AzCATI

Arizona Center for Algae Technology and Innovation

Decision-Model Supported Algal Cultivation Process Enhancement

Award #DE-EE0008906
WBS: 1.3.5.287
BETO Project Peer Review: Advanced Algal Systems
April 4th, 2023

Dr. John McGowen, PI

One little cell, a world of possibilities.

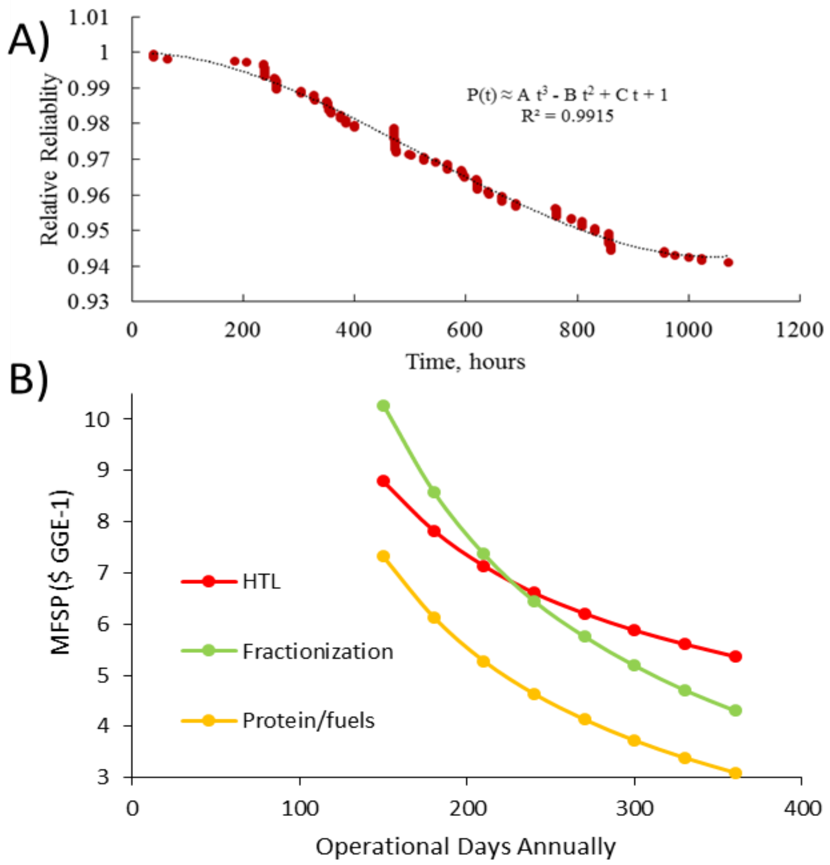
AzCATI Arizona Center for Algae Technology and Innovation

Project Overview: The challenge – culture reliability

Current decision-support models lack critically important, quantitative culture-failure risk data

- Cultivation of microalgae in open raceway ponds is prone to contamination and culture failure
  - Productivity of cultivation systems is thus a critical sustainability parameter
- Culture failure will impact the economics of algal biomass and biofuels ~ reduction in operational days
- How do we quantify cultivation risks and their economic impacts?
  - Semi-continuous versus full-batch cultivation - different risk profiles
  - Unknowns around failure rates for semi-continuous vs. batch operations constitute a critical knowledge gap

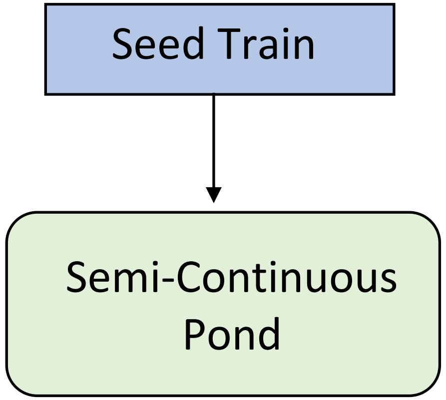
For this project **we generated empirically derived culture-failure risk data** for concurrent TEA/LCA modeling to **quantify the risks associated with culture failure**, and corresponding impacts on sustainability assessed through **sensitivity and scenario analyses**



A) Pond reliability based on data from ATP[3].B) Economic impact of reduced operational days for three different production pathways with an assumed baseline productivity of 25 g m^{-2} d^{-1}. Adapted from Cruce and Quinn (2019).

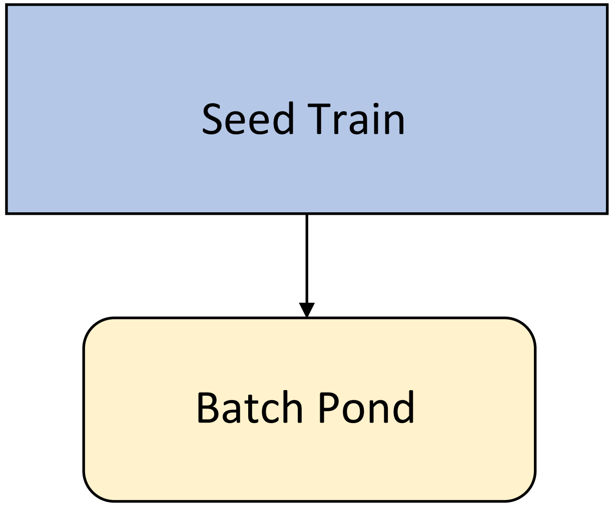
Project Overview: Culture Reliability and Cultivation Mode

Semi-Continuous Pond Operation



- **Lower** costs ~ smaller seed train
- High seed culture exposure time in ponds, more prone to failure
- Low mean time to failure (MTTF) compared to batch mode
- Long scale-up recovery times

Batch Pond Operation



- **Higher** costs ~ larger seed train, lower financial risks
- Minimizes seed culture exposure time, hence, more reliable
- High mean time to failure (MTTF)
- Short scale-up recovery times

The precise advantages and feasibility of either approach is difficult to disambiguate, let alone confidently implement broadly, as cultivation risks are likely to show significant strain-, location-, and seasonal-dependencies. Establishing modeling framework essential for more effective decision support tools and implement feasible solutions

Regardless of operational scenario, algal cultivation at scales that can support commodity-product prices requires:

- Robust cultivars with high productivity and robustness
- Defined agronomic best-practices—including integrated pest-management
- Comprehensive monitoring programs to determine and maintain optimal growth conditions
- *Supporting tools and data-management structures* that are both implementable and effective ($) at enabling data-driven decision-support models for large-scale production

Project Overview: Project Goals

- **Quantify the economic and technical risks** associated with different cultivation strategies and crop protection approaches through **an integrated program of indoor lab studies, cultivation optimization and simulation, multi-scale ‘omics, and robust outdoor cultivation campaigns**
- **Robust TEA, LCA, and biomass productivity modeling** developed to: a) assess progress towards performance targets b) identify critical research and development priorities; and c) evaluate the impact of sub-system technologies at a systems level, allowing for more rapid advancement of those strategies that generate scalable best practices.
  - **Variability and sensitivity analysis through Monte Carlo modeling** understand the **risks** associated with **culture failures** and the **sustainability impact** of **avoidance and mitigation** strategies.
  - Produce a more **integrated and realistic assessment of risks**, the current state of technology, and pathways to BETO’s target of **$3.00 GGE^{-1} and trajectory to $2.50 GGE^{-1}**.
- Through the **development and deployment of a suite of novel real-time sensors** for nutrient and water quality monitoring, **gain better process control** though novel insights, plus the ability to **optimize productivity, robustness, and biomass quality** of our selected high-performance strains.

Approach: Project Tasks

Task 1: Addressing culture failure risks and quantifying impacts (ASU/DTI)

- Multi season cultivation trials comparing (semi)continuous vs. batch operation
- Crop protection through integrated pest management
  - Chemical and physical/mechanical means for crop protection
  - Regulatory aspects/barriers to deployment

Task 2: Integrated Lab to Field to Lab (LFL) to optimize cultivation performance (ASU/LANL)

- Whole culture and single cell phenotyping
  - Developing 96-well plate diagnostics workflow including flow cytometry assays for monitoring culture health
- Environmental simulation with ePBR's based on retrospective scripts
  - Iterative indoor/outdoor flow and 'omics' approach to track and understand culture health/stress as a function of key operational variables (e.g., seed train/culture age and abiotic/biotic crash events)

Task 3: Optimized process monitoring for improving performance (QBI/Burge/ASU)

- Novel sensor development for continuous, real-time monitoring of key cultivation parameters including water quality and nutrients
- Data integration platform to support decision-supported cultivation improvements
  - Goal ➔ AI/machine learning ready data sets with cloud based, open access database and analysis platform

Task 4: Sustainability assessment - coupled TEA and LCA (CSU)

- Concurrent TEA/LCA/resource assessment
- Dynamic thermal and growth model development integrated with crash model

Approach: Team Organization

AzCATI
Arizona Center for Algae Technology and Innovation

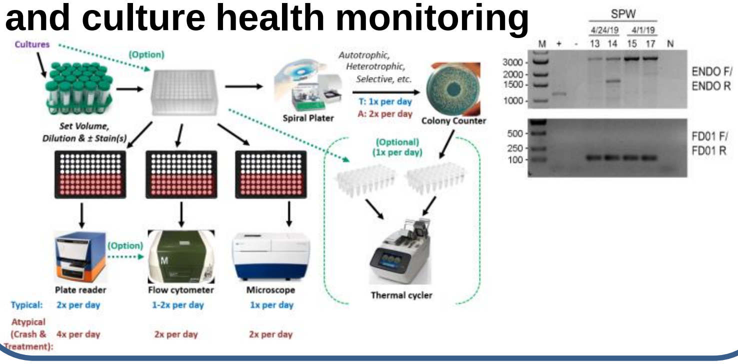
Ira A. Fulton Schools of Engineering
Arizona State University

Project lead

Outdoor cultivation

Task 1

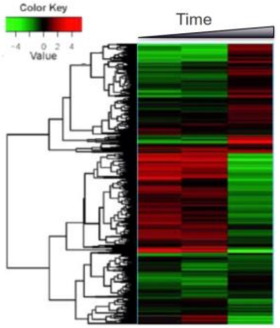
integrated pest management and culture health monitoring



Indoor pond simulation

Los Alamos NATIONAL LABORATORY EST. 1943

'omics'



Single cell phenotyping for culture health

Task 2

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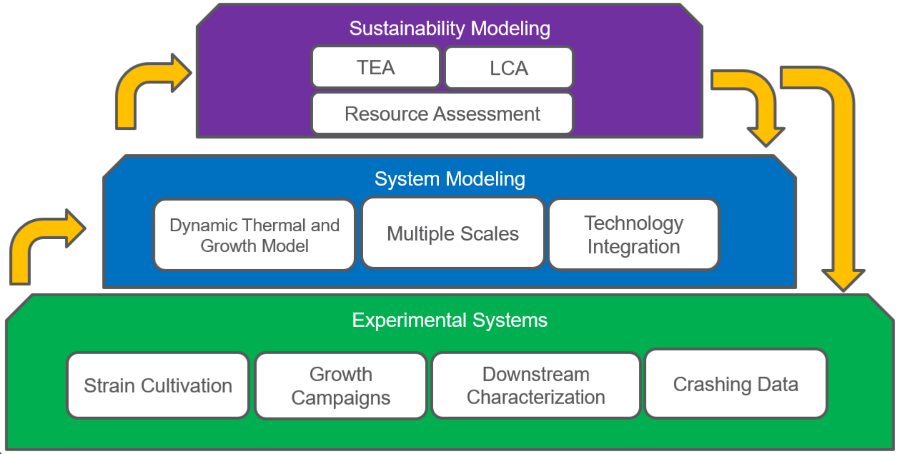
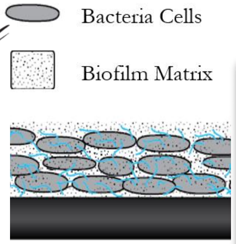
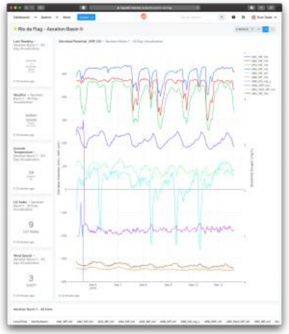


Burge Environmental



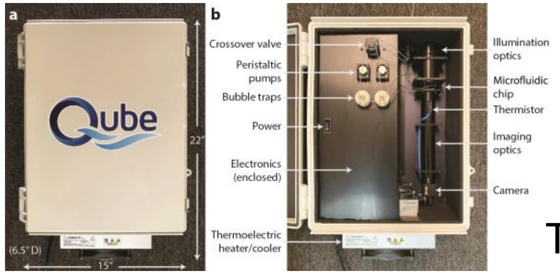
Task 3



Novel microbial sensors/ data integration/analysis

QBI Quantitative BioSciences, Inc.

Novel microbial sensors for real-time nutrient analysis

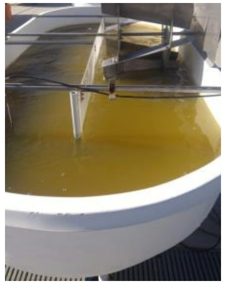


Task 3

DIVERSIFIED TECHNOLOGIES, INC.

Fieldsite deployed PEF

untreated



treated

Pulsed Electric Field (PEF) for crop protection

Task 1

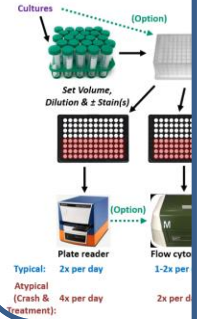
Approach: Team Organization

Ira A. Fulton Schools of Engineering, Arizona State University

Project lead

Outdoor cultivation

integrated
and cultur



Indoor pond simulation

Los Alamos National Laboratory EST. 1943

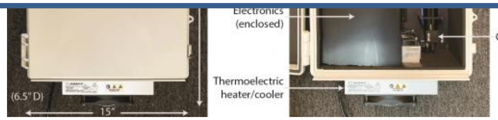
Energy Institute, Colorado State University

Sustainability Modeling

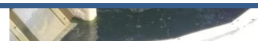
- Monthly and ad-hoc meetings
- Cultivation data including failure rate data to CSU, model outputs guide experimentation
- Grab samples/culture/weather scripts provided to LANL for Task 2 work
- Sensor systems deployed at AzCATI with close coordination with QBI/Burge (Burge attends weekly production meetings)
- Collaboration with other projects
  - Sensor systems development for monitoring cultivation leveraged into other projects (DISCOVR, ADAPT-COST, ASU APEX-DAC)
  - Cultivation data supplied and AzCATI Cloud access for LANL MORE AOP from multiple DOE projects (DISCOVR, ATP3, etc.)
  - All cultivation data migrated into AzCATI Cloud for (eventual) public access

Burge Environmental

Novel microbial sensors/ data integration/analysis



Task 3



Fieldsite deployed PEF

ric
or
crop protection

Task 1

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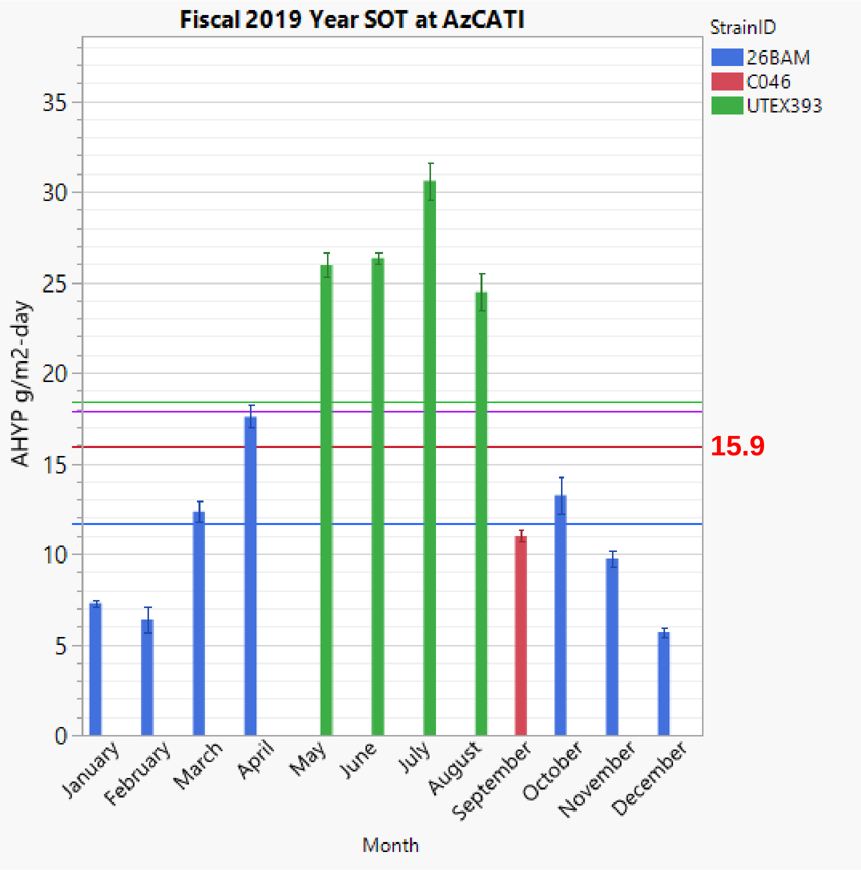
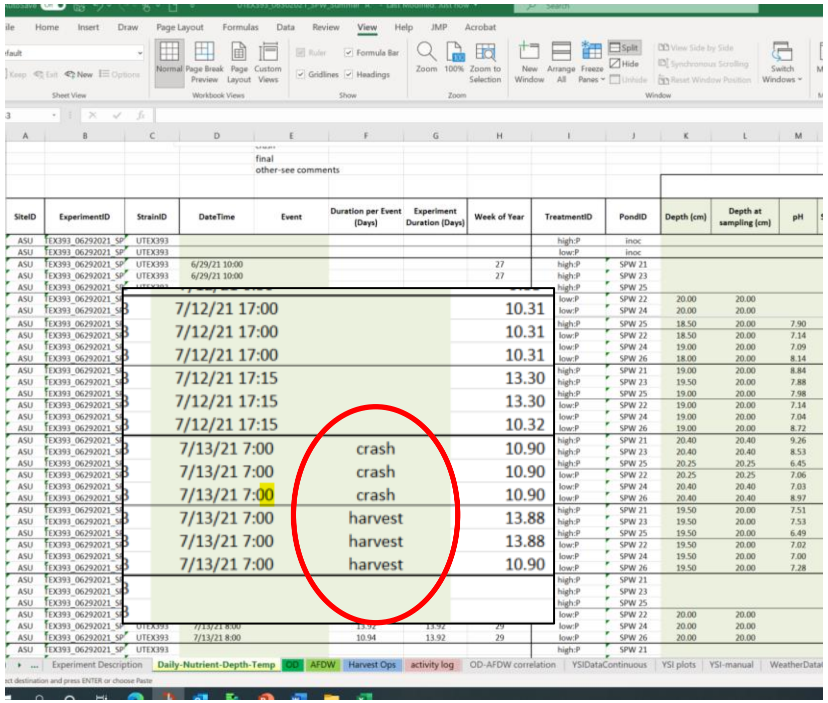
Approach: Go/No Go Decision Points

Go/No Go Decision Point 2 (November 2021): Demonstrate, with one or both operational modes, a minimum 50% improvement in MTTF from the 2018 baseline of **30 days to >/= 45 days**, while meeting or exceeding 2019 SOT productivity baseline for annual average of **15.9 $g \cdot m^{-2} \cdot d^{-1}$.**

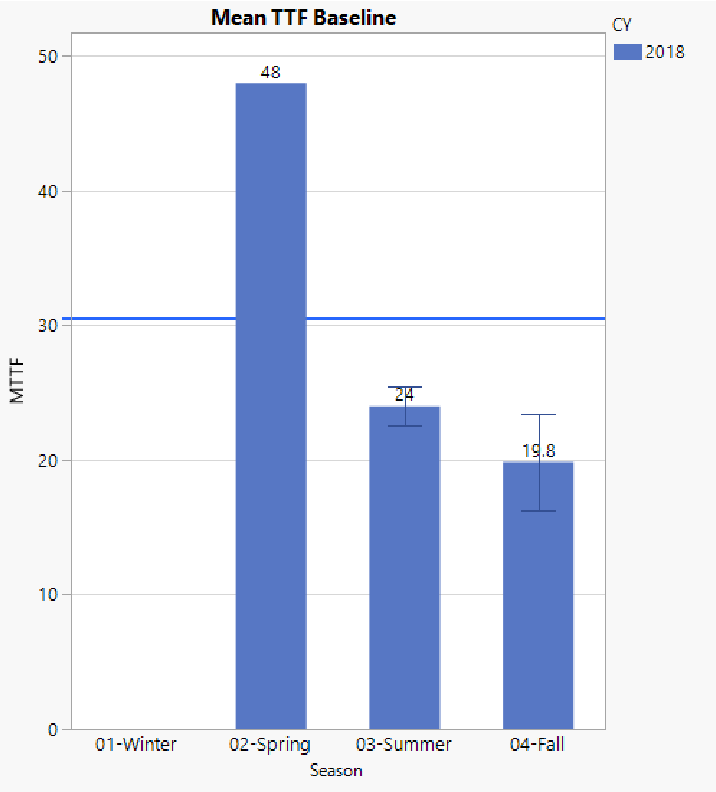
What was our baseline?

