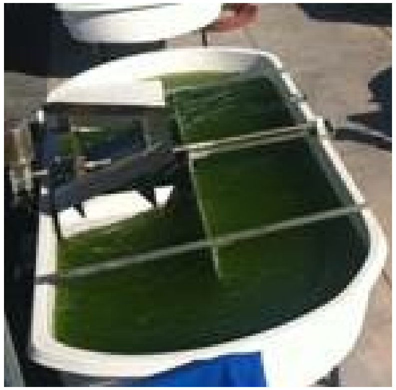


- **Second**: Characterize *P.s.* salinity tolerance
- **Result:** *P.s.* is very broadly tolerant, from 3.5 ppt to 70 ppt (double seawater salinity)
- *Ongoing*: Manuscript submitted on characterization, genome, transformation method

2017 Harvest Productivity for *P. soloecismus*

Picochlorum *grows slowly but did not crash when other strains were crashing*





Harvest productivity

Summary Statistics	
Mean	8.3789474
Std Dev	2.453247
Std Err Mean	0.5628135
Upper 95% Mean	9.5613746
Lower 95% Mean	7.1965201
N	19

Brightfield Microscopy, WT Pico vs GM Pico

100x images, Lugol's staining

A Wildtype
B GOI-OE #3
C GOI-OE #11
D GOI-OE #12