DOE Bioenergy Technologies Office (BETO)
2023 Project Peer Review

Optimizing **S**election **Pre**ssures & Pest Management to Maximize Algal Biomass **Y**ield (OSPREY)

4/3/23
Advanced Algal Systems

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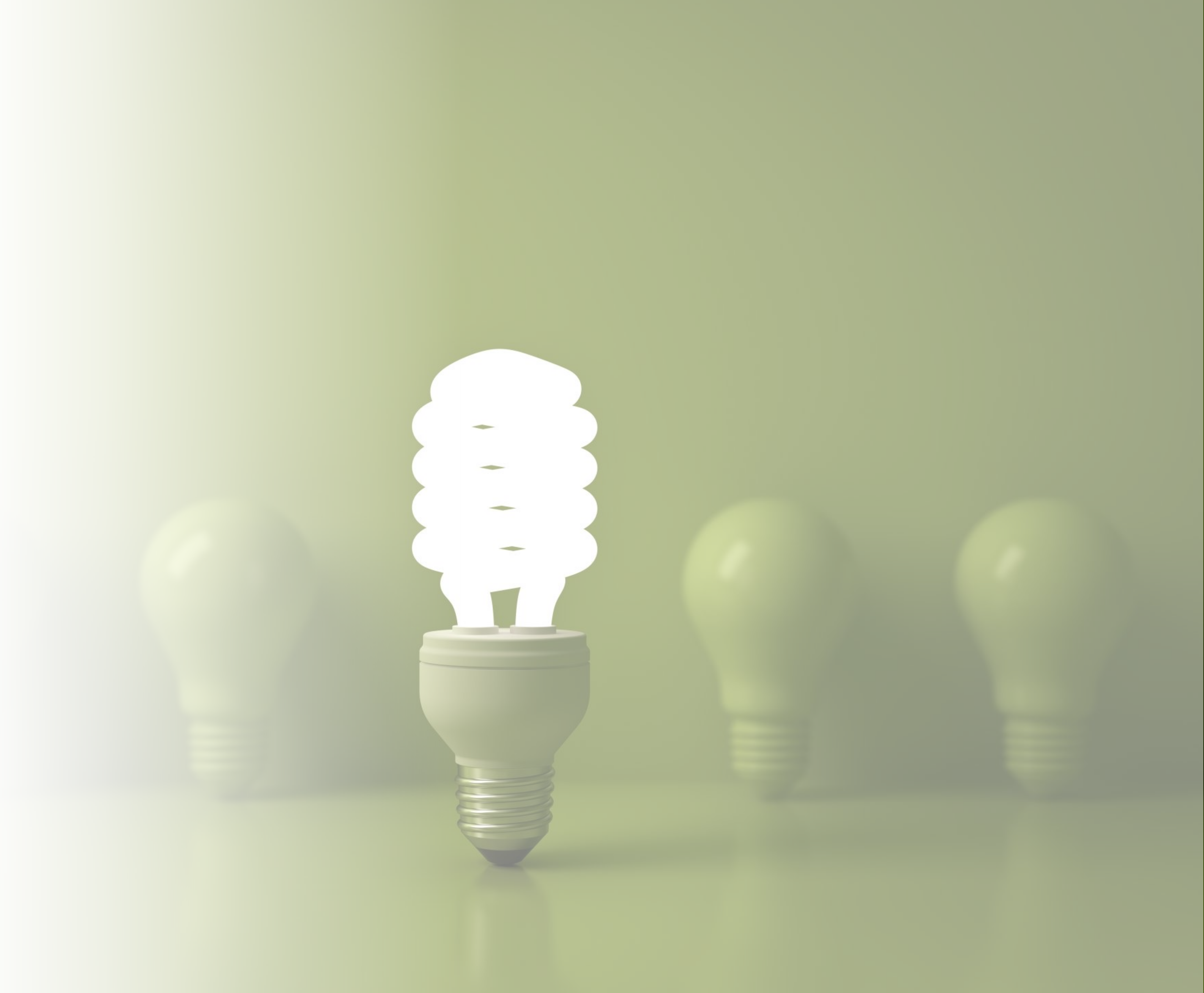
New Mexico Consortium

Project Overview



Trait Drift & Evolution

- Algal industries challenged by poor translatability of lab-scale R&D to commercial systems
- Does the disconnect stem from:
  - a lab-centric approach (lab cultivars, lab-to-field pipeline)?
  - different selection pressures across environments?
- Can we use different selection pressures across sites to:

  (1) generate new strains?

  (2) optimize cultivation practices with information on trait drift and evolution?

We start with a field-adapted industry strain and use field-lab-field iterations.

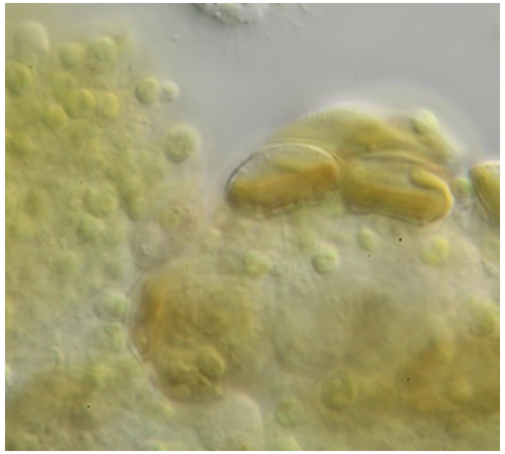
Selection Pressures in the Lab and Field

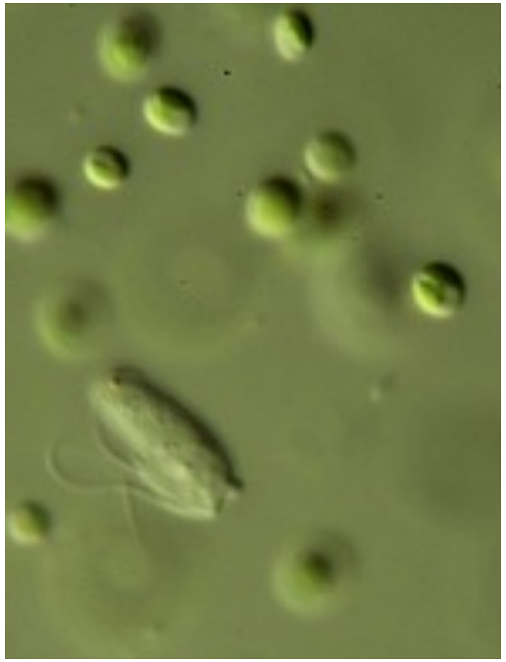
	Lab	Field
Positive pressures (increases desired traits)	NONE	Variable temperature, pests, commercial grade medium, natural sunlight
Negative Pressures (decrease desired traits)	controlled temperature, no pests, permissive growth medium, poor light quality, low light	Frequent harvest, chemical treatment

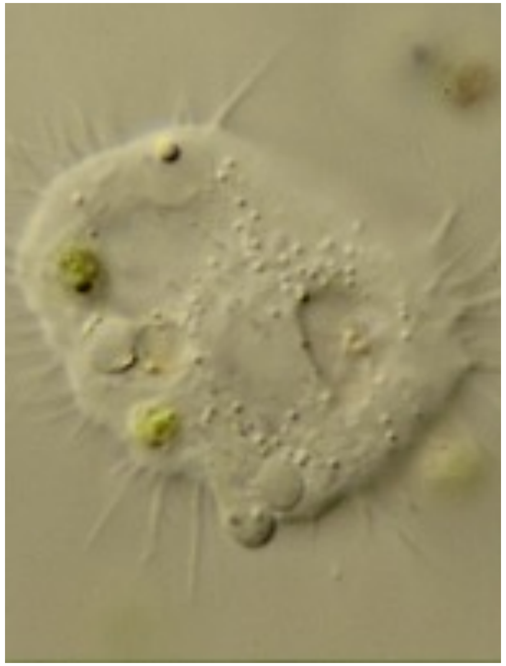
Pest Management

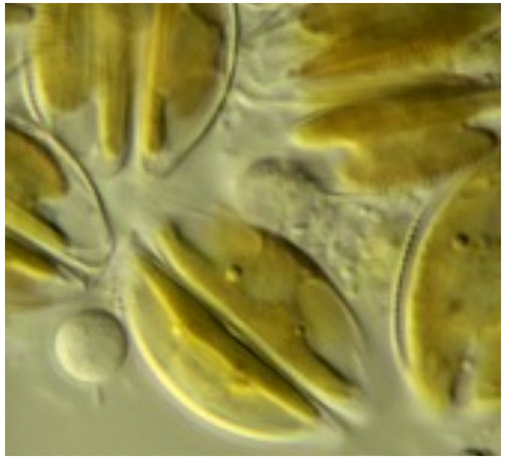
- Pests can be responsible for considerable losses to crops at the commercial scale
- Quick, low-cost pest detection technologies are greatly needed at algal farms
- Can we use new genomic approaches to:
  - (1) detect pests responsible for crashes and periods of low productivity across sites?
  - (2) develop in-field detection technologies?

We use a combination of 16S sequencing and Proximeta® Metagenome Deconvolution/shotgun sequencing to detect pests and develop qPCR tools.





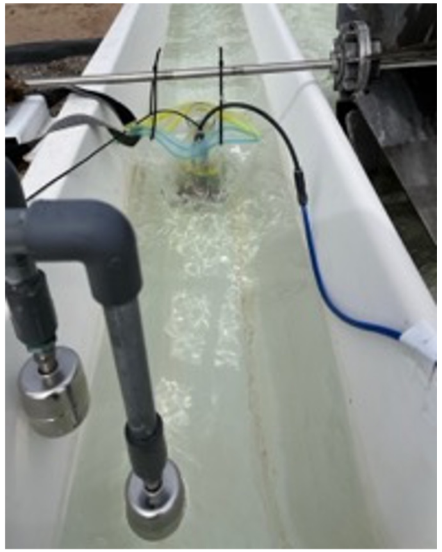


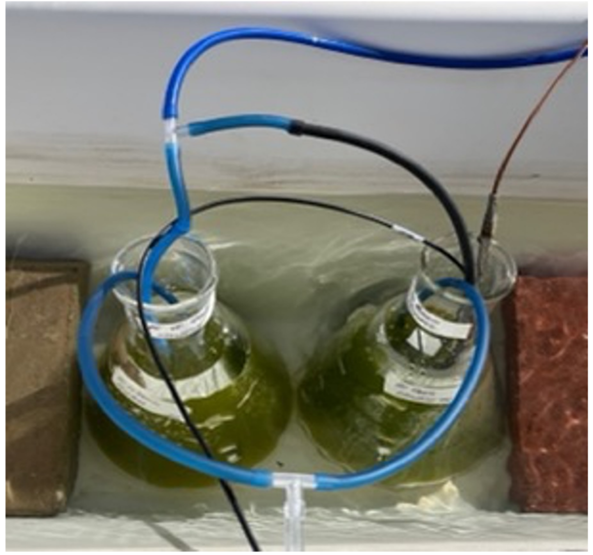


Strain Improvement

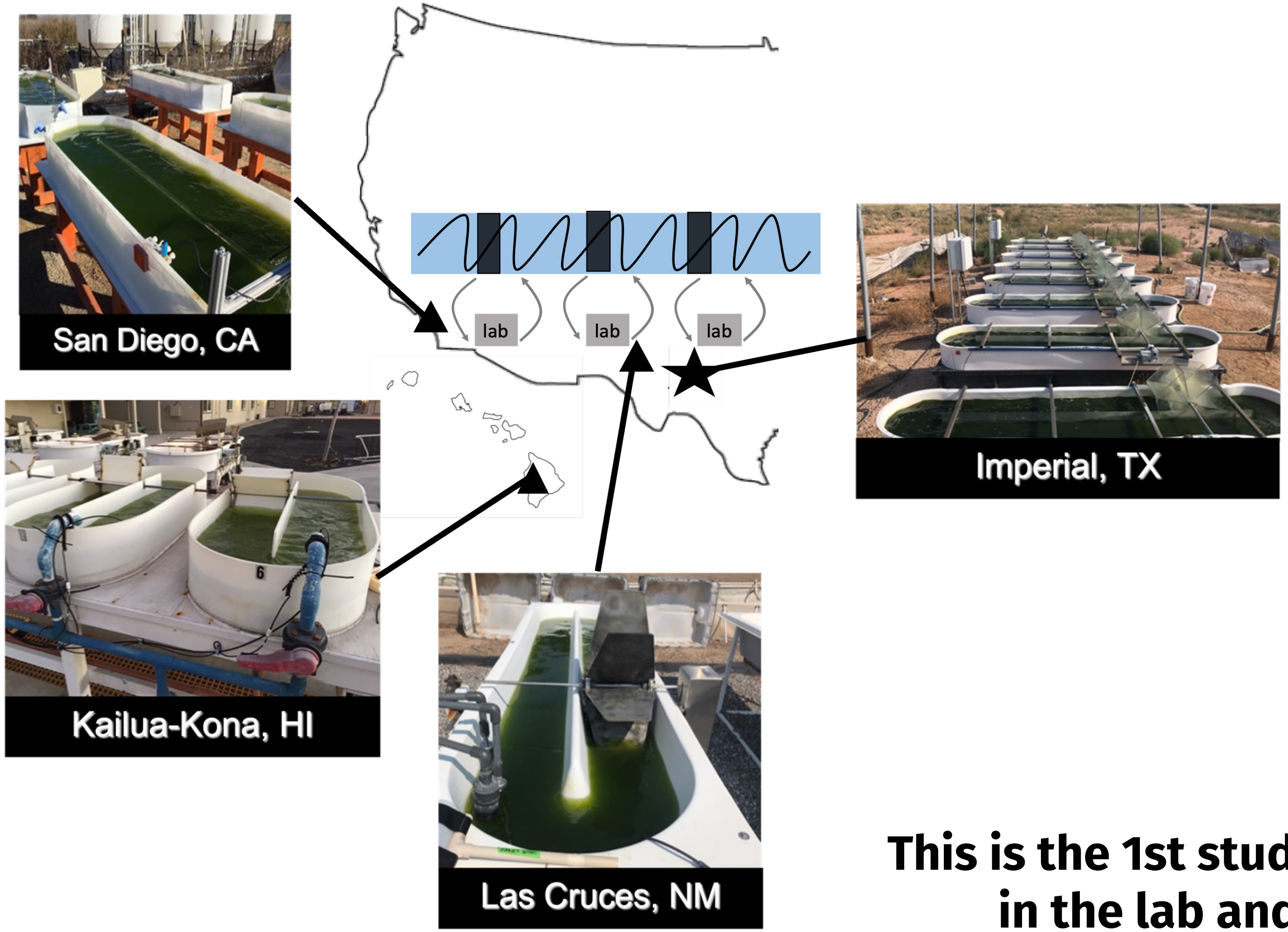
- Strains are often developed/improved in the lab only to fail at the transition to field cultivation
- Can we apply traditional methods to a field-adapted strain to improve:
  (1) productivity?
  (2) robustness?
  (3) biomass composition?
- Can we move traditional approaches to improve strains (e.g., UV and EMS mutagenesis) out into the field?