Standard cultivation format (4.2 m^2 ponds), standard media recipe, all SOPs same standard methodology as in BETO DISCOVR SOT trials.





Annual average MTTF is simply the average of the seasonal TTF values



Harmon, et al, Algal Research 2021
https://doi.org/10.1016/j.algal.2021.102249

Approach: Go/No Go Decision Points

End of Project Goal: (9/30/2023)

Demonstrate, with one or both operational modes, a minimum **100% improvement in seasonal MTTF from 2018 baseline of 20 to >= 40 days for Spring and Summer**, while meeting or exceeding a **50% improvement** over 2018 SOT productivity baseline (**summer and fall seasonal target is 21.8 and 17.0 g/m2-day**, respectively). Achieve compositional improvements of at least 20% to allow a modeled HTL conversion yield of at least **80 gge.**

		Productivity (g/m2/day)	Ash	FAME	Protein*	Carbs**	GGE/ton (AFDW) HTL*
6/12/21 - AM	High_N	23	4.04	5.53	49.82	10.23	64
6/12/21 - AM	Low_N_day1	22	4.01	5.46	48.18	11.49	64
6/13/21 - PM	**Low_N_day2**	**19**	**5.54**	**9.18**	**30.52**	**28.74**	**83**
6/16/21 - AM	Low_N_day5	15	4.73	11.73	25.30	35.94	93

*https://doi.org/10.1016/j.algal.2019.101450

Remaining tasks to meet the end of project goal include Spring and Summer 2023 cultivation runs with 26BAM and UTEX393, respectively including confirming compositional targets met and updates sustainability assessments

Approach: Task 4 Modeling Framework

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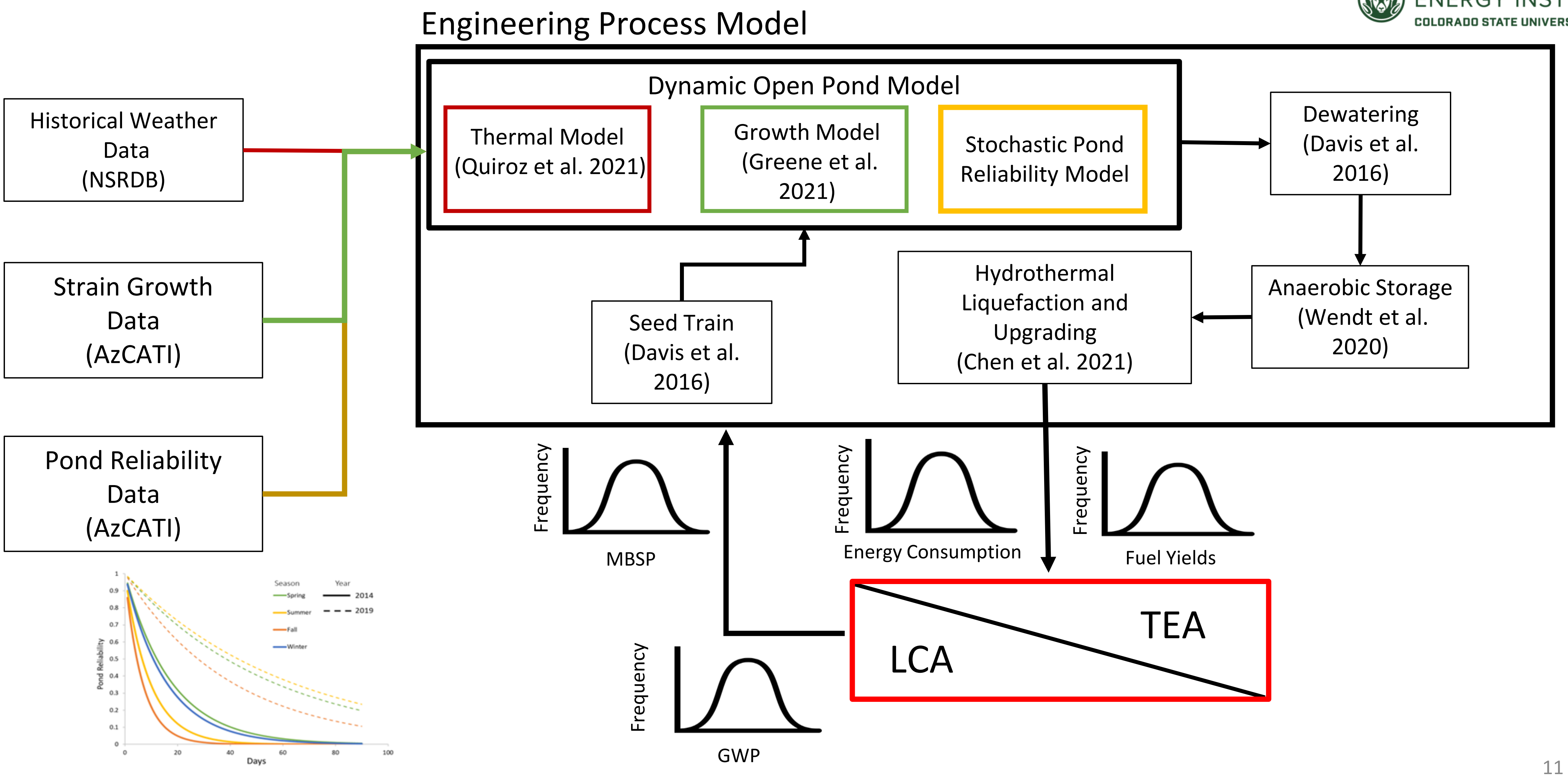

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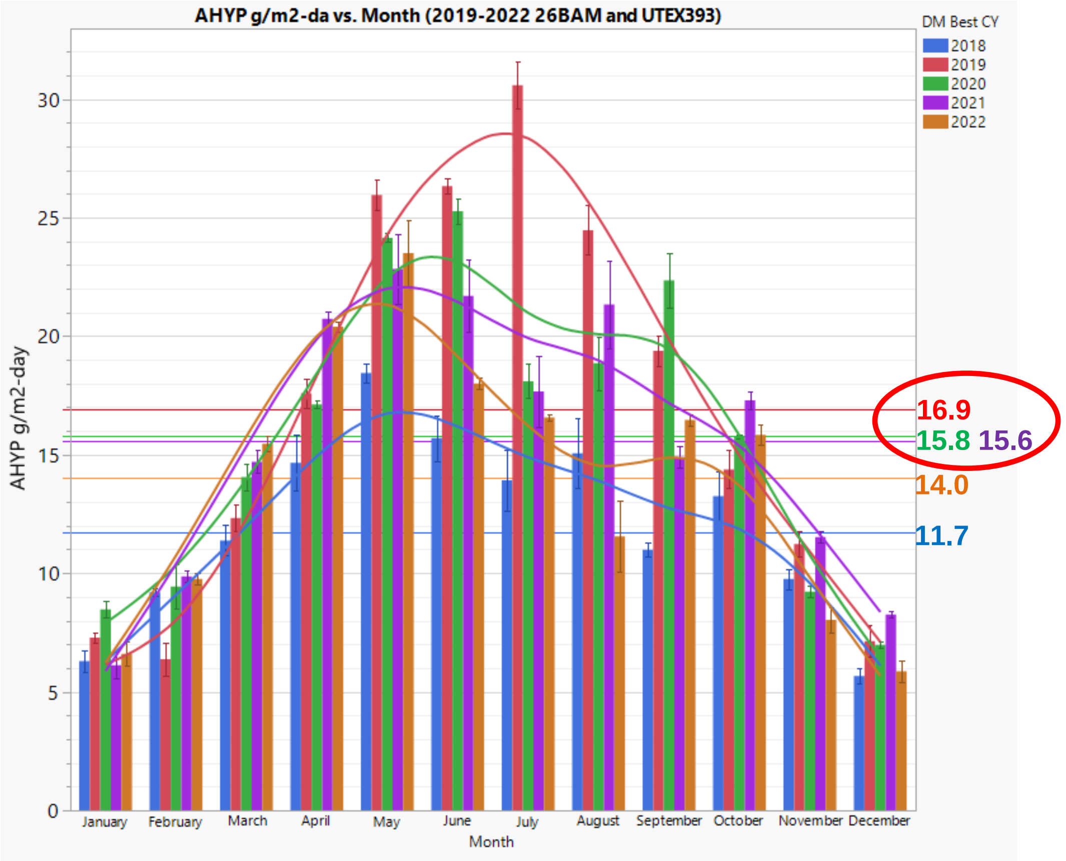
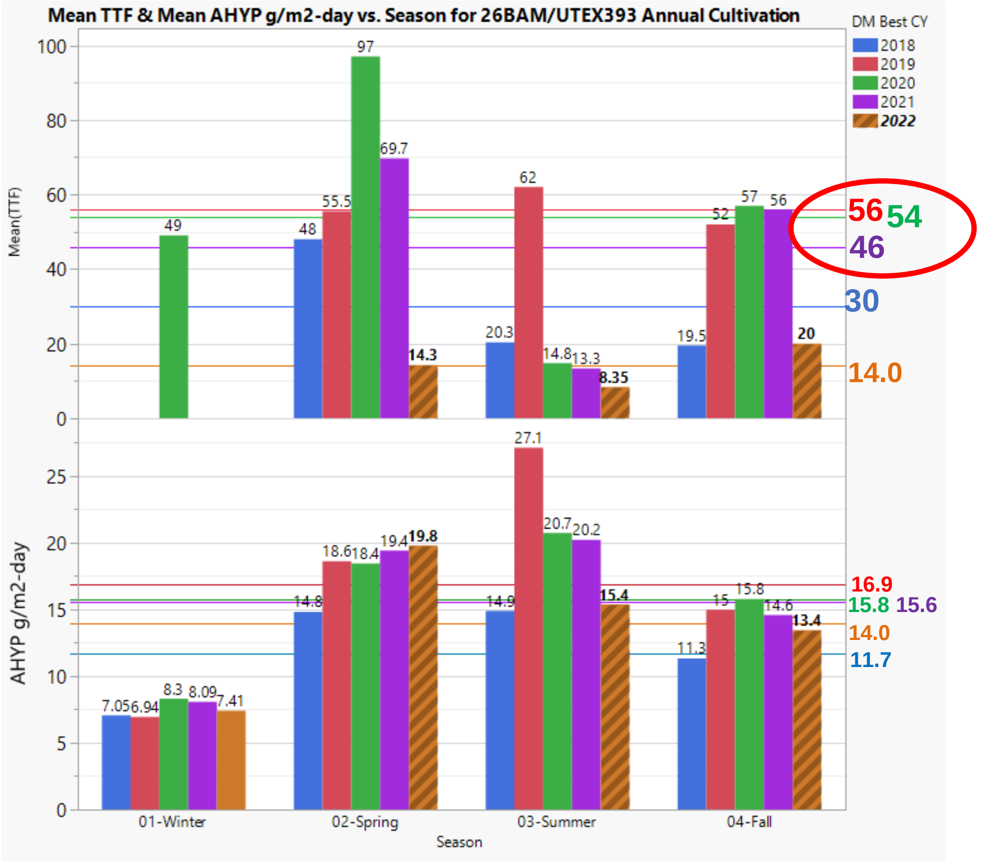
Progress and Outcomes: BP2 Go/No Go Decision Point

Go/No Go Decision Point 2 (GNG met November 2021): Demonstrate, with one or both operational modes, a minimum 50% improvement in MTTF from the 2018 baseline of **30 days to >/= 45 days**, while meeting or exceeding 2019 SOT productivity baseline for annual average of **15.9 g·m^{-2}·d^{-1}.**

Where did we end up?



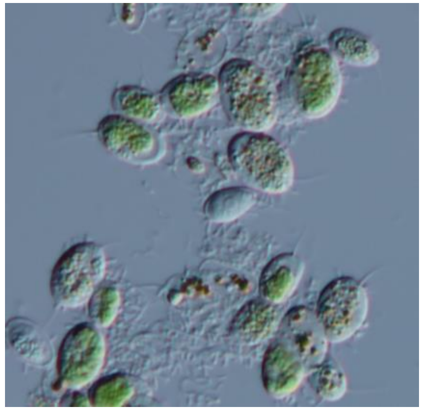
Each bar represents n=3 ponds. Error bars ±1 stdev from mean. Seasonal strain rotation with UTEX in warmer months and 26BAM in cooler months. 2019-2022 data represents full 30-day month productivity except summer months 2021 (minimum 2 weeks).



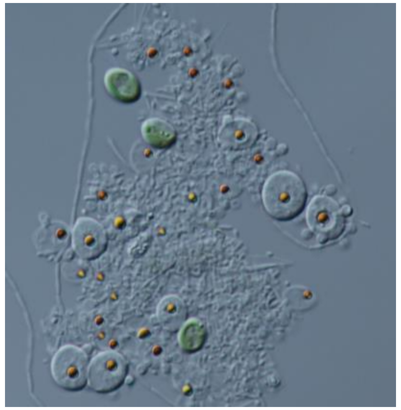
Large decline in TTF and productivity in 2022 warm months – we are heading in the wrong direction. New pest appeared in 2020 affecting UTEX393, and in late 2021 infecting 26BAM (both bacterial).

Progress and Outcomes: Task 1 Cultivation - Historical Pest Threats

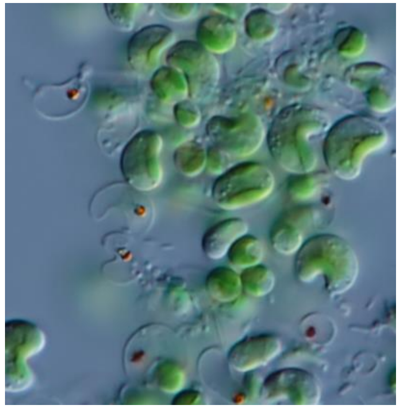
- **Since 2015, AzCATI has observed an increase in fungal parasitoids infecting multiple strains**
  - Freshwater to brackish to full marine media strains
  - Can decimate a culture in days
- **Host specific/obligate parasites**
  - require co-culture for pest models
- **Some fungal parasitoids identified and PCR/RT-PCR protocols for routine monitoring along with pest models established – it's a continuous cycle**
  - New/unidentified parasitoids appear, identified ones disappear and cycle starts anew for pest isolation/ ID/establishing pest model/mitigation/control
  - Lifecycle is understood, interrupting it? Not so much…
- **No effective control strategies up until 2019**
  - Fungicide can be very effective but *strain dependent* and *resistance* can build-up (evidence for this in 2022 with fluazinam) and many strains of interest *show significant growth inhibition* when exposed to fungicide – ***major challenge***
  - pH/dilution rate/salinity – shown to be not effective against aphelids
  - media composition (this needs more work)
  - Seasonally dependent – most active when warm but present and active all year and can crash cultures in winter if mitigation absent
  - Lots remains to be explored for effective mitigation of fungal parasitoids…



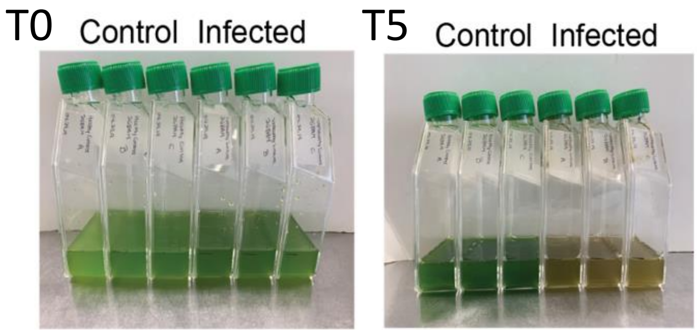
D. armatus infected with unkn. parasitoid



A. obliquus infected with aphelid (FD95)



M. minutum infected with aphelid (HG101)



Infection cycle with 26BAM and HG101 or FD01 aphelids

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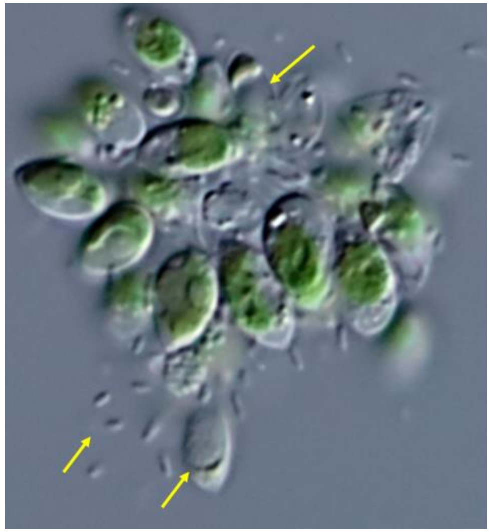
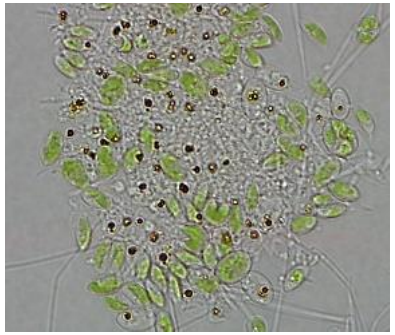
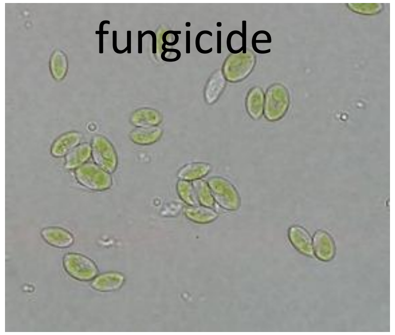
Integrated Pest Management: a continuous cycle

UTEX393 cultivated at AzCATI continuously since Fall 2018

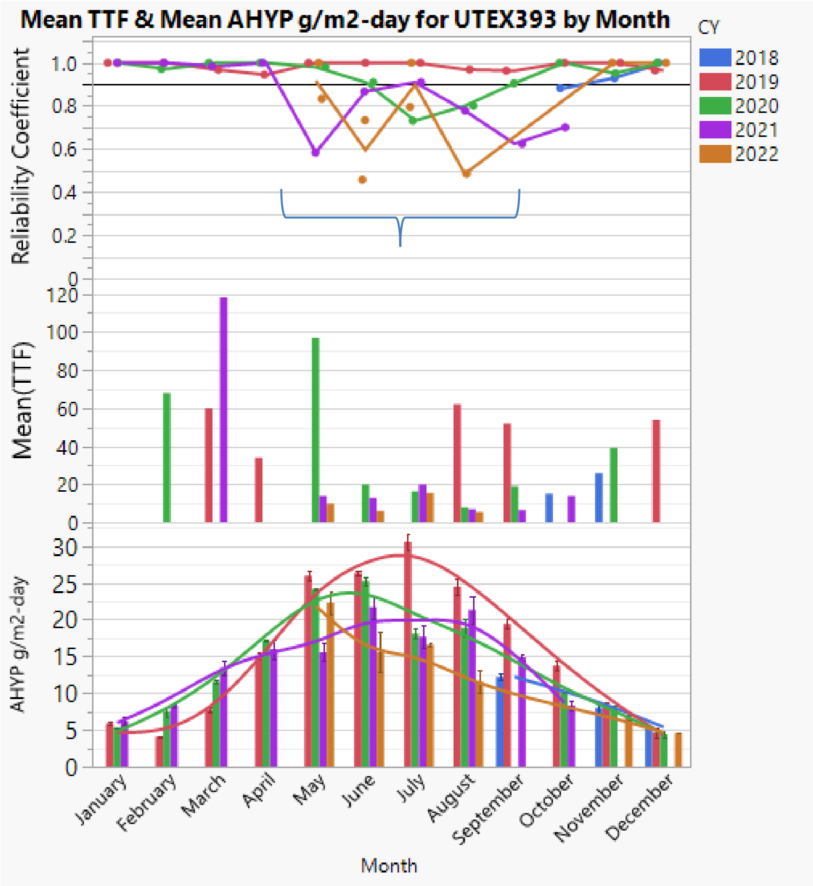
- Rapid culture crashes through Spring 2019
- Fungal parasitoid crash morphology - fungicide mitigation began spring 2019 with success increasing productivity and MTTF
- Highest productivity ever achieved to date with >25 g/m^2-day for 5 months (May-Sept 2019)
  - Increased time to failure (TTF) from 2018 SOT baseline of 30 days to 54 days to ~100 days through Spring 2020
- However, a new contaminant observed in Spring 2020 which looked like a predatory bacterium
- Dropped TTF from over 60 days to **<15 days** and lowered productivity in 2020 and 2021



