DISCOVR

Development of Integrated Screening, Cultivar Optimization, and Verification Research

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Bioenergy Technology Office (BETO) - Peer Review Presentation (March 9, 2021)

Objective of the DISCOVR Consortium Project

Reduce biofuel costs by increasing biomass productivity

Challenge

- A **major driver of algae biofuel costs is productivity**, including culture **resilience** and biochemical **composition**

Project Goals

- **Reduce total microalgae biofuels production costs** by
  - applying an **integrated screening platform** for the **identification of high productivity strains with cellular composition suitable for biofuels and bioproducts for resilient, year-round outdoor cultivation**
  - testing **new concepts** to **increase annual State of Technology (SOT) productivities and reduce the MBSP**, such as
    - improving **biomass productivity (≥20%)**
    - enhancing **biomass intrinsic value** and **value productivity (≥20%)**
    - increasing **crop protection** and **culture stability (≥25% increase** in **mean time between failures)**
  - Use **Techno-Economic Analyses (TEAs) to screen economic feasibility of new concepts** prior to pond trials
  - Evaluate **new strains** and **optimized conditions** in **year-round seasonal SOT trials** at the BETO testbed at AzCATI

Expected Outcomes

- **Increases in annual SOT productivity with concomitant decreases in Minimum Biomass Selling Price (MBSP) to reach BETO 2030 target of $488/T years ahead of schedule**

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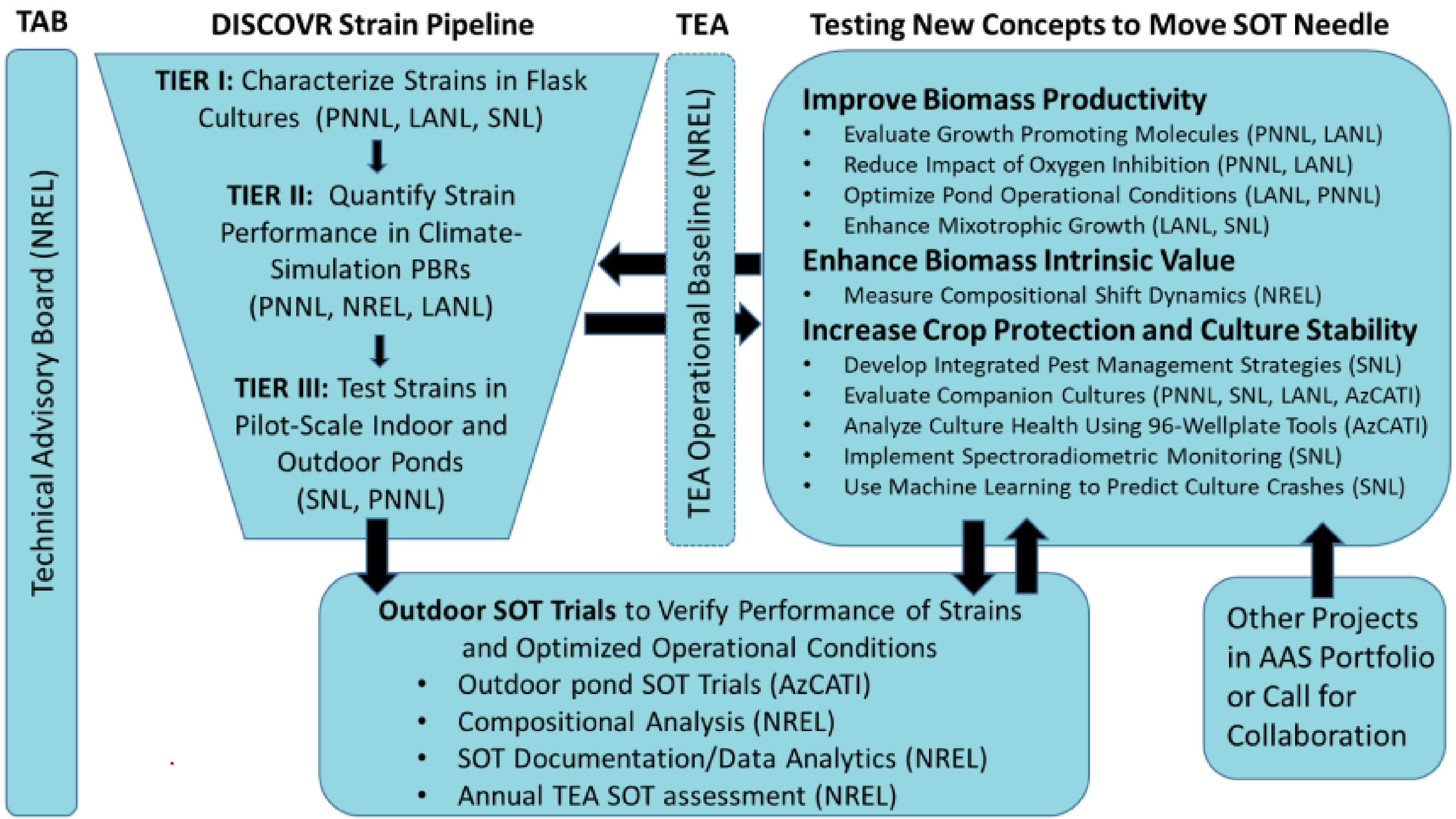
DISCOVR Project Framework and Roles

Identify & evaluate new high productivity strains & innovative approaches to move "SOT" needle



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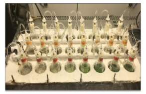
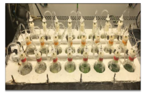
DISCOVR Success Preview: Status of Strain Pipeline

DISCOVR strain pipeline has been successful in down-selecting top strain for SOT trials



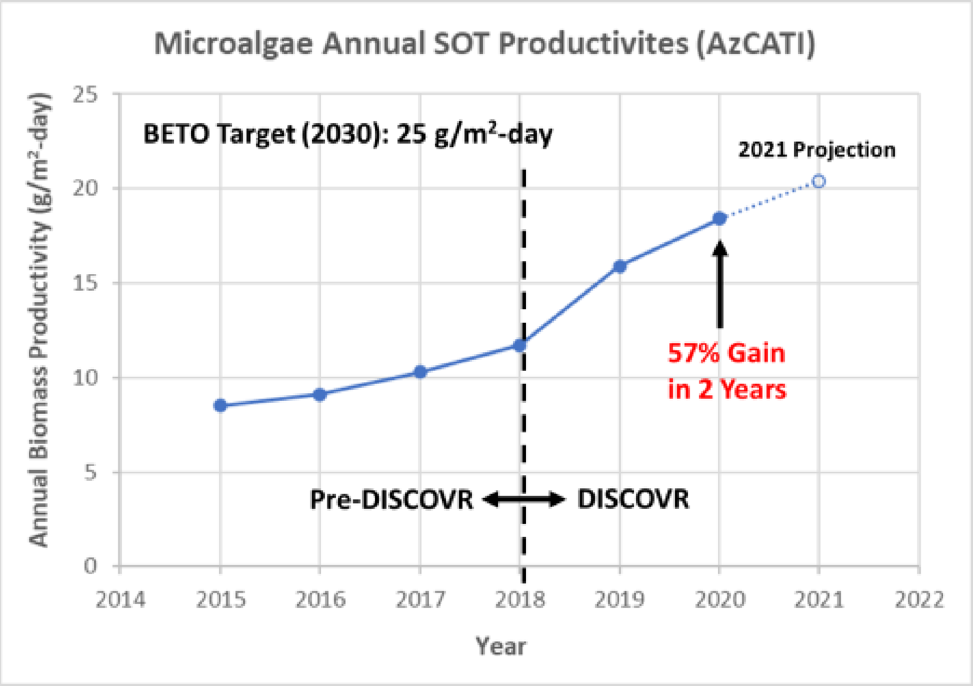

Strain	Temperature + Salinity Tolerance + 16s/18s ID (42 Strains)	LEAPS Photo-Bioreactor Testing (24 Strains)	Outdoor Pond Testing (PAT) (13 Strains)	Outdoor Pond Testing at BETO SOT Testbed (AzCATI) (7 Strains)
Cyanobacterium *AB1* (from Algenol)				
Acutodesmus obliquus DOE0152.z				
Acutodesmus obliquus UTEX393				
Agmenellum quadruplicatum UTEX2268				
Anabaena sp. ATCC 3308.1				
Arthrospira platensis UTEX3086				
Arthrospira fusiformis UTEX2721				
Chlorella antarctica UTEX 1959				
Chlorella autorophica CCMP243				
Chlorella sorokiniana UTEXP13				
Chlorella sorokiniana UTEX BP15				
Chlorella sorokiniana DOE1412				
Chlorella vulgaris NREL 4-C12				
Chlorella vulgaris AZ-1201				
Chlorella sp. UTEX SNO 69				
Chlorococcum littorale UTEX 117				
Chlorococcum sp. DOE1426/UTEX BP7				
Chloromonas reticulata CCALA870				
Chloromonas UTEX SNO11				
Coelastrella sp. DOE0202				
Micractinium sp. NREL 14-F2				

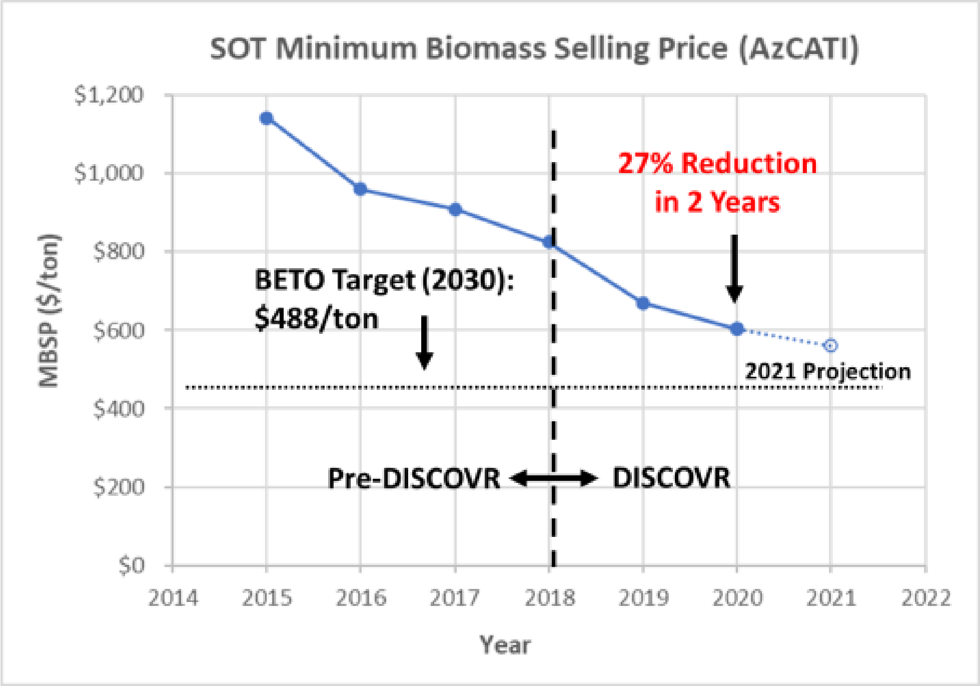



Strain	Temperature + Salinity Tolerance + 16s/18s ID (42 Strains)	LEAPS Photo-Bioreactor Testing (24 Strains)	Outdoor Pond Testing (PAT) (13 Strains)	Outdoor Pond Testing at BETO SOT Testbed (AzCATI) (7 Strains)
Monoraphidium sp. MONOR1				
Monoraphidium minutum 26B-AM				
Nannochloropsis gaditana CCMP1894				
Nannochloropsis oceanica CCAP 849/10				
Nannochloropsis salina CCMP1776				
Oscillatoria cf. priestleyi CCMEE5020.1-1				
Phaeodactylum tricorutum UTEX 646				
Picochlorum renovo NREL39-A8				
Picochlorum celeri WT-CSM/EMRE				
Picochlorum oklahomensis CCMP2329				
Picochlorum soleocismus DOE101				
Porphyridium cruentum CCMP675				
Scenedesmus acutus LRB-AZ-0401				
Scenedesmus rubescens 46B-D3				
Scenedesmus sp. IITRIND2				
Stichococcus minor CCMP819				
Stichococcus minutus CCALA727				
Synechococcus elongatus UTEX2973.1				
Tetraselmis striata LANL 1001				
Tisochrysis lutea CCMP1324				
Tribonema minus sp. MBE				

DISCOVR Success Preview: Gains in SOT Productivity (2018-2020)

Use of DISCOVR strains resulted in 57% increase in SOT productivity and 27% decrease in MBSP





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DISCOVR Management: Relation to BETO AAS Portfolio

Data and new strains are delivered to other projects and community

Strains:
- Culture Collections
- Algae Industry/Academia
- Other BETO Projects

Core Capabilities:
- Environmental Simulation
- Strain Characterization
- Biomass Quality Measurements
- Performance Improvement
- Pond Crashes & Signatures
- Flask-to-field Expertise
- Testbeds

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Downstream Projects:
- Genome Sequencing
- Molecular Toolboxes
- Biomass Storage
- Biomass Conversion
- Nutrient Recycling

Data:
- Outdoor Cultivation
- State of Technology
- Pest Resilience

DISCOVR Management Team and Activities

The cohesive DISCOVR team successfully executes tasks within effective management framework

Michael Huesemann (PNNL)

Lieve Laurens (NREL)

Taraka Dale (LANL)

Todd Lane (SNL)

John McGowen (AzCATI)

Meetings

- Weekly teleconferences with DISCOVR & SOT teams
- Quarterly planning sessions for seasonal SOT trials
- Quarterly meetings with the Technical Advisory Board
- Annual face to face meetings (pre-COVID)

Quarterly Reports and Tracking of Milestones

- Data flows through the PI
- PI tracks milestones and generates all reports with input from technical leads (TLs)
- Technical leads are responsible for achieving task milestones
- Synthesis of results into publication and solutions tracked and mediated by PI

Decision Making

- Decision making is through consensus of PI and TLs
- PI retains ultimate decision-making authority

DISCOVR Management

Critical success factors and risk mitigation strategies

Critical Success Factors

- **Unique state-of-the-art** technical **capabilities** are employed for execution of DISCOVR tasks.
- **Complementary core competencies** of the consortium labs and SOT testbed are **applied together** to make progress towards BETO's **targets**.
- **Effective communication** and **cohesive decision-making across** DISCOVR team.
- **Strong partnership** with outdoor testbed.
- **Cooperation** with **industrial partners** for enhanced impact.

Risk Mitigation Strategies

Risk	Mitigation Strategy
Unable to identify high productivity strains in first round of screening (PNNL).	Identify additional strains from culture collections, industrial partners, and academia.
Strategies for shifting biomass composition do not result in increased value productivity due to decrease in biomass productivity (NREL).	Test additional cultivation conditions for shifting composition and evaluate additional top DISCOVR strains.
Growth promoting molecules (GPMs) are not broadly effective for increasing biomass productivity of DISCOVR/SOT stains (LANL).	There is a chance that GPM addition will be successful in some strains but not others.
New crash agents arise rapidly and dominate SOT testbed, decreasing productivity (SNL)	Simultaneous pursuit of multiple countermeasures: polycultures, bacterial isolates, and crop rotation.

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DISCOVR Management: Go/No-Go Milestones

All DISCOVR tasks are focused on meeting or exceeding the GNG milestones

Go/No-Go Milestones (June 30, 2021):

- Show that for at least 2 out of the 5 DISCOVR new concepts being tested, as well as new strains going through the pipeline, the following improvements under laboratory conditions are achieved:
    - **20% in productivity** (g/L/day or g/m^2/day) relative to control cultures or benchmark strains (***GNG-1***)
    - **20% increase in value productivity** (\$/m^2/day) (i.e., composition-based intrinsic value (\$/g) times biomass productivity (g/m^2/day)) for the top DISCOVR strains (***GNG-2***)
    - or **25% increase in mean time between failure** (MTBF) for crop protection strategies compared to standard crash test (***GNG-3***)

- **Correctly identify crash agents** through anomaly detection tool Pond Sentry for SOT cultivation runs and/or spectroradiometric monitoring w**ith mean early warning of 72 hrs or more before a deleterious event for 75% of cultivation runs** (***GNG-4***).

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DISCOVR Management: Technical Advisory Board

Meetings restructured to address primary risk factors: Focus on single topic

- DISCOVR core team **discusses immediate highest impact topic** to TAB using WebEx on quarterly basis with BETO staff in attendance.
- Presentations are designed to **spark discussion and elicit dialogue** on DISCOVR **critical path elements** and help prioritize research or identify research gaps
  - **Data management**
  - **Crop Protection**
  - **Harvesting Operations**
- Include **10 guiding questions,** open discussion on levers and strategies to test and implement
- Reports will be made publicly available.
- Examples of **TAB recommendations that were incorporated** are in Supplemental Section.

- **<u>2019-2020 TAB members</u>**
  - Rebecca White, Qualitas Health
  - Lou Brown, Synthetic Genomics
  - John Benemann, MicroBio Engineering
  - Valerie Harmon, Harmon Consulting
  - Juergen Polle, Brooklyn College
  - Craig Behnke, Lumen Biosciences
  - Philip Pienkos, Polaris Renewables
  - Phil Lee, Consultant
  - Matt Posewitz, Colorado School of Mines

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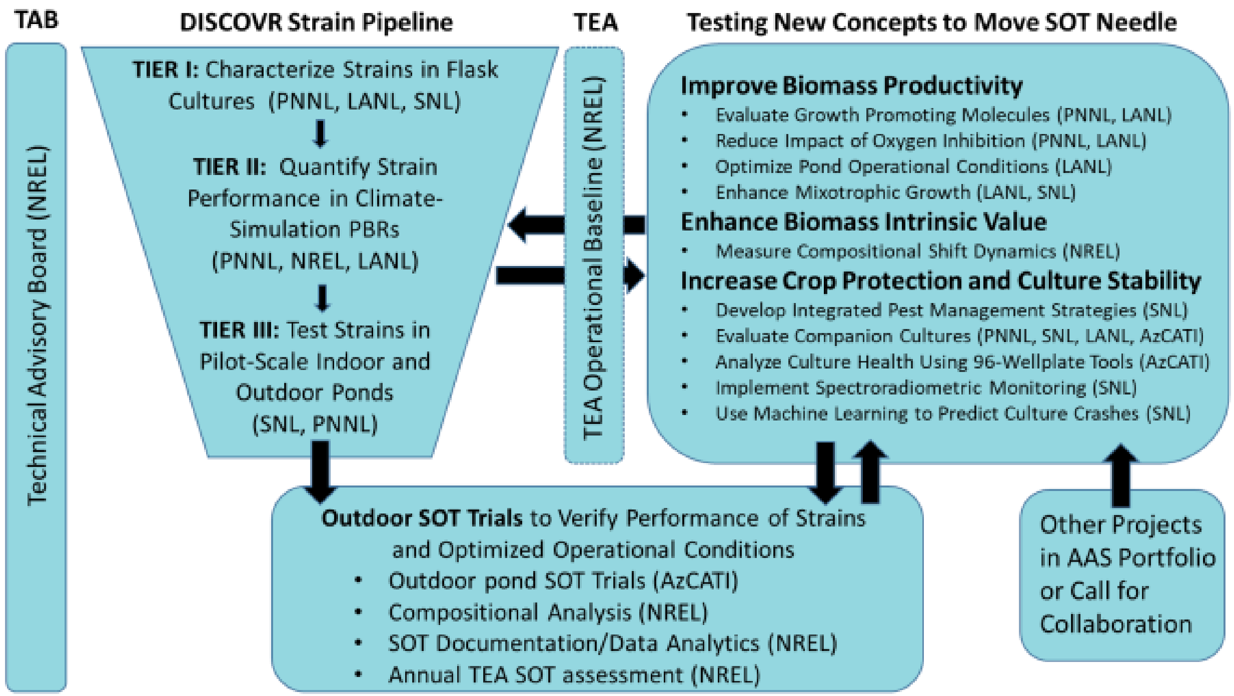
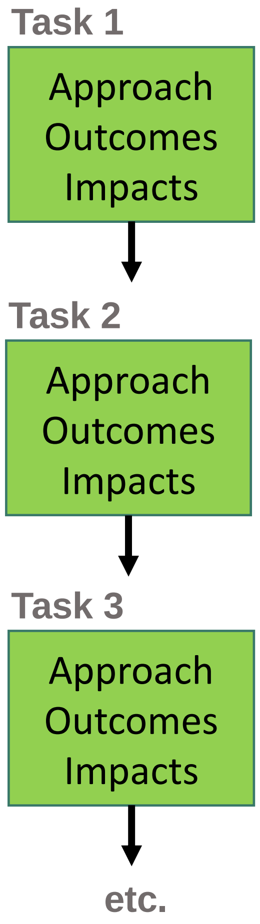
Organization of Presentation on Task Approaches, Outcomes, Impacts

For each DISCOVR task, the respective approach, outcomes, and impacts are presented as a unit





- **Note:** To facilitate the reviewer rating of the different sections (management, approach, outcomes, and impacts), all slides are also organized by section, rather than by task, in a separate PDF file entitled: "**Huesemann_1.3.1.501_DISCOVR-Organized-by-Four-Sections-for-Rating.pdf**".