To our knowledge, our study is the first to deploy “in field mutagenesis”.





Project Objectives



We aim to:

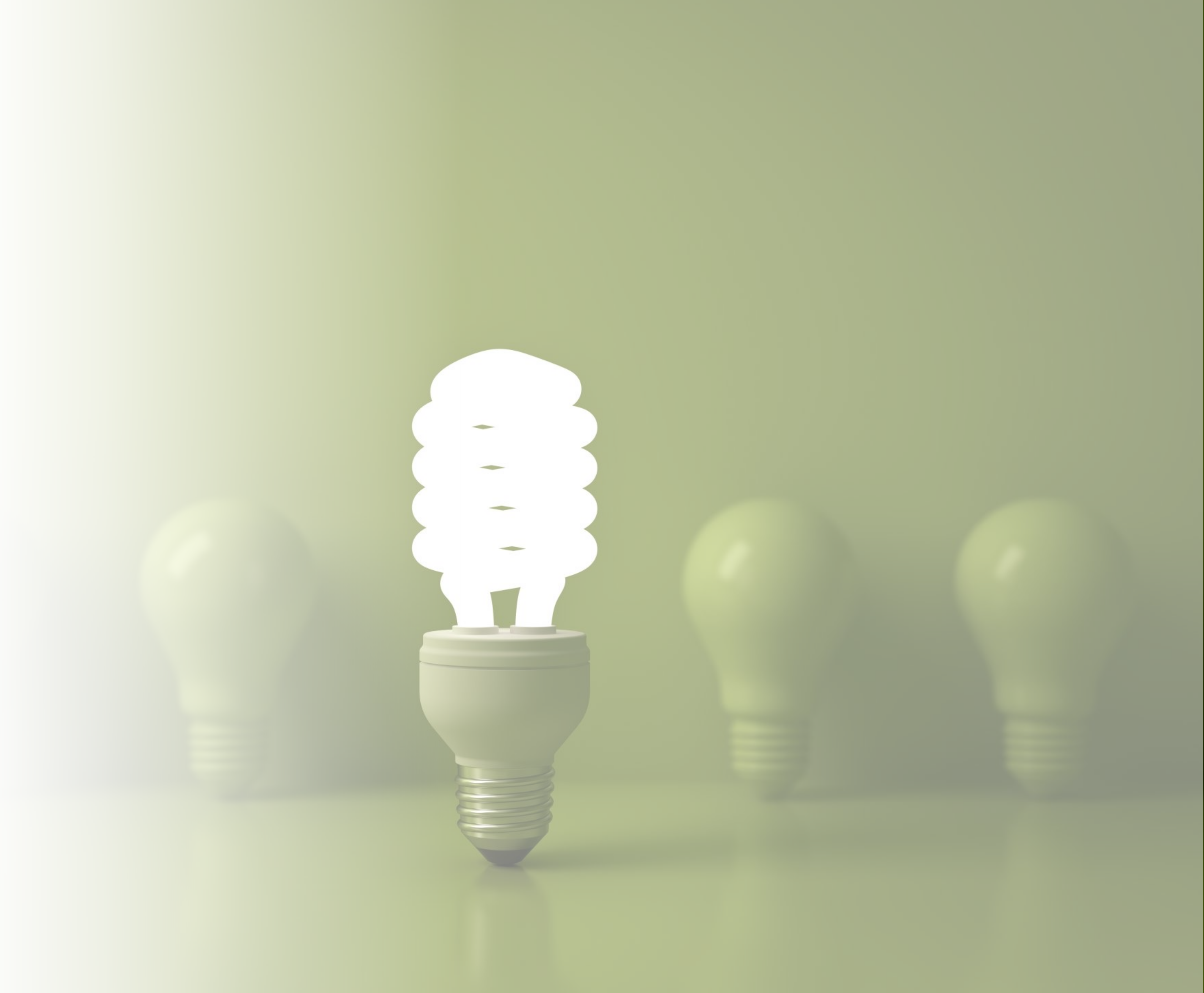
1. Quantify algal trait drift and evolution in the lab and field
2. Isolate new cultivars from field sites
3. Identify new cultivation practices based on trait drift and evolution data
4. Enhance pest monitoring and management
5. Improve productivity, robustness, and biomass composition via mutagenesis and selection

This is the 1st study to quantify algal trait drift and evolution in the lab and field across multiple sites and years.

Fit to DOE BETO Goals and DE-FOA-0002029 AO1

- AO1 objective: by overcoming the challenge in translating results between lab and mass cultures, projects will increase yield, robustness, and quality of cultivation for biofuels and bioproducts
- AO1 areas of interest
  - Indoor/outdoor experimental framework (see Additional Slides)
  - Tools for monitoring cultivation health (fieldable pest-tracking kits, metagenomic database)
  - Improvement of stability and reproducibility (aimed for 50% improvement in harvest & robustness, 20% improvement in conversion yield)

Approach



Long-Term Cultivation

- Isolation of field-adapted industrial production strain
  - Four field sites
  - Three labs
    - Three maintenance modes (batch culture, continuous culture in eBPRs, storage on plates)

Main Project Tasks

- Quantification of algal trait drift and evolution in the lab and field
- Isolation of new cultivars from field sites
- Identification of new cultivation practices based on trait drift and evolution data
- Enhancement of pest monitoring and management
- Improvement of productivity and biomass composition through mutagenesis and selection

Evaluation

- Lab Experiments
- Field Trials
- TEA/LCA work

Long-Term Cultivation

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- **Isolation of new cultivars from field sites**
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Evaluation

- Lab Experiments
- Field Trials

Challenge #1
There is limited trait drift in the field, precluding capture of new field-adapted cultivars and/or identification of new cultivation practices.

Challenge #2
Lab-scale results will not translate to the field.

spoiler alert: selection pressures not strong enough/period not long enough to evolve traits of interest; following BP3 Interim Verification, project scope in last 9 mo changed to remove certain tasks

Long-Term Cultivation

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Go/No-Go Points and Evaluation Metrics

- GNG 1: Technical & Cultivation Readiness Validated (met at end of BP1)
  - Important to show feasibility of and readiness to do work

- GNG 2: Comparison of a mutant and baseline strain; deployment of the beta version of the qPCR tool at the scale of 260L ponds in triplicate at one of the field sites (met at end of BP2)
  - Important to test translatability of the lab-improved field strain and to inform future development of the qPCR tool

- GNG 3: Three lab-to-field iterations that incorporate at least two of the following: trait stack, qPCR tool deployment, and process improvements based on trait drift in the lab and field to reach improvements in productivity, robustness, and biomass composition compared to the baseline *Nannochloropsis* strain (met at end of BP3)
  - Important because it incorporates work from all tasks and requires field-lab-field iterations

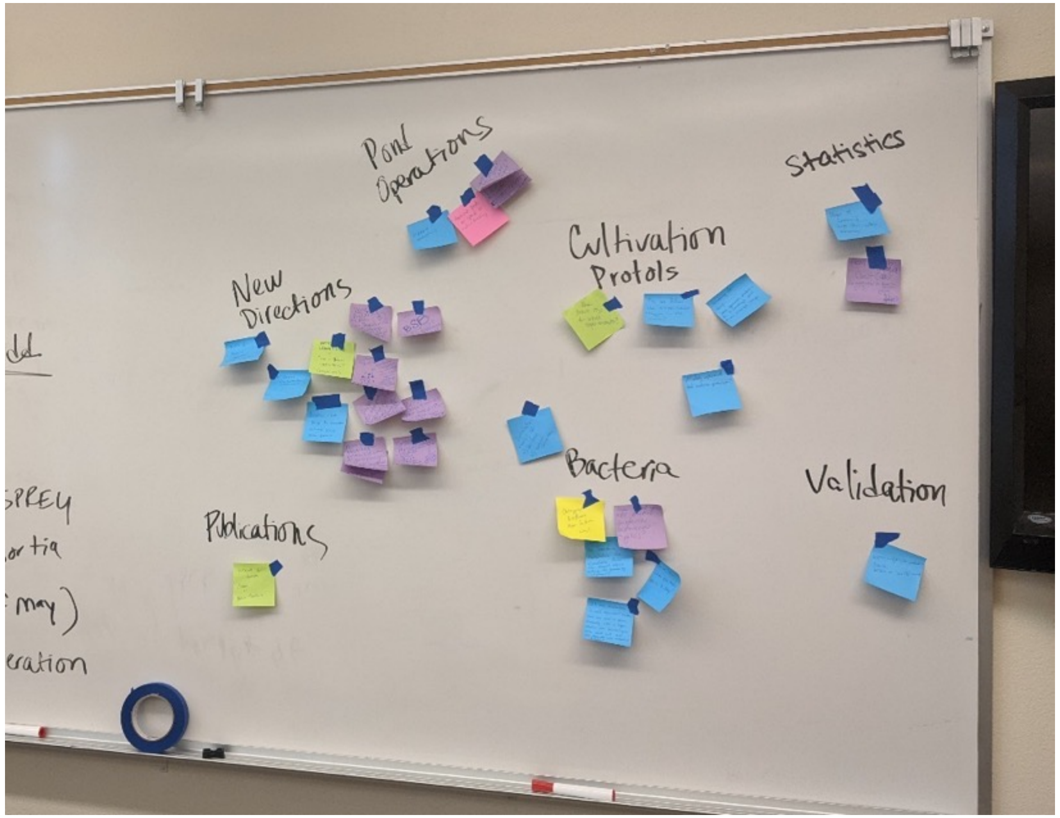
End of Project Goal (revised at Interim Verification): *Synthesize cross-site field data to understand the relative importance of strain adaptation and local drivers on KPPs (productivity, biomass competition), and quantify improvements from deploying qPCR tools across industrial field sites*

Risk Mitigation Analysis and Strategies

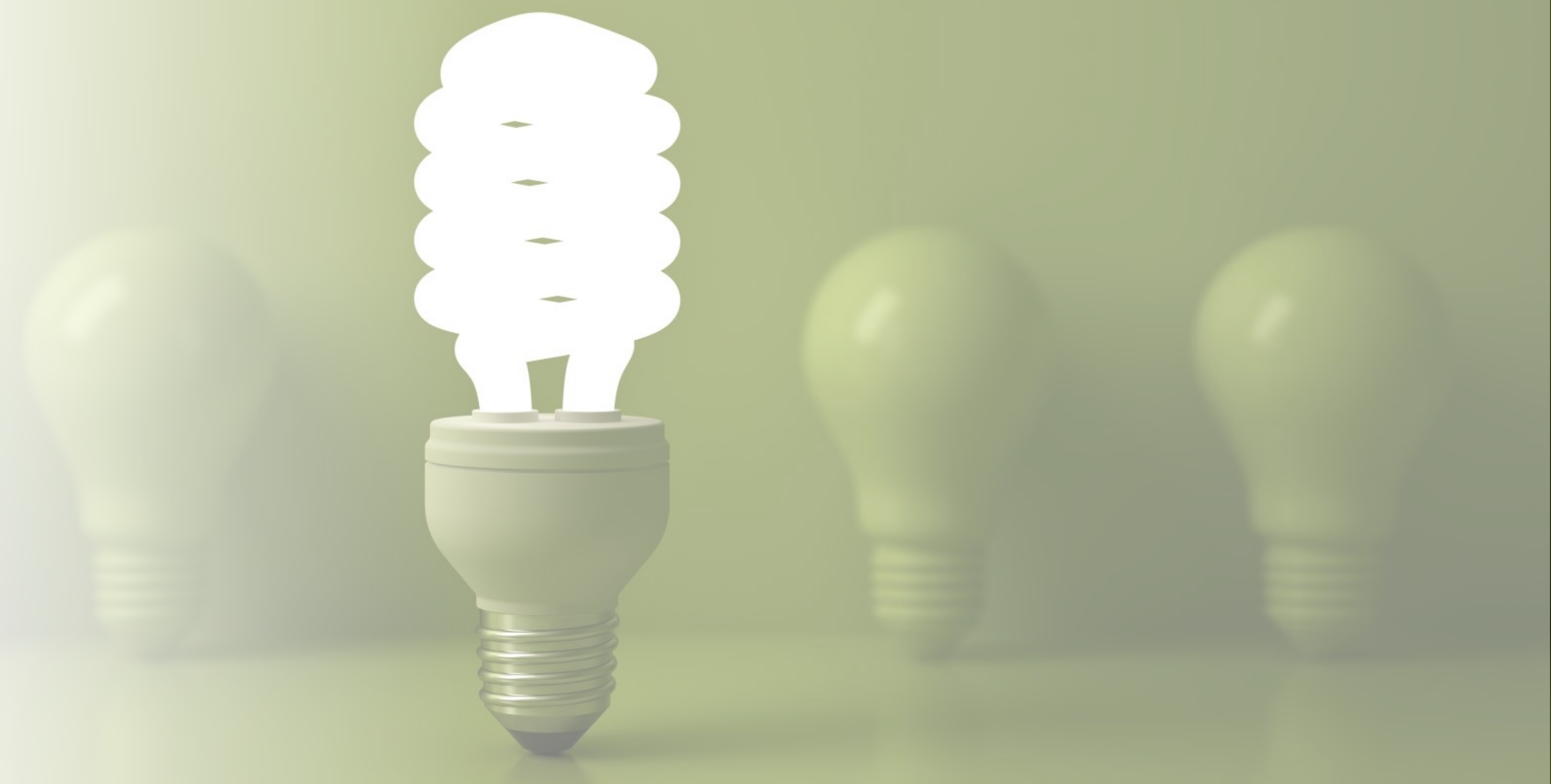
Description	Risk	Impact	Risk Mitigation and Response Strategies
Technical Risks			
Ponds do not crash due to crop protection practices	High	Low	Analyze periods of low productivity, rather than wholesale crashes.
Field strains are lost due to extreme events	Medium	Low	Plate and cryopreserve field cultures every 2 weeks. Re-inoculate ponds from backup field cultures if needed.
Lab strains are lost due to equipment malfunctions	Medium	Low	Plate and cryopreserve lab cultures every 2 weeks. Re-inoculate cultures from backup lab cultures.
Evolution or mutagenesis does not change traits	Medium	Medium	Implement more stringent selection criteria in the lab. Test in-field mutagenesis and selection.
Resource Risks			
Equipment downtime	Medium	Low	Rely on duplicate/shared equipment within and across sites.
Management Risks			
Personnel turnover	Low	Medium	Cross-train personnel.