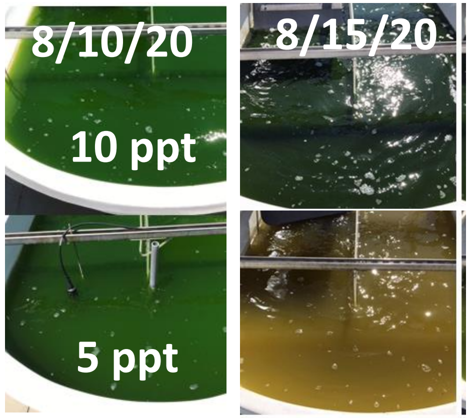




Pre-treatment Post-treatment



- Mitigation with salinity swings increased TTF from ~ 14 days>24 days but cultures still crashed

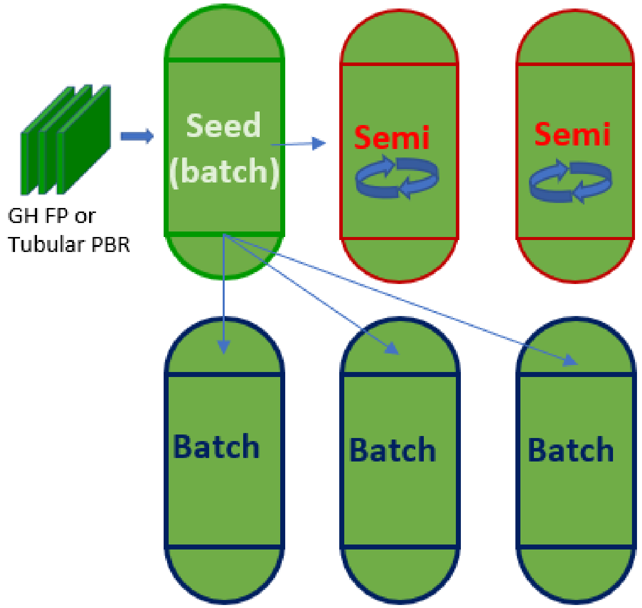


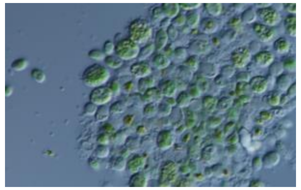
- Chlorine intervention showed mixed results but revisiting
- Pest confirmed as bacterial
  - Crash model established
  - Likely identity has been established (at genus level)
  - PCR/qPCR assays developed
- **Can batch cultivation help to mitigate the effects of this new pest?**

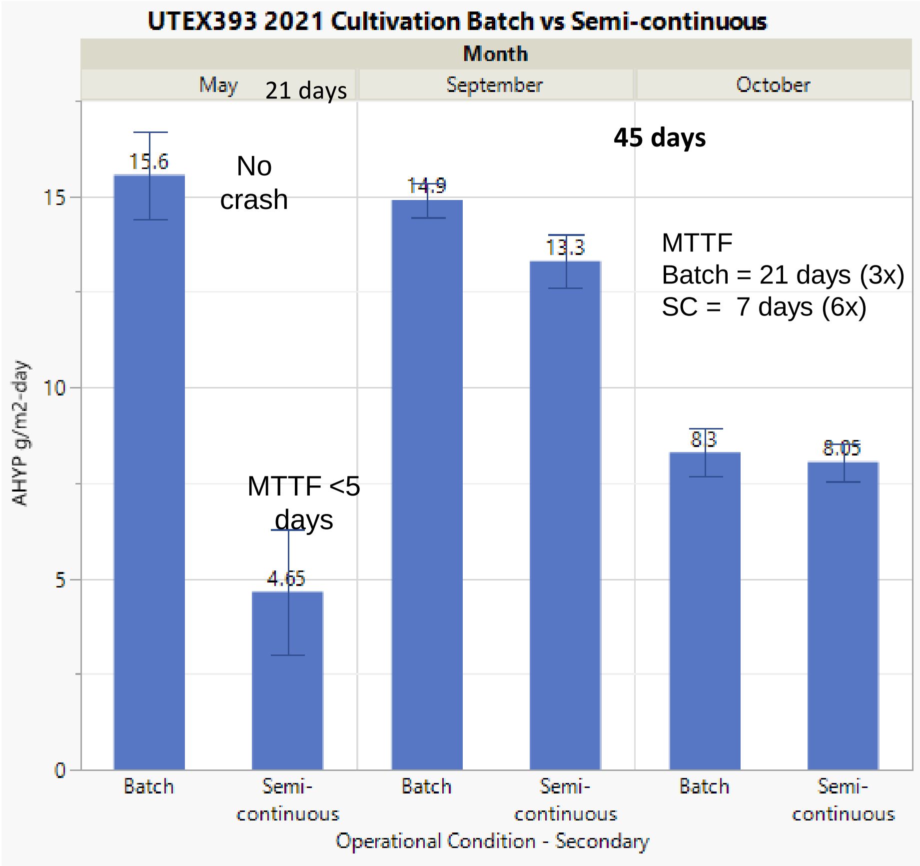
Progress and Outcomes: Task 1

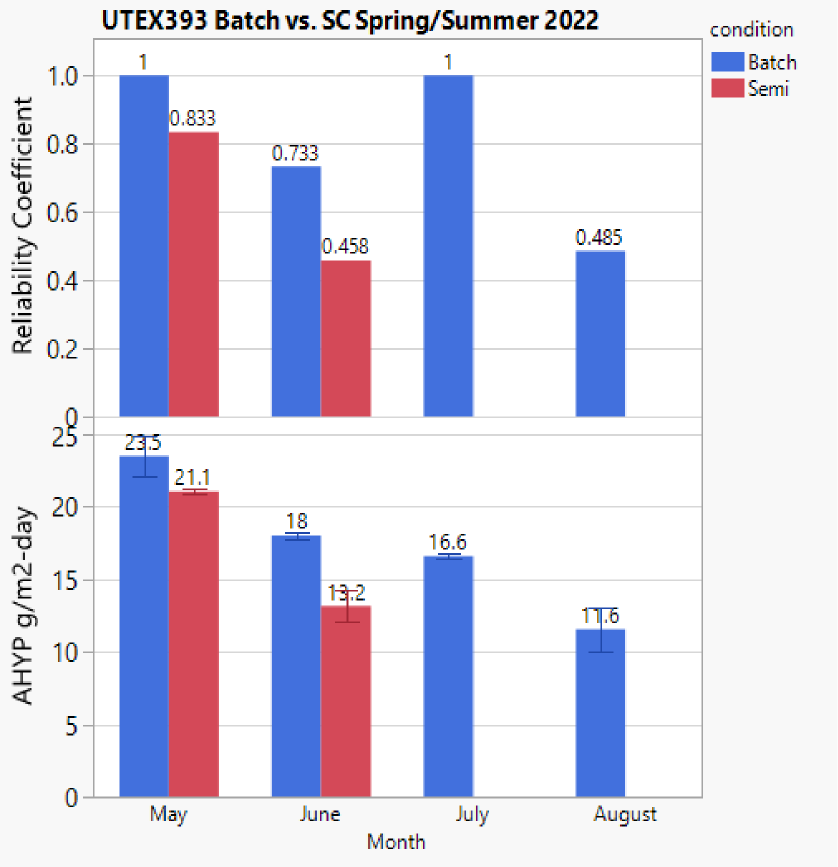
Subtask1.1 Batch vs Semi-cont. cultivation trials

Seed train strategy for batch vs. semicontinuous (SC): Can we avoid crashes and recover productivity (Batch vs SC)?







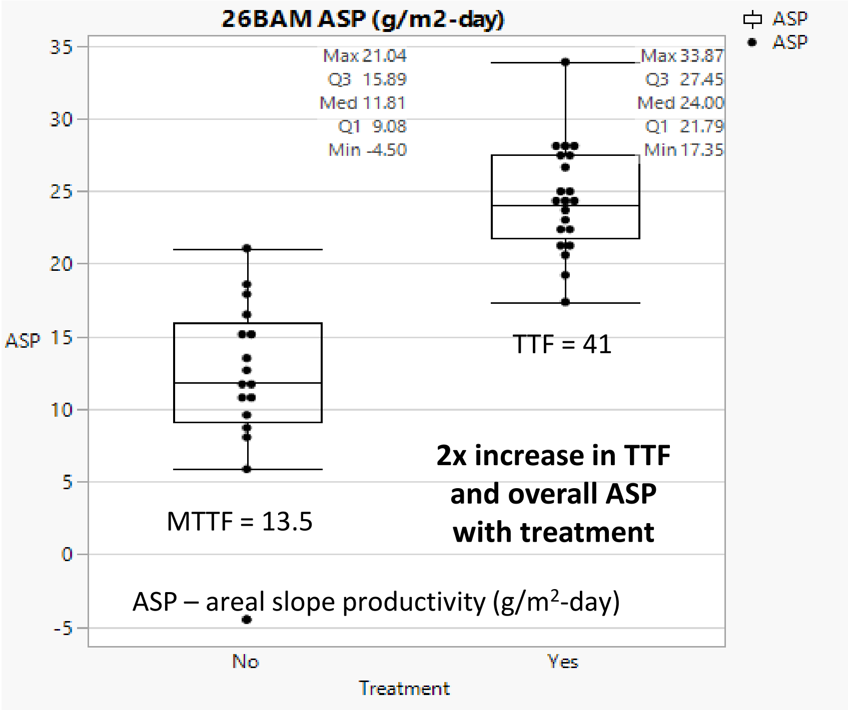


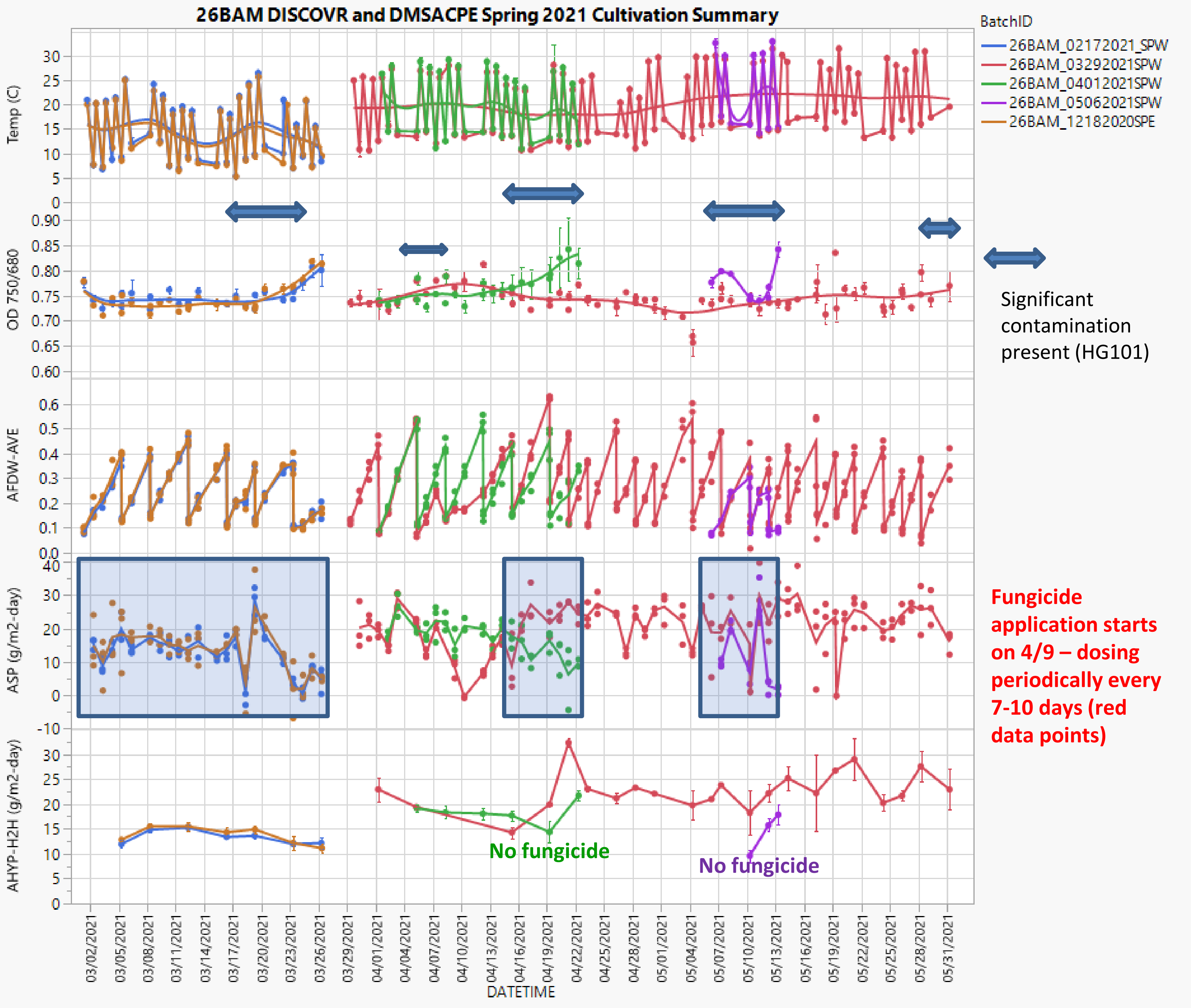
Contribution of biomass from seed ponds excluded

- When contamination pressure high, batch mode has repeatedly shown the ability to extend cultivation days relative to SC
- Demonstrated with both aphelid (not shown) and bacterial contamination in both strains). Typically observed a 20-100% increase in TTF, but bacterial contamination is present and active in both batch and SC and contributing to low productivity even if crash avoided, and by August, crashes not avoided

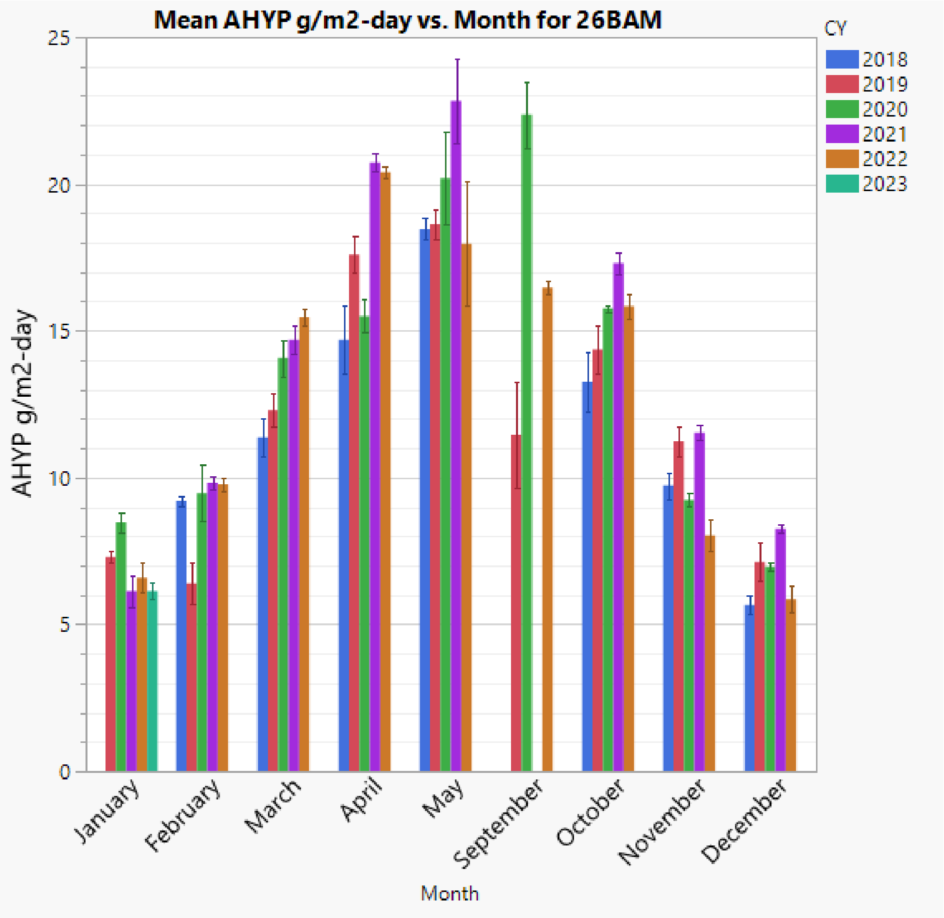
Progress and Outcomes: Task 1.2
Mitigating parasitoid (aphelid) contamination in 26BAM

- 26BAM - top brackish cool weather strain
- Two primary aphelid contaminants observed
  - *A. occidental* matching a Sapphire identified aphelid (FD01)
  - *apheldia* related species (designated HG101)
- Contamination pressure increases as temperatures increase
- Spring 2021 evaluated ASP and TTF as a function of (all semicontinuous):
  - age of culture (early March) – no difference
  - fungicide application (April-May)





Effective chemical treatment regime increased productivity



- Steady increases in productivity year over year for 26BAM
- Aphelids primary source of contamination for 26BAM through 2021.
- Main driver for increased productivity and MTTF was optimization of fungicide application beginning Fall 2020
- Two factors maybe in play for decreased 2022 performance:
  - Early indicators that aphelids may be gaining some pesticide resistance
  - New predatory bacterial contaminant drove down productivity of 26BAM in 2022 (like that affecting UTEX393 since 2020).
  - Prior to late fall 2021, no confirmed bacterial caused crashes observed prior on site in 26BAM cultures

26BAM Seasonal Harvest Productivity g/m²-day

Season	2018	2019	2020	2021	2022	% Increase 2018-2022
Winter	7.4*	6.9	8.3	8.1	7.4	0% (9%)
Spring	14.8	16.2	16.6	19.4	18	22% (31%)
Fall	11.5*	12.4	12.5	14.4*	13.4	17% (25%)
3 Season Avg.	11.3	11.8	13.6	14	12.9	14% (24%)

* Indicates <3 months for season

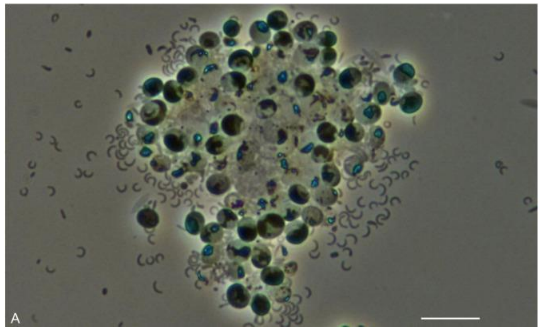
26BAM MTTF Seasonal Summary

Season	2018	2019	2020	2021	2022	% Increase 2018-2021 (2022)
Winter	NC	NC	49	NC	NC	N/A
Spring	48	55	112	70	17	46% (-64%)
Fall	23	21	57	56	20	150% (-13%)

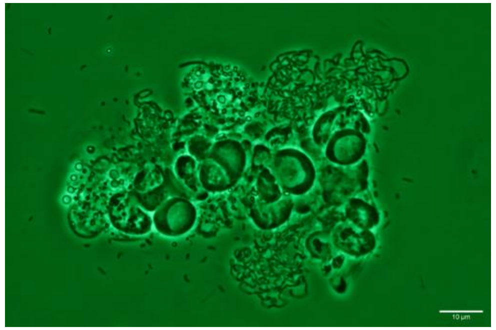
Progress and Outcomes: Task 1.2 IPM – Identification and monitoring of new pest threats