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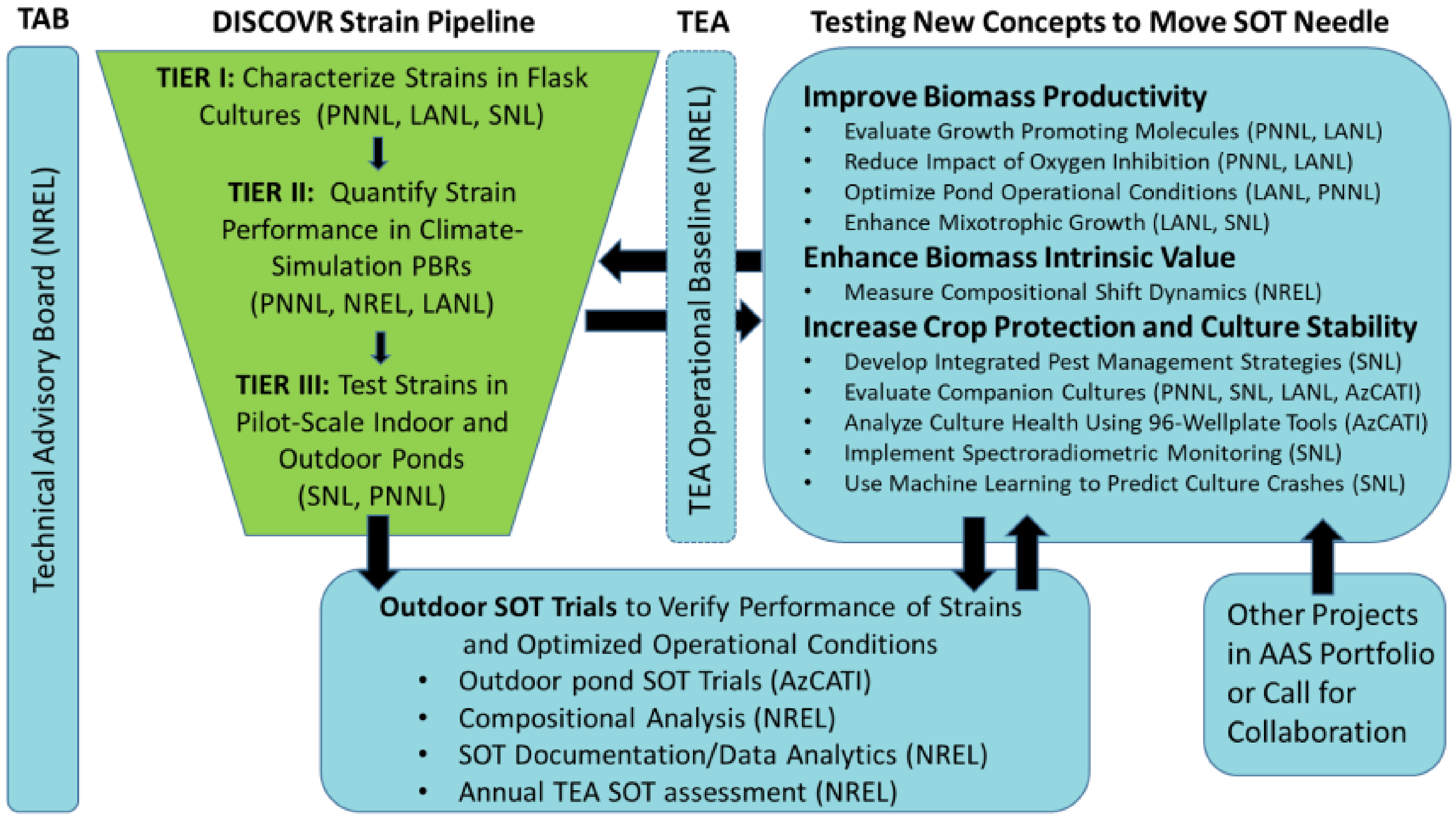
DISCOVR Strain Pipeline Tasks

Approach – Progress & Outcomes - Impacts

Pipeline



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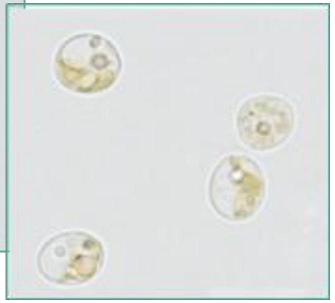
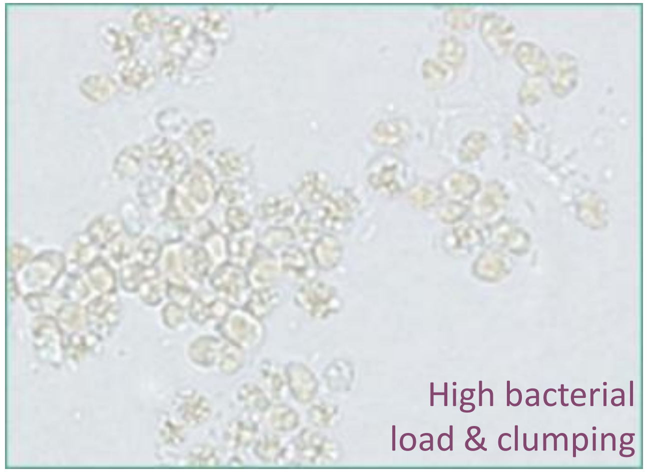
Approach: Strain Identification and Confirmation

Pipeline

Ensuring a known starting point for DISCOVR strains

- **Background/history:** Ongoing effort as part of Tier I Characterization
  - *Algae cultivation expertise used to revive cultures and adapt to DISCOVR media*
  - *Initial flask characterization provides insight into strain ease of handling*
  - *16S(bacterial) and 18S(algae) data informs DISCOVR team on bacterial load and strain identity*
- **Objective:** Revive strains, evaluate bacterial load, confirm strain identity, adapt to DISCOVR media and perform initial characterization, and deliver to PNNL
- **Challenges:**
  1. Strains do not always revive well or grow in DISCOVR media
  2. Some strains have high bacterial load or are a different strain than listed by the culture collection
- **Economic/Technical Metrics:** 16S/18S sequencing of >30 DISCOVR strains.





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Progress & Outcomes: Strain Identification and Confirmation

Pipeline

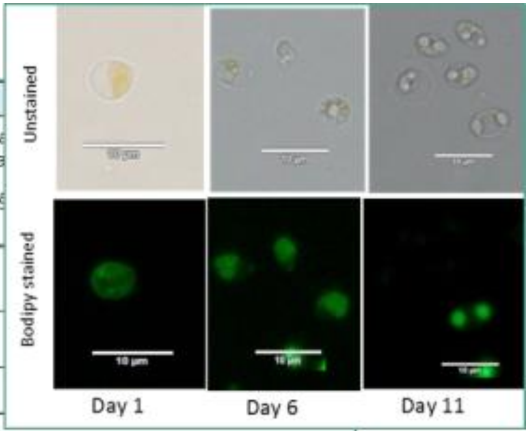
Revived/evaluated 28 strains, sequence checked 33, and delivered 18 to PNNL for screening

Over the course of the project:

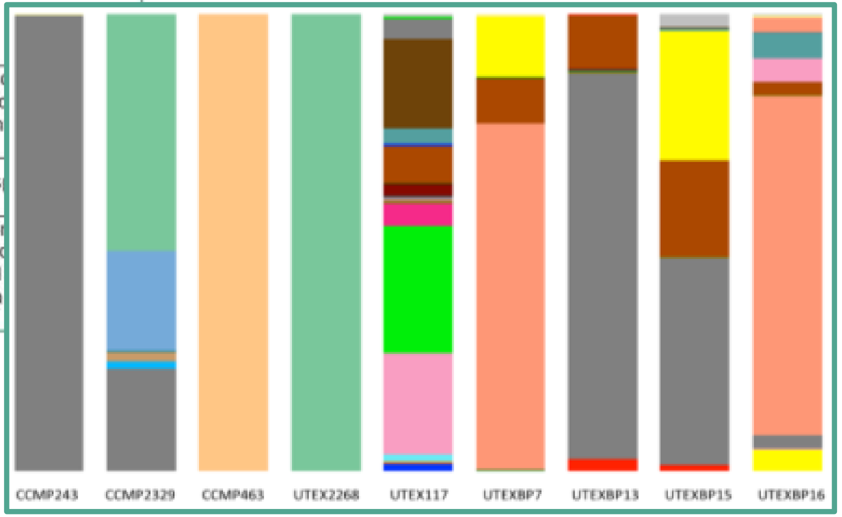
- Identify, order, and revive (**n = 28**)
- Initial growth curves & morphology
- Adapt to media (**22**)
- 16S and 18S sequencing (**33**)
- Clean-up cultures as needed
- Deliver to PNNL (**18**)

Example of a strain tracking sheet

Culture Collection Name	UTEX BP13
Proposed species	*Chlorella sorokiniana* DOE1044 (a green a
Species Identification by 18S	*Chlorella sorokiniana*
Sent to PNNL	Yes
PNNL Screening Status	In progress
Tier I	Yes
Tier II	TBD
Tier III-V	TBD
Media	Grows well in BG11 DISCOVR media
Microscopy	Complete
Growth curves in CO2 chamber	Complete
DNA Isolation	Complete
16S Analysis	9365 sequence counts (1236 were bacterial of the bacterial fraction was from a single bac chloroplast fraction of the counts is consisten identifcation.
18S Analysis	18S is consistent with the culture collection s *C. sorokiniana*.
Basic N depletion and BODIPY staining for flow cytometry	Clear carbon storage upon N depletion, amen cytometry. Lipid bodies interestingly polarized depletion and distribution of staining is broad depletion (11d). Early depletion (6d) shows a straightforward shift in BODIPY stain.



16S data showing that some cultures were free of bacteria (solid bar) and some had a variety and heavy fraction of bacteria in the culture (many colors in one bar)



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Impact: Strain Identification and Confirmation

Pipeline

Reducing risk associated with using culture collection strains in DISCOVR

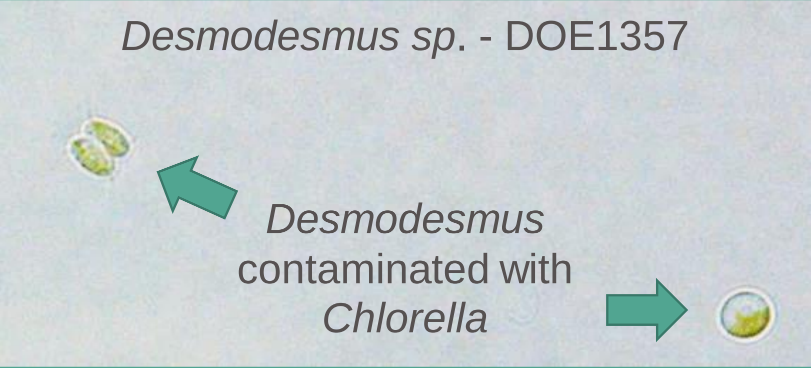
Culture collection strains may have limited characterization or identification data available

- Strains sometimes classified in culture collection by morphology, rather than sequence data
- Bacterial ID & load usually lacking
- Growth on DISCOVR media unknown

Impact

Early strain characterization reduces risk by:

- **Eliminating non-unialgal cultures**
- **Providing information on bacterial load and identity**
- **Establishing growth in DISCOVR media before intensive characterization begins**
- **Important info for future users of strains**



Publication to be submitted in 2QFY21:

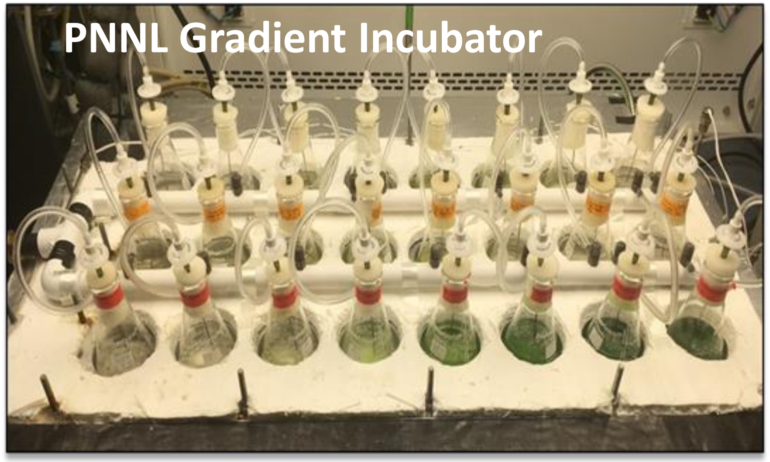
- Huesemann, Edmundson, Gao, Dale, et al. (2021), "Maximum specific growth rate as a function of temperature and salinity for 42 microalgae strains considered for biofuels production in the U.S. Department of Energy DISCOVR consortium project," *Nature Scientific Reports*, to be submitted.

Approach: TIER I Strain Characterization

Pipeline

Temperature and salinity tolerance of each strain is measured in gradient incubators

- **Background/history:** Strain characterization using temperature and salinity gradient incubators
  - *In 2011, as part of the NAABB consortium project, PNNL designed and build a thermal gradient incubator to measure maximum specific growth rates as a function of temperature, for input into the PNNL growth model*



- **Challenges:**
  - Identify the **optimum** medium **salinity** and suitable **growing season** for all candidate DISCOVR strains.
  - Quantify **maximum specific growth rate data** for down-selection to LEAPS (Laboratory Environmental Algae Pond Simulator) testing.

- **Experimental Conditions:**
  - Screening strains in **Salinity Gradient Incubator** at 5, 15, and 35 ppt salinity (25 °C)
  - Testing strains in **Thermal Gradient Incubator** at saturating light intensity from 4 to 45 °C.

- **Economic/Technical Metrics:** Identify optimum medium salinity and suitable growing season. Down-select strains with highest max specific growth rates for subsequent (Tier II) testing in LEAPS.

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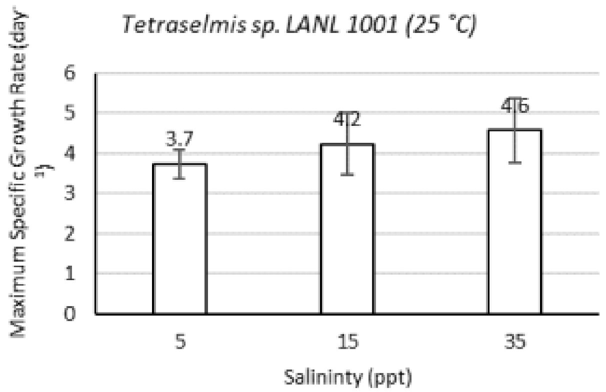
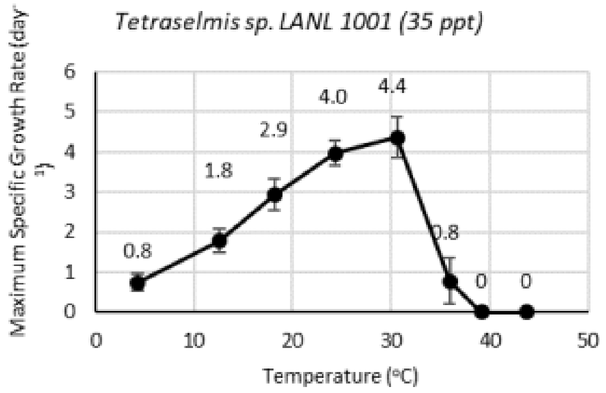
Progress & Outcomes: TIER I Strain Characterization

Pipeline

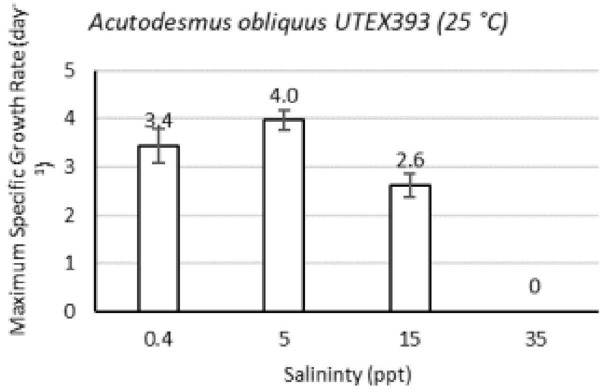
Each strain (42 tested so far) has a unique temperature and salinity tolerance range

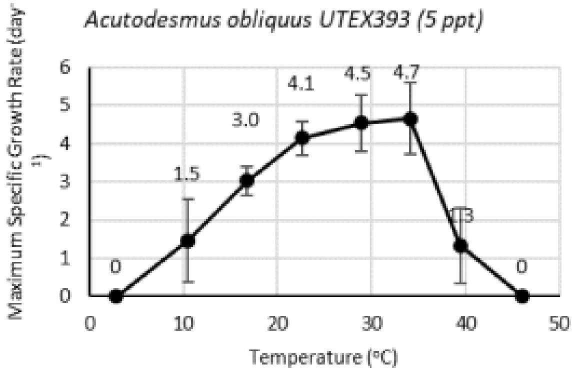
Example #1: Cold Season Strain (Marine)

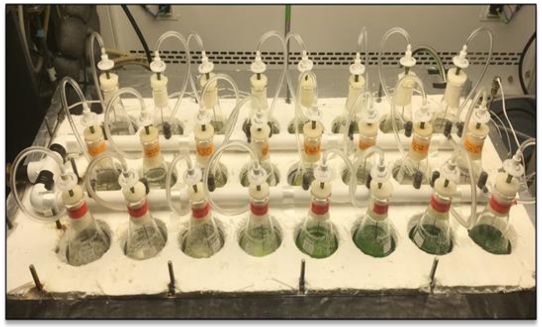




Example #2: Warm Season Strain (Brackish)







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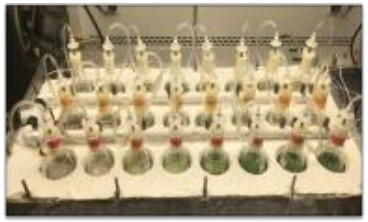
Impact: TIER I Strain Characterization

Pipeline

Identify optimum salinity and suitable growing season, down-select for subsequent LEAPS testing



42 Tier I strains, including three industrial strains, were cultured in PNNL gradient incubators to determine their respective maximum specific growth rate as a function of:

- Temperature
- Salinity

Impact

TIER I strain characterization resulted in:

- **Finding the optimum medium salinity**
- **Identifying the most suitable growing season for given strain**
- **Down-selecting 24 strains for subsequent TIER II testing in LEAPS PBRs**

- **2 publications to be submitted in 2QFY21:**
  - Huesemann, Edmundson, Gao, Dale, et al. (2021), "Maximum specific growth rate as a function of temperature and salinity for 42 microalgae strains considered for biofuels production in the U.S. Department of Energy DISCOVR consortium project," *Nature Scientific Reports,* to be submitted.
  - Krishan, Likhogrud, Cano, Edmundson, Huesemann, McGowen, Weissman, and Posewitz, "*Picochlorum celeri* as a model system for robust outdoor algal growth in seawater", *Nature Communications*, to be submitted.

ExxonMobil ALGENOL BIOFUELS µBIO MicroBio Engineering

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Approach: TIER II Strain Culturing in LEAPS PBRs

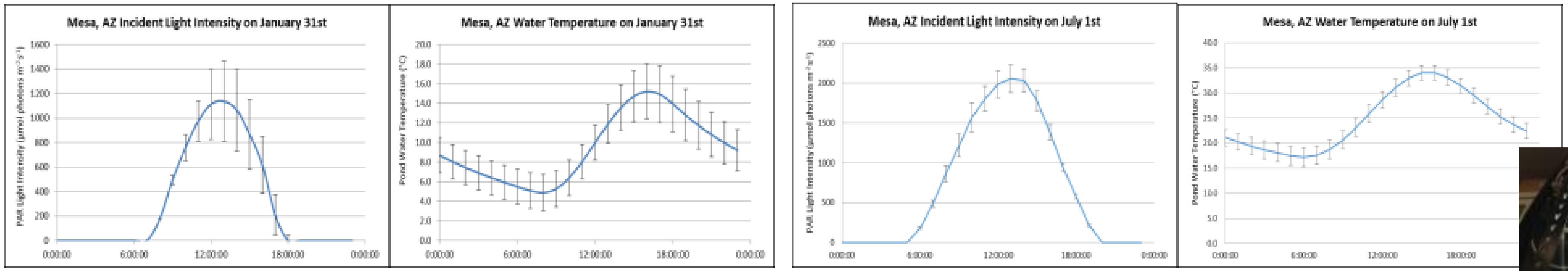
Pipeline

Use unique pond simulator PBR to measure AZ winter and summer season productivity (24 strains)

- **Background/history:** PNNL **Laboratory Environmental Algal Pond Simulator (LEAPS)** PBR
  - *In 2015, PNNL designed the LEAPS photobioreactors which* ***accurately simulate growth in outdoor ponds*** *(Huesemann et al., 2017).*
  - *The LEAPS allows* ***objective comparison*** *of growth/composition of strains using similar light/temp scripts*

- **Challenge:** Quantify Arizona (AzCATI) winter and summer season biomass productivity **under identical climate-simulated culture conditions** and **identify best** (highest productivity) **strains**.

- **AZ Winter and Summer Season Light and Temperature Scripts used in the LEAPS:**



- **Economic/Technical Metrics:** Identify highest productivity winter and summer season DISCOVR strains under identical culture and script conditions.

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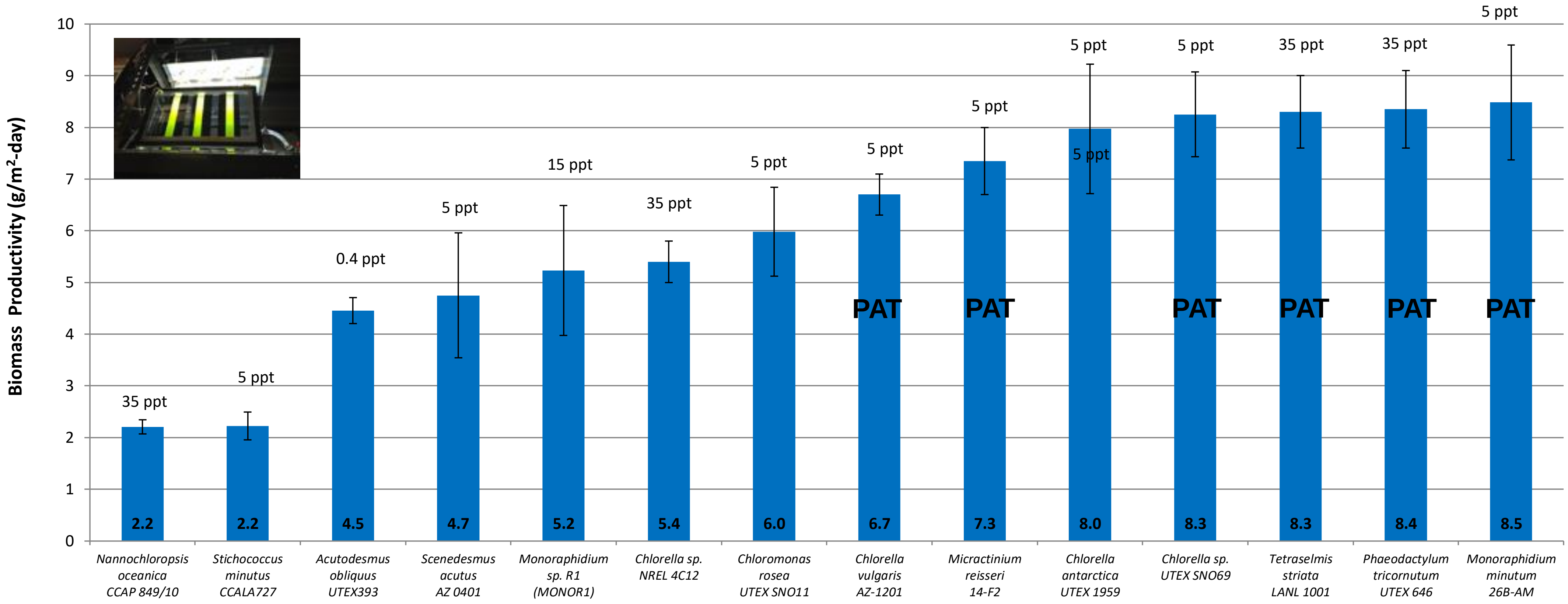
Outcomes: TIER II Winter Strain Culturing in LEAPS PBRs

Pipeline

Winter strains with the highest productivity were tested at the PNNL Algae Testbed (PAT) in AZ



Salinity in parts per thousand (ppt). Error bars are one stdev (n=4).