Project Management

- SOPO, Gantt Chart, Milestones, GNGs, KPPs serve as guidelines
- Frequent communication across team
  - initial PI kickoff
  - task workgroups
  - twice-monthly lead PI calls
  - twice-monthly team calls to share data, evaluate progress on tasks, discuss mitigation strategies
- Project monitoring (calls with DOE every ~6 weeks, quarterly reports, interim verification)

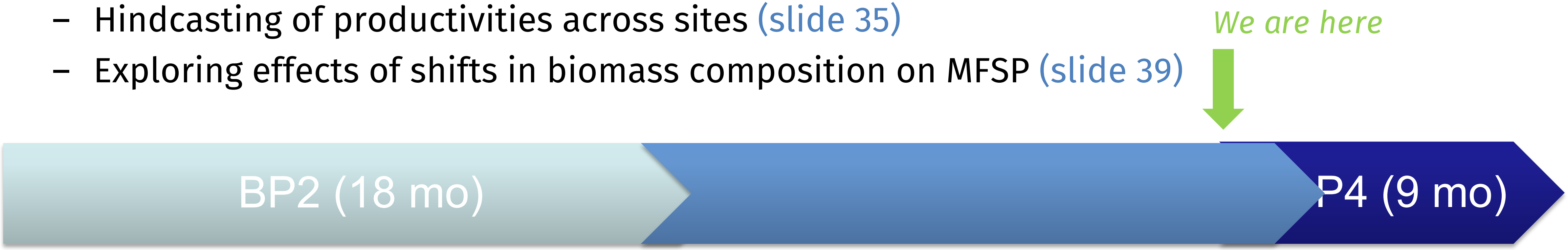


Progress & Outcomes

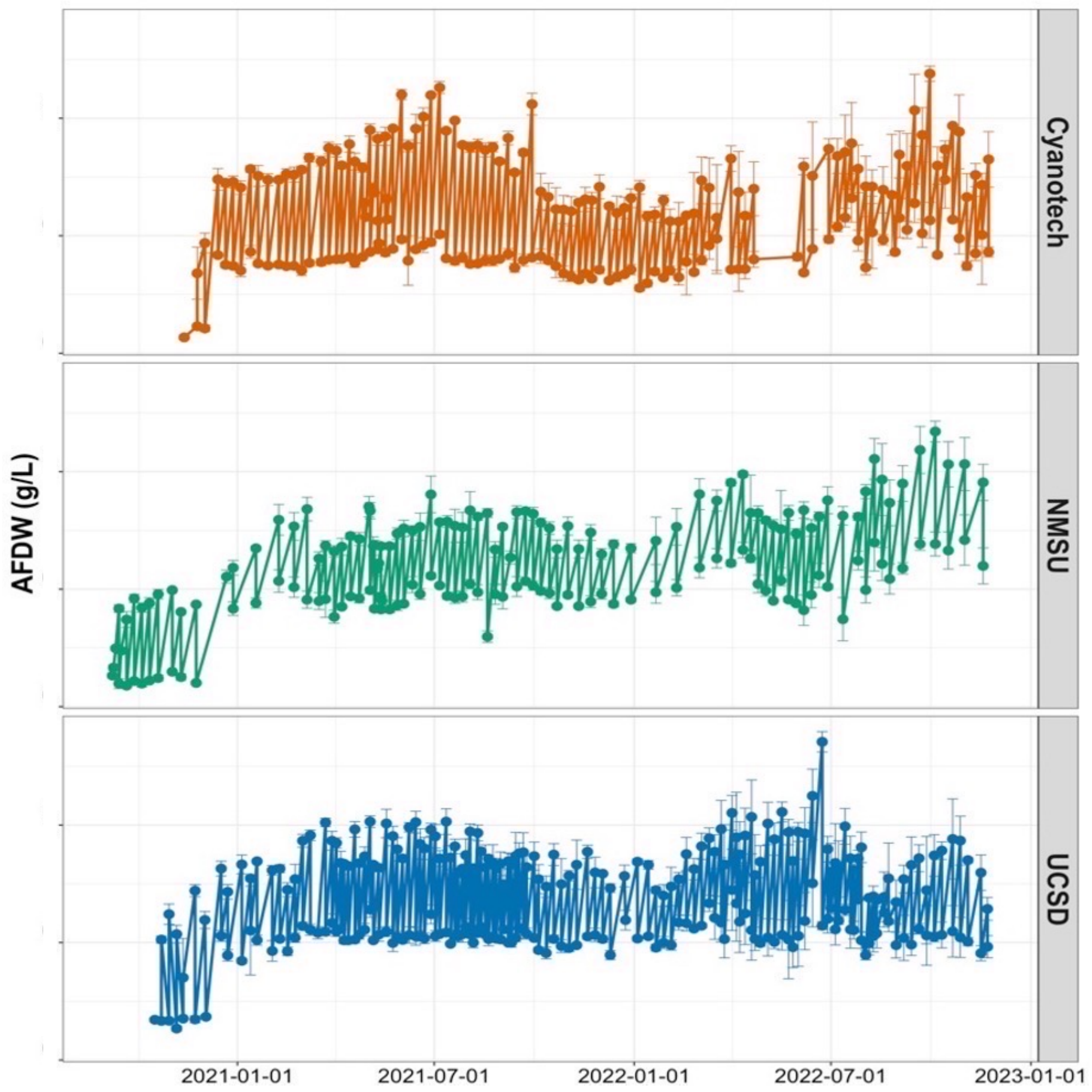


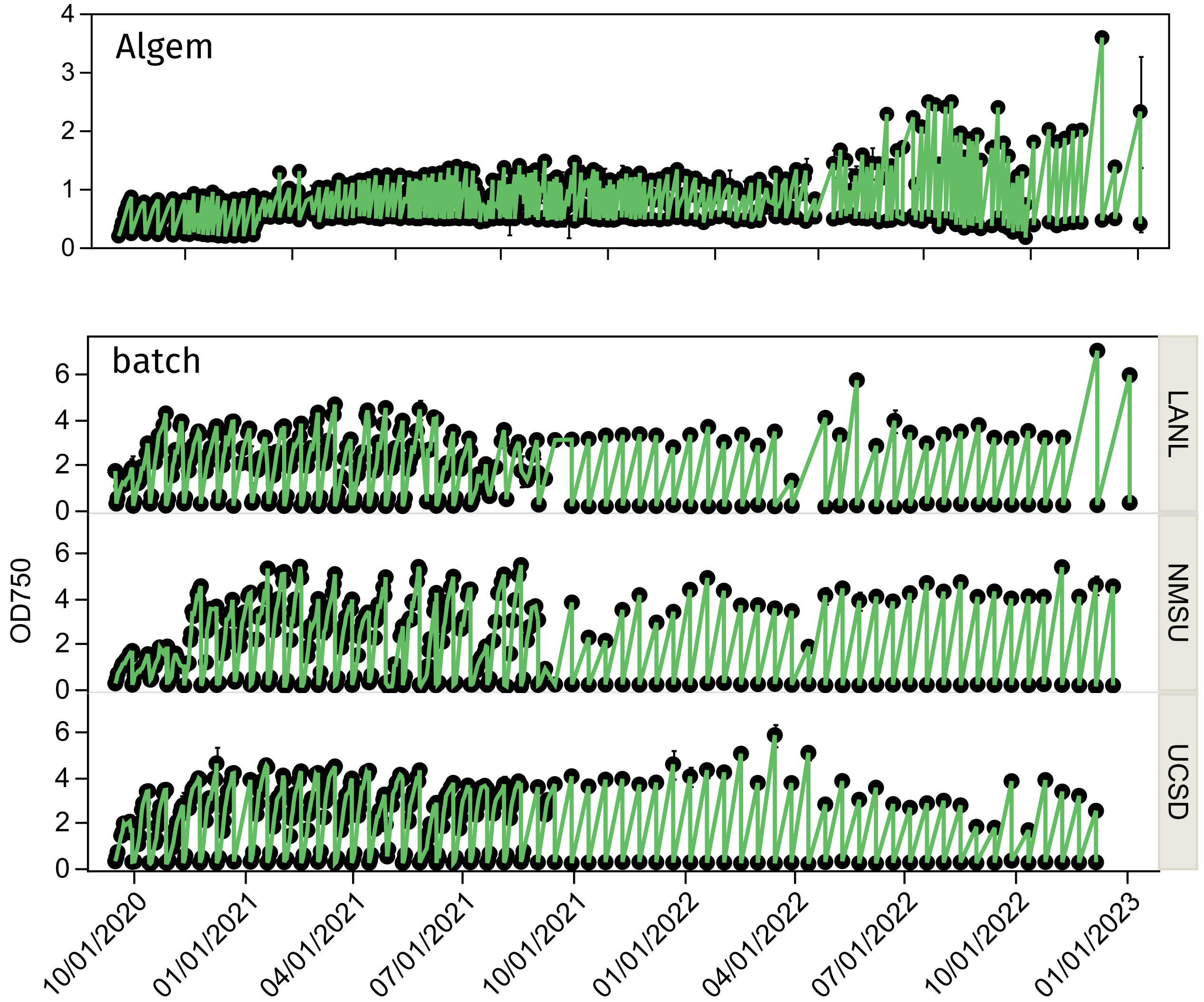
Overview of Progress

- Since Summer/Fall 2020, cultivation of the field-adapted strain at all new field sites and labs without crashes (QH maintained original population) (slide 17)
- Phenotypic and genotypic trait tracking every 6 months (slides 18-20)
- Advanced pest detection and tracking
  - Collected rich 16S dataset, weekly sampling at all field sites (e.g., slide 21)
  - Compared qPCR platforms (cost, ease of use) to identify field-deployable platform (slide 22, 37)
  - Identified novel pests (e.g., slide 21)
  - Used Proximeta® Metagenome Deconvolution/shotgun to inform primer design (e.g., slide 21)
- Ran mutagenesis and laboratory evolution to improve field-adapted strain (slides 23-24)
- TEA/LCA
  - Hindcasting of productivities across sites (slide 35)
  - Exploring effects of shifts in biomass composition on MFSP (slide 39)