- As contaminants identified, PCR assays established for tracking
- Since 2019, actively monitoring via PCR for known fungal parasitoids (mainly aphelids)
- First confirmed predatory bacterial crash in UTEX393 June 2020 and *D. armatus* in September 2020
    - Crash morphology like that reported by Sapphire (Lee et al 2018) for *Nanochlorpsis* sp. infected with a predatory bacteria (FD111) and described as a *Bdellovibrio and like-organisms* (BALO)
    - Using reported PCR primers for FD111, PCR product obtained but not at expected size
    - Sequenced via 16S and it was 96% identical FD111 – became routine proxy while working to isolate/identify AzCATI bacterial pest

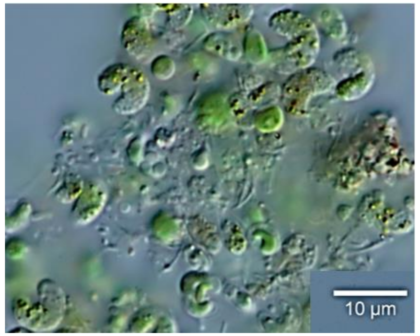
Cells burst from coverslip with “sausage” spilling out



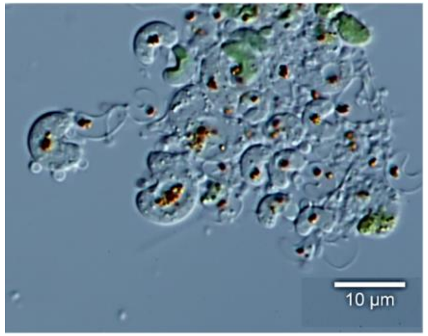
FD111 – Nannochloropsis sp.
From Lee et al



FD111-like positive UTEX393

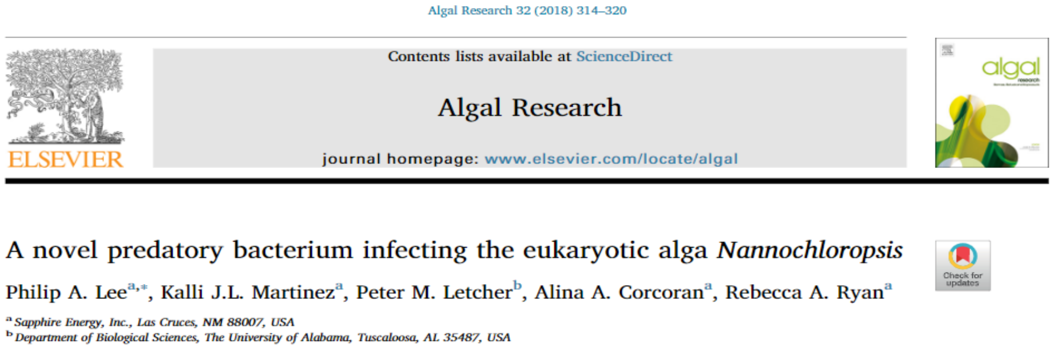


“FD11-like” infected
26BAM (SPW24)



FD01 infected
26BAM (SPW26)

- In Fall 2021, predatory bacteria detected via PCR in samples of 26BAM that had crashed from aphelid infection (HG101). Spring 2022, full on bacterial crashes independent of aphelid infection observed


Algal Research 32 (2018) 314–320

Contents lists available at ScienceDirect

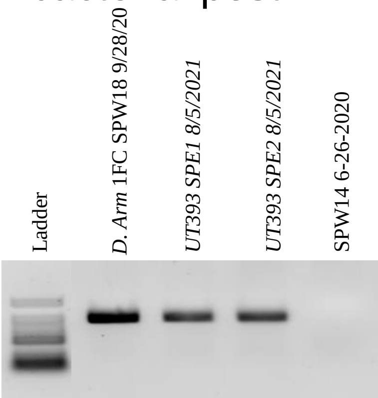
Algal Research

journal homepage: www.elsevier.com/locate/algal

A novel predatory bacterium infecting the eukaryotic alga *Nannochloropsis*

Philip A. Lee[a,*], Kalli J.L. Martinez[a], Peter M. Letcher[b], Alina A. Corcoran[a], Rebecca A. Ryan[a]

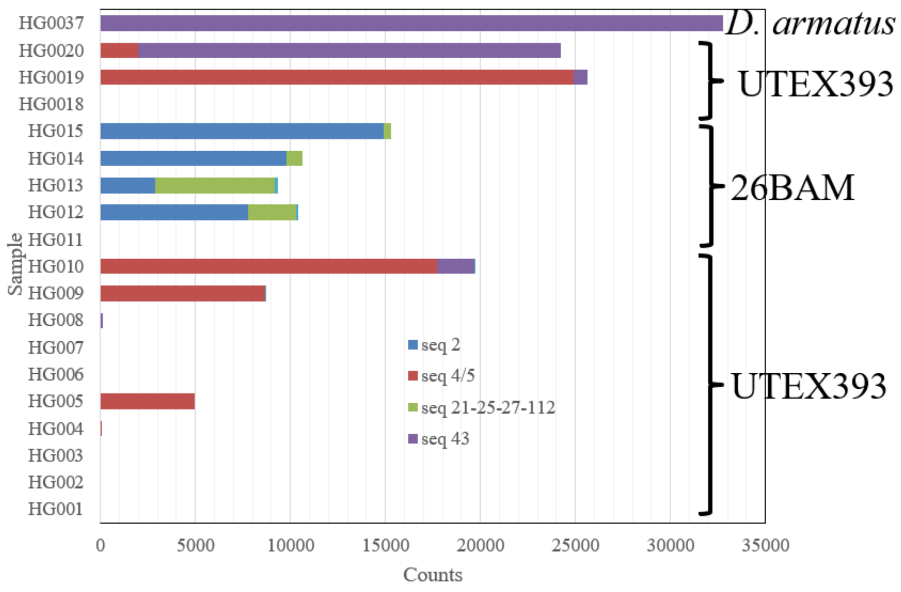
[a] Sapphire Energy, Inc., Las Cruces, NM 88007, USA
[b] Department of Biological Sciences, The University of Alabama, Tuscaloosa, AL 35487, USA



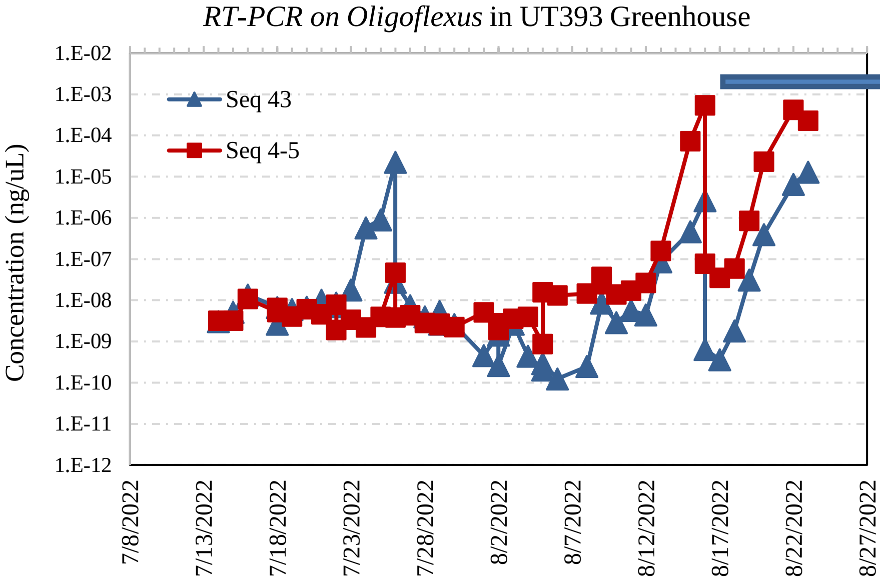
FD111 primers 3F and 2R

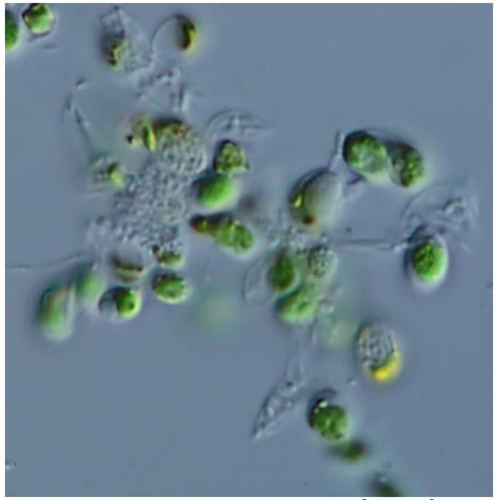
Progress and Outcomes: Task 1.2 IPM – Identification and monitoring of new pest threats

- To aid in identification of new bacterial contaminant(s) a series of samples sent for NextGen Sequencing (NGS: Zymogen 16S, 18S, and ITS.
- From NGS on crashed samples, the most abundant sequences were from the class *Oligoflexia* within the phylum *Proteobacteria* which contains the orders *Bacteriovoracales*, *Bdellovibrionales*, *Oligoflexales,* and *Silvanigrellales*
- *A* set of 4 sequences identified from *Oligeflexia* with highest abundance (2 more abundant in 26BAM samples and 2 more abundant in UTEX393 samples)

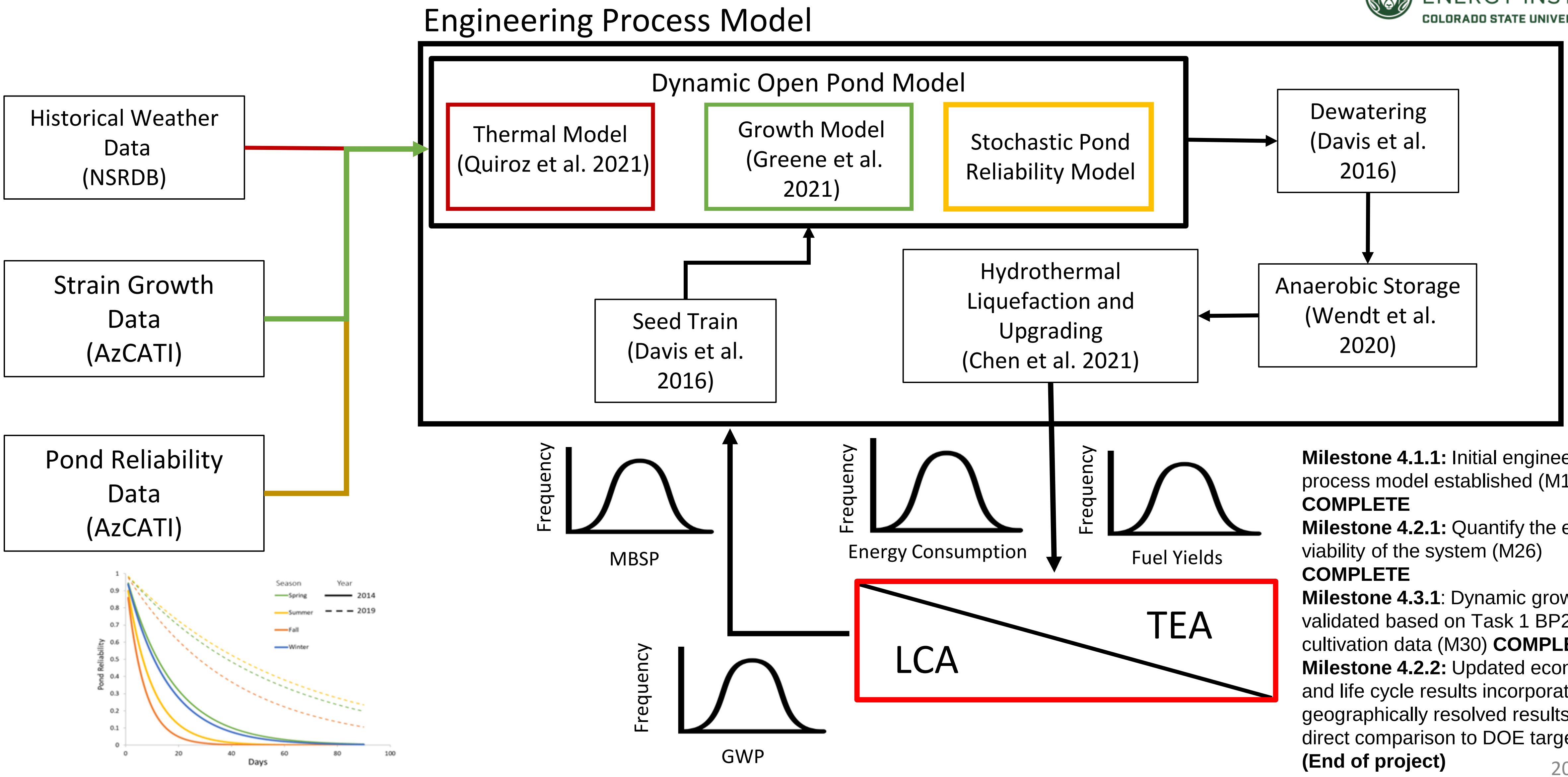


- PCR/qPCR primers designed from NGS data verified trends
- qPCR assays also established for aphelids (FD01 and HG101)




UTEX393 from GH 8/16/2022

Progress and Outcomes: Task 4 Modeling Framework



Milestone 4.1.1: Initial engineering process model established (M12) **COMPLETE**
Milestone 4.2.1: Quantify the economic viability of the system (M26) **COMPLETE**
Milestone 4.3.1: Dynamic growth model validated based on Task 1 BP2 cultivation data (M30) **COMPLETE**
Milestone 4.2.2: Updated economic and life cycle results incorporating geographically resolved results with a direct comparison to DOE targets **(End of project)**

Progress and Outcomes: Task 4

Sustainability assessment - coupled TEA/LCA: Methods – Simulation Framework

Thermal Inputs

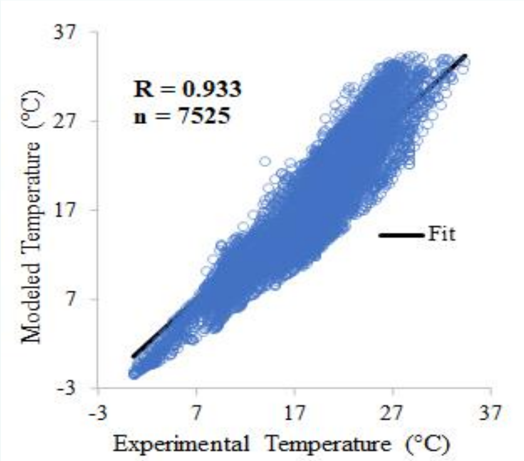
Meteorological Data
- NSRDB (21 years)
- TMY3

Facility Size
- 400-hectares
- 4000-hectares

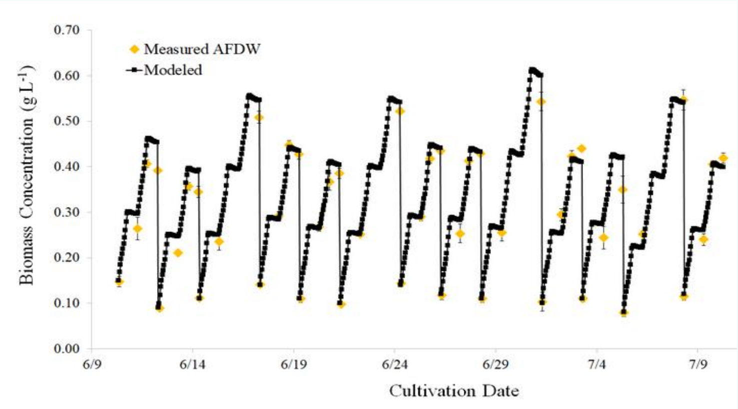
Biological Inputs

UTEX 393
Strain Characterization

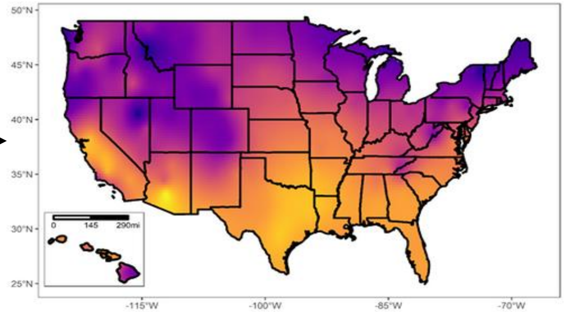
Harvesting scheme



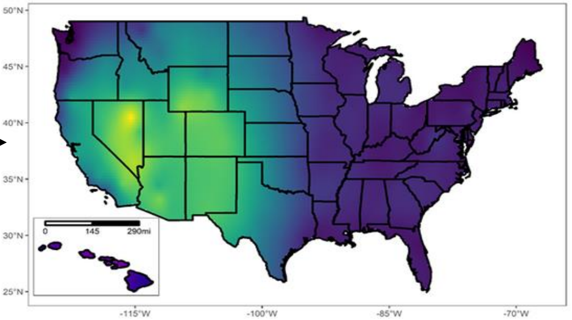
Validated Thermal and Biological Model



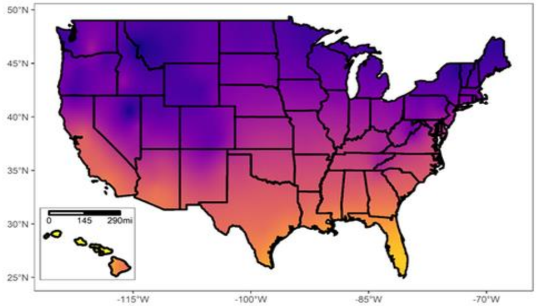
Temperature Tolerance Map



Water Demand Map



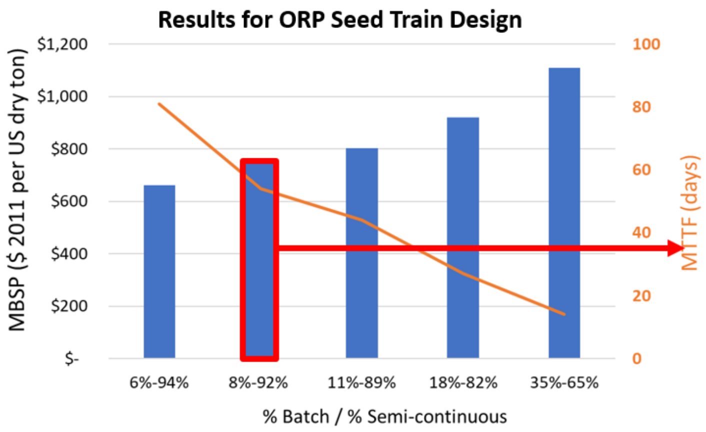
Areal Productivity Map

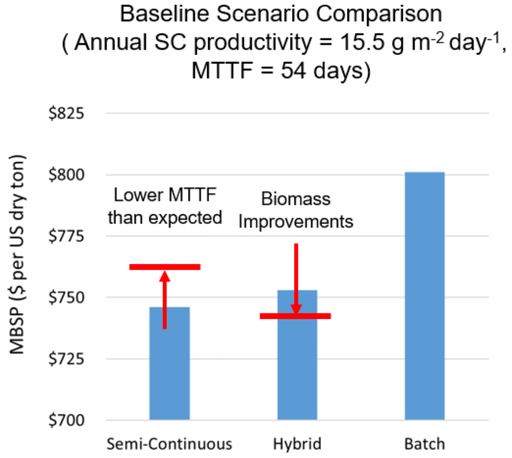


Progress and Outcomes: Task 4 Sustainability assessment - coupled techno-economic and life cycle modeling

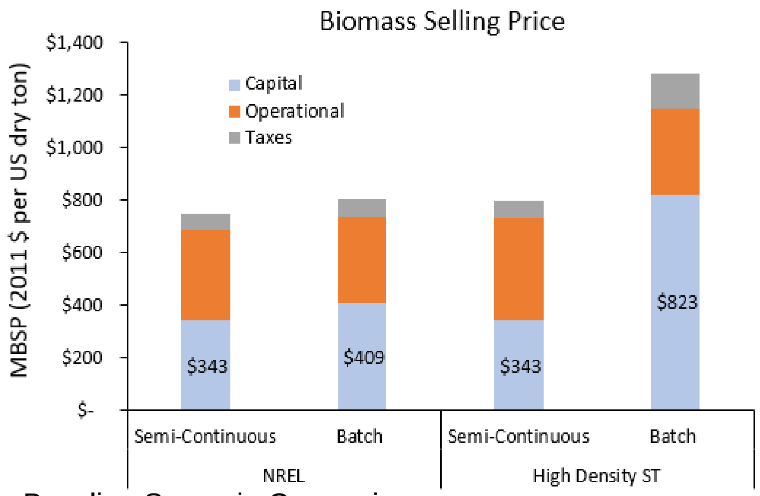
AzCATI
Arizona Center for Algae Technology and Innovation

- Despite higher productivities, high density seed trains economically unviable for inoculating batch scale facilities
- Biomass selling price of semi-continuous cultivation is highly sensitive to the mean time to failure metric.
  - MBSP ($1,106 – $704)
  - MTTF (14 – 65 days)

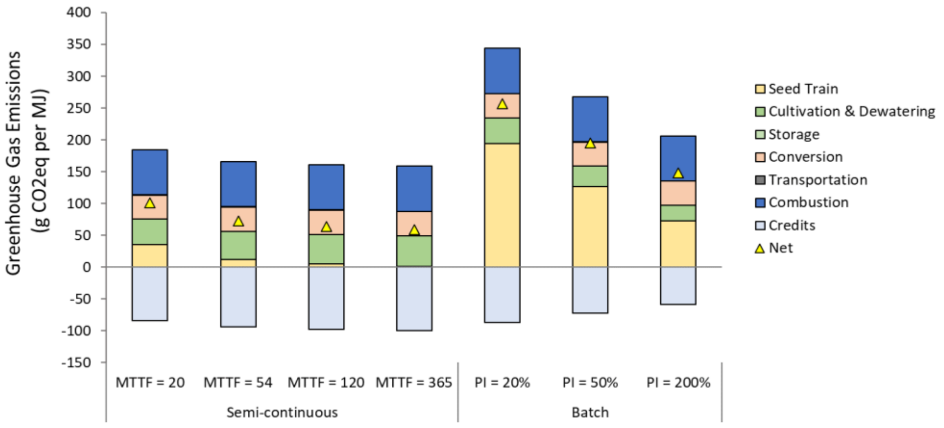




- A hybrid semi-continuous and batch facility could potentially compete economically with semi-continuous only facilities and reduce risks of complete shutdown.