PAT = Tested at the PNNL Algae Testbed

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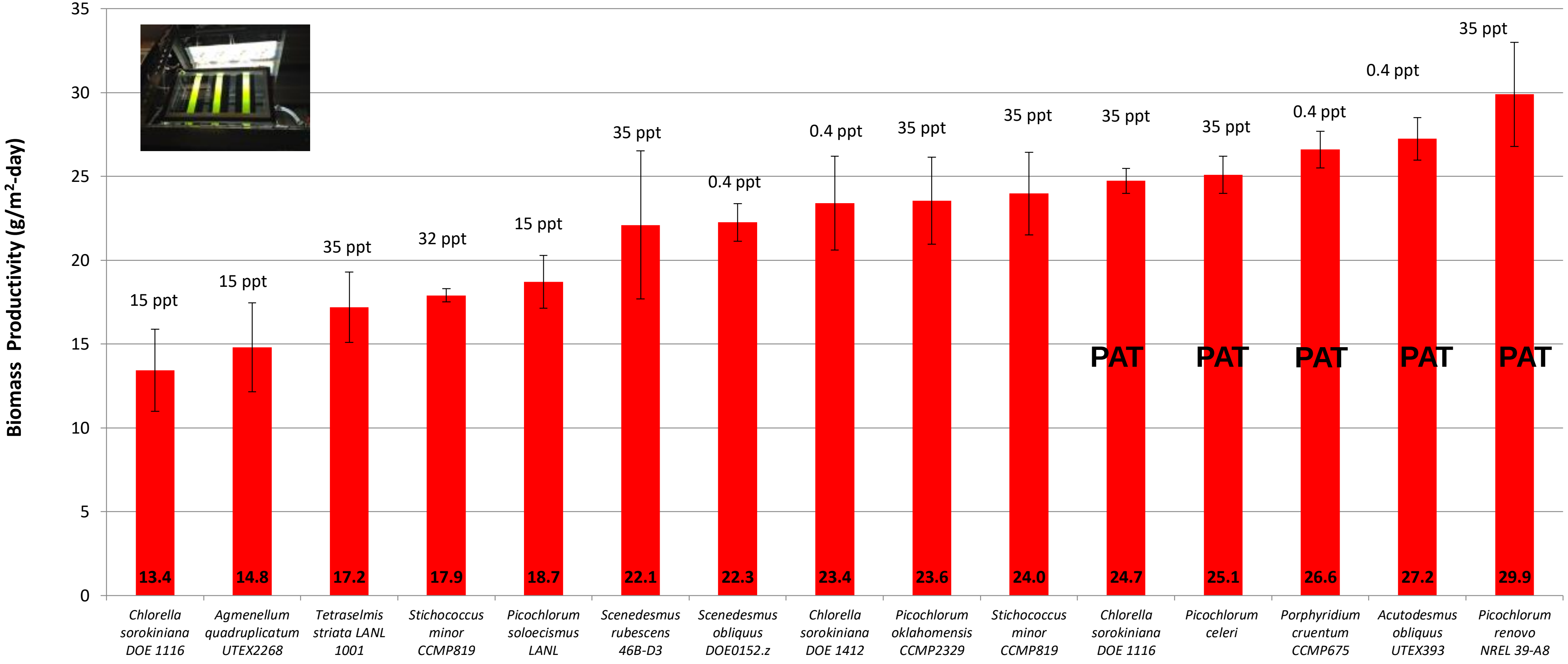
Outcomes: TIER II Summer Strain Culturing in LEAPS PBRs

Pipeline

Summer strains with the highest productivity were tested at the PNNL Algae Testbed (PAT) in AZ



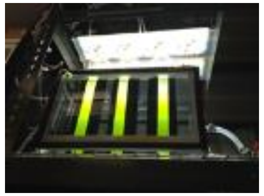
Error bars are one stdev (n=4, with the exception of *Picochlorum renovo*, n=20).

PAT = Tested at the PNNL Algae Testbed

Impact: TIER II Strain Culturing in LEAPS PBRs

Pipeline

Down-select high productivity strains for subsequent testing at the PNNL Algae Testbed in AZ



Top Tier I strains are cultured in LEAPS using identical AZ winter and summer season temperature scripts to comparatively determine:

- Areal biomass productivity
- Biomass compositional shifts upon N-depletion

ExxonMobil

Impact

The highest productivity TIER II strains, including *P. celeri* from ExxonMobil, were down-selected for subsequent testing in AZ outdoor ponds at the PNNL Algae Testbed.

- **2 publications to be submitted in 2QFY21:**
  - Huesemann, Edmundson, Gao, Laurens, et al. (2021), "Areal biomass productivities and biomass compositional shifts in LEAPS climate-simulation photobioreactor cultures of winter and summer season microalgae strains in the U.S. Department of Energy DISCOVR consortium project," *Algal Research,* to be submitted.
  - Beirne, Edmundson, Gao, Huesemann et al., (2021), "Miniaturized Laboratory Environmental Algae Pond Simulator (LEAPS) for higher throughput identification of promising microalgae strains for biofuels production", *Journal of Applied Phycology*, to be submitted.

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Approach: Climate-simulation Culturing of AzCATI Ponds

Pipeline

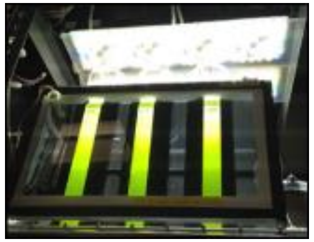
Conduct a comparative validation of all four Labs' respective climate-simulation culturing systems

- **Background/history:** Indoor climate-simulation photobioreactors and raceways at national labs
  - *All four Labs have indoor climate-simulation PBRs (LEAPS, SAGE, ePBRs) and raceways*
  - *A comparative validation using the same set of outdoor pond data had not been carried out to date*

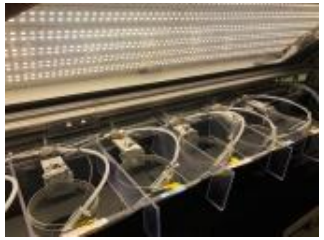
- **Objectives:** For both 26B-AM and UTEX393, measure biomass growth, N-depletion, and shift in biomass composition in all four climate-simulation cultivation systems using AzCATI light and temperature scripts, and compare to respective values measured in AzCATI outdoor pond cultures.

- **Climate-Simulation Culturing Systems at the four Labs:**

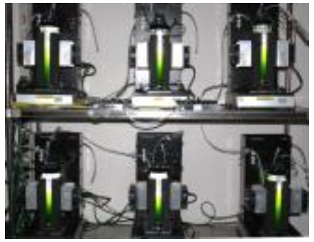
LEAPS (PNNL)



SAGE (NREL)



ePBRs (LANL)



Crash Test Ponds (SNL)

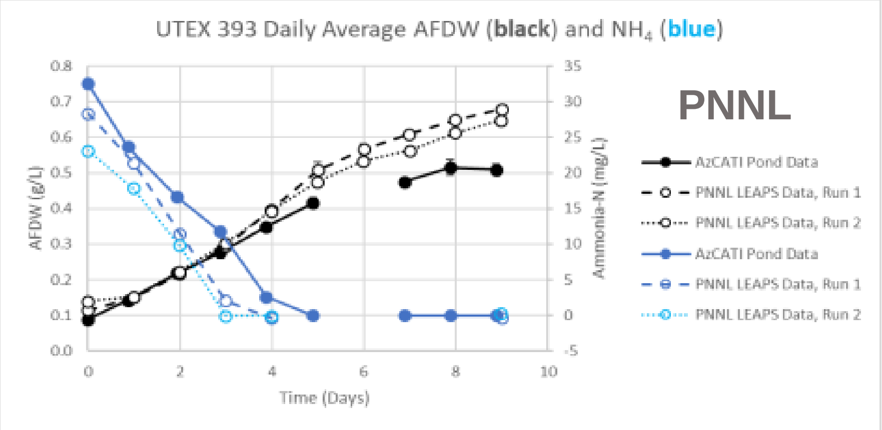
- **Economic/Technical Metrics:** Indoor climate-simulation results for testing new strains and innovative approaches **translate to similar outcomes** in SOT trials at AzCATI **with < 20% error**.

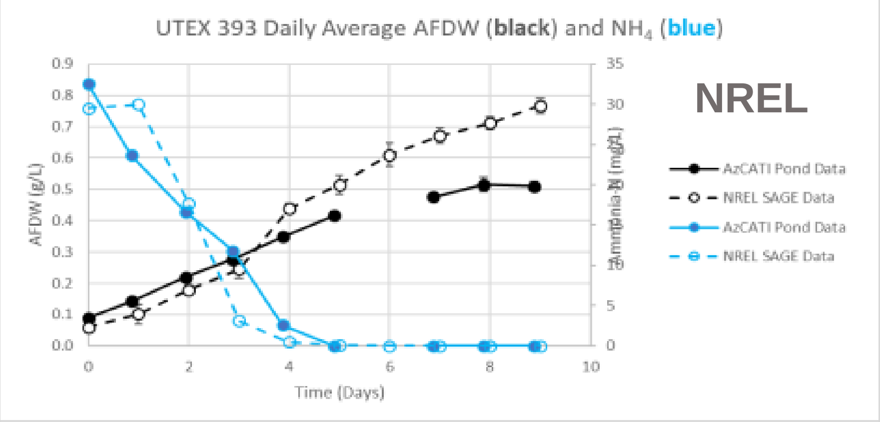
Outcomes: Climate-simulation Culturing of AzCATI Ponds

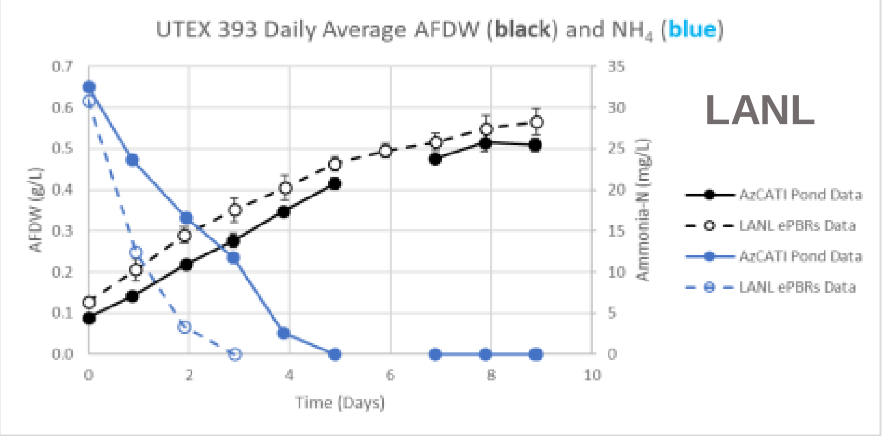
Pipeline

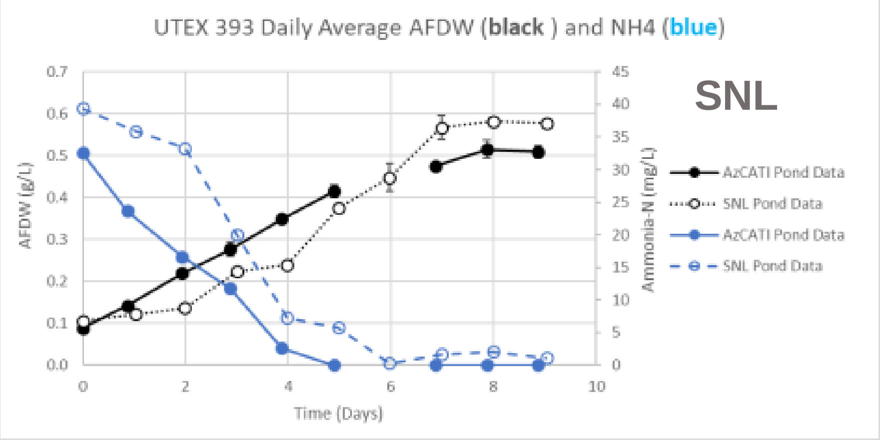
Comparative kinetics of biomass growth and NH_4 depletion for Acutodesmus obliquus UTEX393

- ➢ **Linear-phase productivities were similar (<20% error) to AzCATI ponds in all climate-simulation cultures**









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Impact: Climate-simulation Culturing of AzCATI Ponds

Pipeline

All climate-simulation cultivation bioreactors achieved productivities similar to AzCATI ponds

Repeat the October 2019 outdoor AzCATI pond culture experiments for 26B-AM and UTEX393 in each Lab's respective climate-simulation cultivation system, using AzCATI light and temperature scripts and similar growth media/conditions, and validate using four metrics:

- Linear-phase biomass productivity
- Final biomass yield
- NH_4 depletion kinetics
- Biomass composition

Impact

Following these successful comparative validation experiments, there is now a high level of confidence that results from our four indoor climate-simulation culturing systems for testing new DISCOVR strains and concepts to improve productivity and composition will translate to similar outcomes in SOT trials at AzCATI.

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Approach: TIER III Strain Outdoor Pond Culturing

Pipeline

Top strains are tested at the PNNL Algae Tested (PAT) in Arizona

- **Background/history:** Evaluation of new strains in open outdoor ponds in Arizona
  - *In 2012, PNNL established an outdoor testbed in Arizona consisting of 100 L and 800 L raceway ponds*
  - *The growth performance and stability of strains were evaluated in different BETO funded projects, i.e., the NAABB and RAFT consortium projects, and the AlgaeAirFix Incubator project.*

- **Objectives:** Quantify areal biomass productivity and determine culture stability of TIER III DISCOVR strains, evaluate harvestability via centrifuge, and ship biomass to NREL for compositional analyses

- **Challenges:**
  1. **Compared to LEAPS cultures, outdoor pond cultures are exposed to more stressors:**
     - *More extreme light and temperature fluctuations (including freezing) due to constant weather changes*
     - *Presence of predators (e.g., rotifers), infectious agents (e.g., chytrids) and competitors (e.g., diatoms).*

- **Economic/Technical Metrics:** Down-select highest productivity most stable strains, relative to benchmark strains, for subsequent evaluation at SOT testbed at AzCATI.

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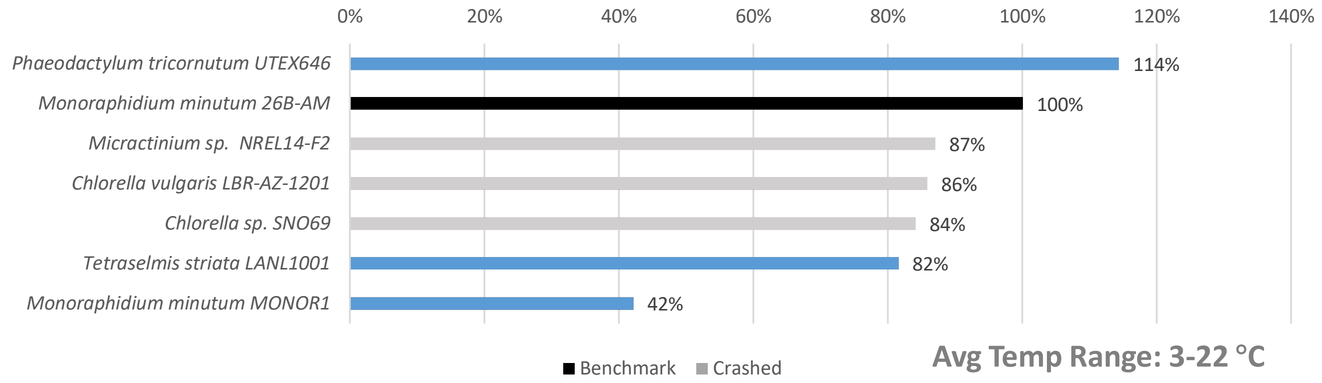
Progress & Outcomes: TIER III Strain Outdoor Pond Culturing

Pipeline

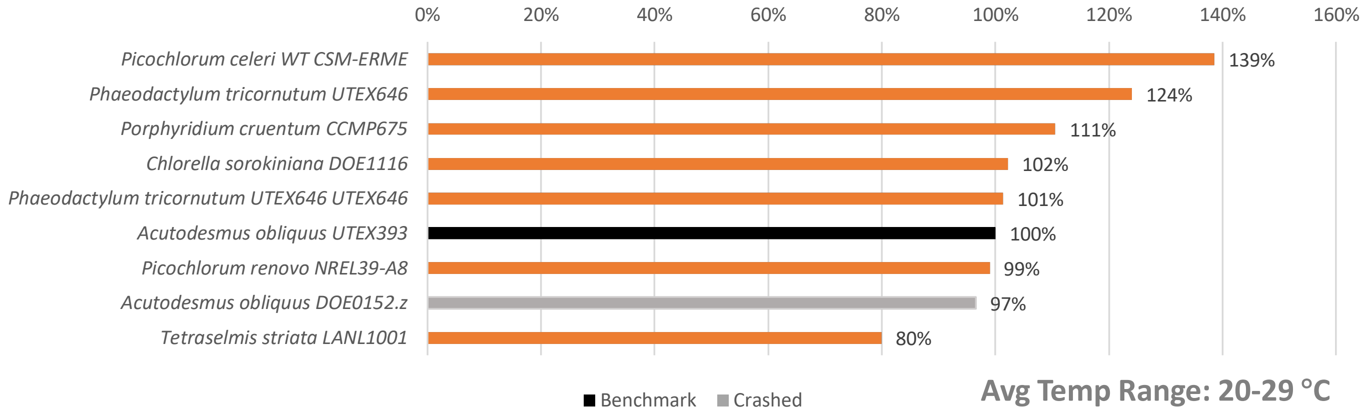
Top 13 strains were tested in 42 duplicate pond runs at the PNNL Algae Tested (PAT) in Arizona

Average Biomass Productivity Relative to Benchmark Strain (Cold Season)



Avg Temp Range: 3-22 °C

Average Biomass Productivity Relative to Benchmark Strain (Warm Season)



Avg Temp Range: 20-29 °C

Strains that had stable growth performance (no crashes) and greater or comparable biomass productivity relative to benchmarks "graduated" for subsequent testing at the BETO SOT testbed at AzCATI.

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Impact: TIER III Strain Outdoor Pond Culturing

Pipeline

Down-select stable and high productivity strains for subsequent testing in SOT trials at AzCATI

Strains with high biomass productivity in the LEAPS PBRs need to be tested under realistic outdoor pond culture conditions to determine their respective

- Areal biomass productivity relative to benchmark
- Stability for sustainable biomass production
- Harvestability
- Biomass composition

Impact

The following DISCOVR strains, including the ExxonMobil strain *P. celeri*, "graduated" at the PAT for subsequent testing in SOT trials at AzCATI:

- ***Monoraphidium minutum* 26B-AM**
- ***Acutodesmus obliquus* UTEX393**
- ***Picochlorum celeri* WT CSM-ERME**
- ***Picochlorum renovo* NREL39-A8**
- ***Micractinium reisseri* NREL14-F2**
- ***Tetraselmis striata* LANL1001**
- ***Porphyridium cruentum* CCMP675**
- ***Phaeodactylum tricornutum* UTEX646**
- **The GNG-1 Milestone "Improve Biomass Productivity by 20% Relative to Control" was met for two strains tested at the PAT.**

DISCOVR Tasks of Testing New Concepts to Move SOT Needle

Approach – Progress & Outcomes - Impacts

New Concepts

TAB

Technical Advisory Board (NREL)

DISCOVR Strain Pipeline

TIER I: Characterize Strains in Flask Cultures (PNNL, LANL, SNL)

TIER II: Quantify Strain Performance in Climate-Simulation PBRs (PNNL, NREL, LANL)

TIER III: Test Strains in Pilot-Scale Indoor and Outdoor Ponds (SNL, PNNL)

TEA

TEA Operational Baseline (NREL)

Testing New Concepts to Move SOT Needle

Improve Biomass Productivity

- Evaluate Growth Promoting Molecules (PNNL, LANL)
- Reduce Impact of Oxygen Inhibition (PNNL, LANL)
- Optimize Pond Operational Conditions (LANL, PNNL)
- Enhance Mixotrophic Growth (LANL, SNL)

Enhance Biomass Intrinsic Value

- Measure Compositional Shift Dynamics (NREL)

Increase Crop Protection and Culture Stability

- Develop Integrated Pest Management Strategies (SNL)
- Evaluate Companion Cultures (PNNL, SNL, LANL, AzCATI)
- Analyze Culture Health Using 96-Wellplate Tools (AzCATI)
- Implement Spectroradiometric Monitoring (SNL)
- Use Machine Learning to Predict Culture Crashes (SNL)

Outdoor SOT Trials to Verify Performance of Strains and Optimized Operational Conditions

- Outdoor pond SOT Trials (AzCATI)
- Compositional Analysis (NREL)
- SOT Documentation/Data Analytics (NREL)
- Annual TEA SOT assessment (NREL)

Other Projects in AAS Portfolio or Call for Collaboration

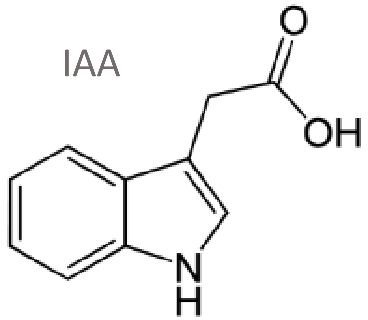
Approach: Growth Promoting Molecules to Boost Productivity