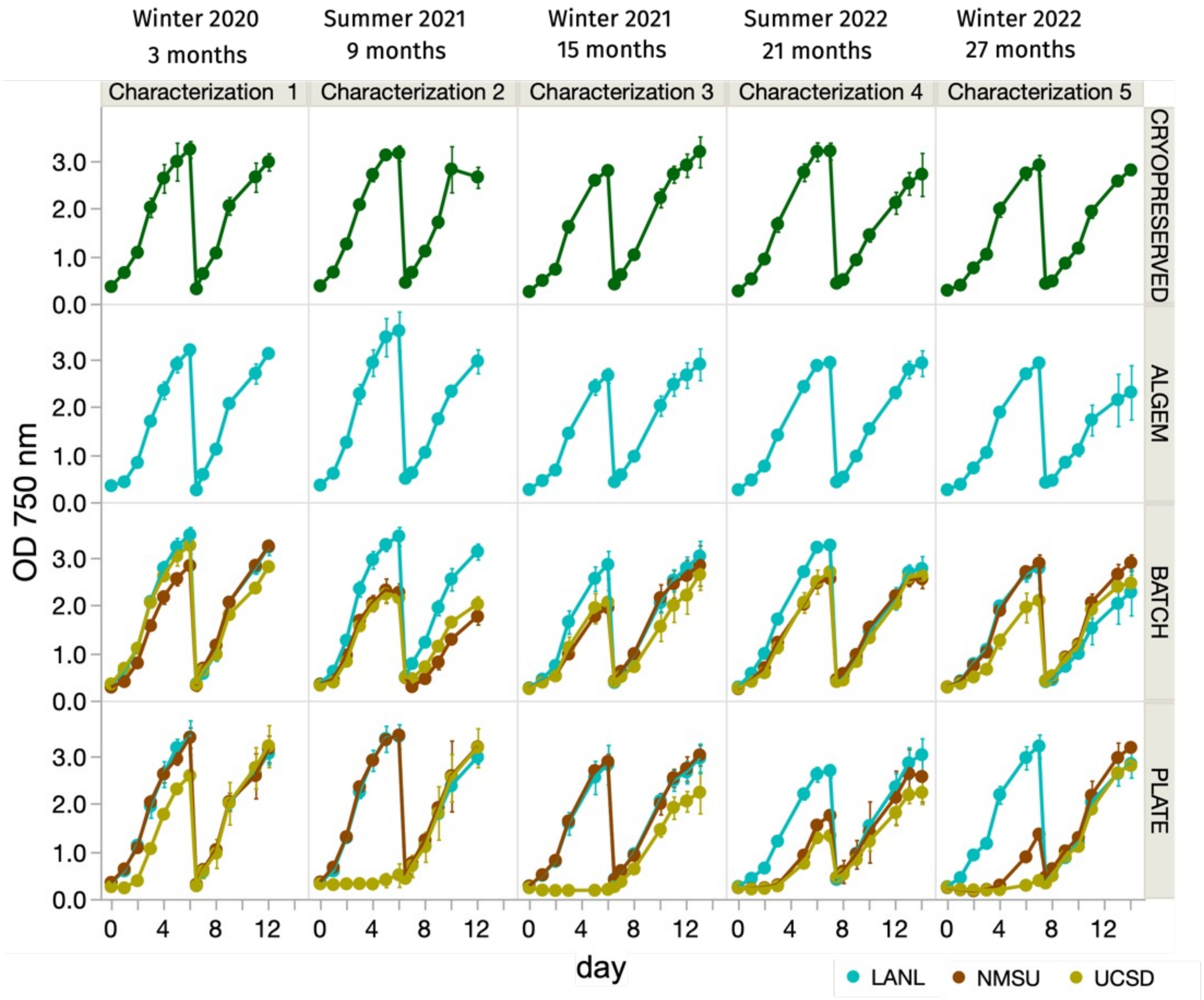


Cultivation Across Different Field Sites, Culture Modes





Cyropreserved and Bioreactor Cultures Most Stable

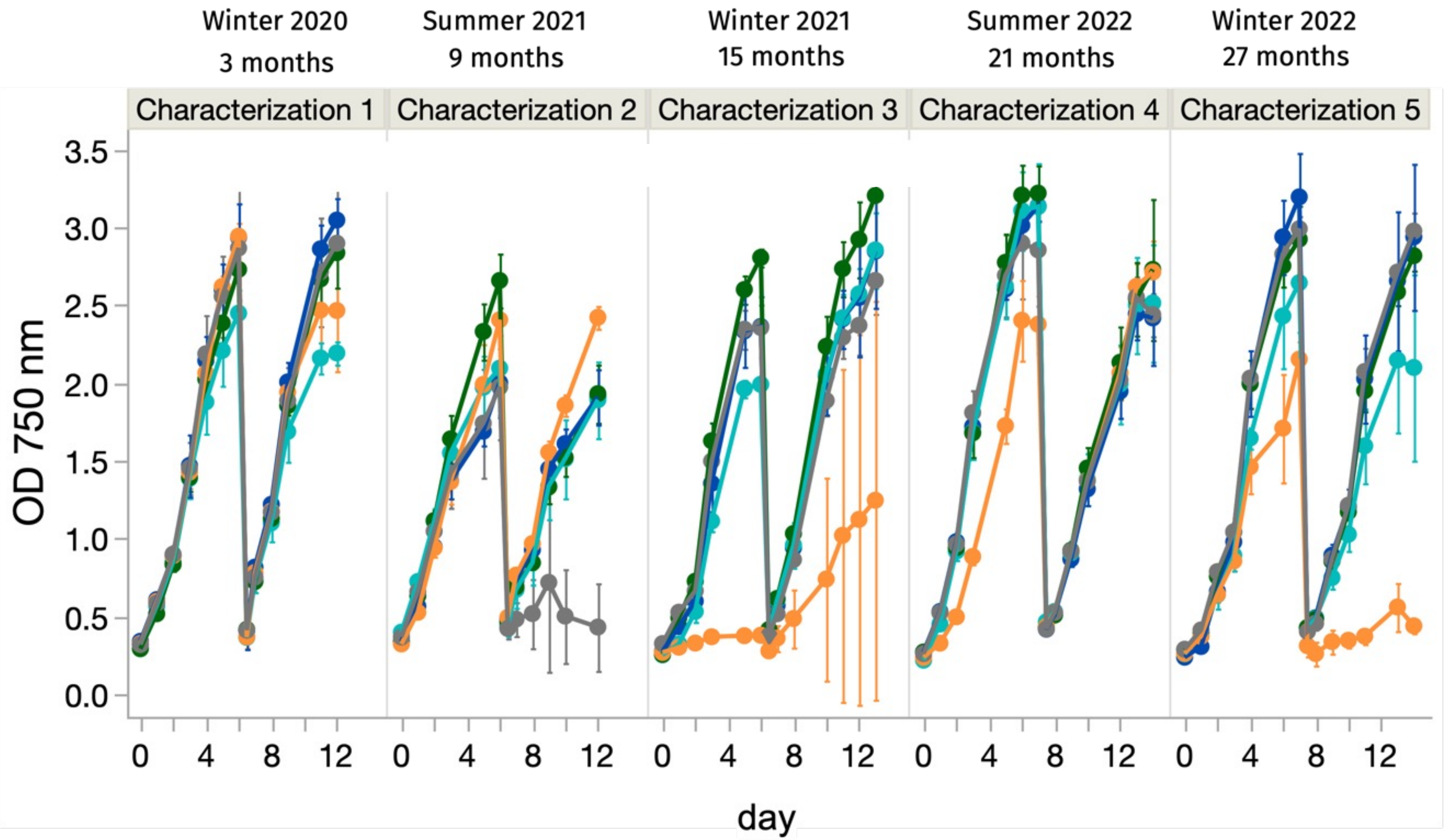


- Cryopreserved and Algem®-maintained strains most stable

- Batch- and plate-maintained strains most variable

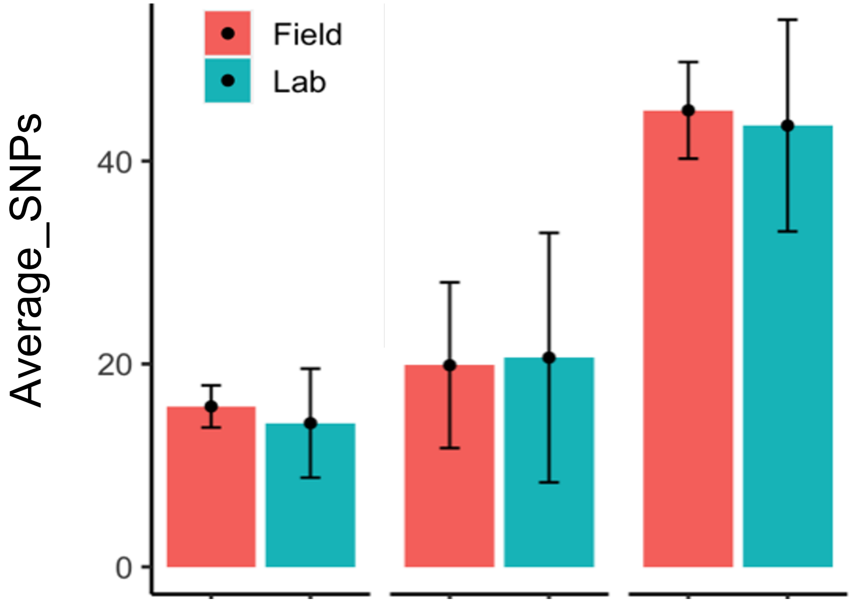
Field-Maintained Cultures Variable

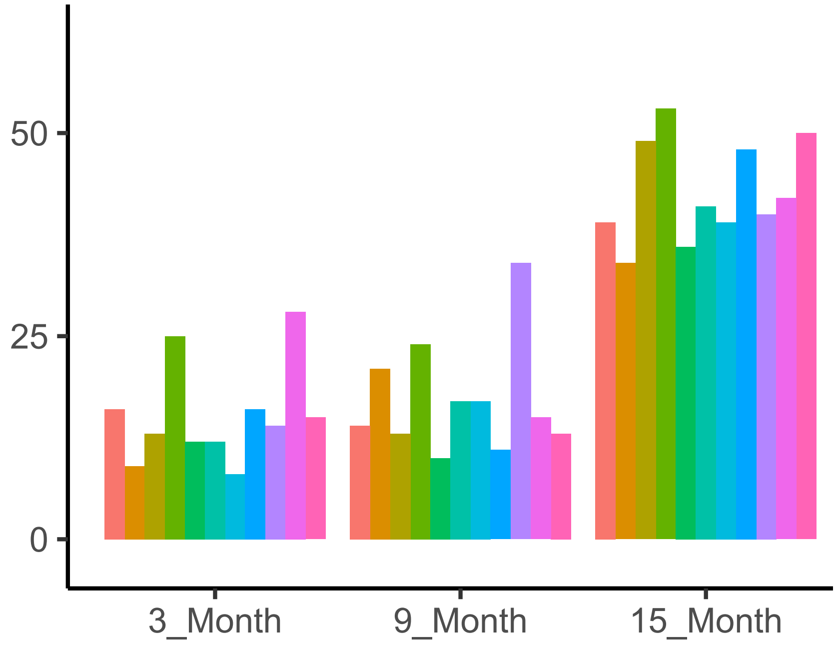
- Compared to the lab-maintained cultures, field cultures exhibited more variability across characterizations
- Variability increased with time



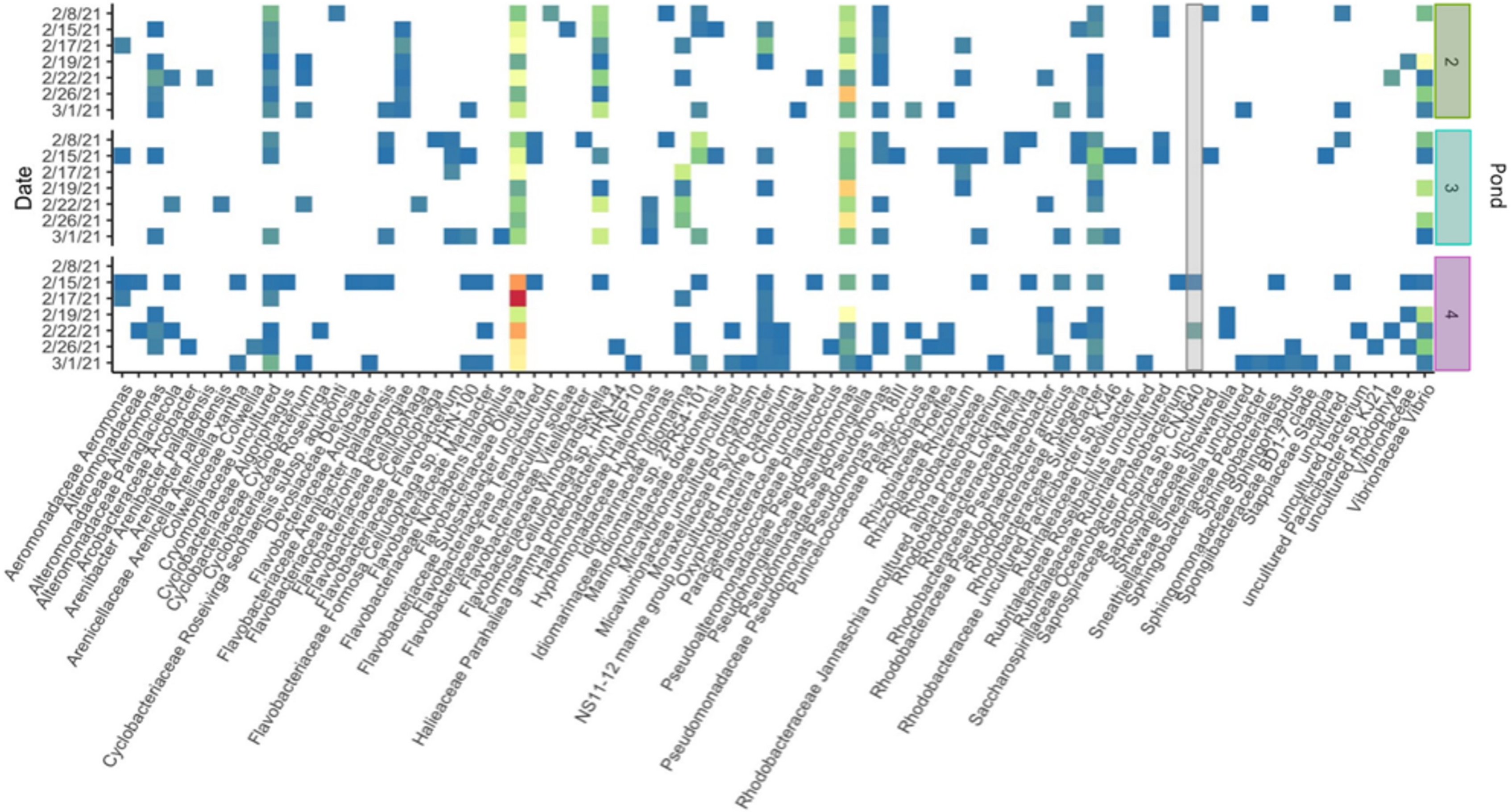
SNPs Increase Through Time in Field and Lab