Baseline Scenario Comparison:
Annual SC productivity = 15.5 g m^{-2} day^{-1} and MTTF = 54 days



- Semi-continuous cultivation presents lower MBSP and environmental impacts than batch

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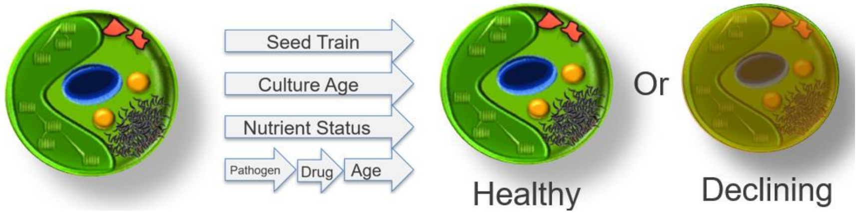
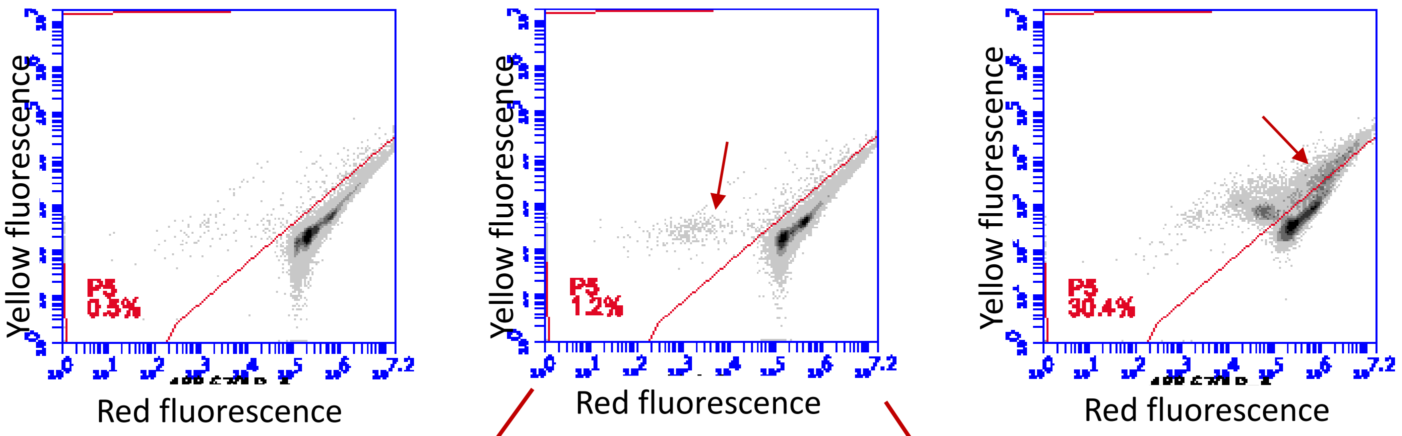
COLORADO STATE UNIVERSITY

Subtask 2.1: Whole culture and single cell phenotyping

- Track various macromolecules and organelles, as well as specific cellular activities/function
- Can be applied to laboratory cultures (ePBR) as well as to samples taken from outdoors
- Analyzed various indoor and outdoor samples including time course series of infection, mitigation and progression of crash events
- Using flow cytometry looked at various intrinsic and physiological stains

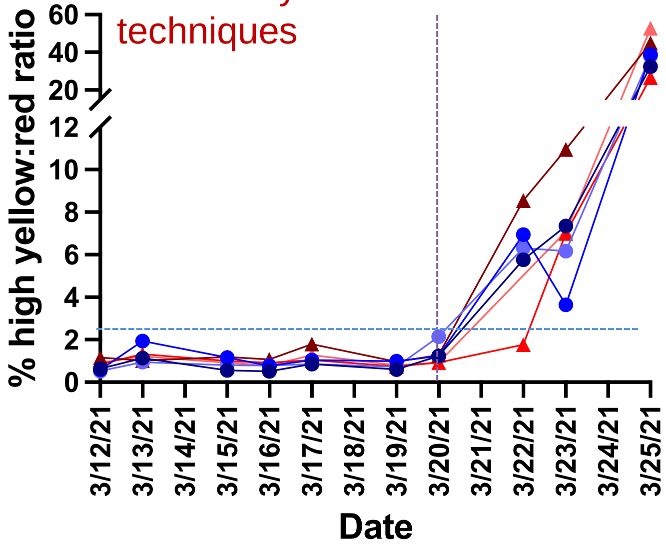


Outdoor cultivation of 26BAM with fungicide intervention



Aphelid infection noted cultures dosed with fluazinam

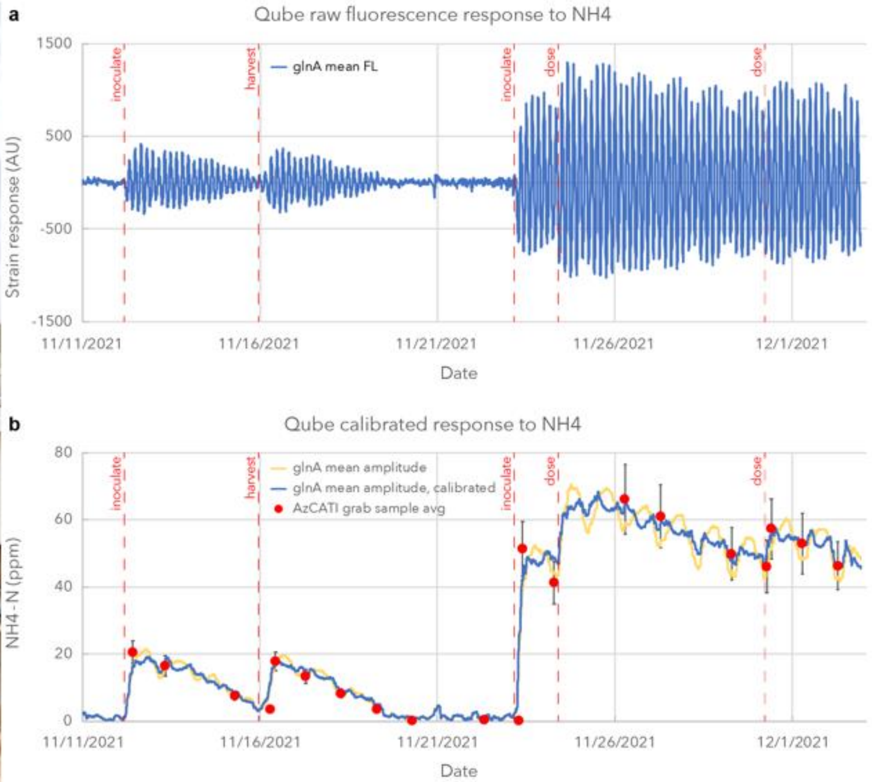
While progression of infection is easily detectable and quantifiable via flow, it does not identify infection earlier than other techniques



AzCATI Arizona Center for Algae Technology and Innovation

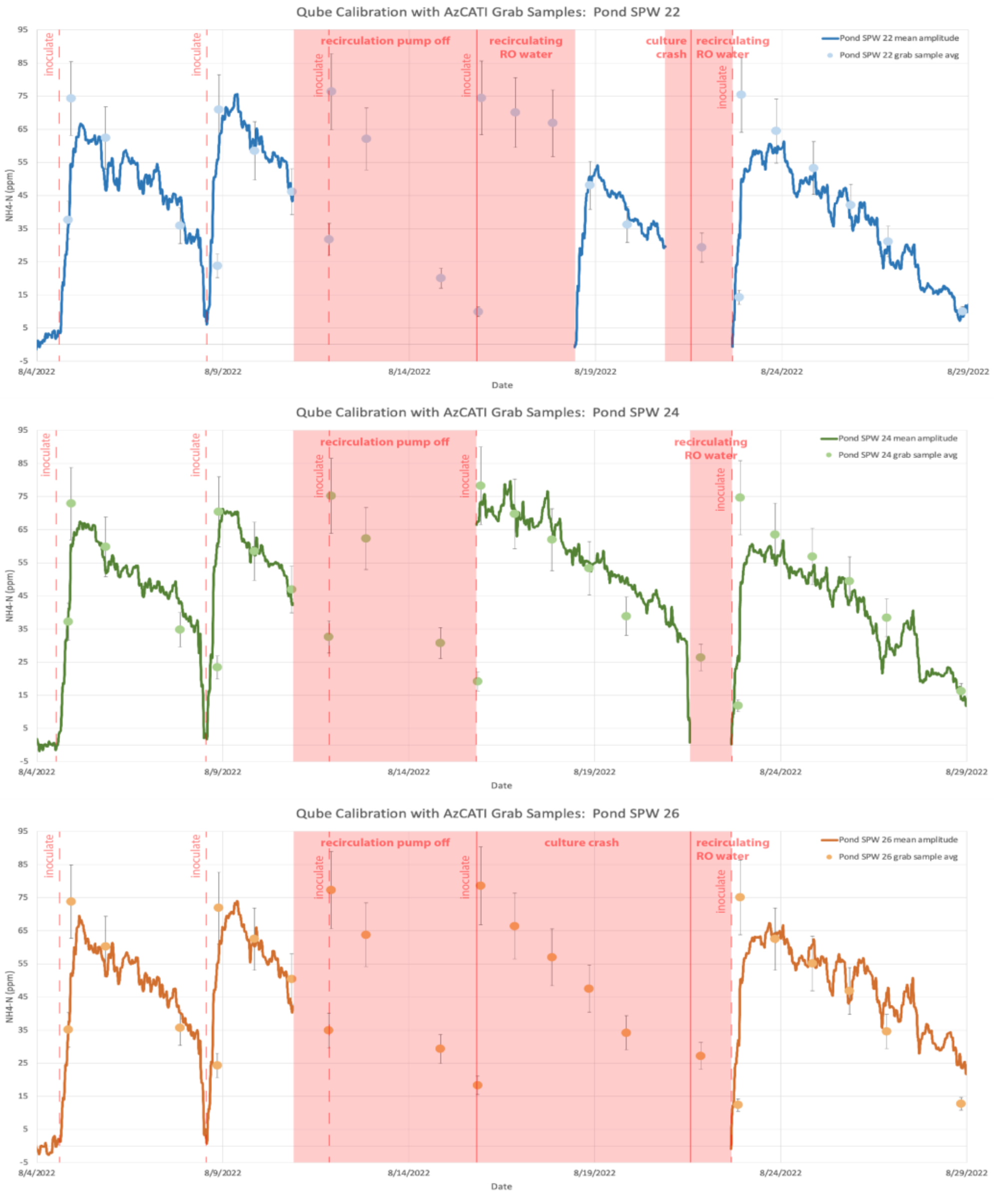
QBI Quantitative BioSciences, Inc.

Progress and Outcomes: Subtask 3.2 Realtime Nutrient Monitoring



- Initial deployment of the Qube: insulated weatherproof shell with integrated fluid handling system with recirculation loop between one outdoor pond and the Qube
- Continuously measured NH_4 in a *Scenedesmus obliquus* (UTEX 393) culture in BG-11 (5 ppt salinity)
- Qube data tracked pond events (dashed red lines), including inoculation, harvesting, and nutrient dosing
- Calibrated Qube data (blue line) matches triplicate standard nutrient analysis (Hach ammonia kit) grab sample data (red circles) within 15% accuracy (error bars) meeting target performance milestones in BP2

Progress and Outcomes: Subtask 3.2 Realtime Nutrient Monitoring



- Pond recirculation downtimes are shaded red

- Qube fluidics and microfluidic chip housing the biosensor strain were reworked to ***monitor 3 ponds in parallel***
- Hardware improvements identified during initial deployments and implemented in BP3
  - Installed Conditioner Module (CM) between each Pond and the Qube (filters, dilutes, and degasses culture)
  - Improved fluidics pumping accuracy and robustness to diurnal changes in culture
  - Remote camera for real-time status remote observations of system physical performance
- Continuously measured NH_4 in a *Scenedesmus obliquus* (UTEX 393) culture ***over 25 days in 3 ponds in parallel***
- Qube data tracks pond events (dashed red lines), including inoculation, harvesting, and nutrient dosing
- Calibrated Qube data (colored lines) matches triplicate Hach grab sample data (colored circles) within 15% accuracy (error bars)
- Remaining milestones before end of project including testing phosphate chip and establish proof of concept for feedback control of nutrient addition

Impact

- Generated data from different cultivation strategies tested side by side under relevant outdoor conditions or quantification of technical, economic and sustainability risks and impacts
- Data will be widely disseminated through an open source platform
- Robust modeling framework with validated dynamic biomass growth model
- 100% Batch cultivation is cost prohibitive without significant gains in productivity for batch cultivation relative to semi-continuous, though a hybrid semi-continuous and batch facility could potentially compete economically with semi-continuous only facilities while reducing risks of complete shutdown.
- Identifying and implementing IPM best practices allowing for high-productivity and robustness under semicontinuous conditions remains a (THE) key goal for cost effective algae cultivation at scale
- Two novel sensor platforms deployed with significant cycles of learning and improvements from actual field-deployed environment – valuable development progression towards more robust product platforms and gaining new insight into culture health in realtime
- Novel pest identified including an aphelid parasitoid (HG101) and potentially several host specific strains of *Oligoflexia* that have proven especially aggressive with two top performing cultivars within BETOS AAS portfolio. Pest models established as well as tools such as qPCR for effective tracking and to aid in development of new mitigation approaches

Summary

- We **quantified the economic and technical risks** associated with different cultivation strategies and crop protection approaches through **an integrated program of indoor lab studies, cultivation optimization and simulation, and robust outdoor cultivation campaigns**
- Developed and deployed of a suite of **novel real-time sensors** for **nutrient and water quality** monitoring with commercial potential, improved process monitoring and control, and pathways identified to reducing costs relative to other real-time sensors platforms.
- **Established multiple pest models** including amoeba, aphelid (2), and bacterial (4)
- Established **PCR and RT-PCR assays for routine monitoring of known contaminants** and to guide identification and development of additional mitigation strategies
- 96-well plate-based techniques for culture health monitoring offer promise streamlining data collection from production ponds but for the assays explored, they did not offer ability to detect contamination or declines in culture health earlier than standard observations such as microscopy but do offer opportunity for increased sample analysis throughput, though cost prohibitive in a production environment
- Generated novel, high-quality, publicly available cultivation datasets a simple set of python libraries to easily access AzCATI datasets pre-merged and structured for modern data-science applications, automated analysis APIs, and controlling experiments.

Quad Chart Overview

Timeline
- May 2020-December 2021 (BP2 target start/end)
  - Formal contracting not completed until October 2020
  - Limited at-risk spending in FY20
- January 2022-September 2023 (BP3 start/end)

	FY Spend to date Costed	Total Award
DOE Funding	$2,950,000	$3,500,000
Project Cost Share	$735,000	$875,000

Funding Mechanism
DE-FOA-002029 FY19 BETO Multitopic FOA
Topic Area of Interest 1: Cultivation Intensification Processes for Algae

Project Partners*
- Los Alamos National Laboratory
- Colorado State University (TEA/LCA/process modeling)
- Burge Environmental (novel sensor development)
- Quantitative Biosciences Inc. (novel sensor development)
- Diversified Technology (crop protection)

Project Goal:
Assess the cost-benefit tradeoff of enhanced "crash-recovery" routes and their impact on biomass productivity and quality and thus economic impact on biomass and biofuel production costs.