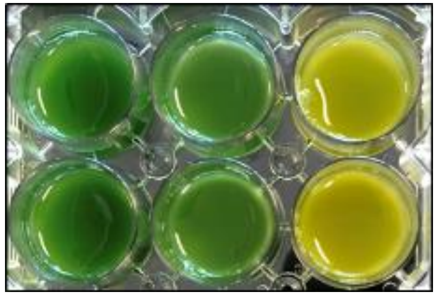
New Concepts

Increasing biomass productivity using phytohormone treatment

- **Background/history:** Growth Promoting Molecules (GPMs) have potential to boost growth and carbon storage
  - *GPMs regularly used in agriculture to alter plant growth and biomass quality*
  - *Algal literature showed GPMs can increase algae growth, but*
    - *DISCOVR strains had not been tested*
    - *Outdoor-relevant conditions had not been tested*
- **Objective:** Examine growth of DISCOVR algae strains in presence of known plant growth promoting molecules, under outdoor-relevant conditions
- **Challenges:**
  1. Cost of GPMs may outweigh benefits to algae productivity
     - *Collaborated with NREL analysis team to identify "tipping points" for costs vs productivity increases*
  2. Effect of GPMs on growth is limited in CO_2 incubator
     - *Conducted experiments in ambient CO_2, then ePBRs*
- **Economic/Technical Metrics:** Aim to boost algae growth and/or biochemical storage by at least 20% under environmental simulation conditions

IAA





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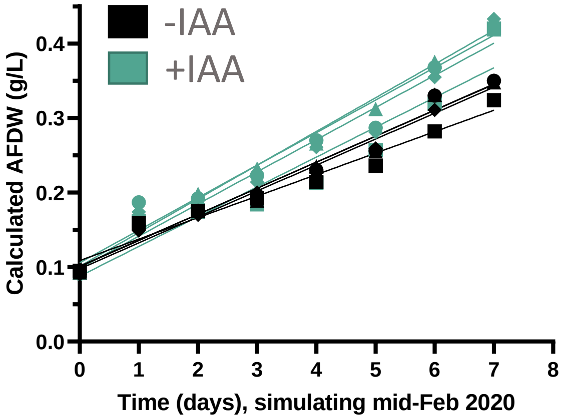
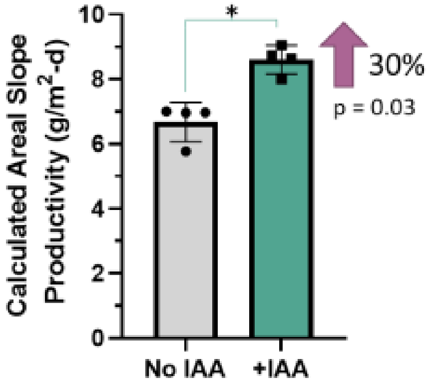
Progress & Outcomes: Using GPMs to Boost Productivity

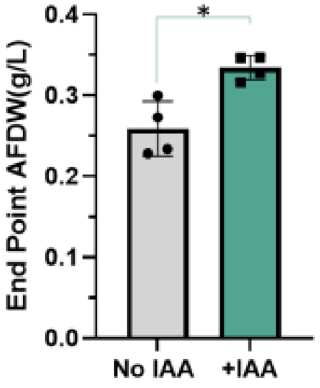
GPMs increases biomass productivity by 30% in winter and can reduce MBSF by 5%

New Concepts

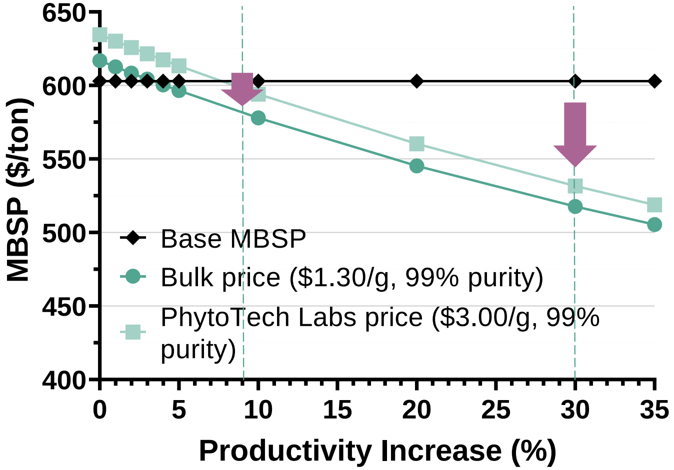
- Test GPM treatment in ePBRs,
- *M. minutum* 26BAM, AzCATI retrospective February script
- +/- 1µM IAA







- Calculated AFDW values over time show 30% increase in growth with IAA
- Slope productivities & final AFDW values show 30% increase with IAA treatment



- TEA shows that a reduction in MBSP could be attained if a productivity increase of ≥2% was observed using 1µM IAA.
- 30% increase *winter* productivity → **>5% cost reduction**
- 30% increase *annual* productivity → **>16% cost reduction**
- **NEXT:** *26BAM +/- GPM testing at AzCATI*

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Impact: Using Growth GPMs to Boost Productivity

Increasing biomass productivity with phytohormones can reduce MBSP

New Concepts

New approaches/ideas need to be tested to determine feasibility of application in outdoor ponds

- GPM data thus far tested in "non-outdoor-relevant" conditions on non-DISCOVR strains
- Cost data lacking

Impact

Observed that GPM treatment on SOT strains:

- **Increases algae productivity in at least cooler months**
- **Modeled costs with 1µM IAA show >5% decrease in MSBP**
- **Contributes to GNG-1: 20% increase in productivity (g/L/day or g/m^2/day)**

Invited Speaker at 2020 Algae Biomass Summit:

- Negi, Carr, Daughton, McGowen, Dale "Effect of phytohormones on improving biomass productivities in algal production strains"

Publication expected in FY21:

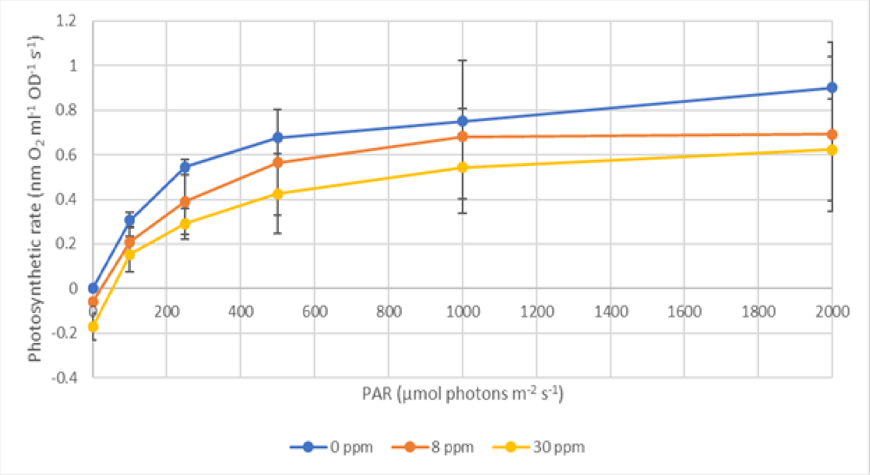
- Negi, Carr, Daughton, Klein, Davis, McGowen, Dale et al. (2021), "Productivity enhancement of microalgae treated with a growth promoting molecule under outdoor-relevant conditions", to be submitted.

Approach: Air Sparging to Remove Inhibitory Dissolved O_2

Increase biomass productivity of UTEX393 by removing inhibitory dissolved oxygen

New Concepts

- **Background/history:** Dissolved O_2 inhibits Photosynthesis of *Acutodesmus obliquus* UTEX393
  - *P-I curve measurements indicated that photosynthetic oxygen production rates for UTEX393 decreased with increasing dissolved oxygen concentration (DO).*
  - *Air-sparging to strip dissolved oxygen from ponds may reduce inhibition and increase biomass productivity.*



- **Objectives:** Sparge raceways (at PAT) from 6 am to 6 pm with air (1%v/v/min) to remove inhibitory dissolved photosynthetic oxygen and increase biomass productivity.

- **Challenges:**
  1. **Air-sparging of large-scale ponds may be cost-prohibitive and increases MBSP.**
  2. **Need to conduct TEA trade-off analysis.**

- **Economic/Technical Metrics: Increase biomass productivity** of DISCOVR strains subject to inhibition by photosynthetic O_2 **by ≥20%.** Impact on MBSP needs to be determined by TEA.

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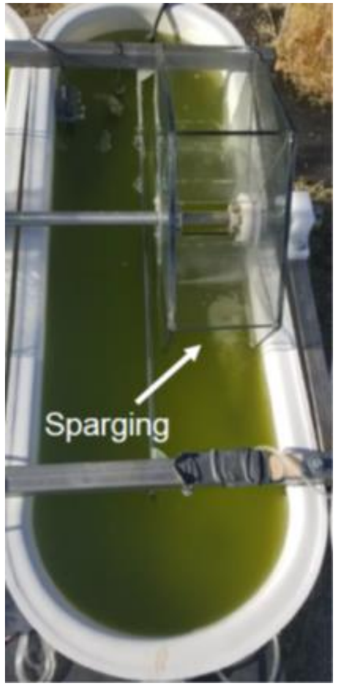
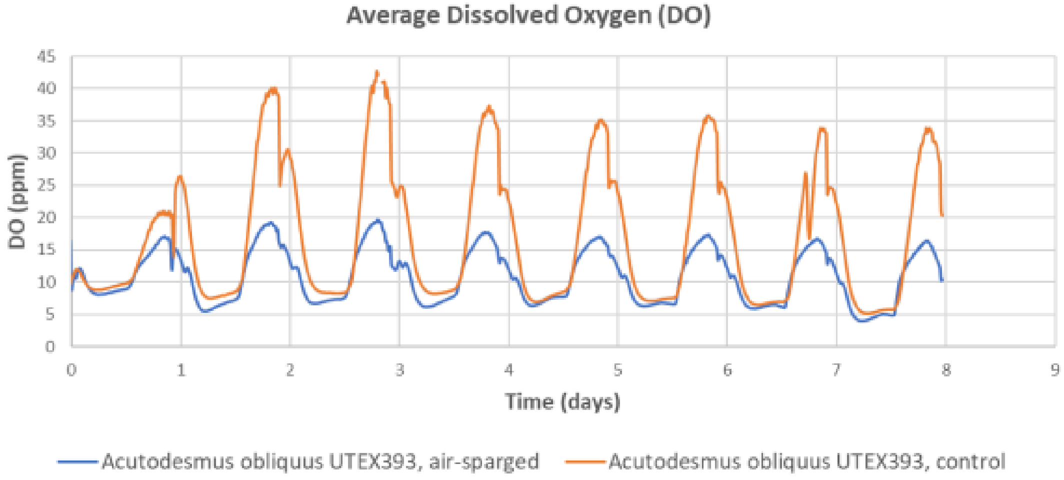
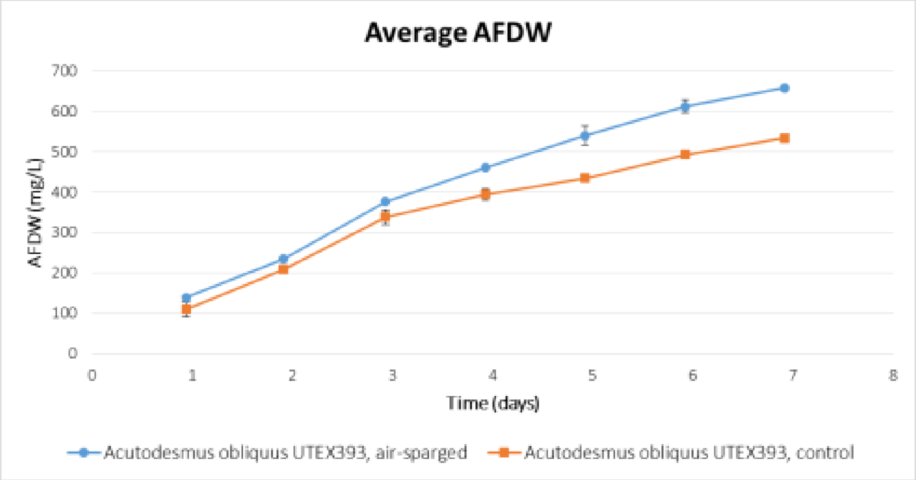
Outcomes: Air Sparging to Remove Inhibitory Dissolved O_2

Sparging UTEX393 cultures decreased DO < 20 mg/L + increased productivity by >50%

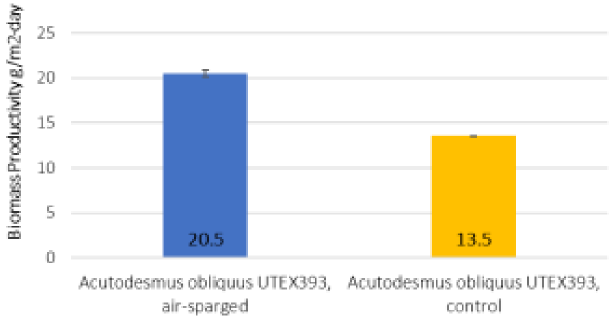
New Concepts







- Increase in biomass productivity by 51%



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Impact: Air Sparging to Remove Inhibitory Dissolved O_2

Sparging UTEX393 cultures increased productivity by >50%, exceeding Go/No-Go

New Concepts

For some DISCOVR strains, inhibition of photosynthesis by elevated dissolved oxygen concentrations can be reduced or avoided by sparging pond cultures with air, stripping excess O_2.

Impact

Air sparging of outdoor pond cultures of *Acutodesmus obliquus* UTEX393 had the following impacts:

- **Dissolved oxygen concentrations decreased below inhibitory levels (<20 mg/L)**
- **Biomass productivity increased by 51% relative to non-sparged controls**
- **The GNG-1 Milestone "Improve Biomass Productivity by 20% Relative to Control" was exceeded and therefore met.**

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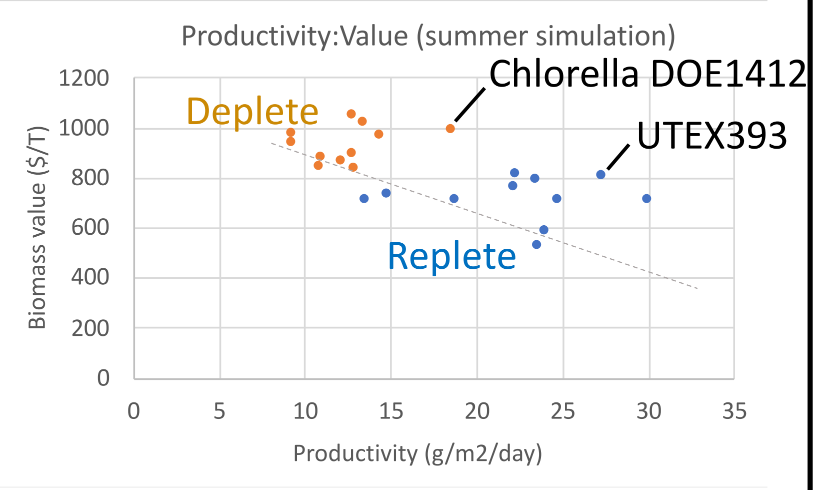
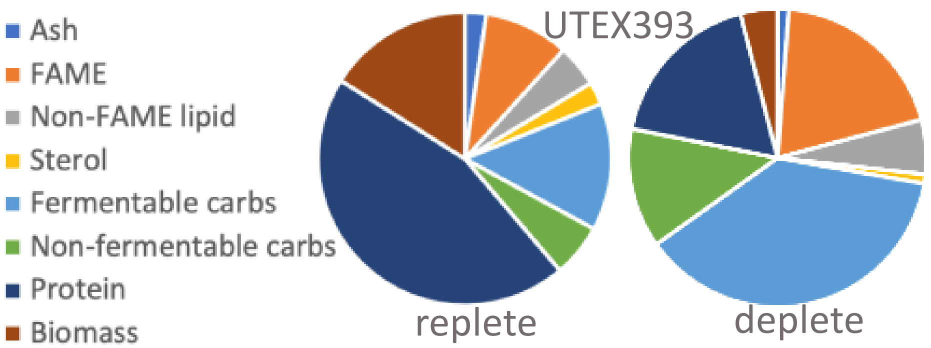
Approach: Manipulating Biomass Composition

Improving biomass value ($/g) and areal value productivity ($/m²-day)

New Concepts

- **Background/history: NREL work showed that species have distinct capacity and rates to shift biomass composition in response to environmental stimuli***
  - Biomass composition was used for ranking strain promise based on areal **Value Productivity ($/m²/day)**
- **Objective:** Improve biomass value productivity by shifting biomass composition to carbon storage products, for valorizating in biomass conversion process
- **Challenges: Composition shift can be accompanied with reduction in growth rates, thereby increasing biomass cost**
  - Tailor and quantify rate of compositional shift to each species, quantify value in CAP conversion
- **Economic/Technical Metrics:** Achieve economic profitability by demonstrating that biomass value exceeds biomass production cost.





*Laurens, 2014, Anal. Biochem, 452:86-95

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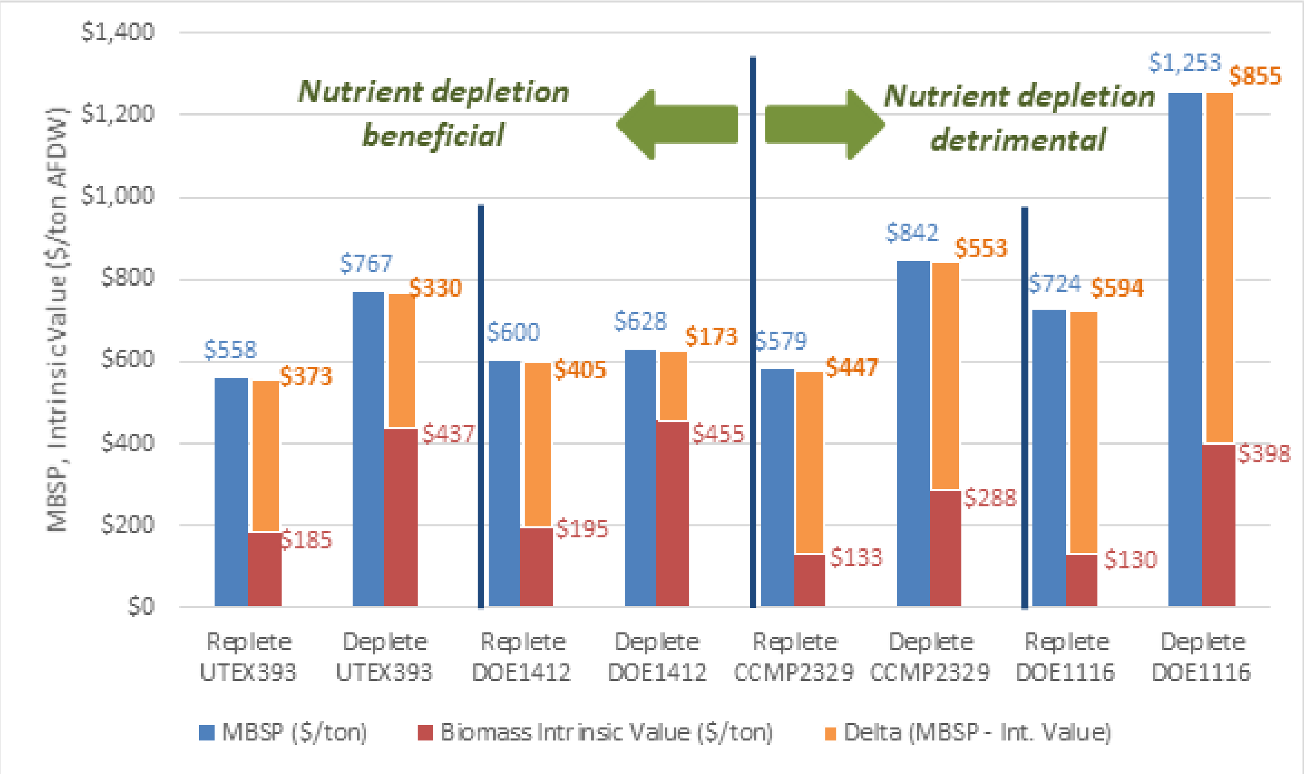
Outcomes: TEA Modeling – Biomass Composition

New approach to algal biomass valorization methodologies

New Concepts

- **Objective:** Provide a rapid means of quantifying the intrinsic value of algal biomass over shifting composition

- Separate NREL projects (Algae TEA, ABC) provided outputs to DISCOVR for new biomass valorization framework
- **Outcome**: Quantified tradeoff between compositional value vs production cost for <u>*one example*</u> CAP case
- Findings leveraged to prioritize DISCOVR priorities, with expansion: other CAP profiles, other strains, more data points vs "replete-deplete" pairs



Value of the biomass linearly correlated to the respective value assigned to the components in their final application after conversion

$$\frac{\$}{T_{biomass}} = \sum a[Lipid_{ash-free}] + b[Carbohydrate_{ash-free}] + c[Protein_{ash-free}]$$

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Impact: Rapidly Manipulating Biomass Composition

Improving biomass value

New Concepts

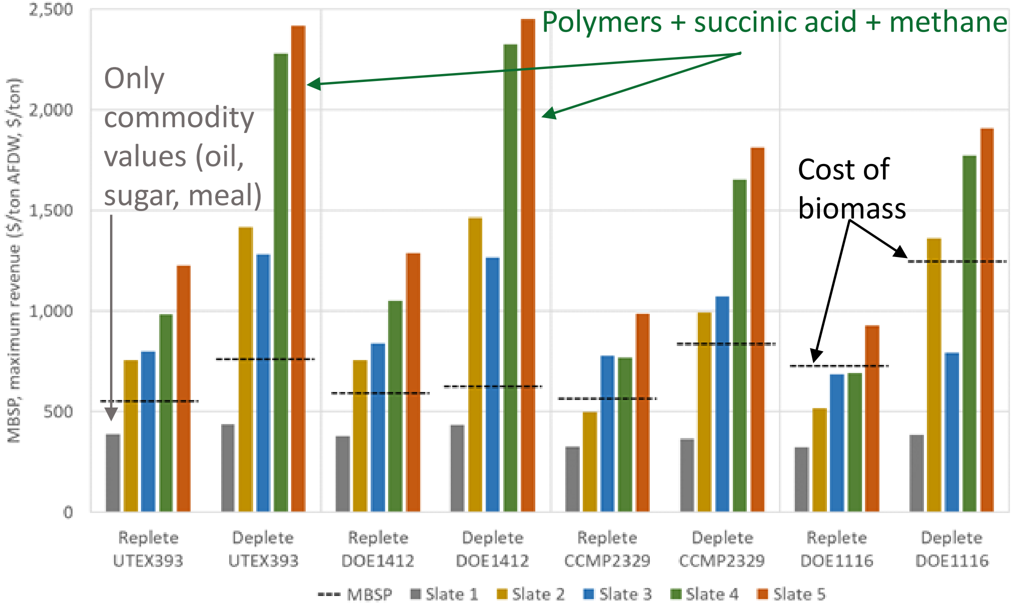
Biomass profiles rich in protein shifted to higher carbohydrates at different rates based on nutrient and environmental conditions

- Response rates critical to minimize impact on productivity

Impact

Top performing strains able to rapidly and predictably shift composition

- **Conversion pathways value carbohydrates as intermediates to high value products and thus rapid compositional response/shift positively impact biomass value productivity**



Combined revenue potential for replete and deplete harvested biomass shows **> $1,000/T margin in value**

- Combined with biomass productivity indicates an **up to 80% increase in Value Productivity (not yet fully cost-burdened for process intensity)**

- Publication to be submitted: Klein, Davis, Laurens, et al., 2021 “Closing the Gap Between Production Cost and Value Accelerates Algae Commercialization Potential” Journal TBD.