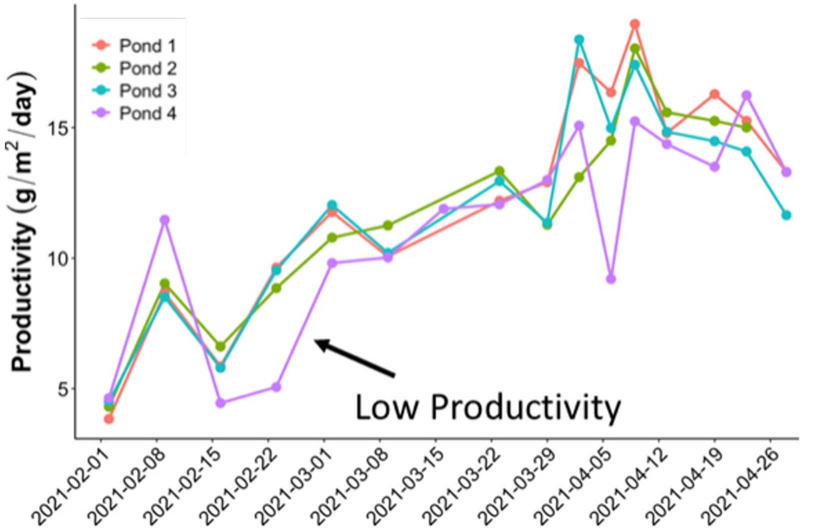
- No difference in acquired mutations between lab- and field-maintained cultures
- Mutations increased through time, with the most mutations present in NMSU and UCSD flasks

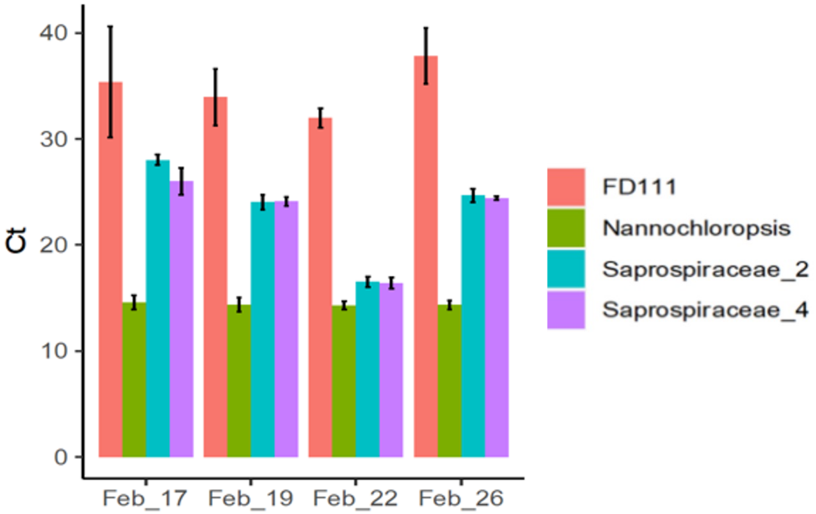




Detection of Predatory Bacteria



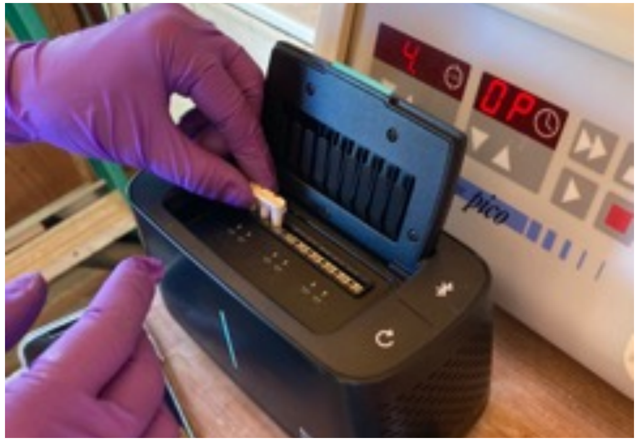




- Metagenomic analysis of low productivity period revealed predatory bacteria
- Developed two qPCR probes that effectively detected pest

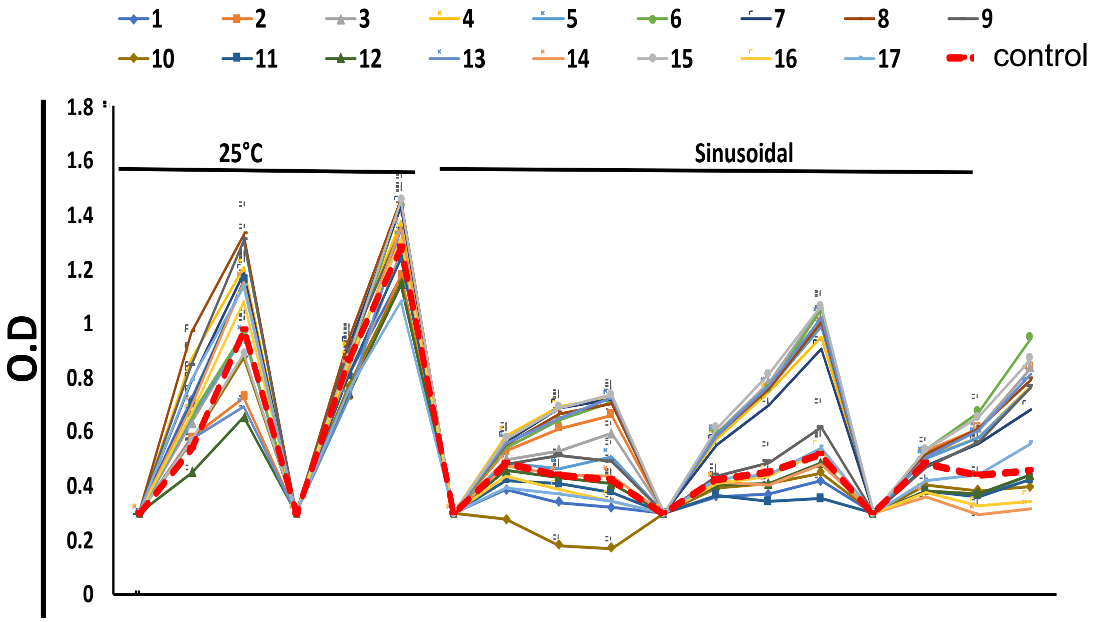
Fieldable qPCR

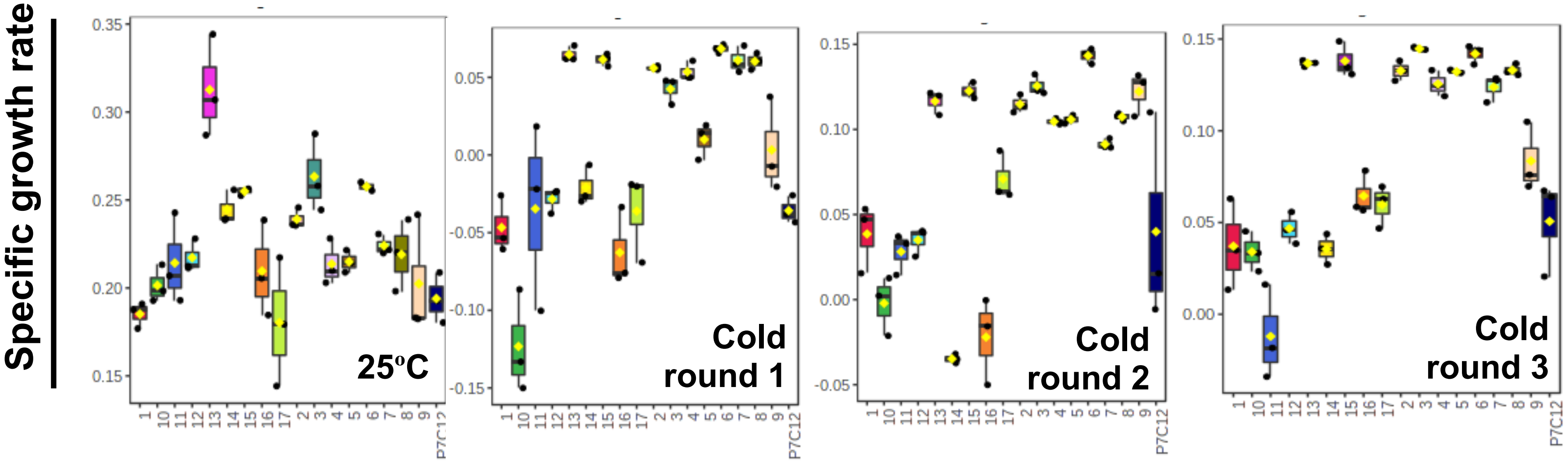
- Compared multiple platforms for cost/ease of use in the field
- Protocols completed in the field (DNA extraction/ purification, qPCR, analysis, generation of results)
- Developed primers for target alga, FD111, Saprospiraceae, a golden flagellate, and additional industry-requested targets (not disclosed)



Improvement of a Field-Adapted Strain in the Lab

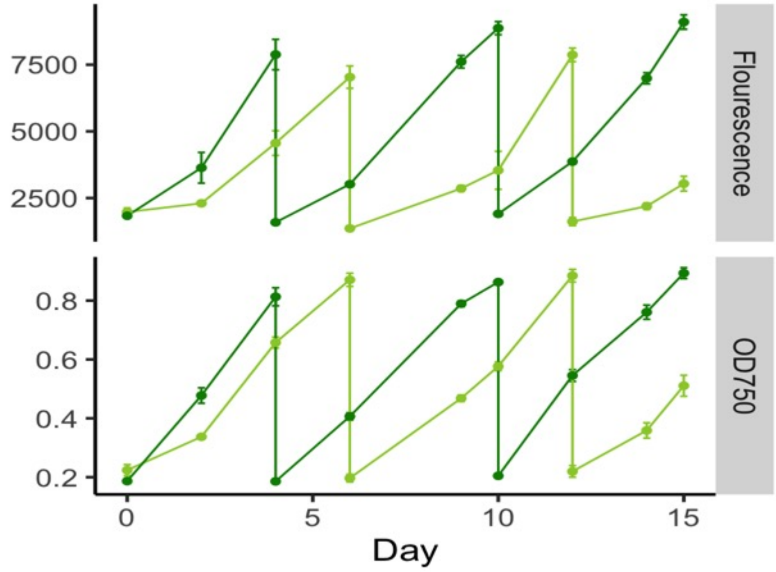
- Several UV mutants outperformed the control culture
- Improved isolates in the 3rd cold grow out exhibited ~50% greater growth rate compared to the control
- Isolate 6 showed a 1.32, 1.18, 2.73, 2.75-fold improvement over control in each of the grow outs respectively

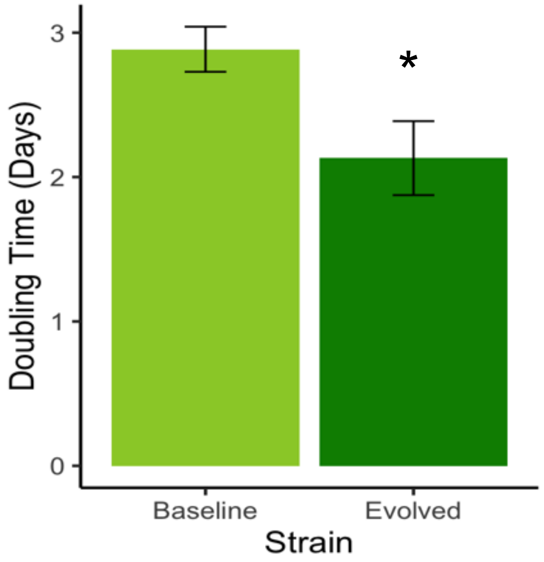


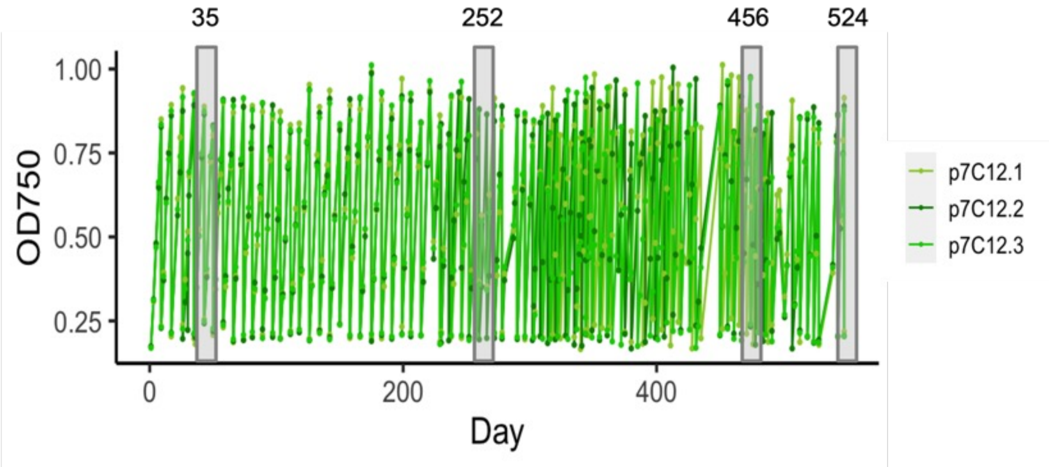


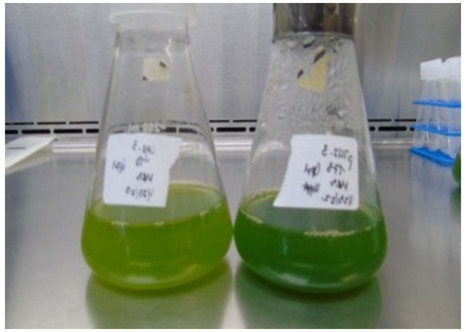
Adaptive Laboratory Evolution of a Field-Adapted Strain