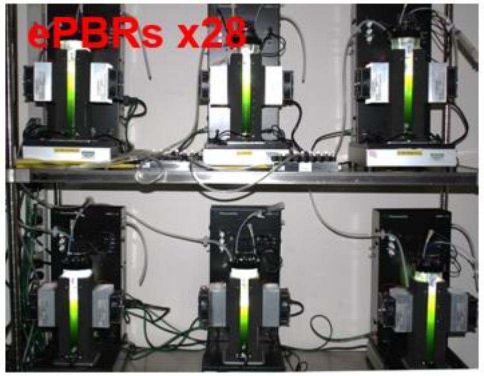
Mid Project Go/No Go:
Increase mean time to failure from 2018 baseline of 30 days to **>/= 45 days**, while meeting or exceeding 2019 SOT productivity baseline for annual average of **15.9 g·m^{-2}·d^{-1}** (November 2021) **– Go/No Go Met**

End of Project Goal:
Demonstrate, with one or both operational modes, a minimum **100% improvement in seasonal MTTF from 2018 baseline of 20 to >= 40 days for Spring and Summer**, while meeting or exceeding a **50% improvement** over 2018 SOT productivity baseline (**summer and fall seasonal target is 21.8 and 17.0 g/m2-day**, respectively). Achieve compositional improvements of at least 20% to allow a modeled HTL conversion yield of at least **80 gge.**

DMSACPE Team Members

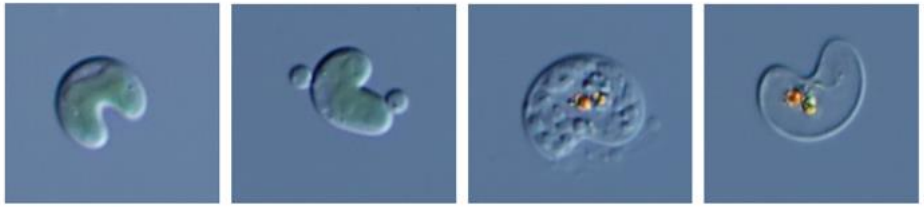
Task 1 ASU Team
John McGowen (PI), Peter Lammers (Co-PI),
Jessica Forrester, Jason Potts, Emilie Smith, Pedro Caballero
Richard Malloy, Nick Murray

Task 1 DTI
Mike Kempkes



Task 2 ASU Team
Taylor Weiss (Co-PI),
Henri Gerken, Mauricio Gonzalez, Aaron Geels, Raafay Jafri

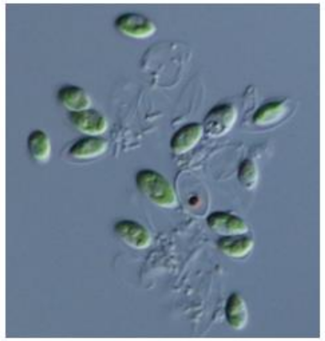
Task 2 LANL Team
Taraka Dale (Co-PI) Claire Sanders (Co-PI), Carol "Kay" Carr

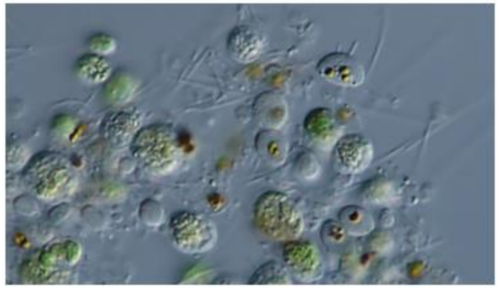


Task 3 Burge Env.
Evan Taylor (Co-PI), Brian Ford, Dave Baker, Chad Ripley, Scott Burge

Task 3 QBI
Natalie Cookson (Co-PI), Michael Ferry, Scott Cookson

Task 4 CSU
David Quiroz, Jason Quinn (Co-PI)





Additional Slides: Dissemination of Project Results

Publications, Presentations

1. *"Geographical assessment of algal productivity and water intensity across the United States."* D. Quiroz, J.M. Greene, J. McGowen, J.C. Quinn., *Algal Research*, 2021, 102483, DOI: 10.1016/j.algal.2021.102483.
2. *Regionalized Life-Cycle Water Impacts of Microalgal-Based Biofuels in the United States* David Quiroz, Jonah M. Greene, and Jason C. Quinn. *Environmental Science & Technology* 2022 *56* (22), 16400-16409 DOI: 10.1021/acs.est.2c05552
3. *"Biodiversity and disease risk in an algal biofuel system: An experimental test in outdoor ponds using a before-after-control-impact (BACI) design"* Spenser L. Widin, Kia M. Billings, John McGowen, Bradley J. Cardinale. *Plos One 2022,* doi.org/10.1371/journal.pone.0267674
4. *"Cultivation reliability and its impact on the economics and sustainability for algae-based products. What data is needed?"* McGowen, J., invited talk, presented at Algae Biomass Summit, September 2020.
5. "*Economics and Optimization of Inoculum Systems Operations" Quiroz, D. and Quinn, J. presented at the Algae Biomass Summit, September 17, 2020.*
6. *"Improving Decision Making in Day-to-Day Algae Cultivation: Quantifying and Managing Risks to Increase Product Yield" John McGowen, Presented at ABO Conference October 2021*
7. *"A Geographical and Temporal Assessment of the Water Requirements and Temperature Tolerances for Large-Scale Cultivation of Microalgae"* Quiroz, D. and Quinn, Presented at Algal Biomass, Biofuels and Bioproducts Conference, June 2021
8. *"A Geographical and Temporal Assessment of the Water Requirements for Large-Scale Cultivation of Microalgae"* Quiroz, D. and Quinn, J. Abstract accepted for oral presentation at the International Symposium of Sustainable Systems and Technology, June 2021
9. ***"****Techno-economic analysis of a combined semi-continuous and batch cultivation platform incorporating seed train economics"* Quiroz, D. and Quinn, J. Abstract accepted to the Algae Biomass Summit, September 2021
10. *"Geographically and temporally resolved thermal evaluation of commercial-scale open raceway ponds"* Quiroz, D. and Quinn, J. Abstract accepted to the Algae Biomass Summit, September 2021
11. *"Geographical water footprint analysis of algal systems compared to conventional biomass feedstocks"* Quiroz, D. and Quinn, J. Abstract accepted to the Algae Biomass Summit, September 2021
12. *Algae Cultivation at the Arizona Center for Algae Technology and Innovation – Generating Data to Support Algal TEA/LCA and the Broader Modeling Community" John McGowen presented at* Algae Biomass Summit , October 2022.
13. *"Sustainability assessment of semi-continuous and batch cultivation of algal biomass"* Quiroz, D. and Quinn, J., McGowen, J., Weiss, T. Lammers, P. Algae Biomass Summit , October 2022.
14. *"Algae Cultivation at the Arizona Center for Algae Technology and Innovation – Generating Data to Support Algal TEA/LCA and the Broader Modeling Community"* McGowen, J. Algae Biomass Summit, October 2022.
15. *"Fungal and Bacterial Parasites: Characterization, Identification and Effect on Algal Productivity in Outdoor Cultivation*" John McGowen, Henri Gerken, Aaron Geels, Raafay Jafri, accepted for poster presentation Algal Biomass, Biofuels and Bioproducts Conference, June 2023

Additional Slides

- **Responses to Previous Reviewers' Comments:**
- It appears that the project may be diverging away from its original project goal and end of project milestone. The summaries of progress that are presented seem to reflect the use of some different methods and techniques to achieve results. If so, these types of benefitting adjustments are suitable when the are made in efforts to improve the project and better reflect BETO's goals. It is important that the concept of crop insurance begins to be explored and addressed. The basic steps of assembling monitoring and decision data, the methods of its collection, and the application of predictive and probabilistic techniques is a good place to start.
  - *Response: Our overall project task structure, milestones and end of project goal remain as agreed upon with BETO. We are continuing to explore the regulatory space for use of different agents for crop protection and a slide discussing this task is in the supplemental slides. Our project team has participated in two public panels, one on crop insurance as part of the Algae Biomass Summit in fall of 2020 and a BETO sponsored workshop on crop protection in spring of 2021. Our project team plans to engage appropriate stakeholders and share our data on risk and cultivation failure modeling broadly and believe it will be a key, foundational data framework for algae crop insurance.*

- TEA outputs will be a critical measur of any contamination control strategy. Use of the NREL TEA or compatibility with it allows for the most direct comparisons of innovations made in this project to the SOT. The project addresses one of the least well understood risks to algae biofuel development in a way that can be a foundation for iterative improvement. Direct comparisons of different cultivation strategies are rare and TEA outputs are most useful for comparing different strategies as opposed to determining real world value of investments in algae cultivation. These new inputs will strengthen the TEA as a comparative tool and help highlight other work like this that can be done to reduce risk without creating new inventions.
  - *Response: ASU/AzCATI believe in open collaboration across our project portfolio and we are sharing data, samples, pest models, etc. very broadly within the BETO stakeholder community. Our project partners for this project, in particular AzCATI and LANL are also partners with both Sandia and NREL on DISCOVR and CSU is building the TEA/LCA with the NREL Farm Model as its basis to allow seamless exchange of findings.*

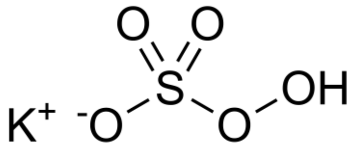
Progress and Outcomes: Task 1

Subtask1.2.1 Chemical treatments and media optimization to control pests

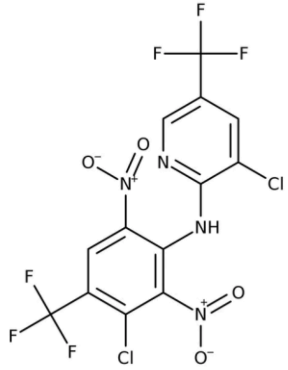
Control Agents: Batch vs. Semi-Continuous

- Sterilizing biocides kill microorganisms non-specifically, may offer greater utility in batch-modes of operation, and are **not** regulated as pesticides
- Pesticides which target specific organisms, offer greater utility in semi-continuous modes of operation, and are **strictly** regulated

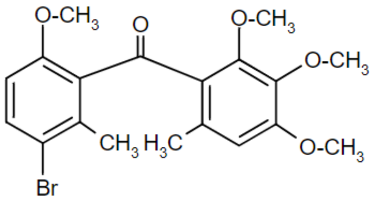
Commercial Name	Virkon®	Secure®	Vivando®
Category	biocide	fungicide	fungicide
Active Ingredient	"Oxone"; pentapotassium bis(peroxymonosulphate) bis(sulphate)	Fluazinam; 3-chloro-N-(3-chloro-2,6-dinitro-4-trifluoromethylphenyl)-5-trifluoromethyl-2-pyridinamine	Metrafenone; 3'-bromo-2,3,4,6'-tetramethoxy-2',6-dimethylbenzophenone
Mechanism of Action	Peroxy compound (i.e., oxidizes)	Uncoupler of oxidative phosphorylation	Likely disrupts actin cytoskeleton organization regulation
Global Usage	Global	Global, but banned in Norway	**EU and US approval as of 2022**
Restrictions	Not approved for outdoor or aquatic use (yet)	Not approved for aquatic applications	Not approved for aquatic applications
Degradation	Hydrolysis, photolysis; very sensitive to heat and salinity	Hydrolysis, photolysis	Photolysis; pH/temp stable and "non-biodegradable"
Aquatic DT_{50} (abiotic)	pH 4 = 800 h pH 7 = 145 h pH 9 = 2.8 h *20 ºC, pH 8 seawater = 5.5 hours* *20 ºC, pH 8 freshwater = 215 hours*	pH 7 = 42 d pH 9 = 6 days	24 h dark: 157.3 days 12:12 h light/dark: 6.2 days 24 h light: 5.5 days
Aquatic DT_{50} (biotic)	Tests are not required, due to rapid abiotic decomposition	~8 hours aerobic and anaerobic	12:12 h light/dark: 5.2 days
Assay	Colorimetric peroxide assay	LC/MS/MS (EPA MERID No. 48635802)	LC/MS (BSAF)



Oxone



Fluazinam



Metrafenone

Progress and Outcomes: Task 1

Subtask1.2.1 Chemical treatments and media optimization to control pests

Milestone 1.2.1.1: Literature review and initial risk assessment of potential pest control agents and top candidates identified for indoor crash assays (M9) **Complete**.

Milestone 1.2.1.3 Data-supported risk assessment of aquatic algal pest control agents and regulated implementation scenarios, including economic and environmental impacts (M30)

Toxicological risk assessment = hazard identification, dose-response assessment, exposure assessment, and risk characterization

Two reports will be generated with different impacts intended, but assessing the externality of **feasibility** is a common criteria

The intent of these reports is **not** to advocate for the use of pesticides, but rather detail what commercial use would actually entail

Toxicological Risk Assessment[2]

Report #1 (M9) - **COMPLETE**

- Primarily for internal consumption, establishing the parameters of further inquiry
- Summary of primarily public information (e.g., literature)
- Will contain a general framework for feasibility concerns facing any algal pesticide (e.g., fungicide) use
- Will contain a specific framework around fluazinam as a highly relevant pesticide example
- Will contain recommendation(s) for further pesticide examples which should be included in laboratory research, with special attention to provisioning pesticide-mechanism rotation
- Results will help guide laboratory studies toward highest impact activities, including protocol developments that will generate regulatory-relevant data

Report #2 (M30) - **PENDING**

- Primarily for external consumption, establishing specific contents for further engagement (DOE, EPA, manufacturers, etc.)
- Combination of public information and new research
- Will specifically incorporate the results of LCA and TEA modeling specific to the value and impacts of pesticides
- Will contain the broader framework for algal pesticide use, using project pesticide(s) as a documented example
- Intended to outline the necessary details of applying any pesticide to commercial algal practice

Progress and Outcomes: Task 1

Subtask1.2.1 Chemical treatments and media optimization to control pests

US 20140378513A1

(19) **United States**
(12) **Patent Application Publication** (10) **Pub. No.: US 2014/0378513 A1**
McBride et al. (43) **Pub. Date: Dec. 25, 2014**

(54) **USE OF FUNGICIDES IN LIQUID SYSTEMS**

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(86) PCT No.: **PCT/US2012/060120**

§ 371 (c)(1), (2), (4) Date: **Apr. 11, 2014**

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C12Q 1/68 (2006.01)
A01N 43/40 (2006.01)

(52) **U.S. Cl.**
CPC *C12N 1/12* (2013.01); *A01N 43/40* (2013.01); *A01N 43/56* (2013.01); *C12Q 1/6895* (2013.01); *C12Q 2600/16* (2013.01)
USPC **514/352**; 435/6.12; 506/2; 435/6.15

(57) **ABSTRACT**

The present disclosure provides methods to detect pests in liquid culture systems for the growth of microalgae. The disclosure further provides methods to treat and control pests in a liquid system and for methods to increase yields of microalgae grown in a liquid culture systems. Methods are provided for the growth, monitoring, treatment and harvesting of microalgae from liquid culture systems.

[0298] Crop protective action is indicated by the threshold C_t for each continuously monitored pond. Upon indication, a first fungicide is added at a predetermined concentration (Headline® 1 ppm, Omega® 0.5 ppm, Thiram® 1 ppm) by a licensed applicator and monitoring is continued. If the C_t threshold is reached again, a different fungicide (second fungicide) is added at a predetermined concentration (Headline® 1 ppm, Omega® 0.5 ppm, Thiram® 1 ppm) by a licensed pesticide applicator and monitoring is continued. To avoid the development of resistant pests, fungicides are rotated based on the mode of action. For example, three fungicides are rotated in outdoor ponds: Headline® (Pyraclostrobin) and Omega® (Fluazinam) and Thiram®-42WP (Thiram®). Headline® is a strobilurin and acts to inhibit the respiratory chain. Omega is a pyridine fungicide which acts to inhibit cellular energy production. Thiram® is a sulfide which acts on multiple sites in the respiratory pathway. Effectiveness of treatment is monitored using both molecular and non molecular means post treatment (FIG. 7).

Critical Limitations of the Sapphire Energy Patent

Patent specifically listed (and status as of 2023):

1. Fluazinam (Norway: banned 2010; US/EU/Canada: **use restricted, but banning unlikely**)
2. Pyraclostrobin (US: sharply limited uses in 2022; **"highly toxic to algae" [algal genotoxicity suspected*]**)
3. Thiram (EU: banned 2019; **US: ban considered with registration review continuing in 2023**)
4. Chlorothalonil (CAN/NZ: use restricted 2018; EU: banned 2019; **US registration review delayed until 2023**)
5. Dithianon (EU: "toxic to algae"; acute 72 h EC_{50} = 0.09 ppm; **US: no approved usage as of 2013**)
6. Dodine (US: "highly toxic to algae"; acute 120-hour EC_{50} 0.95 ppb; **restrictions since 2016 and no new uses**)
7. Dibromocyanoacetamide (US: "toxic to algae"; acute E_rC_{50} = 0.9 ppm; limited use biocide)

IMPACT - Except for fluazinam, the greater feasibility of the identified compounds appears extremely limited This has potential serious consequences as single fungicide use likely to drive resistance in pests.

*Liu, Xiaoxu, et al. "Acute toxicity and associated mechanisms of four strobilurins in algae." *Environmental toxicology and pharmacology* 60 (2018): 12-16. DOI: 10.1016/j.etap.2018.03.021

Progress and Outcomes: Task 1

Subtask1.2.1 Chemical treatments and media optimization to control pests

- Based on current EPA review schedules, no immediate updates to antimicrobial candidates are expected
- *Virkon (oxone) testing is underway, though the available formula for use has changed*
- More explicitly antimicrobial compounds (e.g., surfactants) have not yet been substantiated

Syndel

What is the difference between Virkon® Aquatic and Virkon-S? Virkon® Aquatic was first made available in the USA in May of 2007 and it is specifically designed for use in fish culture while Virkon-S is designed for use in the production of poultry, swine, and other types of agriculture. Virkon® Aquatic does not contain an indicator dye or perfume as found in Virkon-S. The dye and perfume have been removed to make the product more suitable for use around fish and other aquatic life. Virkon® Aquatic has a formulation based on the same active ingredients as Virkon-S but with inert ingredients that are designed for use in aquatic applications. There are also important EPA labeling differences between Virkon® Aquatic and Virkon-S. The EPA approved label for Virkon® Aquatic contains expanded aquaculture claims and applications and is approved for use in aquaculture. Virkon-S is no longer recommended for use in aquaculture and its label will no longer carry EPA approval for aquaculture claims.

Known Agent Updates: Oxone and Fluazinam

- Reassessments of fluazinam globally have not resulted in any recent regulatory changes and more appear unlikely for the next 10-15 years, making it viable for the foreseeable future
- The EPA has even issued new registration and label approvals for more fluazinam products in the US (ex. AX FLUAZINAM), but none are currently commercially available for testing
- *Fluazinam remains in testing, but more compounds of diverse mechanisms of action are needed for rotation*

U.S. ENVIRONMENTAL PROTECTION AGENCY
Office of Pesticide Programs
Registration Division (7505P)
1200 Pennsylvania Ave., N.W.
Washington, D.C. 20460

NOTICE OF PESTICIDE:
_X_ Registration
___ Reregistration
(under FIFRA, as amended)

EPA Reg. Number:	Date of Issuance:
89167-74	6/26/20

Term of Issuance: Unconditional

Name of Pesticide Product: AX FLUAZINAM

ACCEPTED
06/26/20
Under the Federal Insecticide, Fungicide and Rodenticide Act as amended, for the pesticide registered under EPA Reg. No. 89167-74

FLUAZINAM | GROUP 29 | FUNGICIDE

AX FLUAZINAM[TM]

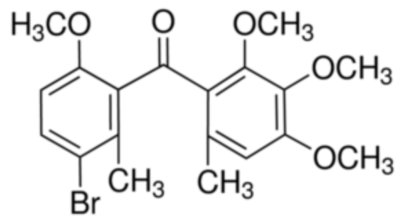
Active Ingredient:	(% by weight)
Fluazinam*	40.0%
Other Ingredients:	60.0%
Total	100.0%

*3-chloro-*N*-[3-chloro-2,6-dinitro-4-trifluoromethyl)phenyl]-5-trifluoromethyl-2-pyridinamine (CA)

Progress and Outcomes: Task 1

Subtask1.2.1 Chemical treatments and media optimization to control pests

- **Metrafenone appears to be an extremely promising new fungicide, as it appears extremely safe at all levels of current testing**
- **Mechanism of action is unsubstantiated,** but "*results suggest that the mode of action of metrafenone interferes with hyphal morphogenesis, polarised hyphal growth and the establishment and maintenance of cell polarity. Metrafenone likely disturbs a pathway regulating organisation of the actin cytoskeleton*."
- *Very new* (no registered uses in 2006), with mushrooms and various fruits now approved in the US and EU



Pest Management Science — Pest Manag Sci 62:393–401 (2006)

Metrafenone: studies on the mode of action of a novel cereal powdery mildew fungicide†

Krystina S Opalski,[1] Stefan Tresch,[2] Karl-Heinz Kogel,[1] Klaus Grossmann,[2] Harald Köhle[2] and Ralph Hückelhoven[1]*

- Registrations in AUS, CAN, and US as of 2022
- Stable across pH, aquatic photolysis (half-life = 3.1 days) to CO_2, slightly hydrophobic, demonstrated sub-ppm susceptibility across standard algae/cyanobacteria
- Intend to incorporate into final studies immediately, *if* compound can be sourced (expected for Spring/Summer 2023 testing)
- **IMPACT – A second agent may allow for altering applications to avoid resistance and or open up available algae strains due to tolerance**

New Agent Update: Metrafenone

2020-2021 Registration Review Schedule for Docket Openings, Preliminary Work Plans, and Final Work Plans for Conventional Cases (as of 03/15/2021)

Docket Opening/Preliminary Work Plans	Final Work Plans
Quarter 1 FY2021	
	• Chlorantraniliprole
Quarter 2 FY2021	
• Metrafenone	• Aminocyclopyrachlor • Anthraquinone • Tolfenpyrad
Quarter 3 FY2021	
Quarter 4 FY2021	
	• Metrafenone

Progress and Outcomes: Task 1

Subtask 1.2.2: Physical treatments for algal pest control - Pulsed Electric Field (PEF)

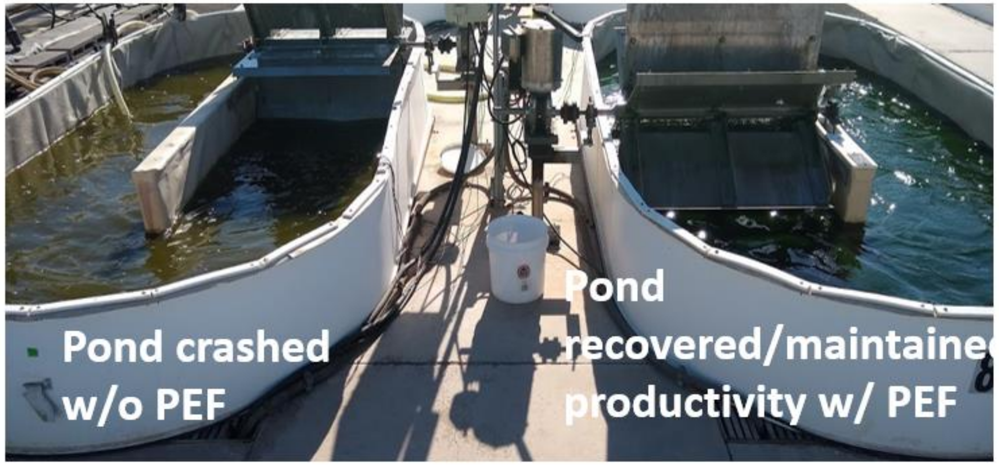
Impact - Established a working and repeatable pest model with amoeba in outdoor open pond systems.