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Approach: Increase Crop Protection and Culture Stability

New Concepts

Develop integrated pest management strategies

- **Background/history:** SNL's capabilities in crop protection (pest identification and countermeasures were developed over a series of BETO funded projects (Pond Crash Forensics, ATP3, TABB, PEAK)
  - *Developed and demonstrated methods for detection and genetic identification of pond crash agents.*
  - *Developed strategies for control of pest species (e.g., rotifers) that are cost free and not dependent on chemical treatment.*
- **Objective:** Develop an integrated pest management strategy to control grazers and parasites, through a combination of algal strain improvement, industrial ecology, counter-measures, and crop protection technologies and strategies. Goal is to deploy strategies at the SOT testbed to improve seasonal productivity.
- **Challenges:**
  1. **Recapitulation of crashes and isolation of agents**
     - *Agents may be obligate pathogens or parasites complicating efforts at isolation and characterization.*
  2. **Identification of agents**
     - *Sequencing analysis of pond crashes provides presumptive identifications only; that must be confirmed by further analysis.*
- **Economic/Technical Metrics:** Development of integrated pest management strategies that result in increases in productivity and Mean Time Before Failure (MTBF) by at least 33%

Outcomes: Identification of SOT Pond Crash Agent

We have identified the agents responsible for pond crashes

New Concepts

- ***SNL is actively investigating a number of deleterious species isolated from AzCATI culture material.***
- *We have identified a number of pest species*
- *We have developed in house assays*
- *We are characterizing the agents and developing countermeasures*
- *We herein describe the work carried out for one of these pest species.*

- ***Koch's postulates for pond crashes***
- *SNL carried out Microbiome analysis of crashed 26B-AM ponds from AzCATI*
- *Identified Amoeboaphelidium sp as putative agent for Monoraphidium minutum 26B-AM.*
- *Carried out PCR analysis of pond crash samples with Amoeboaphelidium specific probes.*

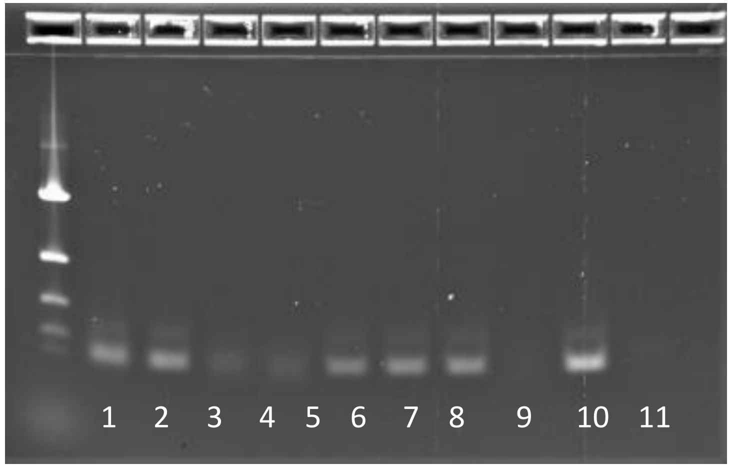
MiSeq FD01 Hits

		SPW15 6/4/18	SPW10 7/23/18	SPW12 7/23/18	SPW14 7/23/18	SPW10 10/23/18	SPW12 10/23/18	SPW13 4/24/19	SPW14 4/24/19	SPW16 4/1/19
OTU 11	Amoeboaphelidium sp. PML-2014 isolate FD01					29274	42642	280	2519	15301

PCR on Pond Samples with FD01 primers

Pond samples

2000
800
400
200
100



1. DNA Ladder
2. spw10 (10/23)
3. spw12 (10/23)
4. spw15 (6/4)
5. spw12 (7/23)
6. spw13 (10/23)
7. spw14 (10/23)
8. spw16 (10/23)
9. *26BAM* control
10. spw12 plug 8
11. No template

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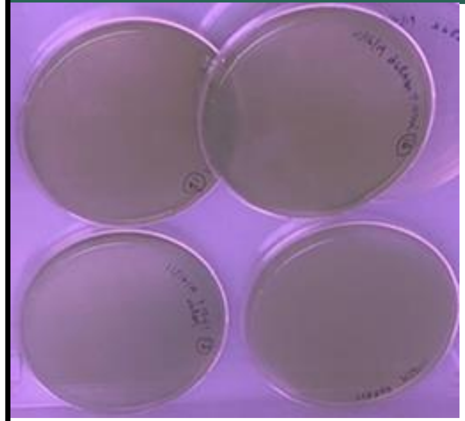
Outcomes: Agent Isolation and Crash Recapitulation

Isolated crash agents and created crashes on demand under controlled conditions

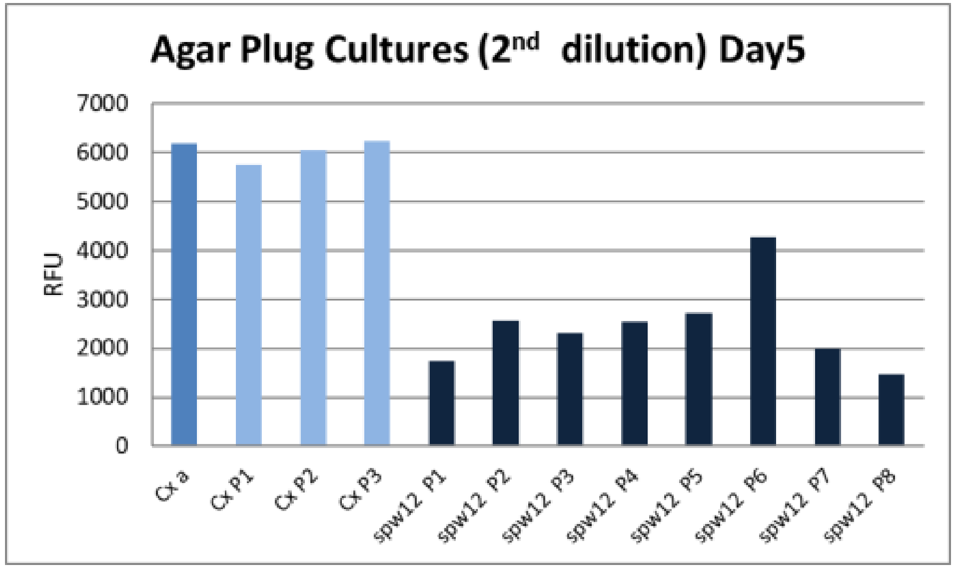
New Concepts



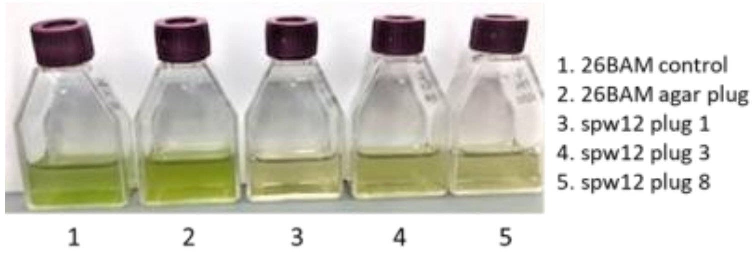
- *Agents were isolated by plating on algal lawn.*
- *Zone of clearing were cored creating agar plugs*

Agar plug cultures

Second dilution of agar plug cultures into fresh *26BAM* to recapitulate crashes



The first dilution of the agar plugs were diluted 1:10 into fresh *26BAM*. This is the fluorescence on day 5 after *26BAM* inoculation. Samples are: *26BAM* controls (medium blue), *26BAM* agar plugs (light blue), and spw12 plugs (dark blue).



- *Individual isolates were used to recapitulate crashes.*
- *Isolates that were sufficient to crash cultures we identified by PCR and sequencing*
- *Isolates were then used to create crash assays for the development of countermeasures*

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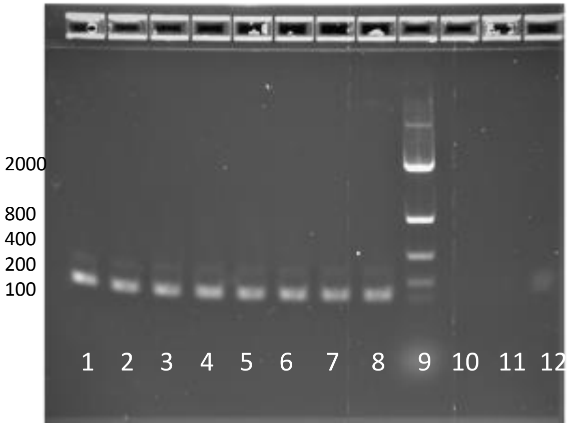
Progress & Outcomes: Development of Countermeasures

We are characterizing "crash parameters" and developing preventative strategies

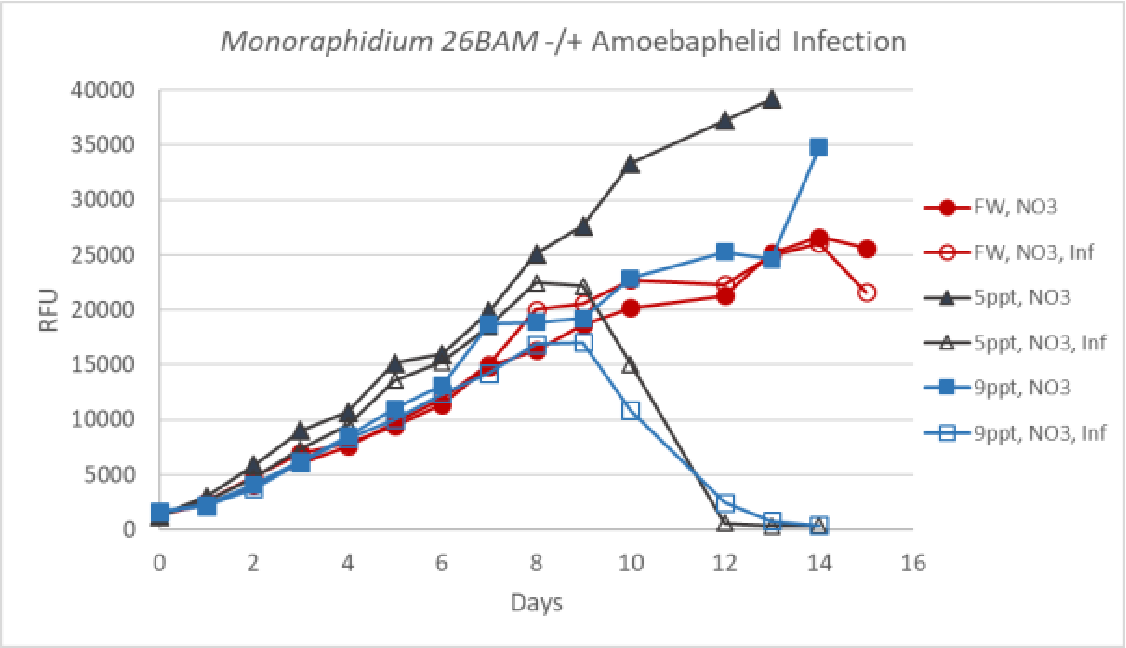
New Concepts

- **Identified isolated agents capable of causing pond crashes**
  - *Isolated Amoeboaphelid was identical to that found in crashed ponds (confirmatory identification)*
- **Developing countermeasures for prevention or delaying of Amoeboaphelid mediated crashes**
  - *Identified algal culture conditions that strongly delay the onset of crashes*
  - *Testing algal/bacterial co-cultures for the ability to inhibit Amoeboaphelid growth*
  - *Developing resistant strains of algae.*

Agar plugs from spw12 recapitulated crash



1. spw12 agar plug 1
2. spw12 agar plug 2
3. spw12 agar plug 3
4. spw12 agar plug 4
5. spw12 agar plug 5
6. spw12 agar plug 6
7. spw12 agar plug 7
8. spw12 agar plug 8
9. DNA Ladder
10. No template
11. 26BAM agar plug 2
12. 26BAM agar plug 3



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Impact: Integrated Pest Management

Increasing mean time to failure without increasing cost of production

New Concepts

Annualized productivity is limited by pond failure due to biocontaminants.

- Options for treatment are often limited and can contribute to the cost of production.
- Because of expense, current crop protection strategies are often limited to treat upon detection strategies.
- Improved strategies would be integral to the production system and prevent crashes

Impact

Development of integrated pest management strategies reduces risk by:

- **Developing multiple mechanisms of crop protection.**
- **Creating a defense in depth that can compensate for the failure of one or more strategies.**
- **Creating low or zero cost method and strategies for crop protection.**

- **1 peer-reviewed publication:** Fisher, C.L. and **Lane, T.W.** 2019 Operational, Prophylactic, and Interdictive Technologies in Algal Crop Protection. In *Grand Challenges in Algae Biotechnology* (*ed* P.H. Rampoletto and H. Armin) Springer. Pages 35-70 https://doi.org/10.1007/978-3-030-25233-5_2
- **Additional publications will be submitted in Q2**: Lane, Hagerstrand, and Lane "Characterization of the Resistance of DISCOVR Algal Strains to Multiple Deleterious species" to be submitted to *Algal Research.*
- *Lane and Lane "Isolation, Growth Characterization and Host Specificity Determination of of a novel Amoeboaphelid isolated from Pilot-Scale Algal Production Ponds".*

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Approach: Spectroradiometric Monitoring

An early-warning system for a broad array of pond pests

New Concepts

- **Background/history:** SNL's spectroradiometic monitoring approach was originally developed & demonstrated for assessment of algal biomass & pigment optical activity (SNL LDRD→BETO ATP[3]).

Innovation

  - All optical detection, only requires line-of-sight
  - Rapid (~ 5 min) temporal resolution
  - Functional detection of pest presence based on optical properties of algae and/or pest
- **Objective:** Extend approach to automated detection of a broad array of pests indoor and outdoor
- **Challenges:**
  1. **Deployment at the CA Testbed provides unique challenges compared to outdoor**
     - *A generalized analysis approach to enable lab-to-field & indoor vs. outdoor transferability.*
     - *Indoor LED illumination has poor spatial/angular homogeneity and lacks near-infrared.*
  2. **Automated operation over a broad array of pests, strains & conditions**
     - *Requires extensive pest/algae characterization*
     - *Modular code design, improved interface, automated change detection algorithms*
- **Economic/Technical Metrics:** Early detection facilitates mitigation, ↑productivity and ↑MTBF

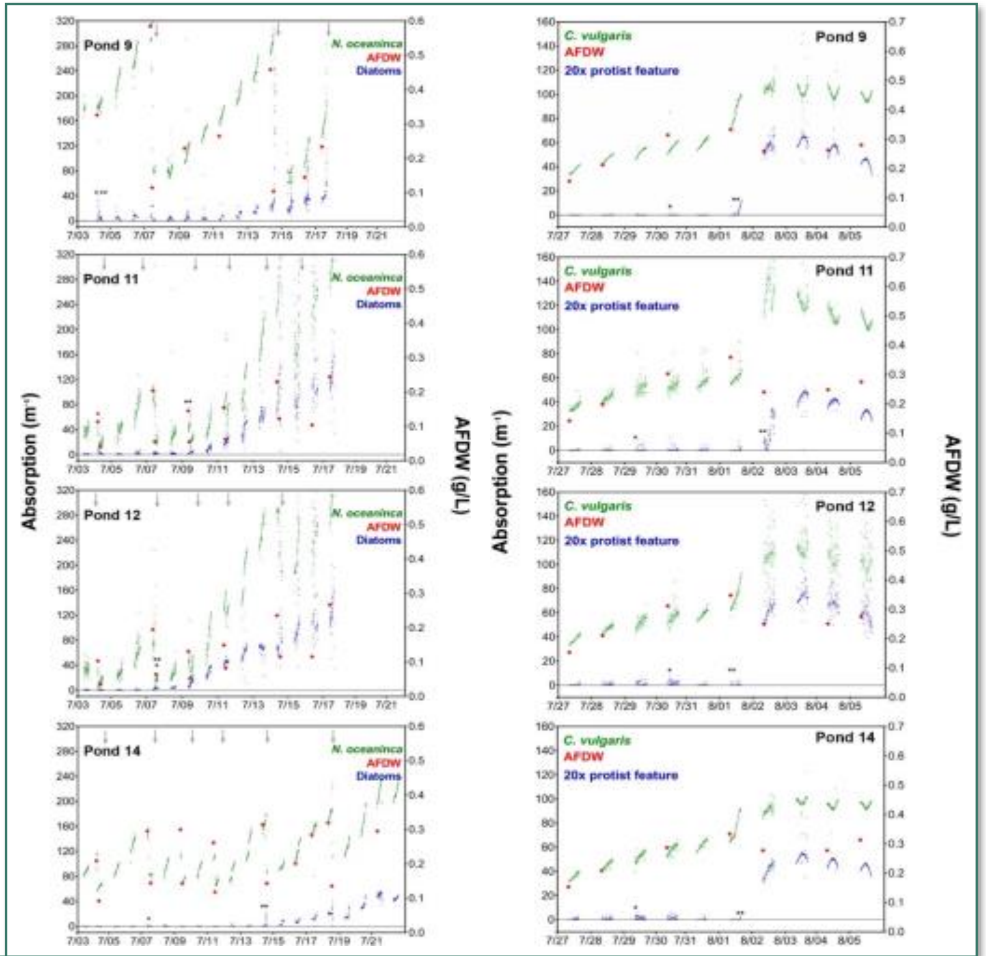
Progress and Outcomes: Spectroradiometric Monitoring

New Concepts

Validated pest detection capability

- **Key technical accomplishments:**
  - Successfully validated pest detection capability
    Reichardt et. al. *Algal Research* **51**, 102020 (2020)
  - Showed early detection (up to 3 days) for two algal/pest pairs in outdoor cultivation at AzCATI as compared to molecular sequencing and microscopy
    - Diatoms invading *N. oceanica*
    - Poteriochromonas grazing on *C. vulgaris*
  - Identified a potential “treatment” window between initial and sustained pest presence

- *Signatures expected to be indicative of class of pest and extendable to detect other diatoms and grazing events → Additional algal/pest pairs in progress*



Analysis of spectroradiometric data from 4 replicate ponds at AzCATI demonstrates the ability to monitor invasion from diatoms (left column) and predation by Poteriochromonas (right column).

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Impact: Spectroradiometric Monitoring

Detect-to-warn & detect-to-treat

New Concepts

Current assessments of pest presence are:

- Based upon grab samples
- Requires on-site laboratory & skilled support staff
- Not scalable to large-scale farms

Impact

Spectroradiometric monitoring will enable semi-automated, broad-area-identification of pest presence in algal factory farms earlier & more economically than current methods

Early detection facilitates mitigation strategies leading to enhanced productivity & longer times between failure.

Milestone progress:

- FY20, Q3 – met, automated software
- FY21, Q1 – in prog/on target, anomaly algorithm
- FY21, Q3 – in prog/on-target, monitor 4 strains, detect anomalies

- **1 collaborative peer-reviewed publication**
  - Reichardt, Maes, Jensen, Dempster, McGowen, Poorey, Curtis, Lane, and Timlin, "Spectroradiometric detection of competitor diatoms and the grazer *Poteriocrhomonas* in algal cultures," *Algal Res* **51**, 102020 (2020).
- **2nd publication to be submitted in Dec/Jan** timeframe: Atencio, Maes, Hipple, and Timlin, "Susceptibility of two saltwater strains of *Chlorella sorokiniana* to *Vampirovibrio chlorellavorus*," to be submitted to *Algal Res* (2020).
- **4 conference presentations (1 invited)**

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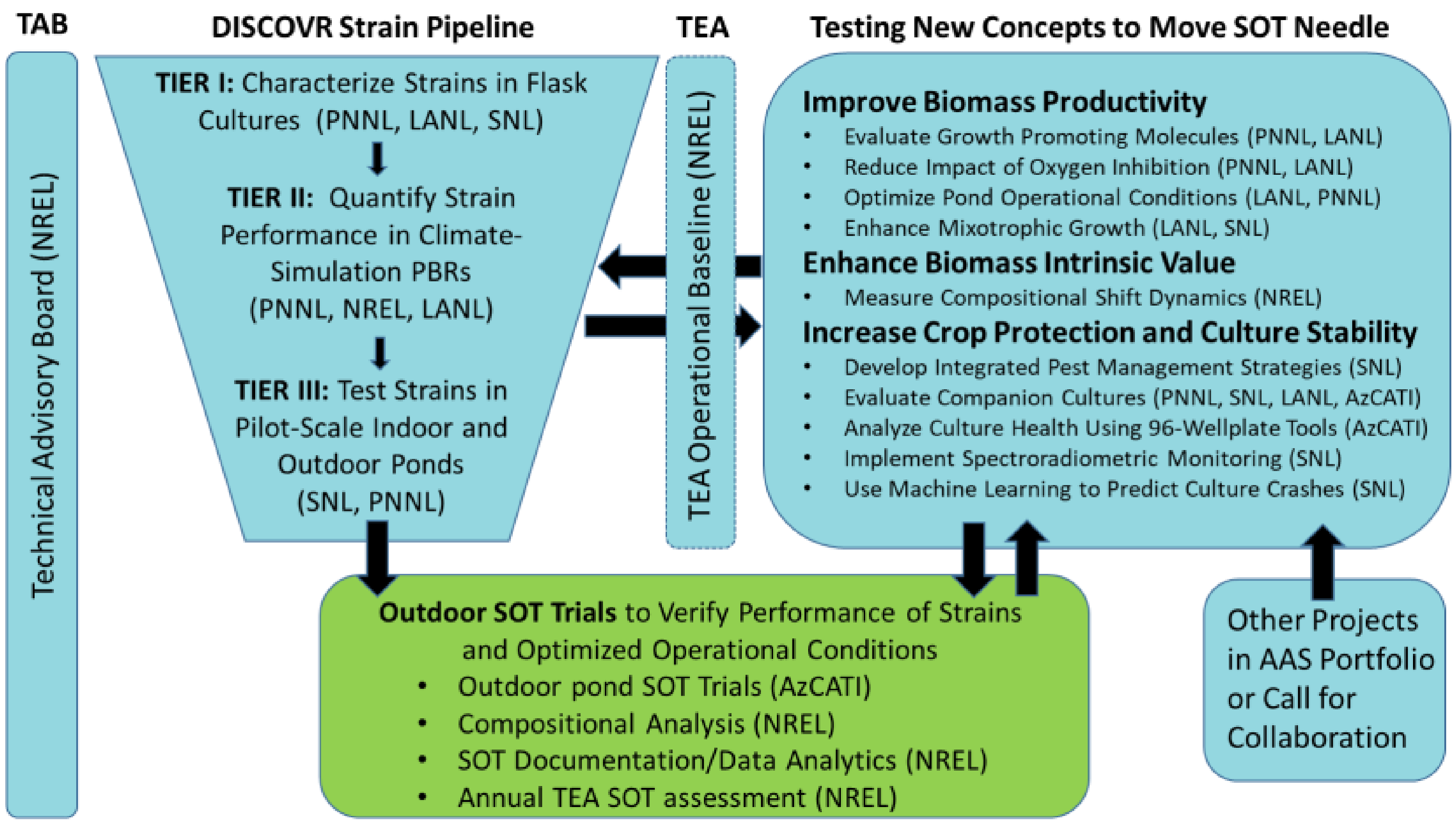
Outdoor State of Technology (SOT) Trials

Approach – Progress & Outcomes - Impacts

SOT Trials



Approach: State of Technology Cultivation Trials

Robust experimental framework for conducting trials based on > 7 years of experience

SOT Trials

- **Background/history:** *SOT cultivation framework established under ATP^3 (2013-2018) and continued under DISCOVR (2018-present).*
- **Objective:** *Conduct year-round, outdoor cultivation trials to determine monthly/seasonal/annual biomass productivity.*
- **Challenges:** *Implementing effective and robust crop protection and integrated pest management strategies remains the most significant challenge to achieving AND maintaining high productivity.*
- **Economic/Technical Metrics:** *The SOT cultivation trials are THE main source of data for the determination of current MBSP and MFSP allowing for AAS to measure progress towards the 2030 goals of achieving* ***25 g/m^2-day*** *annual average productivity and MFSP* ***<$2.5 GGE****.*

Standard 4.2 m^2 open raceway ponds (ORP) for SOT cultivation trials at AzCATI. Twelve (12) ORP's dedicated to DISCOVR seasonal cultivation trials allowing up to four experimental conditions to be run simultaneously in triplicate.