- Evolved populations of baseline strain displayed faster growth rate and greater fluorescence at generation ~180



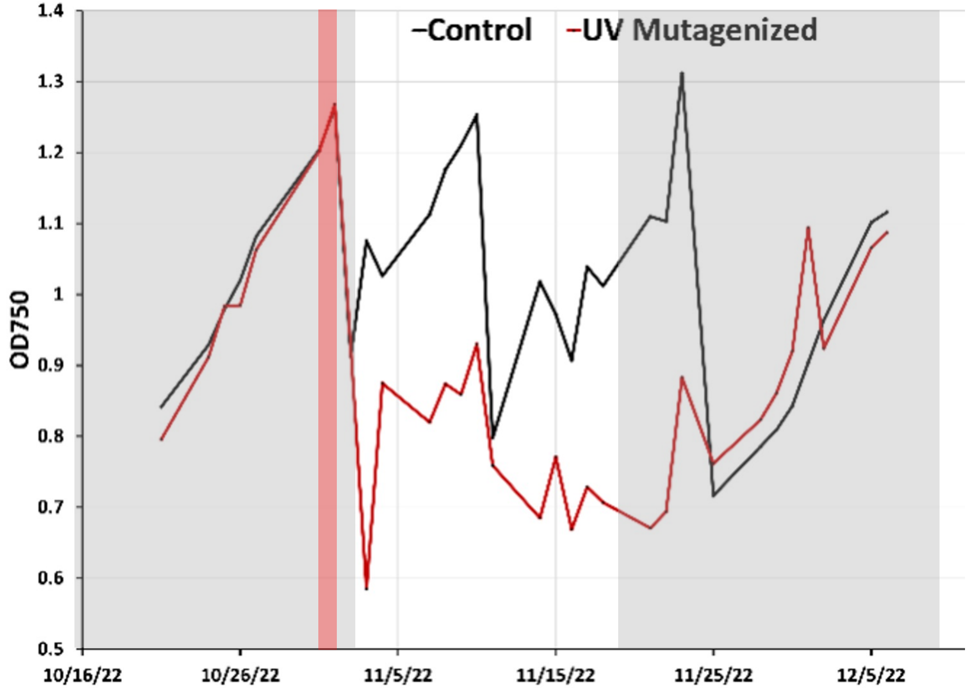


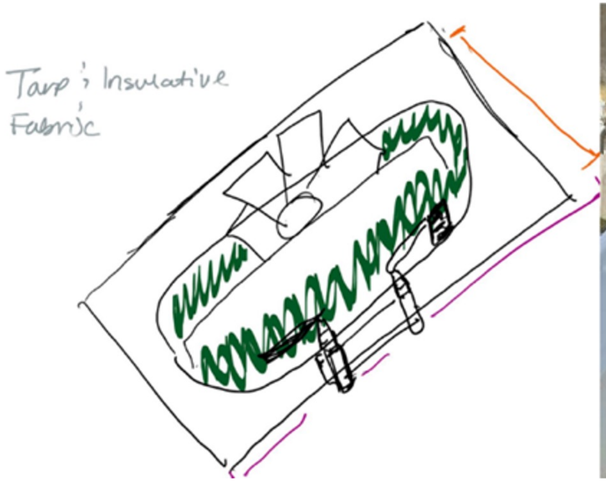


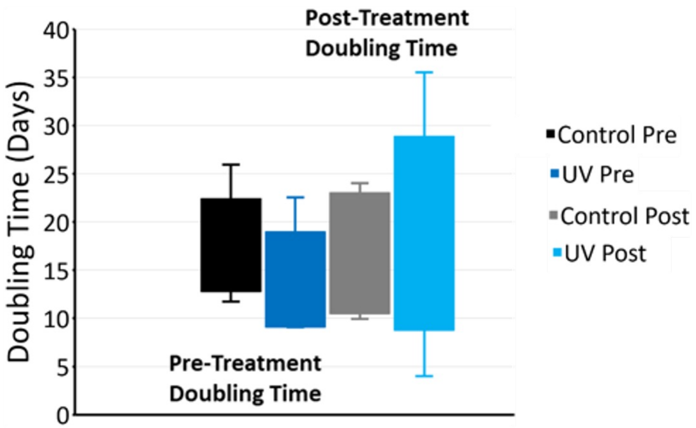


In Field Mutagenesis as New Strategy

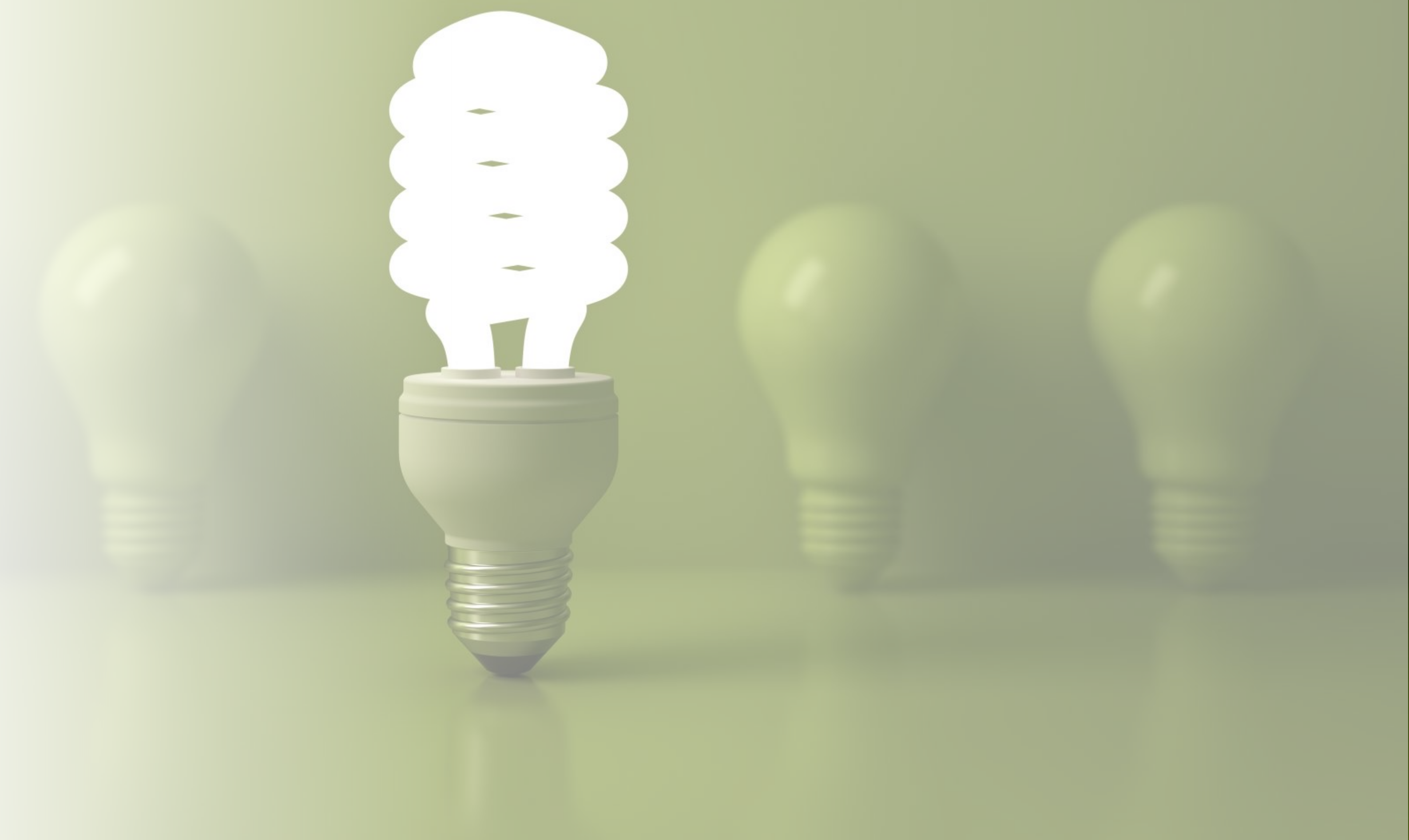
- Does large-scale UV mutagenesis overcome lab bottlenecks
- Do the field-adapted mutants outperform lab mutants?
- Continued treatment and evaluation for productivity and pest resistance







Outputs, Impact, and Summary



Outputs and Impact

Process or Deliverable	Impact
Cross-site cultivation	Year-round productivity data (3+years) to support decision making for consideration of new industrial locations/farms
Field-deployable qPCR tool	Deployed at remote field site by entry-level technicians Multiplexed tool allows for rapid detection of industry-requested targets (targets not disclosed)
Metagenomic database of pests across diverse sites	Data from three sites will be made available, will inform development pest management tools for other researchers
Metagenomic data	Informative to industrial downstream processes (data not shown due to IP)
In-field mutagenesis	Potential to generate stable phenotypes for field cultivation
Manuscripts, presentations, final report	Dissemination to the broader scientific community

Summary

This is the 1st study to quantify algal trait drift and evolution in the lab and field across multiple sites and years. We found the cryopreserved strain is more stable and productive than field-maintained and lab-maintained strains in common garden experiments.

We:

(1) Developed qPCR tools developed for the target alga, FD111, Saprospiraceae and additional industry-requested targets

(2) Developed hot and cold mutants through mutagenesis/selection and adaptive laboratory evolution

(3) Documented lab-scale improvements, but these did not translate to the field

(4) Developed an in-field mutagenesis pipeline to improve translatability between the lab and field.

Project Partners and Team

LEAD INSTITUTIONS

NMC: Alina Corcoran, Ahlem Jebali, Heather Martinez, Stephanie Getto, Gurpreet Nagi

LANL: Shawn Starkenburg, Monica Sanchez, Thomas Biondi, Matthew Green

ACADEMIC PARTNERS

CSU: Jason Quinn, Jonah Green

NMSU: Omar Holguin, Harman Kaur, Marwah Sobhan

UCSD: Jonathan Shurin, Isidora Echenique, Ugbad Farah

INDUSTRIAL PARTNERS

Qualitas Health: Jakob Nalley

Cyanotech Corp: Charley O’Kelly, Julia Gerber

Phase Genomics: Steve Eacker, Ivan Liachko

Timeline:

- Project Start: 1/1/2019
- Project end date: 1/30/2024

	FY20 Costed	Total Award
DOE Funding	$505,876	$4,999,470
Cost Share	$215,171	$1,290,354

TRL at Project Start: 2
TRL at Project End: 3

Project Goal: We aim to use the long-term cultivation of a field adapted strain at four distinct field sites and in three laboratories to understand trait drift and adaptation, enhance pest management, and improve strain productivity and robustness

End of Project Milestone: Synthesize cross-site field data to understand the relative importance of strain adaptation and local drivers on KPPs, and quantify potential improvements from deploying qPCR tools across industrial field sites

FY19 BETO Multi-Topic FOA
AOI 1: Cultivation Intensification Processes for Algae (CIPA)

Project Partners

- Los Alamos National Laboratory (Los Alamos, NM)
- Cyanotech Corporation (Kailua-Kona, HI)
- Phase Genomics, Inc. (Seattle, WA)
- Qualitas Health, Inc. (Imperial, TX)
- Colorado State University (Fort Collins, CO)
- New Mexico State University (Las Cruces, NM)
- University of California San Diego (San Diego, CA)

Additional Slides

Responses to Previous Reviewers' Comments

The previous peer review showed no significant questions/criticisms. However, reviewers:

(1) wanted to see more details on the approach to develop new strains and process improvements by measuring trait drift and evolution;

(2) were curious if lab-based UV/selection approaches would be fruitful; and

(3) suggested the impact on the BETO mission could have been explained more thoroughly.

We address these criticisms in the following slides.

(1) Details on Approach

- Our vision was that unique selection pressures of each outdoor system would allow us to develop robust cultivars and facilitate process innovations with broad geographic applicability. Cultivars from distinct sites (e.g., with improved temperature tolerance) would be isolated and then deployed at other sites. To date, we have not identified strains with improved phenotypes. We conclude that selection pressures have not been strong enough/time not long enough for the improved traits. Another possibility is that the 5+ years in cultivation prior to the prior start allowed for this phenotypic plasticity.

- As an example illustrating process innovations, if a particular field or lab-maintained strain showed greater productivity or stability than the baseline strain, we could recommend seeding/scaling procedures to enhance field performance. Conversely, if maintaining cultivation strains in the laboratory negatively affected strain performance, we would recommend against this process. Indeed, our project demonstrated that cryopreserving a strain preserved its growth phenotype **(see Progress and Outcomes slide # 18)**. Using a cryo-stock for scaling is not a universal practice industrially.

(2) Utility of UV/Selection Work

- Reviewers wondered if the UV/selection work would show improvements. Improvements were shown to be dramatic in the lab **(see Progress and Outcomes slides # 23 and #24)**, but did not translate to the field **(see Additional Slide #38)**.

- As part of EOBP2 Interim Verification, the Verification team recommended pushing the boundaries of this work by having greater kill rates and greater selection pressures in the lab. This work has begun.