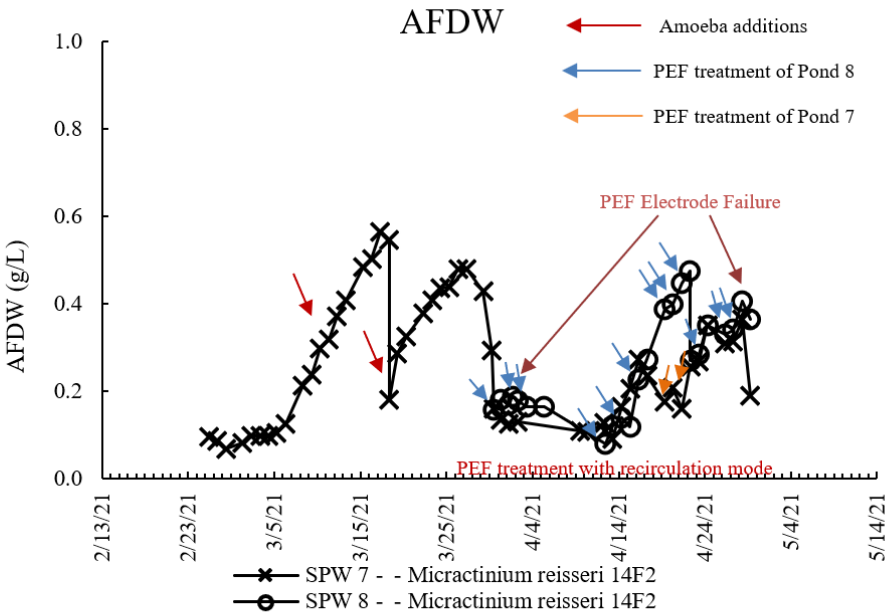
Verified ability of PEF to recover failing pond and control amoeba populations over time

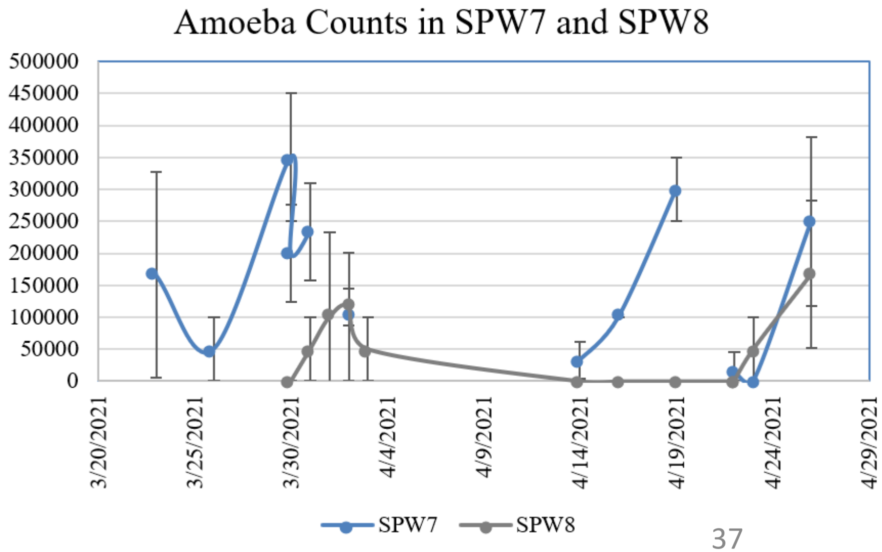
Future work to extend to other strains than 14f2

3/31/2021

Ponds 7 and 8







Progress and Outcomes: Task 1

Subtask 1.2.2: Physical treatments for algal pest control - Pulsed Electric Field (PEF)

- Evaluated using PEF treatment to mitigate pond crashes due to amoeba in *Micractinium* 14F2 cultures with a repeatable pest model.
- Salinity plays a major role in the ability of *Micractinium* 14F2 to survive PEF treatment at voltages that inhibit amoeba.
- However, higher salinities leads to increased wear and tear on electrodes and leads to higher heat rise in cultures.
- Initial cost analysis indicates that the method of PEF treating algae on transfers during scale up is a more cost-effective approach toward mitigating contamination.
- IMPACT – Viable approach for controlling certain grazer populations but most effective for batch operations and or ensuring seed train remains as healthy as possible before production. Unlikely to scale if treating production ponds through recirculation.

Condition 10 kV 20 μsec	Batch Culture / Transfer Mode Freshwater	Batch Culture / Transfer Mode Saltwater	Recirc Mode Freshwater	Recirc Mode Saltwater
Volume Treated	111000 L	111000 L	5M Liters	5M Liters
kJ / L	4.4	90	4.4	90
L / kW-hr	818,000	40,000	818,000	40,000
kW-hrs for PEF	0.13	1.24	6.11	125
Cost / kW-hr	$0.13	$0.13	$0.13	$0.13
Cost of PEF Electricity	**$0.02**	**$0.16**	**$0.79**	**16.25**
Pump Efficiency	0.4 kW-hr / 1000L	0.4 kW-hr / 1000L	0.4 kW-hr / 1000L	0.4 kW-hr / 1000L
kW-hr to pump liquid	44.4	44.4	2000	2000
Price to Pump liquid	**$5.77**[1]	**$5.77**[1]	**$260**	**$260**
Total Cost to PEF treat	$5.79	$5.93	$260.79	$276.25

1. Since transfer mode uses a pump to transfer between ponds, there is minimal additional cost to perform this transfer through the PEF.

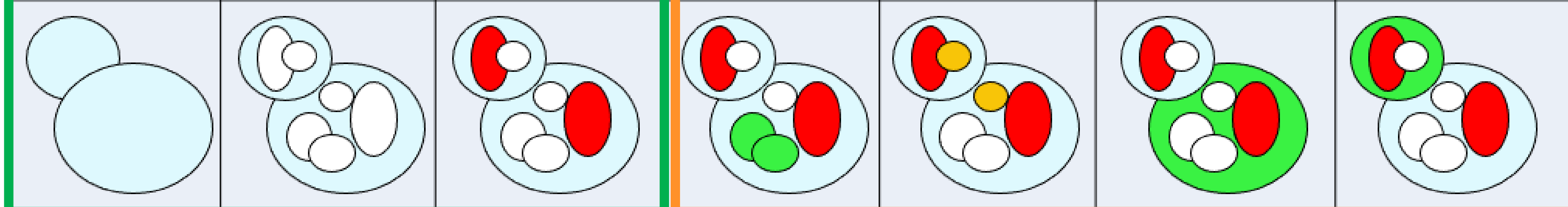
- A kW-hr of electricity is 3.6 MJ (1000 J/s * 3600 secs) and costs $0.13. A kW-hr can treat 818 kL of pond at 4.4 kJ / L (freshwater), In saltwater, A kW-hr can treat 40kL at 90 kJ / L so the cost would be $16.25 for the PEF electricity.
- Recirc mode will also burn through far more electrodes since electrode life is based on volume treated independent of continuous or batch.

DIVERSIFIED TECHNOLOGIES, INC.

Progress and Outcomes: Task 2

Flow Cytometry Measurements

Intrinsic parameters			Physiological stains			
Forward Light Scatter	**Side Light Scatter**	**Red Fluorescence**	**Green Fluorescence**	**Orange Fluorescence**	**Green Fluorescence**	**Green Fluorescence**
Size	Granularity	Chlorophyll Autofluorescence	Lipids (when stained)	DNA (when stained)	Metabolic Activity (when stained)	Reactive Oxygen Species (when stained)



Steadman Tyler, C.R., Sanders, C.K., Erickson, R.S., Dale, T., Twary, S.N. & Marrone, B.L. 2019. Functional and phenotypic flow cytometry characterization of Picochlorum soloecismus. *Algal Res.* 43:101614.

Huesemann, M., Dale, T., Chavis, A., Crowe, B., Twary, S., Barry, A., Valentine, D. et al. 2017. Simulation of outdoor pond cultures using indoor LED-lighted and temperature-controlled raceway ponds and Phenometrics photobioreactors. *Algal Res.* 21:178–90.

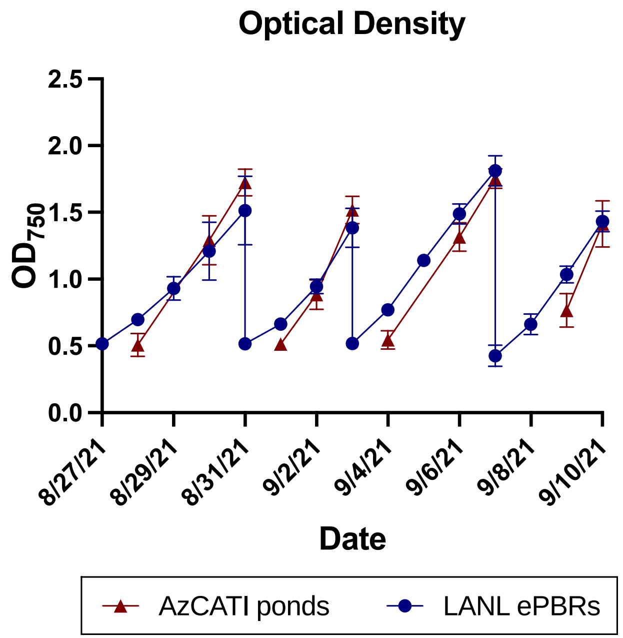
Sanders, C.K., Hanschen, E.R., Biondi, T.C., Hovde, B.T., Kunde, Y.A., Eng, W.L., Kwon, T. et al. 2021. High-quality genome assembly and phylogenetic analyses support reclassification of the oleaginous marine species Nannochloris desiccata (Trebouxiophyceae, Chlorophyta), formerly Chlorella desiccata. *Under Revis.*

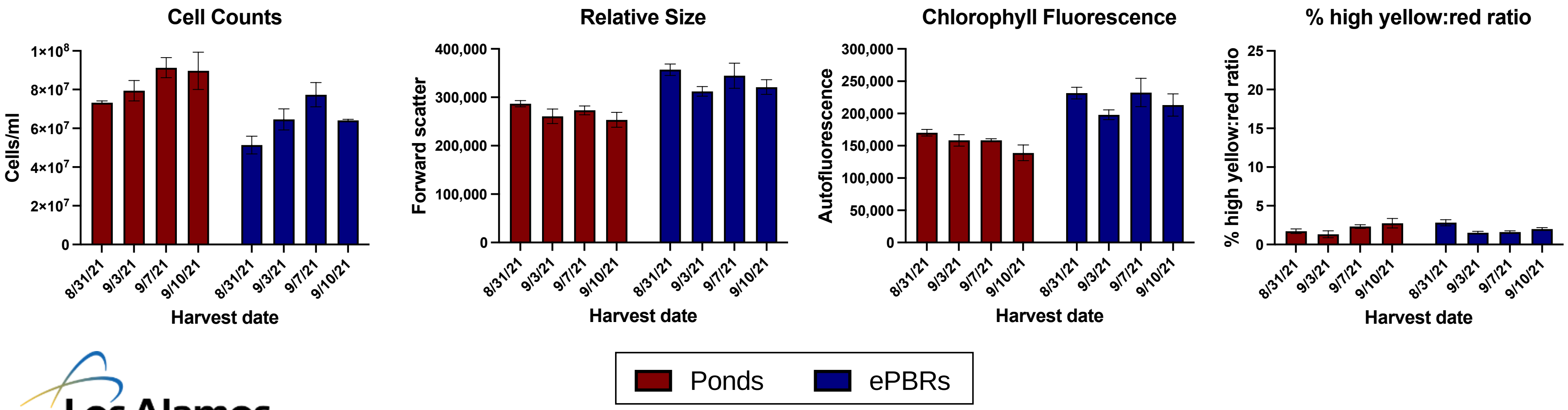
Progress and Outcomes: Task 2

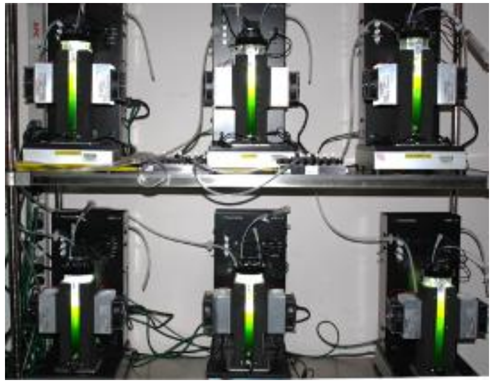
Subtask 2.2: Environmental Simulation with ePBRs and Transcriptomics

Picochlorum soloecismus pond and ePBR growth

- Growth patterns in ponds and ePBRs match
  - Slightly larger slopes in ponds
- Good simulation of growth for *P. soloecismus*
- ePBR cultures have lower cell counts, but larger cells as compared to ponds
- Patterns between harvests are similar



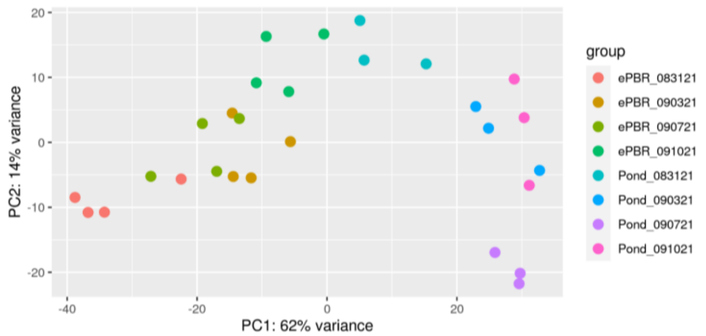




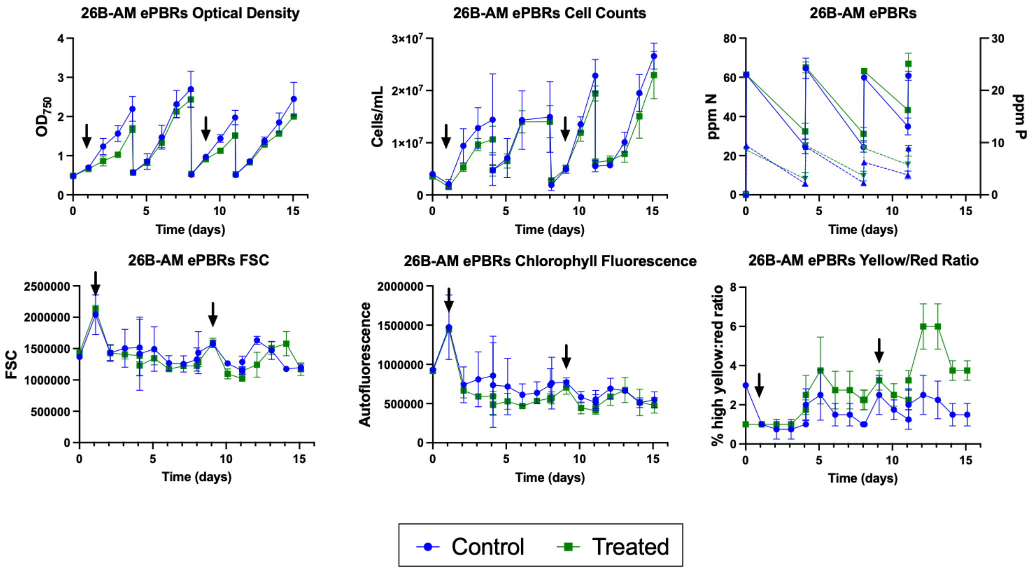
Progress and Outcomes: Task 2

Subtask 2.2: Environmental Simulation with ePBRs and Transcriptomics

- *Picochlorum soloecismus*
  - Conduct RNAseq on samples from 4 harvests in both ponds and ePBRs
  - Samples analyzed – significant variation between indoor and outdoor
  - Additional experimentation in progress to identify if sampling time variation affected variability for outdoors or represent real differences based on environment
- *Scenedesmus obliquus* (UTEX393)
  - Seed train, including indoor, helix and greenhouse flat panels
    - both saw deterioration
  - Ponds with and without crashing
  - Green house and seed pond untreated and treated with fluazinam
  - Bacterial contamination high – fungal parasitoids minimal
  - Samples have been collected but not yet run



PCA analysis of transcriptomic reads for harvest day samples from Ponds at AzCATI and ePBRs at LANL. looking at the number of genes that show very significant changes in expression (log2FC of >4 or <-4) there are very few in the ePBRs, but more in the ponds and between the ponds and the ePBRs.



Quantitation of effect of standard dosing of fluazinam (0.5 ppm) on growth and recovery of 26BAM under outdoor simulated conditions. Running in ePBRs allowed for looking at effect under relevant environmental factors but in absence of any biotic influence such as pests.

Progress and Outcomes: Task 3

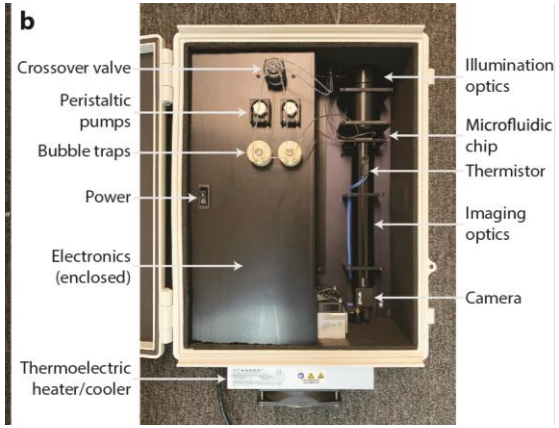
Optimized process monitoring for improving performance

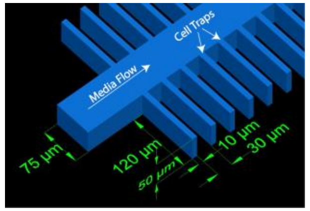
Subtask 3.1 and 3.2

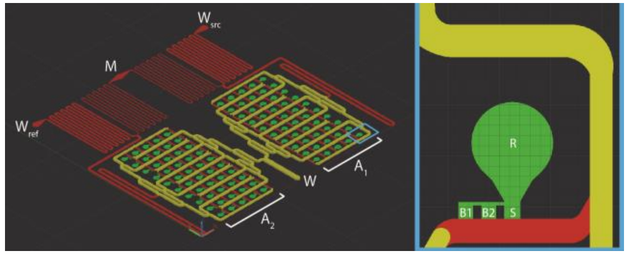
- Novel sensor development for continuous, real-time monitoring of key cultivation parameters including water/culture quality (3.1) and nutrients (3.2)

MiProbe system and example of current sensor deployed

- Novel, continuous operation, real-time data/results
- MiProbe measures electron potential on electrode surface populated with a biofilm made up of endemic species of microbes.
- ‘Microbial Potential’ responds to changes in the environment from the perspective of the biofilm.
- Redox changes, photosynthesis, biomass (e.g. Ash Free Dry Weight / MLSS / BOD / COD), nutrient loading, presence of biocidal compounds/events can be monitored in real-time.
- Very LOW-COST sensors, easy to deploy remotely (low power – solar battery typical)







- Novel, continuous operation, real-time data/results
- GFP based microbial microfluidic sensor genetically engineered to respond to different analytes (e.g., heavy metals, nitrate/ammonia/phosphate)
- Housed in environmental enclosure with optics, hardware, software all integrated to sustain cell growth and perform image acq./processing

Milestone 3.1.1: Deliver and install MiProbe systems at AzCATI and LANL. **COMPLETE**
Milestone 3.1.2: Operational protocols for deploying and maintaining MiProbes systems with initial demonstration of >/= 85% correlation to one or more production metrics. **COMPLETE**
Milestone 3.2.1: Qube systems deployed at AzCATI **COMPLETE**

Progress and Outcomes: Task 3

Optimized process monitoring for improving performance

Subtask 3.3: Data Integration Tool Development. AzCATI-cloud Python Libraries

Burge Environmental

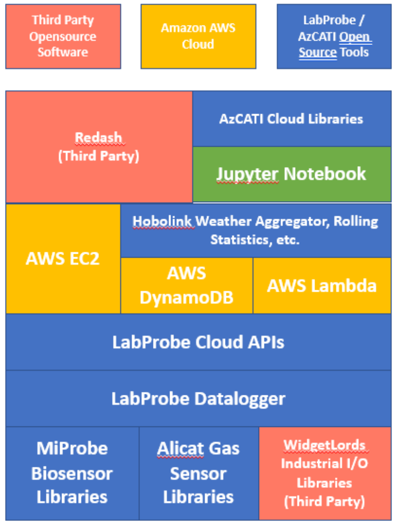
Goal: Provide researchers, technicians, and students a simple set of python libraries to easily access AzCATI datasets pre-merged and structured for modern data-science applications, automated analysis APIs, and controlling experiments.