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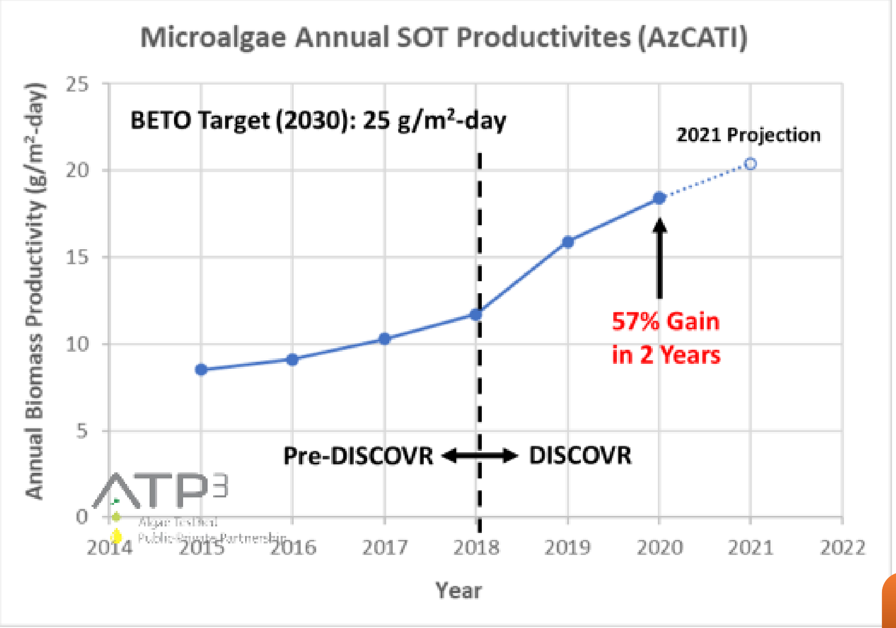
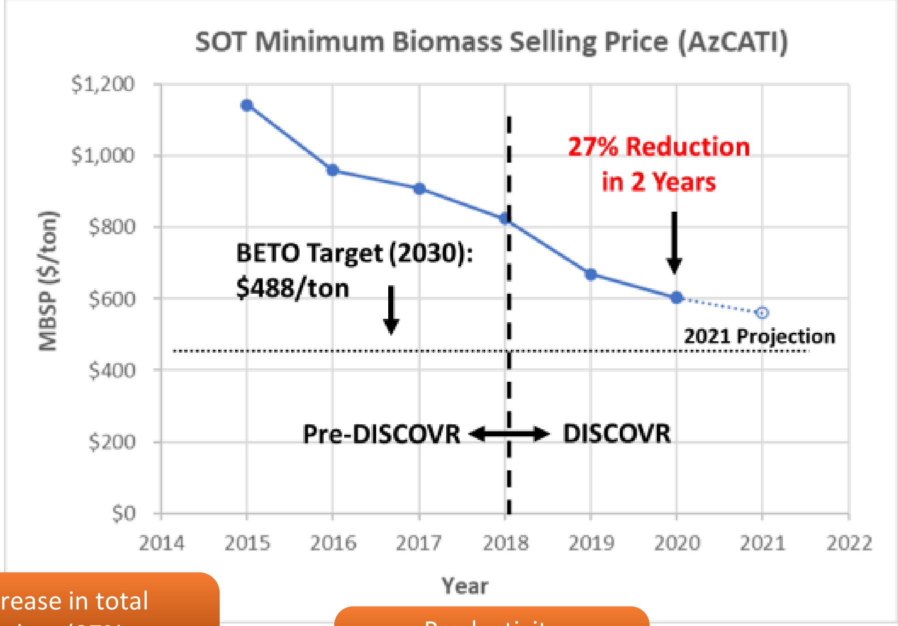
Progress & Outcomes: SOT Cultivation Trials

SOT Trials

Use of DISCOVR strains resulted in 57% increase in SOT productivity and 27% decrease in MBSP





8% increase in total cultivation days (97% - now exceeds 90% SOT basis)

Productivity improvements driven by fall, winter, summer

		FY2019 SOT						FY2020 SOT						
Season	Month	Productivity g/m2-day	AFDW at Harvest g/L	Strain	Days	Ops cond	Avg. Stdev	Productivity g/m2-day	AFD[illegible] Harvest g/L	Strain	Days	Ops cond.	[illegible] St[illegible]	% Gain/Loss YOY
Fall	September	11.0	0.29	C046	16	20cm/SC	**11.4**	**19.4**	0.35	UTEX393	29	20cm/SC	**15.0**	32.0%
	October	13.3	0.38	26BAM	23	20cm/SC	1.8	**14.4**	0.35	26BAM	30	20cm/SC	4.1	
	November	9.8	0.57	26BAM	27	20cm/SC	RSD = 16%	**11.2**	0.36	26BAM	28	20cm/SC	RSD = 27%	
Winter	December	5.7	0.58	26BAM	38	10cm/SC	**6.5**	**7.1**	0.35	26BAM	34	20cm/SC	**8.3**	28.9%
	January	7.3	0.52	26BAM	24	10cm/SC	0.8	**8.5**	0.72	26BAM	31	10cm/SC	1.2	
	February	6.4	0.43	26BAM	28	10cm/SC	RSD = 12%	**9.4**	0.60	26BAM	28	10cm/SC	RSD = 14%	
Spring	March	12.3	0.68	26BAM	31	10cm/SC	**18.6**	**14.1**	0.58	26BAM	31	10cm/SC	**18.5**	-0.9%
	April	17.6	0.66	26BAM	28	10cm/SC	6.9	**17.1**	0.33	UTEX393	32	20cm/SC	5.2	
	May	26.0	0.46	UTEX393	28	20cm/SC	RSD = 37%	**24.2**	0.33	UTEX393	30	20cm/SC	RSD = 27%	
Summer	June	26.3	0.44	UTEX393	27	20cm/SC	**27.1**	**27.1**	0.40	*P. Celeri*	19	20cm/SC	**31.6**	16.4%
	July	30.6	0.48	UTEX393	30	20cm/SC	3.1	**31.9**	0.49	*P. Celeri*	30	20cm/SC	4.4	
	August	24.5	0.37	UTEX393	28	20cm/SC	RSD = 11%	**35.8**	0.56	*P. Celeri*	31	20cm/SC	RSD =	
					328		15.9				353		18.4	15.4%

Impact: State of Technology Cultivation Trials

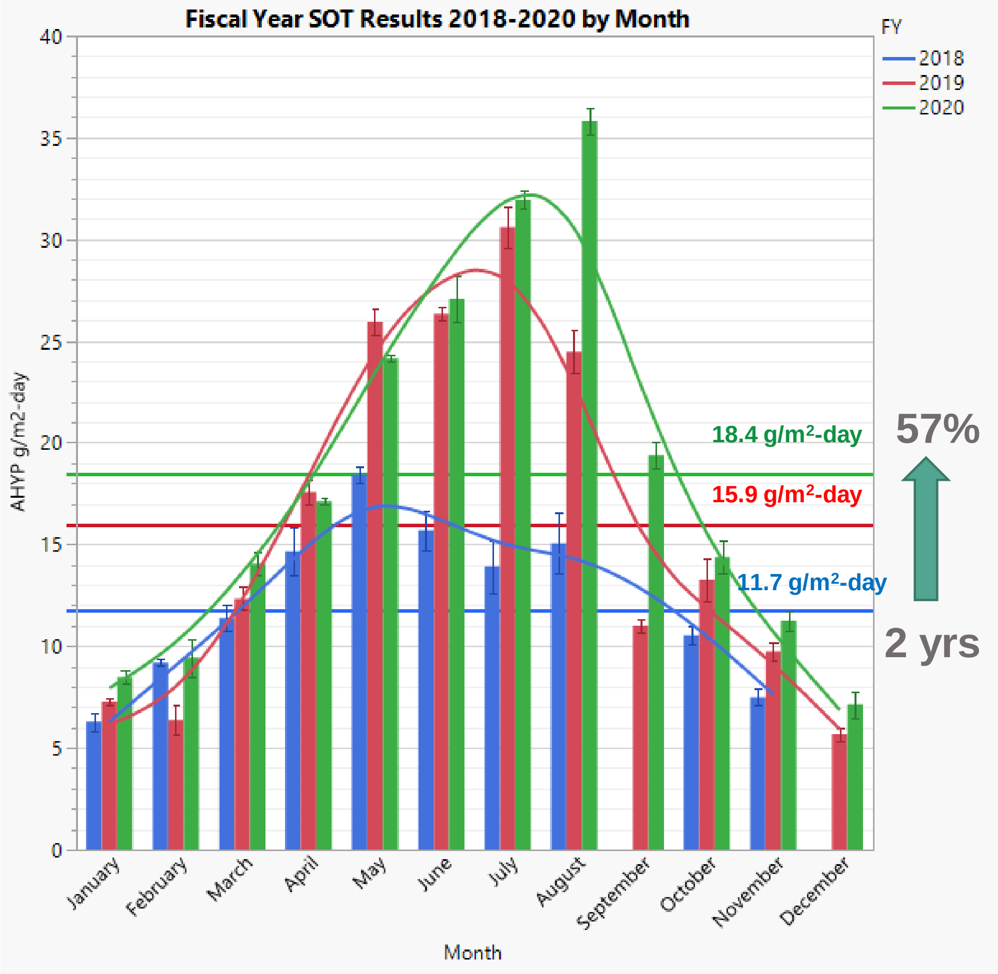
Outdoor verification of best strains/approaches with primary focus on improving productivity

SOT Trials

Current year over year cultivation trials have yielded steady progress over last three annual cycles.

- Improvements in biomass productivity realized thru a combination of
  - improved cultivars from the DISCOVR pipeline
  - maturing operations and experience with those cultivars
  - successful pest management approaches leveraged from other AAS portfolio projects
- Increased annual average to **>18 g/m²-day** with a Summer season average in excess of **31 g/m²-day**
- Increased operational days (up-time) from 218 → 328 → 356 for 2018-2019-2020.

Impact



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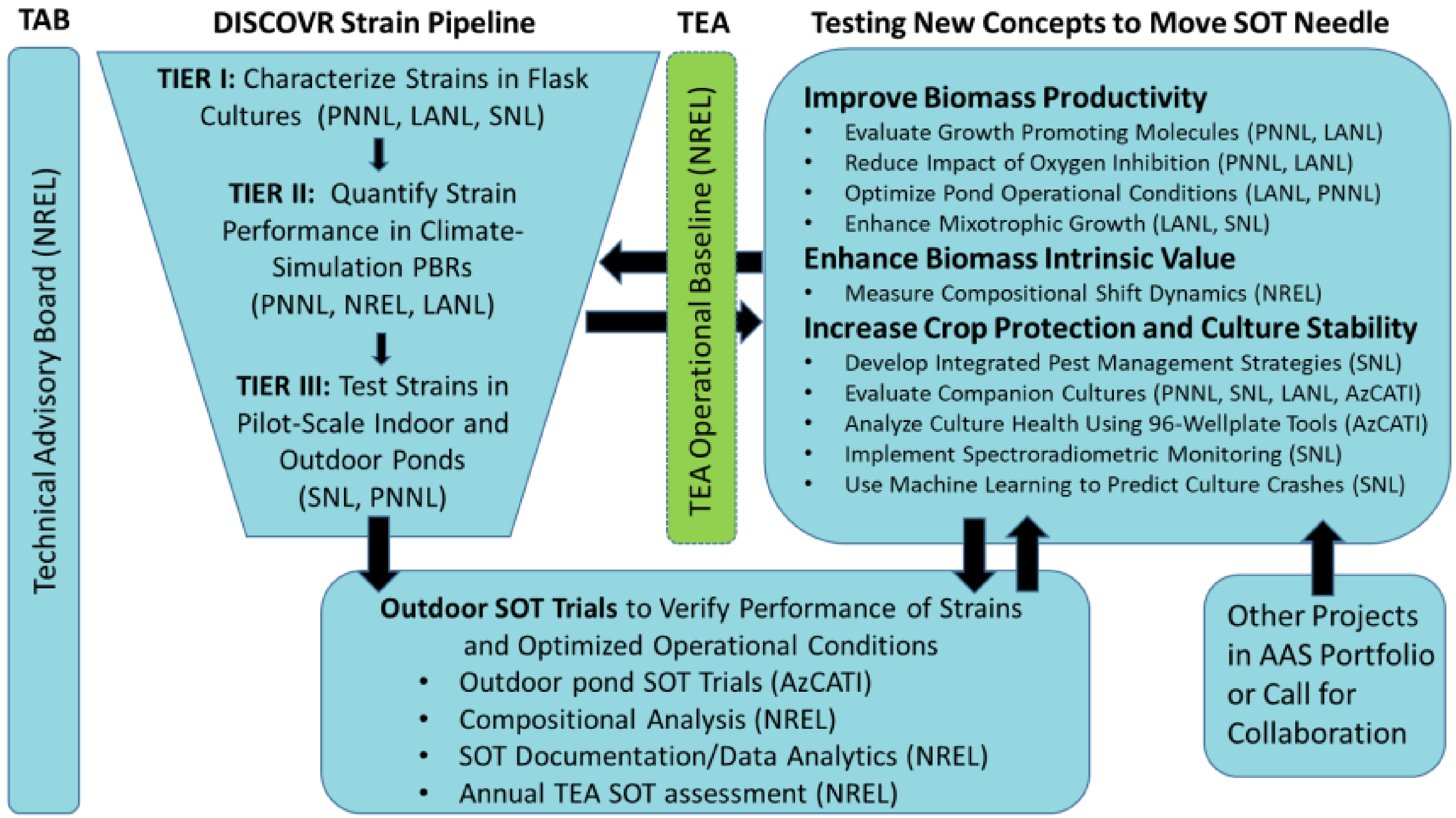
DISCOVR Techno-Economic Analysis (TEA) Modeling

TEA

Approach – Progress & Outcomes - Impacts



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Approach: Technology Economic Analysis (TEA) Modeling

TEA

Benchmark operational performance and screening to prioritize future work

Objective

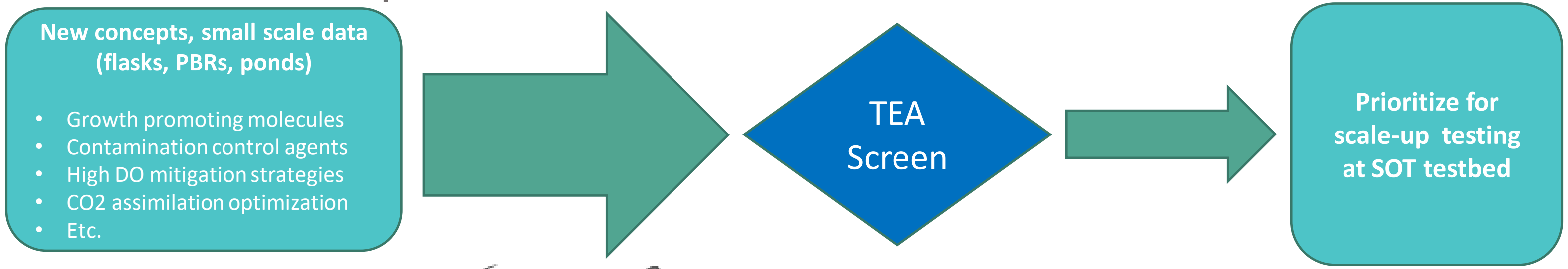
- Inform DISCOVR team on the impact of experimental achievements by **translating technical performance to economics**
- TEA interface linking DISCOVR data outputs to Algae Platform SOT inputs; expand upon SOT to reflect DISCOVR operations in more granularity
- Support the definition of **research priorities** based on largest cost drivers

Approach

- Utilize TEA as a screening tool as part of DISCOVR pipeline to evaluate small-scale data, prioritize scale-up experiments moving to test-beds
- Establish a pre-n^{th}-plant "operational baseline" to expand on n^{th}-plant SOT benchmarks that capture DISCOVR methods/accomplishments – quantify future DISCOVR improvements as reductions in biomass costs

Challenges

- Data gaps for operations outside DISCOVR scope that impact integrated model results (e.g., dewatering)
- Scale-up of test-bed data to commercial scale model farm (5,000 acres)



Impact: TEA Modeling

TEA

Quantify Economic Impact of DISCOVR Progress

Current "n^th^-plant" SOTs provide high-level TEA benchmarks to Algae Platform, but are limited in granularity:

- Fixed pond uptime (330 days/year)
- No inclusion of contamination frequency, inoculum train design specifics
- No explicit costs included for contamination control
- Salt blowdown disposal costs based on fixed assumptions for strain salinities, seasonal evaporation rates
- Fixed biomass compositions

Impact

- Operational baseline TEA provides an additional layer of granularity and context to operational details behind *n^th^*-plant SOTs:
  - Strain salinity tolerance
  - Local evaporation rates/blowdown disposal needs
  - Seasonal strain compositions
  - Contamination control costs
  - Inoculum train design specifics
  - Data for pond depths applicable for scale-up
  - Efficacy for low-cost dewatering
- Supplements other Platform activities to better understand the gap between current operational practices and future *n^th^*-plant needs, how to reduce the gap in the future
- Solicit input from industry to verify the "right" questions are being asked and addressed through TEA modeling, cost trends/drivers are in line with industry observations

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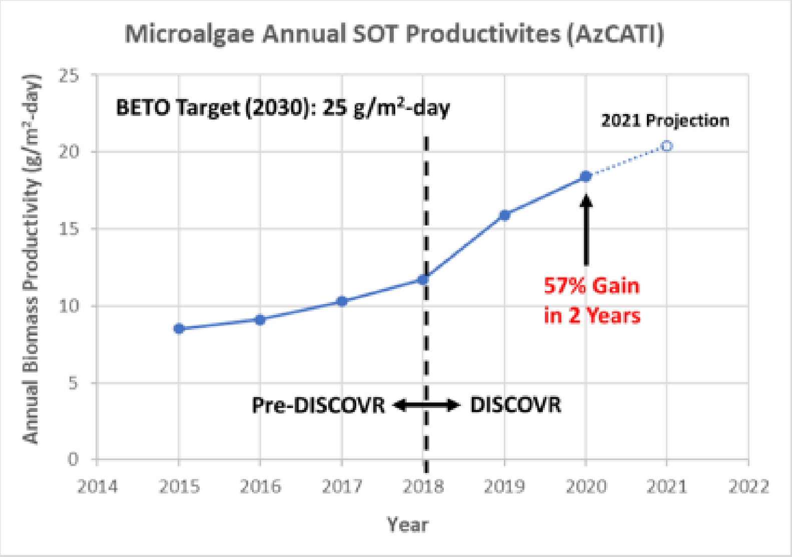
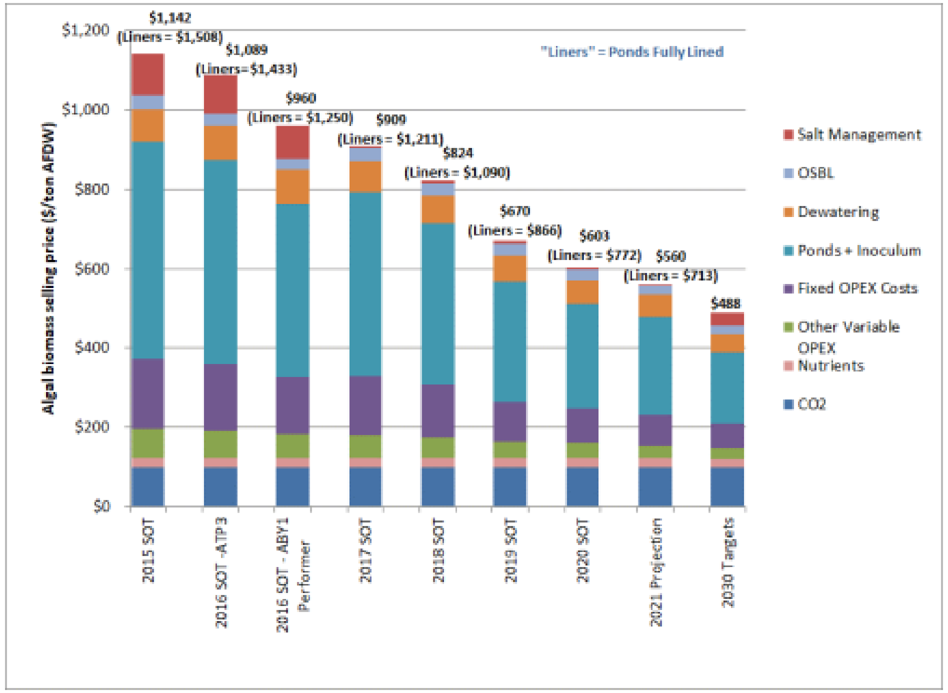
Progress and Outcomes: TEA Modeling