(3) Link to BETO mission

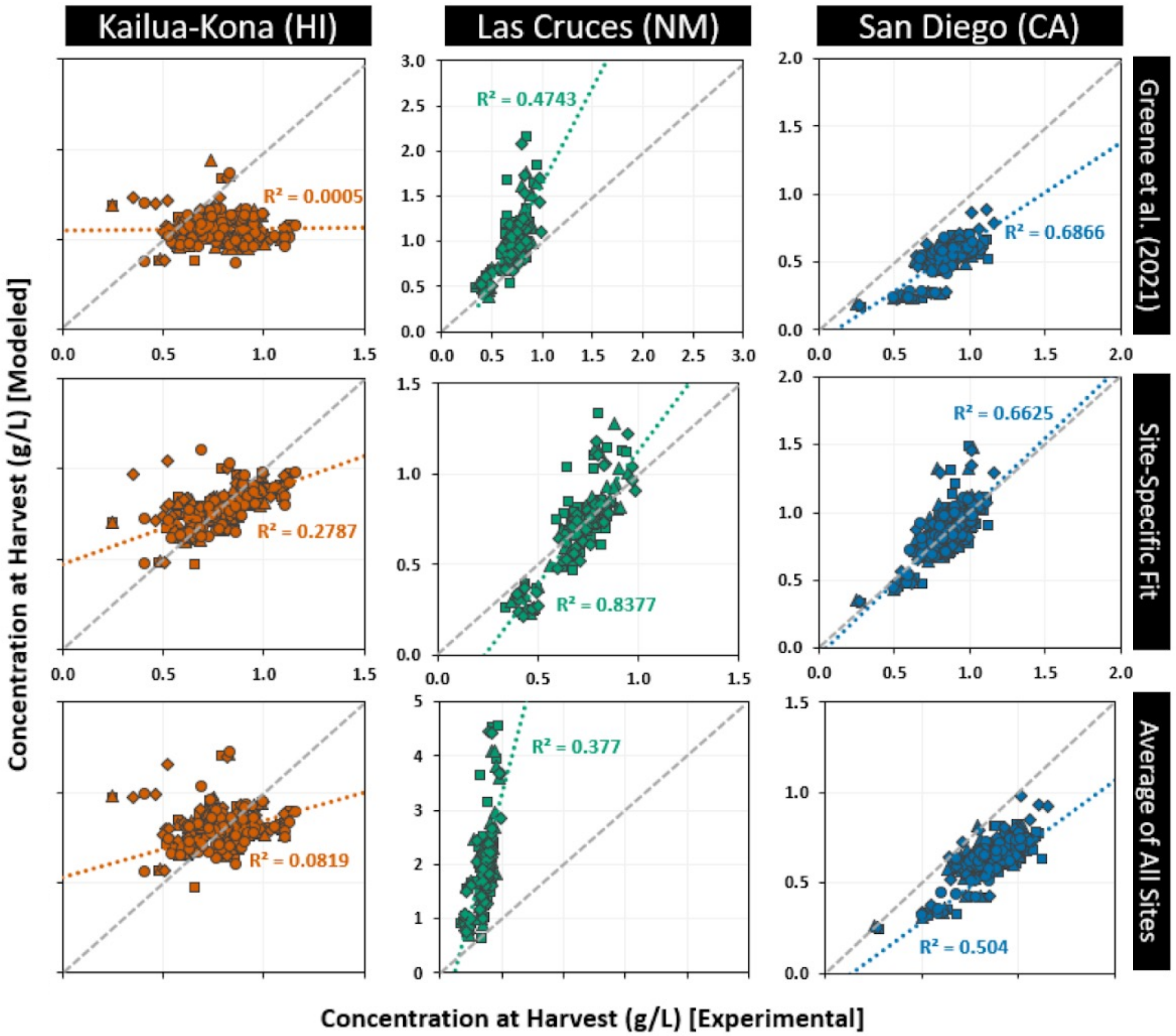
- In our last review, the FOA targets were listed in our impact slide: a 50% improvement in harvest yield based on AFDW (g m^2 d^{-1}), a 50% improvement in robustness based on stability metrics (e.g., high-productivity cultivation days, pond uptime), and a 20% improvement in conversion yield. During EOBP2 Interim Verification, the project was rescoped to focåus data compilation, analysis, synthesis and pest management given the lack of trait drift/evolution. Even with this rescoping, the project meets the BETO goals and the goals of the initial FOA **(see Progress and Outcomes slide # 7)**.

Hindcasting Highlighted Local Drivers of Biomass Productivity

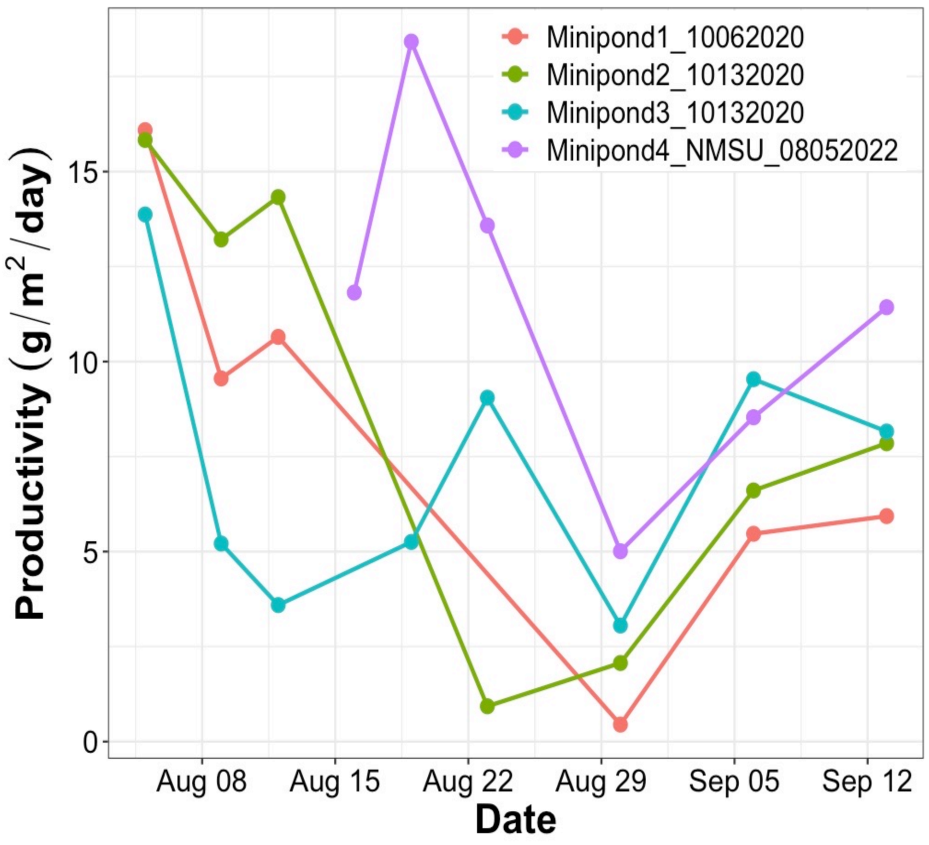
- Hindcasting using strain parameter values from literature (top row) resulted in the lowest modeling accuracy

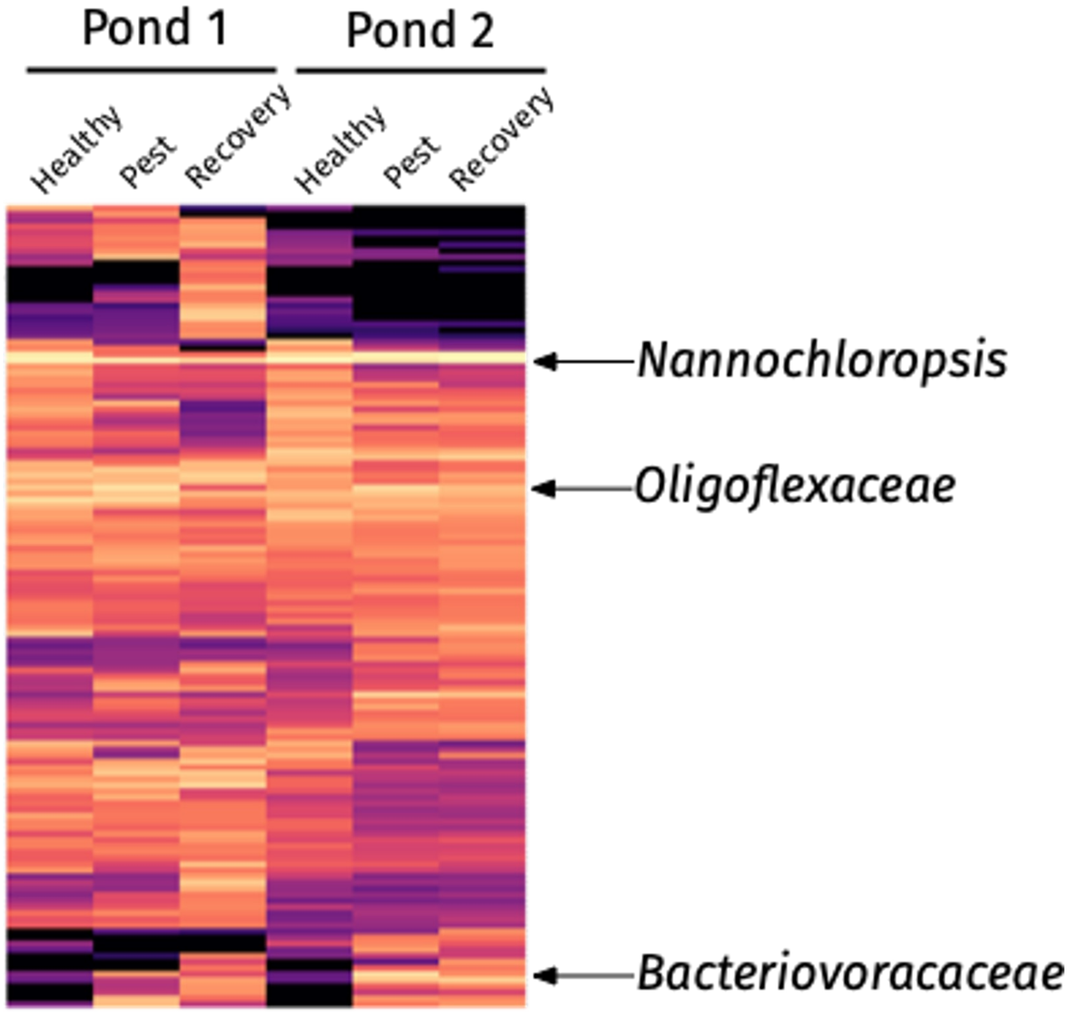
- Greater modeling accuracy achieved when using site-specific strain parameters
  - dark respiration rate
  - optimal temperature

- Data point to strain adaptation or site variables not otherwise controlled (e.g., water source)