Basic AzCATI Python Data Libraries:

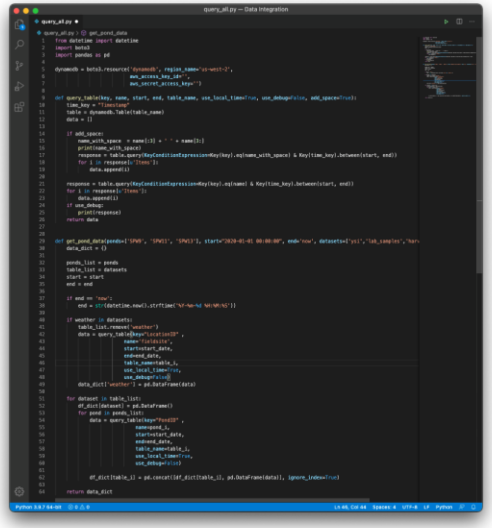
query_table() – Returns dataframe for a specific dataset for a given key and optional time-period.

get_pond_data() – Returns an object of dataframes of any combination of datasets for any combination of ponds for any date range where data exists by aggregating query_table() results.

AzCATI Cloud Software Stack



The actual function code



Example Code:

```
from azcati_cloud import get_pond_data, query_table

utex393_replicates = get_pond_data(ponds=[“SPW13”, ”SPW11”, “SPW9”], start=“2021-01-01”, end=“2021-05-05”)
```

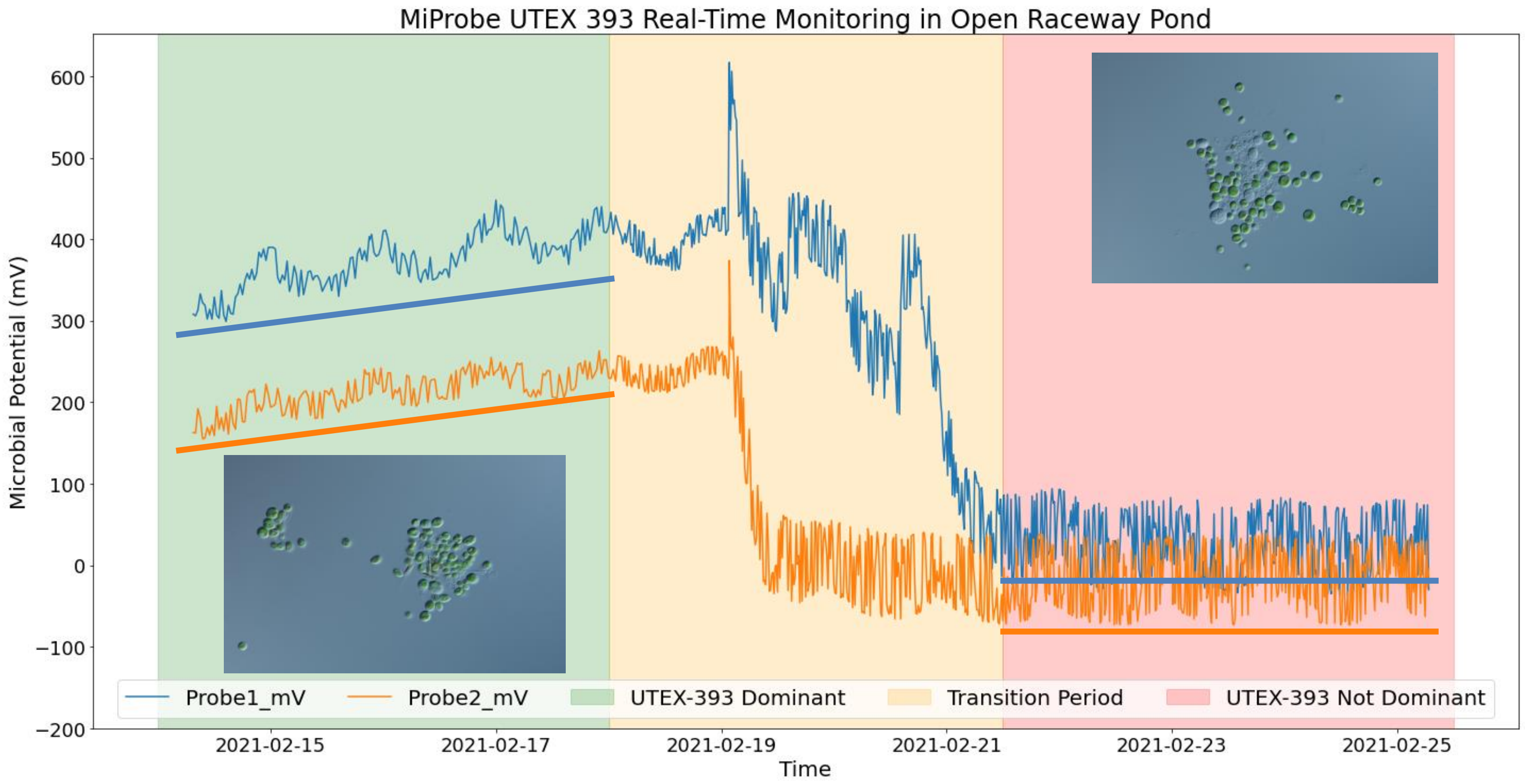
These 2 lines of code are all a researcher needs to start applying data-science tools to the utex393_replicates datasets. The data is already merged, organized, and formatted.

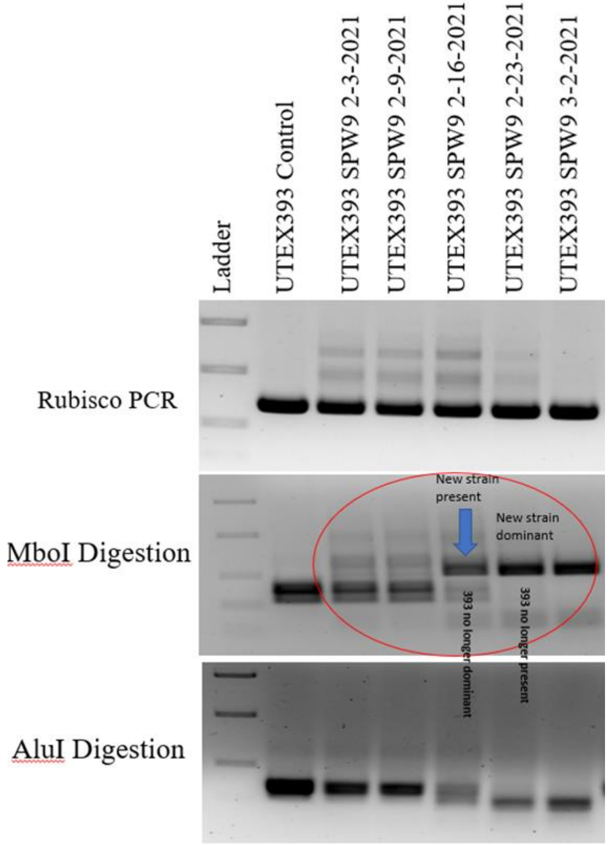
Impact - AzCATI’s entire cultivation history (ATP3/DISCOVR/DMSACPE, future projects) being migrated into AzCATI Cloud by end of Summer 2023

AzCATI
Arizona Center for Algae Technology and Innovation

Progress and Outcomes: Task 3

Optimized process monitoring for improving performance





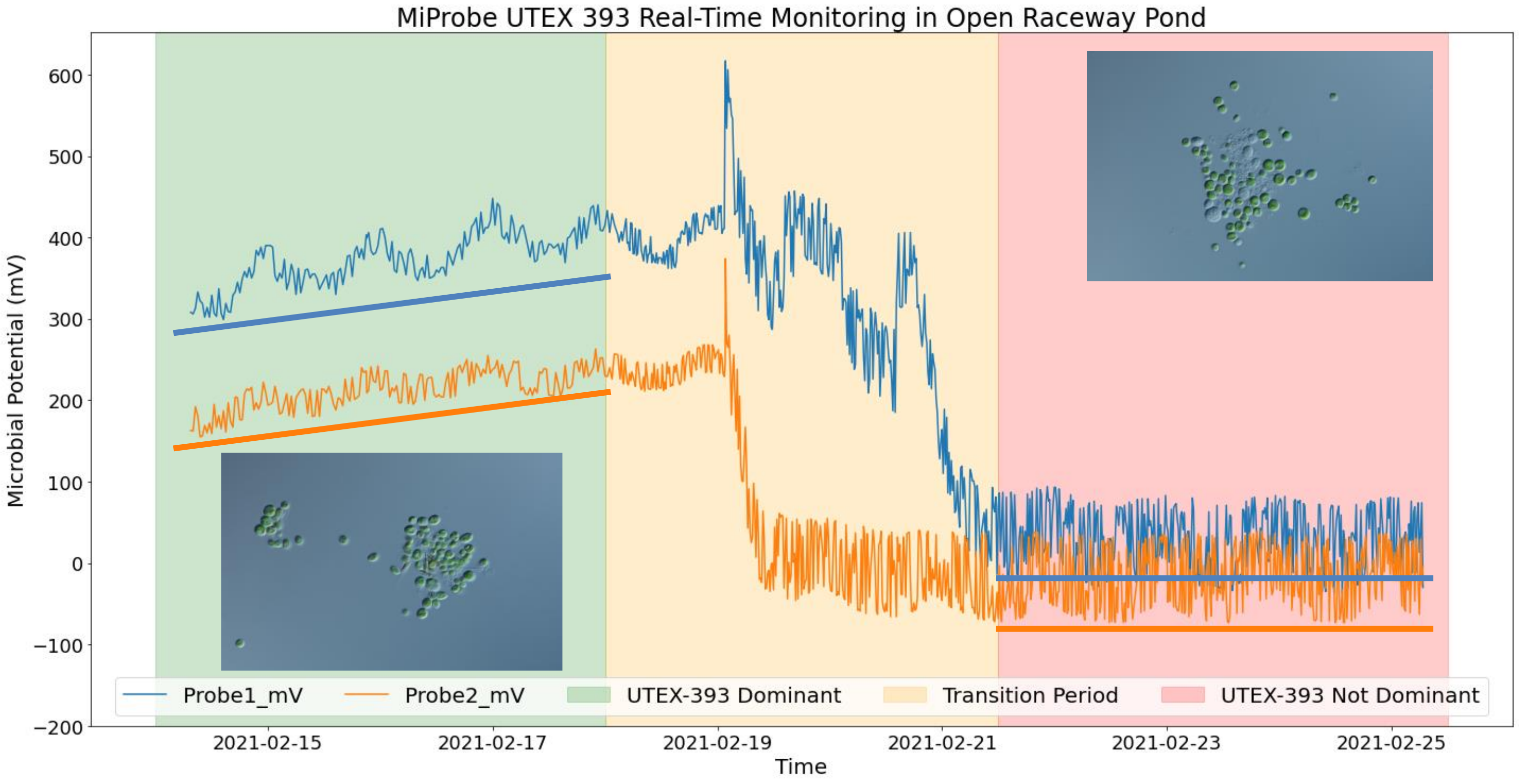
DNA PCR & CAPS Analysis

- A UTEX393 pond was monitored by MiProbes at different depths
- A total pond displacement event was recognized *in real-time* by trendline deviance
- Due to morphological similarities of invading algae species – *not detected by routine microscopy.*
- The pond did not crash nor exhibit obvious aberrant behavior; only retrospective PCR analyses days later revealed what had occurred.

IMPACT: Real-Time Detection of Strain Displacement

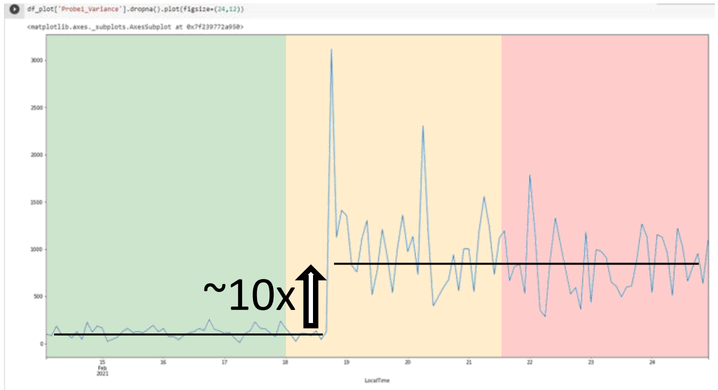
Progress and Outcomes: Task 3

Optimized process monitoring for improving performance

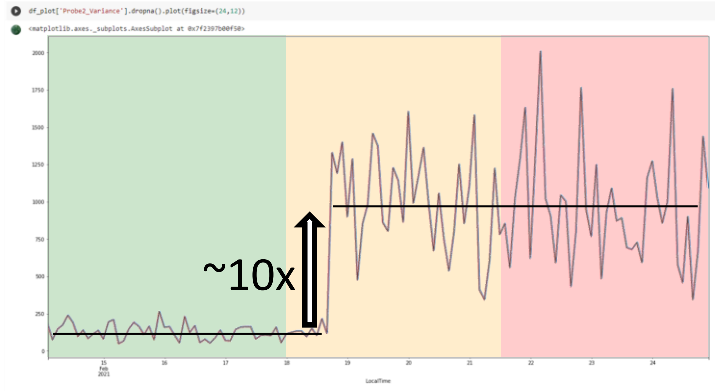


Residuals vs. Time

Probe 1



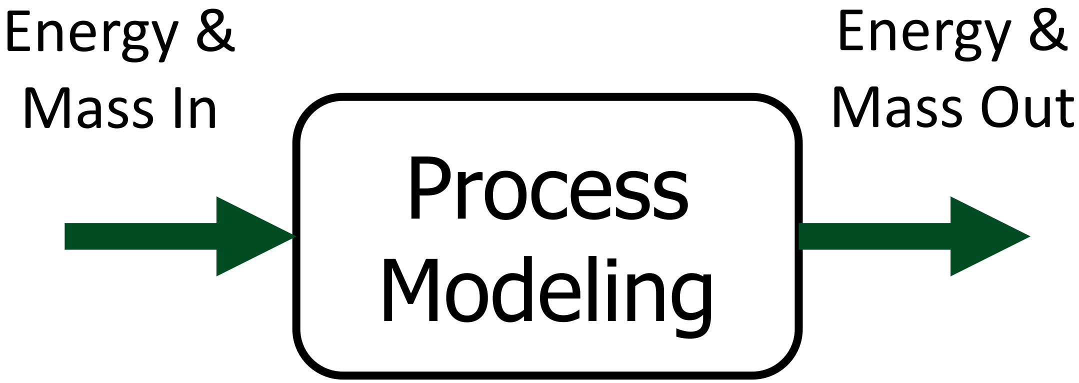
Probe 2



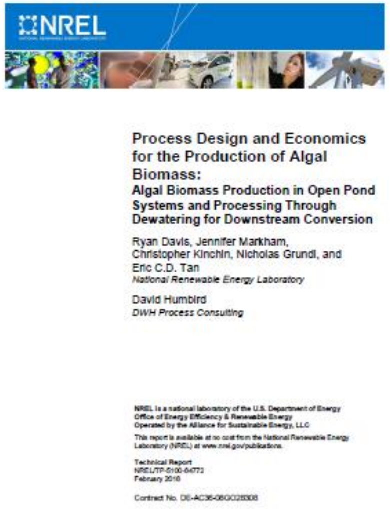
- A distinct shift in residual fits coincided with the takeover of the second algae algal species
- Intrusions by contaminating algae species may be rapidly distinguishable

IMPACT: Strains May Be Differentiable (“Fingerprinting“)
Indoor MiProbe setup established for liquid cultures as well as a plate-based format for MiProbe established to further explore this work outside the scope of the current project.

Techno-Economic Analysis

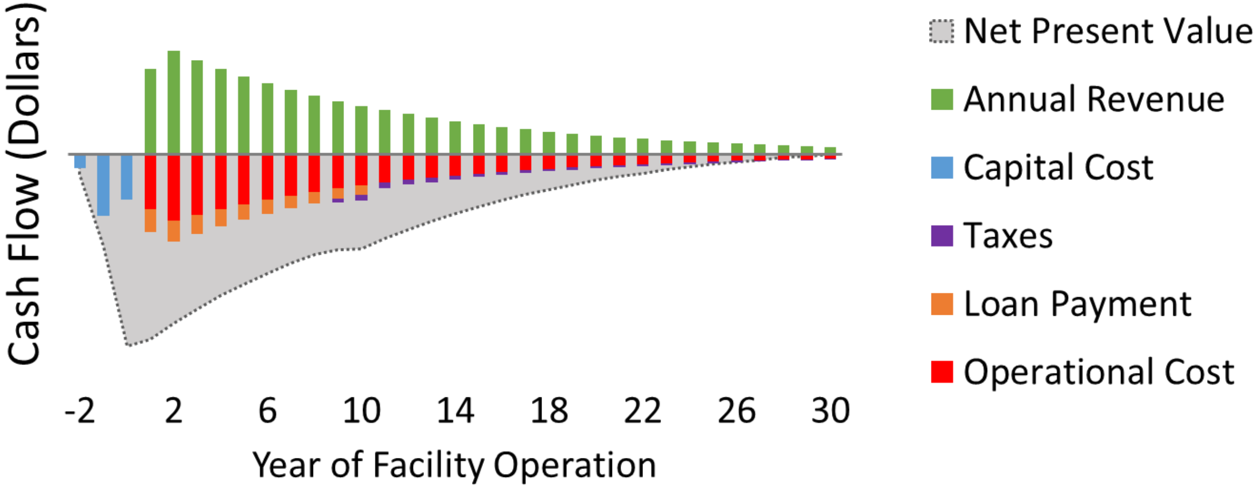


Capital and Operational Expenses



Nth Plant Assumptions

- **30-year life**
- **10% IRR**
- **MACRS Depreciation**
- **NPV=0**



Life Cycle Assessment

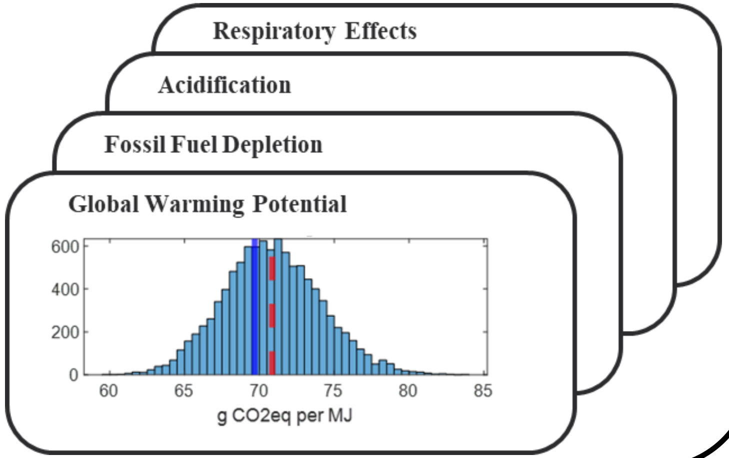
Energy & Mass In → **Process Modeling** → Energy & Mass Out

<u>Life Cycle Inventory Data</u>

ecoinvent

GREET LIFE-CYCLE MODEL

<u>TRACI Impacts</u>



<u>LCA Assumptions</u>

- **Well-to-wheel**
- **IPCC AR6 – 100-year GWP**
- **Functional unit: energy unit of fuel**
- **Electricity impacts at a FERC region level**