TEA

Inform annual State of Technology (SOT) benchmarking updates

- **Objective:** Translate experimental developments from the DISCOVR consortium to economic performance on n^{th}-plant modeling assumptions





- Tracking SOT benchmarks over the years and making projections to determine required yearly productivity improvements
- Linkage to Algae Platform TEA project (formally handles SOTs)
- 2020 *vs.* 2019 SOT = 10% reduction in minimum biomass selling price (MBSP) @ 15% productivity improvement
- 2021 projection vs 2020 SOT = 7% reduction in MBSP @ 11% productivity improvement

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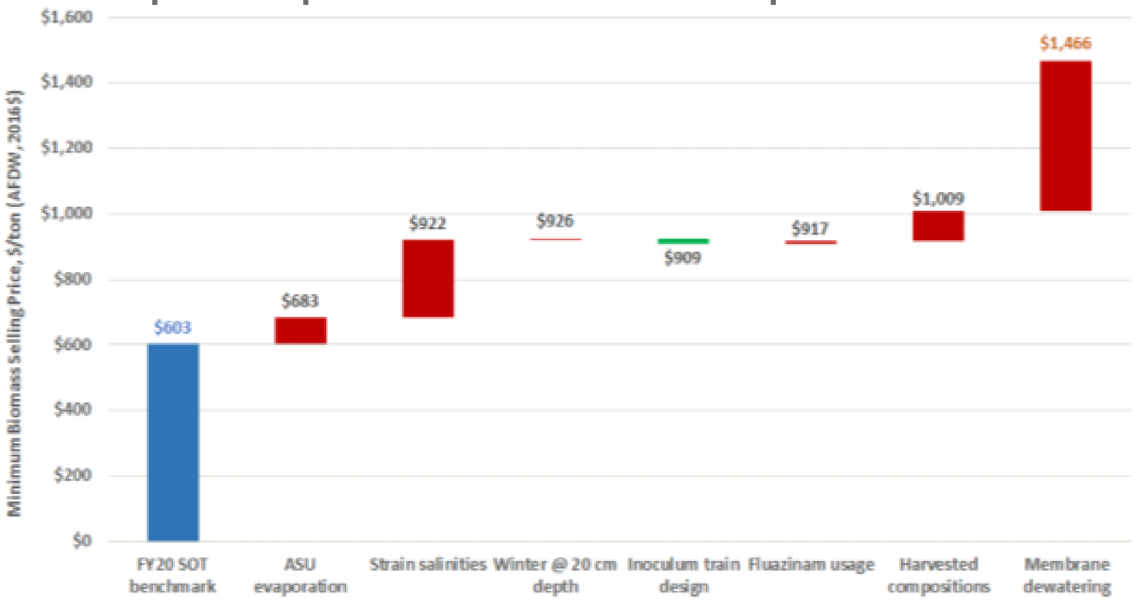
Progress and Outcomes: TEA Modeling

Establish "pre-nth plant" model of DISCOVR "operational baseline"

- **Objective:** Adjust the TEA framework to account for more data granularity brought by DISCOVR (as a supplement to *n*th-plant SOT efforts)

Specific updates relative to 2020 nth-plant SOT basis



- Key drivers = membrane dewatering *(inability for primary settling)*, strain salinities *(blowdown disposal costs)*, harvested compositions *(N/P nutrients)*
- Findings will be used to guide future DISCOVR priorities and to quantify future progress for details not otherwise captured in formal SOT

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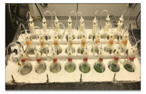
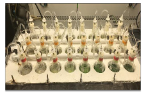
Summary I: DISCOVR Strain Pipeline - Status

DISCOVR strain pipeline has been successful in down-selecting top strain for SOT trials

Strain	Temperature + Salinity Tolerance + 16s/18s ID (42 Strains)	LEAPS Photo-Bioreactor Testing (24 Strains)	Outdoor Pond Testing (PAT) (13 Strains)	Outdoor Pond Testing at BETO SOT Testbed (AzCATI) (7 Strains)
Cyanobacterium *AB1* (from Algenol)				
Acutodesmus obliquus DOE0152.z				
Acutodesmus obliquus UTEX393				
Agmenellum quadruplicatum UTEX2268				
Anabaena sp. ATCC 3308.1				
Arthrospira platensis UTEX3086				
Arthrospira fusiformis UTEX2721				
Chlorella antarctica UTEX 1959				
Chlorella autorophica CCMP243				
Chlorella sorokiniana UTEXP13				
Chlorella sorokiniana UTEX BP15				
Chlorella sorokiniana DOE1412				
Chlorella vulgaris NREL 4-C12				
Chlorella vulgaris AZ-1201				
Chlorella sp. UTEX SNO 69				
Chlorococcum littorale UTEX 117				
Chlorococcum sp. DOE1426/UTEX BP7				
Chloromonas reticulata CCALA870				
Chloromonas UTEX SNO11				
Coelastrella sp. DOE0202				
Micractinium sp. NREL 14-F2				

Strain	Temperature + Salinity Tolerance + 16s/18s ID (42 Strains)	LEAPS Photo-Bioreactor Testing (24 Strains)	Outdoor Pond Testing (PAT) (13 Strains)	Outdoor Pond Testing at BETO SOT Testbed (AzCATI) (7 Strains)
Monoraphidium sp. MONOR1				
Monoraphidium minutum 26B-AM				
Nannochloropsis gaditana CCMP1894				
Nannochloropsis oceanica CCAP 849/10				
Nannochloropsis salina CCMP1776				
Oscillatoria cf. priestleyi CCMEE5020.1-1				
Phaeodactylum tricorutum UTEX 646				
Picochlorum renovo NREL39-A8				
Picochlorum celeri WT-CSM/EMRE				
Picochlorum oklahomensis CCMP2329				
Picochlorum soleocismus DOE101				
Porphyridium cruentum CCMP675				
Scenedesmus acutus LRB-AZ-0401				
Scenedesmus rubescens 46B-D3				
Scenedesmus sp. IITRIND2				
Stichococcus minor CCMP819				
Stichococcus minutus CCALA727				
Synechococcus elongatus UTEX2973.1				
Tetraselmis striata LANL 1001				
Tisochrysis lutea CCMP1324				
Tribonema minus sp. MBE				

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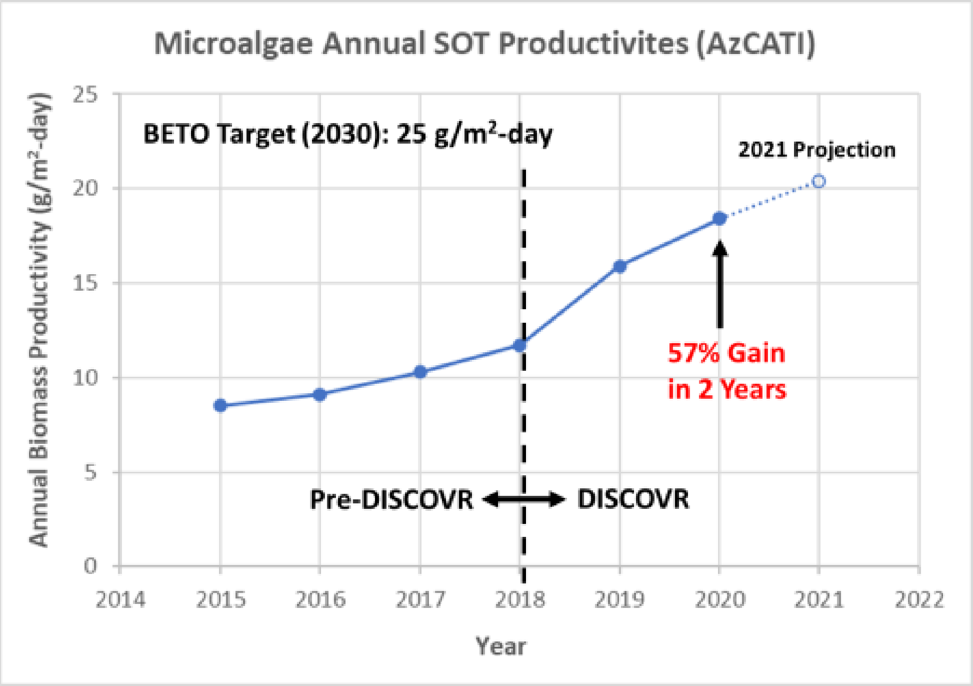
Summary II: Success Highlights

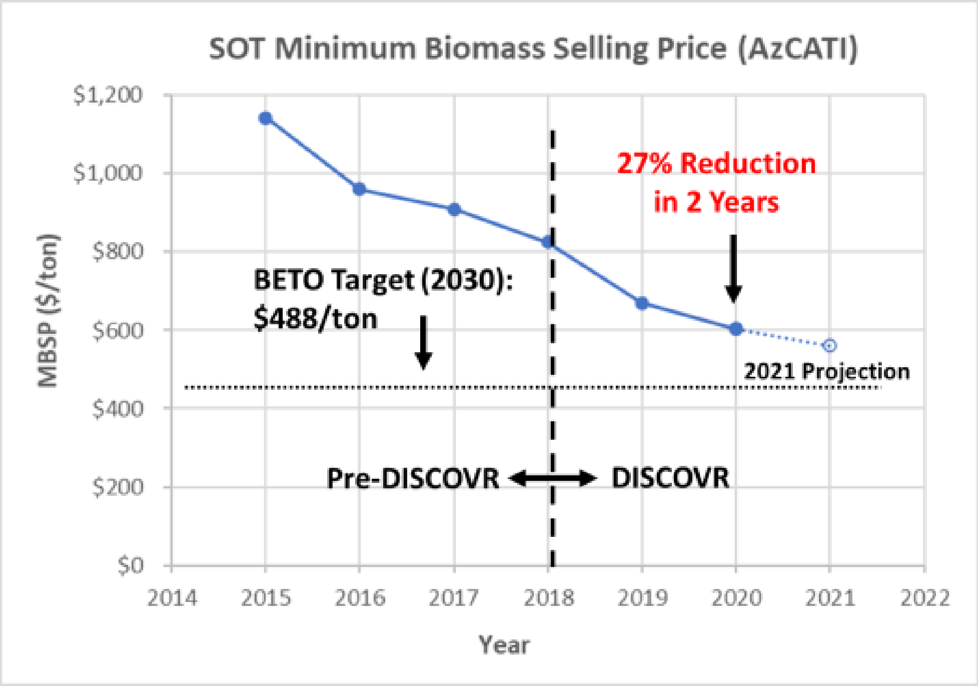
DISCOVR is BETO's flagship consortium project to rapidly meet productivity and cost targets

- Identified **stable high productivity winter & summer season strains** for deployment at **AzCATI**
- **Validated performance** of respective **climate simulation culturing systems** at each Nat Lab
- The **plant hormone IAA increased** simulated winter season **productivity of 26B-AM by ca. 30%**
- **Air-sparging** of ponds reduced **DO** and increased the **productivity of UTEX393 by ca. 50%**
- **Mixotrophic studies** with corn stover hydrolysate showed a **50% increase in SOT strain growth**
- **Demonstrated improvement in value productivity for UTEX393 upon nutrient depletion**
- **Identified, isolated,** and **characterized agents responsible for crashes** during **SOT trials**
- **Spectroradiometric method** demonstrated **predation detection ≥ 2 days before amplicon seq.**
- **Machine learning algorithm** had **100% accuracy to predict crashes with >72 hrs warning**
- Used **TEA modeling to identify key cost drivers** and **guide** future **DISCOVR research priorities**

Summary III: Gains in SOT Productivity (2018-2020)

Use of DISCOVR strains resulted in 57% increase in SOT productivity and 27% decrease in MBSP





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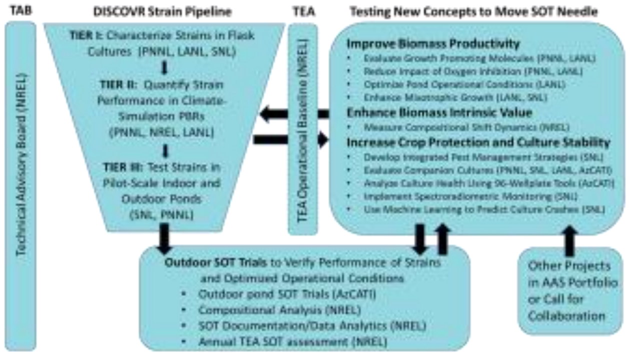
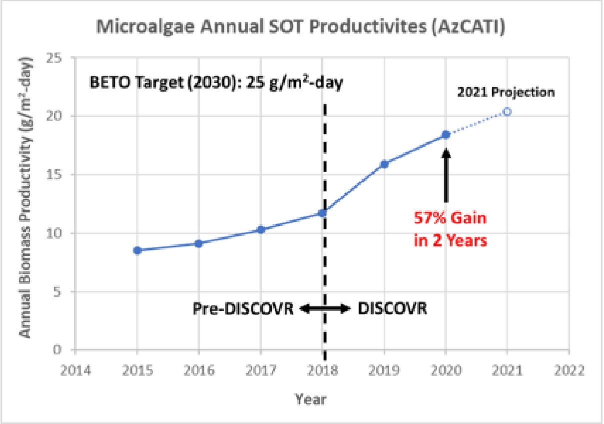
DISCOVR Summary: Management-Approach-Results-Impacts

Highly coordinated and innovative research to rapidly meet BETO productivity and cost targets

- **Management:** **Weekly conference calls** by the **highly cohesive DISCOVR team** and quarterly guidance by the **Technical Advisory Board** ensure **effective execution** of all coordinated tasks.
- **Approach:** The coordinated approach consists of **identifying stable and high productivity strains via the DISCOVR pipeline**, testing innovative concepts to **improve productivity**, **enhance biomass value**, and **increase crop protection and culture stability**, and **conduct year-round SOT trials** using crop-rotation at AzCATI.



- **Results and Outcomes:**
  - **Successful execution** of the **DISCOVR strain pipeline** (42 strains entering)
  - **Growth promoting molecules & air-sparging increased productivity >30%**
  - **Compositional shifts increased value productivity of UTEX393**
  - **Improved prediction and prevention of pond crashes**
- **Impacts:** **57% increase in annual SOT biomass productivity in 2 years** (11.7 to 18.4 g/m^{2}-day), equivalent to a **reduction in MBSP by 27%**, from $824/ton to $603/ton.



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Supplemental Slides Section

Supplemental: Acknowledgements (staff in addition to listed on title slide)

DISCOVR is a highly collaborative effort with many contributors

- **BETO ALGAE TEAM**
  - Dan Fishman
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  - Madeline Lane
- **LANL**
  - Bridget Daughton
  - Hanji Dauligault
  - LANL 'omics Team
- **SNL**
  - Pamela Lane
  - Cameron Kunstadt
  - Anita Sumali
  - Danae Maes
  - Lauren Atencio
  - Tyler Hipple
  - Deanna Curtis
  - Parshant Rai
- **AzCATI**
  - Laura Marshall
  - Madison Clar
  - Aaron Geels
  - Jason Sandoval
  - Jason Potts
  - Clara Missman
  - Richard Malloy
  - Mark Seger
  - Pete Lammers

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Supplemental DISCOVR Management: Quad Chart

Budgets, barriers addressed, project goals, and end of project milestone

Timeline

- October 1, 2016
- September 30, 2022 (2nd 3 Year Cycle)

	FY20	Active Project
DOE Funding	(10/01/2019 – 9/30/2020) **$3,000K**	3 Year Budget **$9,250K**

Project Partners

PNNL: 24%
NREL: 23%
SNL: 21%
LANL: 13%
AzCATI: 19 %

Barriers addressed

- Aft-B: Sustainable Algae Production
- Aft-C: Biomass Genetics + Development
- Aft-E: Algal Biomass Characterization

Project Goals

- Use the DISCOVR Strain Pipeline to deliver **new high-productivity strains** for robust annual outdoor cultivation.
- Test **new concepts** using DISCOVR Strain Pipeline to move the SOT needle via **improvements in biomass productivity, biomass value**, **crop protection and culture stability** (see Figure 1)**.**
- Verify performance of strains in outdoor **SOT Trials** at AzCATI.

End of Project Milestone

Employ new strains and novel concepts that were successfully demonstrated in the laboratory to **achieve at least 10% year over year annual productivity improvement (g/m^2/day) at SOT testbed**, relative to the pre-DISCOVR 2018 annual SOT productivity of 11.7 g/m^2/day, and normalized for climate anomalies (**PNNL, LANL, NREL, SNL, AzCATI**).

Funding Mechanism

AOP funding to each respective Lab. NREL subcontract to ASU.

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Supplemental Management: Responses to 2019 Peer Review

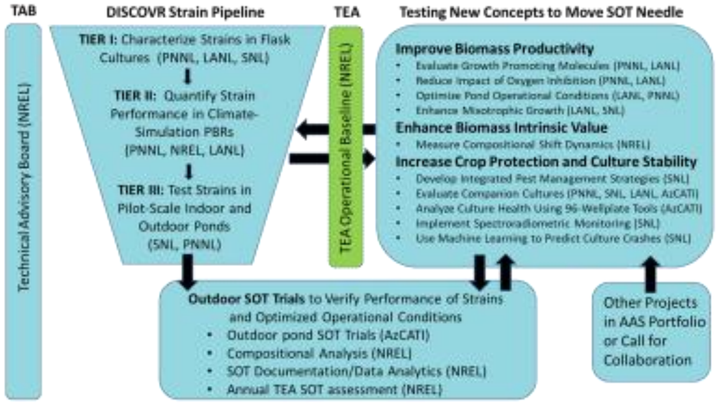
An Operational Baseline TEA task was included for the current 3-year DISCOVR project cycle

Concerns:

- “Their future efforts need to focus on TEA/LCA modeling”.
- “No data within the last three years has contributed to any TEA work currently.”
- “Completing TEA and LCAs for most promising strains will be crucial for quantifying the benefits.”
- “All the data that is being generated from this consortium would greatly add valuable datasets and complexity to TEA and biomass production models but not sure if the group will have enough time to complete a TEA.”

Responses:

- **We have included a specific TEA task for the current 3-year DISCOVR cycle.**
- **Annual increases in SOT productivities are translated into MBSP reductions.**
- **A TEA “Operational Baseline” is carried out to incorporate further details specific to operational approaches taken by DISCOVR. For example, characteristics specific to each strain being cultivated such as composition, salinity tolerance, contamination events, and contamination control measures.**
- **TEAs are also being conducted to screen economic feasibility of new concepts prior to deployment in outdoor ponds, as well as to quantify the “intrinsic value” vs production cost of algae across compositional profiles.**



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Supplemental Management: DISCOVR Timeline

Historical overview of consortium trajectory

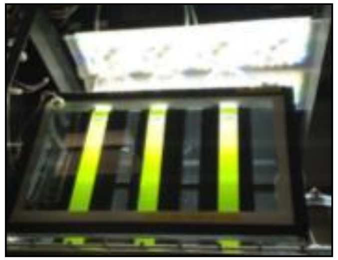
FY16 | FY17 | FY18 | FY19 | FY20 | FY21 | FY22

Merit Review Cycle (FY17–FY19) | Merit Review Cycle (FY20–FY22)

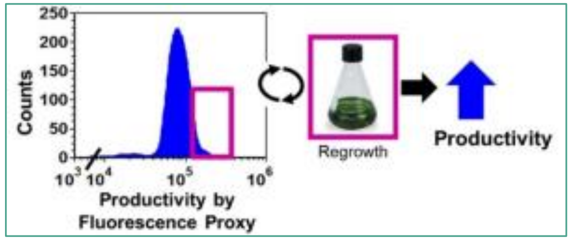
- **Interconnected Research between indoor and outdoor cultivation**

Initiation of Consortium (PNNL, LANL, NREL, SNL)

Strain characterization selection pipeline for increased productivity using indoor reactors



Strain adaptation salinity tolerance and biomass composition



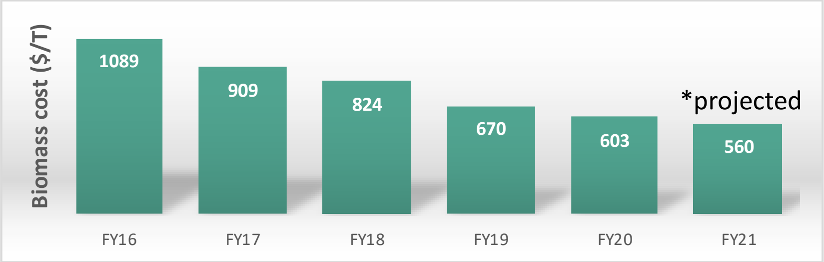
Strain adaptation pest and contamination resilience

Outdoor Validation for SOT TEA calculation

\+ Support for long-term outdoor cultivation testbed

\+ Operational Baseline SOT to rapidly evaluate sensitivities and guide research priorities

\+ Strong Advisory Board engagement to remain relevant to community interests



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Supplemental Management: DISCOVR Call for Collaboration

DISCOVR Focus Areas

- Develop and implement a pipeline as a standardized and validated strain characterization process.
  - *Purpose*: Evaluate algae strains for their potential as future algae biomass/biofuels/bioproducts production strains.
- Facilitate the execution of BETO's annual Algae State of Technology (SOT) experimental effort.
  - *Purpose*: Demonstrate progress towards outyear biomass productivity goals as outlined in the BETO MYPP.

We recognize that the algae industry and research communities are also continuously developing new strains and cultivation methods, which are important for driving progress of the field as a whole.

Goal

- Offer an opportunity for DISCOVR and the algae community to work together to incorporate the best algae strains, operational/cultivation strategies, and crop protection strategies into DISCOVR and the SOT.