Detection of Oligoflexaceae at UCSD



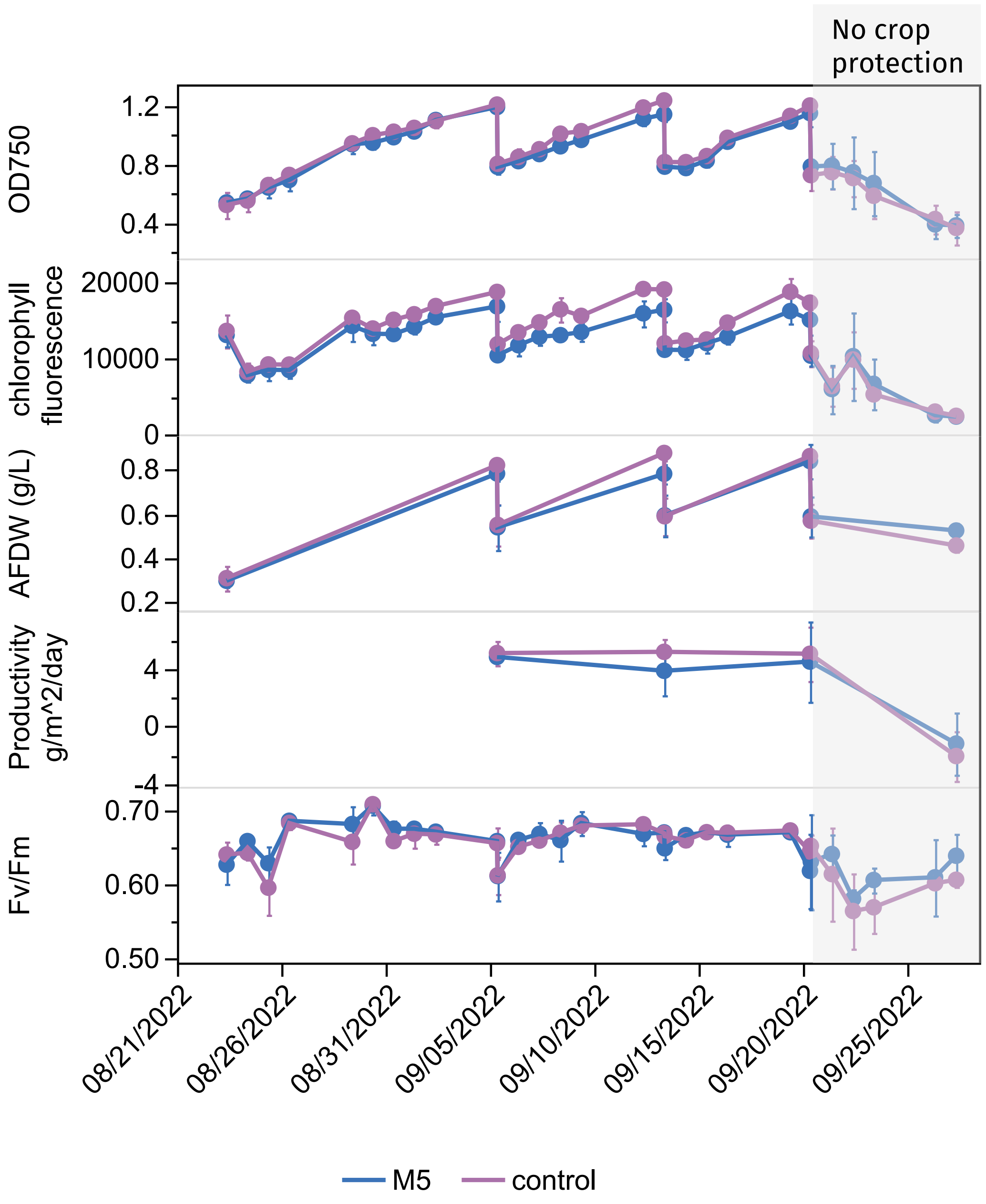


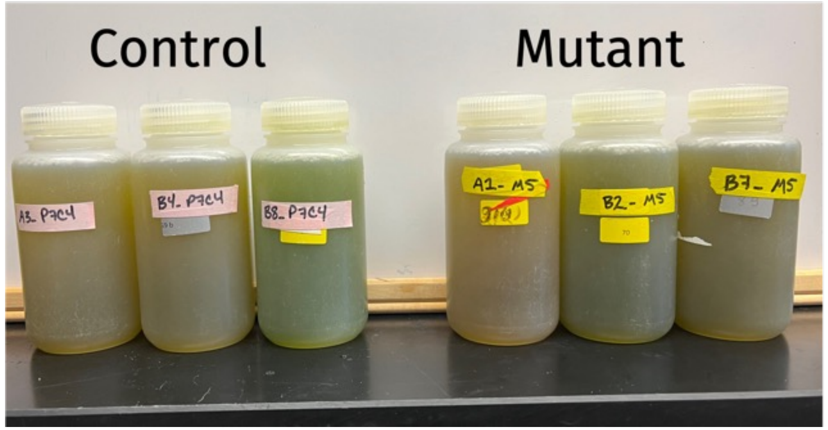
- *Oligoflexaceae* is a class of the phylum *Bdellovibrionota* a group containing well described predatory bacteria

Query of field-deployable qPCR instruments

Name	Developer	Number of Samples	Cost	Size	Weight	SYBR Green
Open qPCR	Chai	16	Single Channel = $4,999, Double = $6,999	11 x 9.5 x 7.5 in	9 lbs	✅
Q qPCR	QuantaBio	48	Four Channel = $17,260 (says 2 Channel is available but can't locate)	5.1 x 5.9 x 5.9 in	4.5 lbs	✅
PrimePro	Techne	48	$19,290	13.6 x 12.2 x 12.6 in	30 lbs	✅
UF-150 GENECHECKER	Victoria Scientific	10	$7,348	7.9 x 7.9 x 4.9 in	7 lbs	✅
Maverick	Anitoa Systems	4 or 8 or 16	X	6.5 x 4.5 x 3 in	2.1 or 3.2 lbs (4 and 8 well)	✅
Mic qPCR cycler	Biomolecular systems	48	$19,290	5.1 x 5.9 x 5.9 in	4.4 lbs	X
Biomeme	Franklin	9	$9,950 ($5,950 for one Channel - green)	"Hand held"	2.2 lbs	✅
Liberty 16	Ubiquitome	16	X	8.35 x 4.33 x 4.53 in	7.05 lbs	✅
Hunter qPCR	Instant Labs	10?	X	6.77 x 16.22 x 8.66 in	11.9 lbs	X

Lab Improvements Did Not Translate to Field Systems





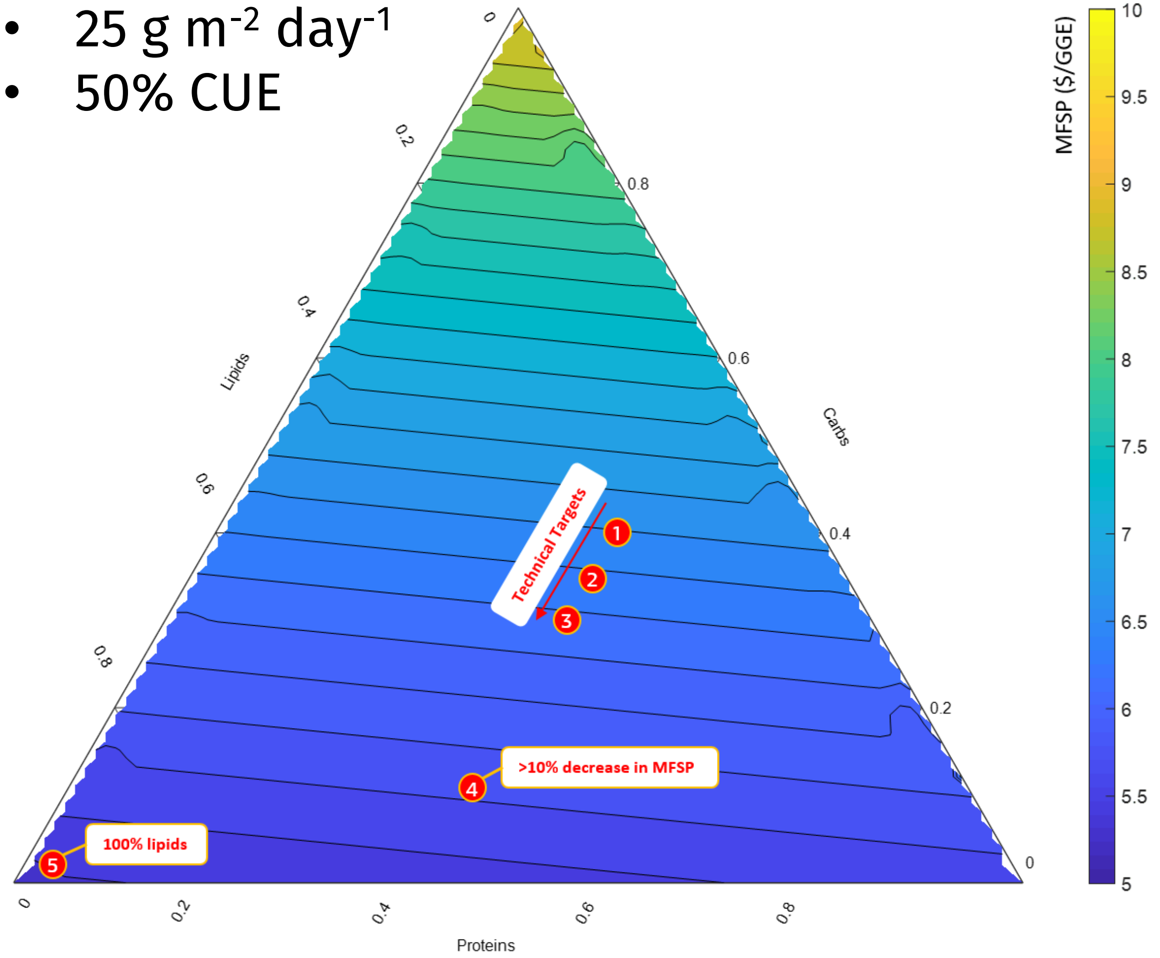
- Two low temperature mutants (winter 2021)
- One high temperature mutant (summer 2022)
- One low temperature mutant (winter 2022)

Concurrent TEA and LCA

ML 6.1: Economic impact of carbon partitioning (increase in lipids and decrease in carbs) on HTL performance and the MFSP (Minimum Fuel Selling Price)

Major Assun

- $650 per
- 25 g m^{-2} day^{-1}
- 50% CUE



Technical Targets

Composition (AFDW)		MFSP
1	Proteins: 40 wt% Lipids: 20 wt% Carbohydrates: 40 wt%	$6.57 per GGE *Baseline*
2	Proteins: 40 wt% Lipids: 25 wt% Carbohydrates: 35 wt%	$6.41 per GGE *-2.4%*
3	Proteins: 40 wt% Lipids: 30 wt% Carbohydrates: 30 wt%	$6.26 per GGE *-4.7%*
4	Proteins: 40 wt% Lipids: 50 wt% Carbohydrates: 10 wt%	$5.74 per GGE *-12.6%*
5	Proteins: 0 wt% Lipids: 100 wt% Carbohydrates: 0 wt%	$5.42 per GGE *-17.5%*

Moving from 20 – 50% lipids (Point 1 to Point 4) we see a 7% increase in biocrude yield and 12.6% decrease in MFSP

GNG 3: Three lab-to-field iterations that incorporate at least two of the following: trait stack, qPCR tool deployment, and process improvements based on trait drift in the lab and field to reach improvements in productivity, robustness and biomass composition compared to the baseline *Nannochloropsis* strain

LAB-FIELD ITERATIONS	IN FIELD
cold selected mutants --> outdoor field trials --> indoor characterization	QH strain ---> NMSU
hot selected mutant --> outdoor field trial --> indoor characterization	NMSU strain --> UCSD
field strains --> sorted and scaled --> field trial	pest pond (NMSU and Cyanotech)
QH strain --> cryopreserved, scaled--> outdoor field trial	outdoor mutagenesis (NMSU)
outdoor field strains --> characterization in lab (no improvements found)	
outdoor mutant pools --> characterization in lab	

- qPCR tools developed, deployed at NMSU, in process at Qualitas Health
- process improvement: reseeding, in field mutagenesis
- trait stacking: ploidy on cold and hot tolerance

Technical Conclusions

- Cryopreserved strain is most stable and productive
- Without dramatic crashes, the biological causes of low productivity can be complex
- Use of field-adapted cultivar does not improve translatability in lab-to-field transitions
- Selection pressures are not strong enough and/or time not long enough to generate strains with dramatic improvements in productivity, stability or biomass composition

Publications, Patents, Presentations, Awards and Commercialization

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2. Echenique, I. U. Farah, M.R. Sanchez, A. Jebali, G. Kaur Nagi, M. Balleza, H. Martinez*, J. Nalley, C. O'Kelly, Omar Holguin. A.A. Corcoran, Shawn R. Starkenburg, J. Shurin. Local adaptation of Nannochloropsis produces site-specific temperature-growth curves. Poster Presentation. Algae Biomass Summit. October 2021. Virtual.
3. Greene, J.M., D. Quiroz, A.A. Corcoran, J. Nalley, J.C. Quinn. Integrating regionally and temporally resolved microalgae growth rate modeling with pond reliability metrics to accurately model the economic and environmental performance of algae cultivation at scale, Oral Presentation. Algae Biomass Summit. October 2021. Virtual.
4. Greene, JM, D Quiroz, S Compton, JC Quinn. "Modeling algae cultivation at scale: Productivity, pond reliability, and resource consumption across the United States." International Symposium on Sustainable Systems and Technology (ISSST); Pittsburgh, PA; June 2022.
5. Greene, JM., M Belleza, A Corcoran, I Echenique, J Gerber, S Getto, O Holguin, A Jebali, H Martinez, J Nalley, C O'Kelly, SR Starkenburg, J Shurin, JC Quinn. "Hindcasting of algal productivity in open raceway ponds to quantify the impacts of various pest pressures." Algae Biomass Summit – Algae Biomass Organization (ABO); Virtual Conference; Sept – Oct 2022.
6. Jebali, A., M.R Sanchez, K.Y. Mak, I. Echenique, H. Martinez, S. Getto, M. Balleza, F. Omar Holguin, B. Hovde, J. Nalley, C.J. O'Kelly, J. Shurin, S.R. Starkenburg, Alina A. Corcoran. Phenotypic and genotypic characterization of a field-adapted Nannochloropsis strain. Oral presentation. Algae Biomass Summit, October 3-28, 2022. Virtual..
7. Jebali, A., M.R. Sanchez, E.R. Hanschen, S.R. Starkenburg, and A.A Corcoran. 2022. Trait drift in microalgae and applications for strain improvement. Biotechnology Advances 60: 108034. https://doi.org/10.1016/j.biotechadv.2022.108034
8. Jebali, A., M.R. Sanchez*, I. Echenique, M. Balleza, H. Martinez, J. Nalley, C.J. O'Kelly, F.O. Holguin, J. Shurin, S.R. Starkenburg, A.A. Corcoran. Phenotype characterization of field-adapted Nannochloropsis for the evaluation of trait drift and evolution in lab and field cultures. Poster Presentation. Algae Biomass Summit. October 2021. Virtual.
9. JFarah, U., Echenique-Subiabre, I., Sanchez, M., Jebali, A., Kaur, H., Nalley, J., O'Kelly, C., Holguin, O., Corcoran, A. A., Starkenburg, S., Shurin, J. The Evolution of the Adaptive Landscape in Nannochloropsis sp. Poster Presentation. Algae Biomass Summit. October 2022. Virtual.
10. Kaur, H. Improvement of field adapted Nannochloropsis oceanica strain performance and composition. Graduate Research and Arts Symposium, NMSU. Oral presentation and lightening talk. November17, 2022.

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11. Sanchez, M.*, A. Jebali*, I. Echenique*, E. Denning, M. Balleza, J. Nalley, C. O'Kelly, J. Shurin, A.A. Corcoran, S.R. Starkenburg. Characterization of trait drift in the field and laboratory: shifts in microbial community composition. Oral Presentation. Algae Biomass Summit. October 2021. Virtual.
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15. You Mak, K., Sanchez, M., Echenique, I, Farah, U., Shurin, J., Gerber, J., Balleza, M., O’Kelly, C.J., Martinez, H.*, Jebali, A., Corcoran, A.A., Hovde, B., S.R. Starkenburg. Characterizing microbial communities in long-term algal ponds across sites. Sequencing to Function: Analysis and Application for the Future (SFA^{2}F). Poster Presentation. Santa Fe, NM, June 21-23, 2022