Approach

- Release a Call for Collaboration to solicit strains, tools, and techniques to help achieve BETO's aggressive technical and economic targets for algae bioenergy production.

Outcome

- Accelerate the development and implementation of "the best of the best" algae technologies to foster the growth of the bioeconomy and facilitate the realization of cost effective algae biofuels and bioproducts.

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Supplemental Managmt: DISCOVR Call for Collaboration - Report 2019

Executive Summary

In order to encourage interaction between external stakeholders and DISCOVR, a "Call for Collaboration" was released by LANL, PNNL, SNL, and NREL. This effort differed from the Directed Funding Opportunities of other BETO consortia, in that there were no specific funds set aside for any collaborations. Instead, the team discussed research ideas with external stakeholders and determined if some of the concepts could be tested within the existing scope/framework of the project. This Call resulted in 10 responses, which are detailed further below. Of these 10, two initially were selected to write full proposals, and ultimately one company submitted a proposal. The team is excited to work with Aequor and are working out the agreement details.

Lessons Learned

Although the lack of funds to external partners is consistent with the recent actual DFOs, not having a specific budget for the Labs for this call did complicate the planning of the proposals. We had to be sure that any proposed work could be easily layered into ongoing scope, rather than having the freedom to develop new, but related, scope with the external partner.

One lesson learned was that we tried to have a rolling call, with no particular deadlines. With everyone's busy schedules, this approach resulted in the team deprioritizing this effort. We've proposed to associate a deadline with future Calls, in order to bring the Call, reviews, proposals, and selection front-and-center to the team.

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Supplemental DISCOVR Management: Technical Advisory Board

Advice incorporated in long-term planning

- **Medium formulation**
  - Cost of DISCOVR medium vs. others
  - Build up of sulfate based on ammonium capture and water recycle
  - Use vitamins in screening medium; assume microbial production outdoors
- **Growth studies**
  - Consider possible additive impact of high temperatures, high light and salt concentrations
  - Worthwhile shifting from pond crash analysis to pond protection strategies
  - Care must be taken to disinfect ponds after crashes to avoid carryover of crash agents
  - Robust discussion on nutrient depletion studies considering goal of finding highest biomass productivities
- **Strains**
  - Shift focus to halotolerant strains
  - Solicit strains from academia and industry

Included in current merit-review cycle work proposal:

Media optimization captures N:P ratio and tailored salinity for each species

Rapid crash detection research and empirical testing of pesticides to extend cultivation

Nutrient depletion time course research ongoing to quantify biomass value

***Picochlorum celeri* is rapid summer producer strain (originating from CSM)**

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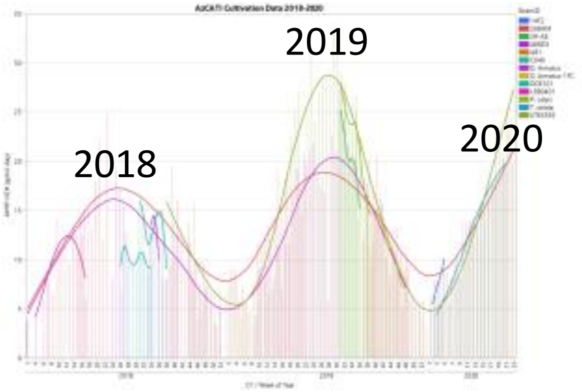
Supplemental Managmt: Technical Advisory Board Restructure in FY20

Quarterly meetings – single topic deep dive



- **June 2020: Data Management and Dissemination**
  - **Value**: DISCOVR data set provides long term cultivation baseline
  - **Challenge:** Shortening time to make SOT data available (publications)
  - **Solution**: Open access platform for data deposit, e.g. *OpenEI.org*
  - **Gap**: Data collection on carbon and nutrient balance is missing
- **September 2020: Implementable Crop Protection Strategies**
  - **Value**: Data available on top strains' pest resilience and susceptibility
  - **Challenge**: Pesticides availability limited to extend long-term cultivation
  - **Solution**: early detection and immediate response, or prophylactic stabilization of cultures
  - **Gap**: actionable strategy to link with early detection
- **December 2020: Harvesting Operations Priorities**
  - **Value**: Reducing harvesting operation cost and energy intensity large impact on SOT MBSP
  - **Challenge**: SOT modeled operations not connected to experimental data collection and selection pressure high with reliance on transient cell structural parameters, e.g. flocculation
  - **Solution**: collect empirical data on settling, flocking and filtering capacity and flow rates
  - **Gap**: not much information on flocculation and experimental data on distinct harvesting susceptibility of top performing strains (e.g. *Picochlorum*)

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Supplemental Management: Tie-In BETO Projects

WBS #s of projects that tie into the inputs and outputs of DISCOVR

Tie-Ins	WBS #s
Resource Assessment & Environmental Simulation	1.3.4.101
Biomass Characterization	1.3.2.001
Productivity Improvement	1.3.1.120, 1.3.2.610, 1.3.2.103
Pond Crashes & Signatures	1.3.2.631, 1.3.1.103
Testbeds	1.3.5.101
Genome Sequencing	1.3.1.600, 1.3.1.111, 1.3.2.110
Molecular Toolboxes	1.3.1.112, 1.3.1.100, 1.3.1.001
Biomass Storage	1.3.3.100
Biomass Conversion	1.3.4.201, 1.3.5.202
Nutrient Recycling	1.3.5.203
State of Technology	1.3.5.200, 1.3.1.200
CO_2 Utilization	1.3.2.601
Integrated Process Improvements	1.3.5.302, 1.3.5.270, 1.3.4.288

Supplemental Approach: DISCOVR Future Work

Future research encompasses multiple approaches to rapidly meet productivity and cost targets

- Continue to **identify stable and high productivity strains** using the **DISCOVR pipeline**
- Evaluate the **effects** of various **growth hormones** on the **productivity of top DISCOVR strains**
- Investigate **luminostat control** for **increasing productivity in ponds** for top DISCOVR strains**.**
- Test **addition of plant substrates** for **increasing productivity in raceways supplied with CO_2**
- Use environmental simulation (ePBRs) to **optimize of AzCATI outdoor operational conditions**
- **Quantify rate of compositional shifts** and **value productivity** for **top DISCOVR strains**
- **Improve mean time between failure** by **developing integrated pest management**
- **Improve pond crash prediction** using **spectroradiometric monitoring** and **machine learning**
- Study **complementary cultures** of top DISCOVR strains for **increased outdoor pond stability**
- Continue **TEA modeling to** further refine **operational baseline** and **quantify progress vs FY20 benchmarks,** and **guide future DISCOVR priorities**
- Conduct **SOT trials** using top DISCOVR **strains** and **innovations** to **meet BETO targets**

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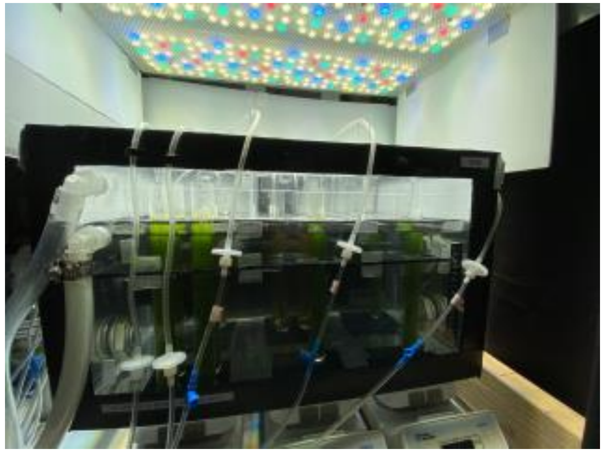
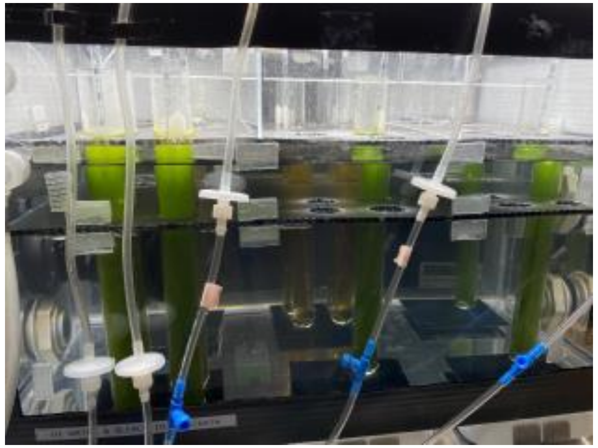
Supplemental Approach: TIER II Strain Culturing in mini-LEAPS

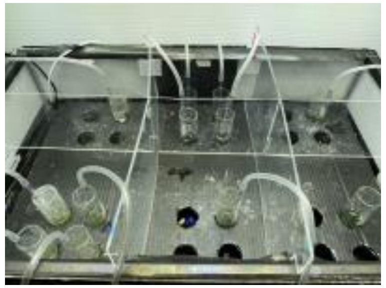
Pipeline

Use next generation LEAPS – the mini-LEAPS – for screening 24 strains/conditions simultaneously

- **Background/history:** PNNL **mini-LEAPS** PBR
  - *In order to increase the number of strains and experimental conditions that can be tested simultaneously using a given light and temperature script, a mini-LEAPS with 24 columns was designed and validated.*
  - *This increases the number of strains and conditions that can be tested by a factor of 4, relative to the LEAPS which has only 6 columns.*
  - *The mini-LEAPS was used in FY20 to evaluate new DISCOVR winter season strains at different salinities.*







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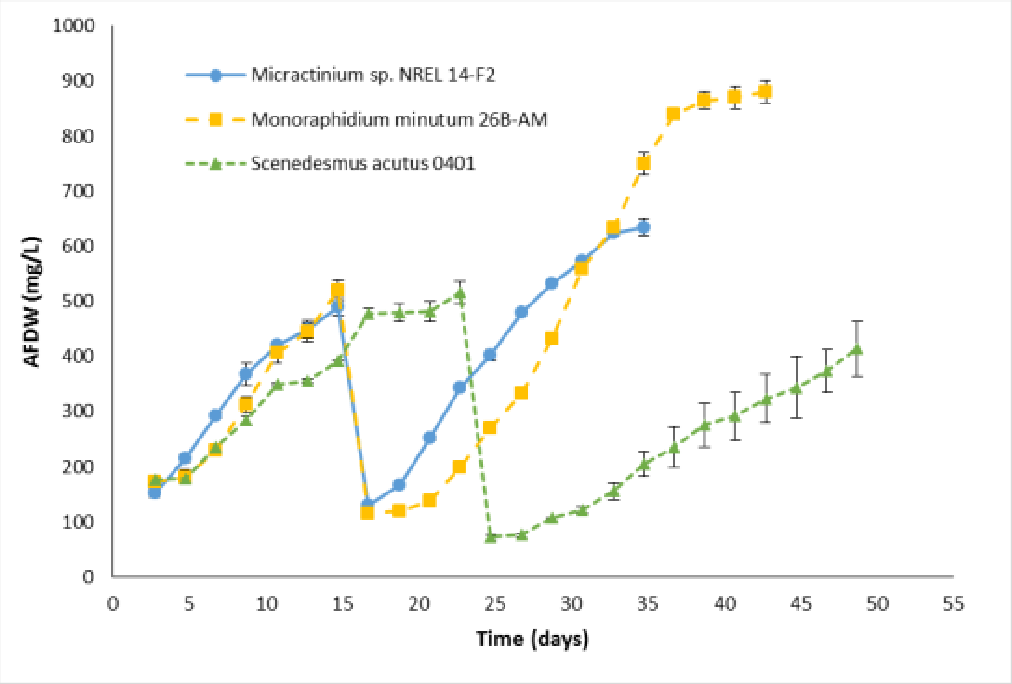
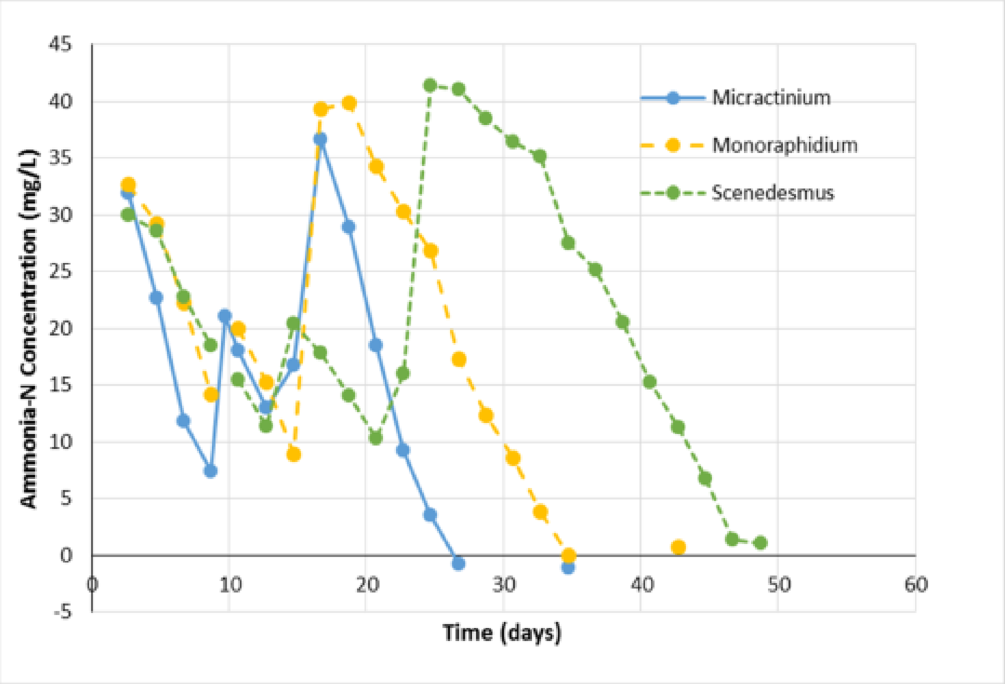
Supplemental Outcomes: Typical Results - LEAPS Experiments

Pipeline

AFDW and NH_4-N vs. time for nutrient-replete and nutrient-deplete growth phases





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Supplemental Outcomes: Publications and Presentations

Results from DISCOVR were widely communicated to algae community

Publications

1. Fisher and Lane (2019), “Operational, Prophylactic, and Interdictive Technologies in Algal Crop Protection”. In Grand Challenges in Algae Biotechnology (eds P.H. Rampoletto and H. Armin), Springer. Pages 35-70.
2. Reichardt, Maes, Jensen, Dempster, McGowen, Poorey, Curtis, Lane, and Timlin (2020), “Spectroradiometric detection of competitor diatoms and the grazer Poteriocrhomonas in algal cultures,” *Algal Research*, 51, 102020.
3. Huesemann, Knoshaug, Laurens, Dale, Lane, McGowen, Davis, Timlin, Poorey, Reichardt, Edmundson, and Pienkos (2021), “Development of Integrated Screening, Cultivar Optimization, and Verification Research (DISCOVR): A Highly Coordinated Research-Driven Approach to Improve Microalgal Productivity, Composition, and Stability for Commercially Viable Biofuels Production”, *Algal Research*, to be submitted.
4. Krishan, Likhogrud, Cano, Edmundson, Huesemann, McGowen, Weissman, and Posewitz (2021), “Picochlorum celeri as a model system for robust outdoor algal growth in seawater”, *Nature Communications*, to be submitted.
5. Huesemann, Edmundson, Gao, Dale, et al. (2021), “Maximum specific growth rate as a function of temperature and salinity for 42 microalgae strains considered for biofuels production in the U.S. Department of Energy DISCOVR consortium project,” *Nature Scientific Reports*, to be submitted, 2021.
6. Huesemann, Edmundson, Gao, Laurens, et al. (2021), “Areal biomass productivities and biomass compositional shifts in LEAPS climate-simulation photobioreactor cultures of winter and summer season microalgae strains in the U.S. Department of Energy DISCOVR consortium project,” *Algal Research*, to be submitted.
7. Beirne, Edmundson, Gao, Huesemann et al., (2021), “Miniaturized Laboratory Environmental Algae Pond Simulator (LEAPS) for higher throughput identification of promising microalgae strains for biofuels production”, *Journal of Applied Phycology*, to be submitted.
8. Negi, Carr, Daughton, Klein, Davis, McGowen, Dale et al. (2021), “Productivity enhancement of microalgae treated with a growth promoting molecule under outdoor-relevant conditions”, to be submitted.
9. Klein, Davis, Laurens, et al. (2021) “Closing the Gap Between Production Cost and Value Accelerates Algae Commercialization Potential”, to be submitted.
10. Lane, Hagerstrand, and Lane (2021), “Characterization of the Resistance of DISCOVR Algal Strains to Multiple Deleterious Species”, *Algal Research*, to be submitted.
11. Lane and Lane (2021), “Isolation, Growth Characterization and Host Specificity Determination of a novel Amoeboaphelid isolated from Pilot-Scale Algal Production Ponds”, to be submitted.
12. Atencio, Maes, Hipple, and Timlin (2021), “Susceptibility of two saltwater strains of *Chlorella sorokiniana* to *Vampirovibrio chlorellavorus*,” *Algal Research*, to be submitted.

Presentations

1. Huesemann, Edmundson, Gao, Dale, Barry, Laurens, Pienkos, et al. "DISCOVR: Development of Integrated Screening, Cultivar Optimization, and Verification Research." 9thInternational Conference on Algal Biomass, Biofuels and Bioproducts, Boulder, CO, 17-19 June 2019.
2. Lane, Timlin, Reichardt, Poorey, Lane, Hagerstrand, “A survey of microalgal resistance to algal pond crashes,” International Conference on Algal Biomass, Biofuels and Bioproducts, Boulder, CO, 2019.
3. Huesemann, Edmundson, Gao, Dale, Barry, Laurens, Pienkos, et al. "DISCOVR: Development of Integrated Screening, Cultivar Optimization, and Verification Research." Algae Biomass Summit, Orlando, FL, 16-19 September 2019.
4. Poorey, “iPredictome: Data Science Toolkit for Predictive Modeling for Health Anomalies”, DTRA Chemical Biological Defense S&T Conference, November 2019.
5. Edmundson, Huesemann, Gao, Dale, Negi, Lane, Poorey, Timlin, et al. "The Algae DISCOVR Project: Multi-tier screening of algae as feedstocks for the bioproducts of the future." American Association Advancement of Science Annual Meeting, Seattle, Washington, February 2020.
6. Huesemann, Edmundson, Gao, Dale, Barry, Laurens, Pienkos, et al. "DISCOVR: Development of Integrated Screening, Cultivar Optimization, and Verification Research." Algae Biomass Summit, September 2020.
7. Negi, Carr, Daughton, McGowen, Dale “Effect of phytohormones on improving biomass productivities in algal production strains”, Algal Biomass Summit, September 2020.

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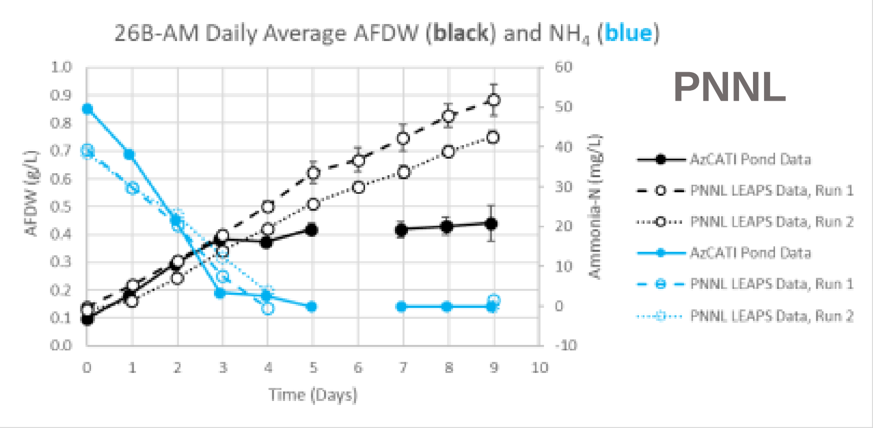
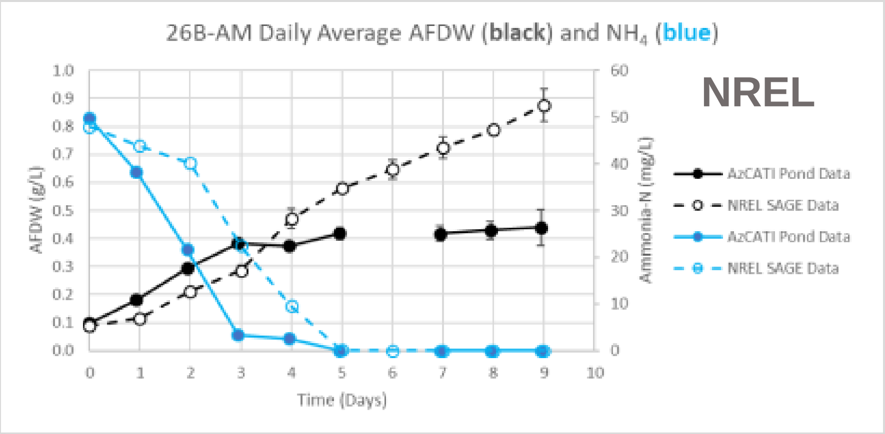
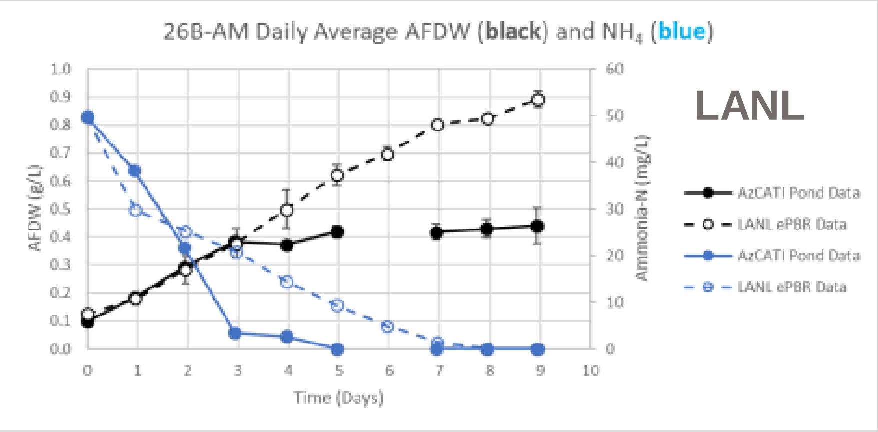
Suppl. Outcomes: Climate-simulation Culturing of AzCATI Ponds Pipeline

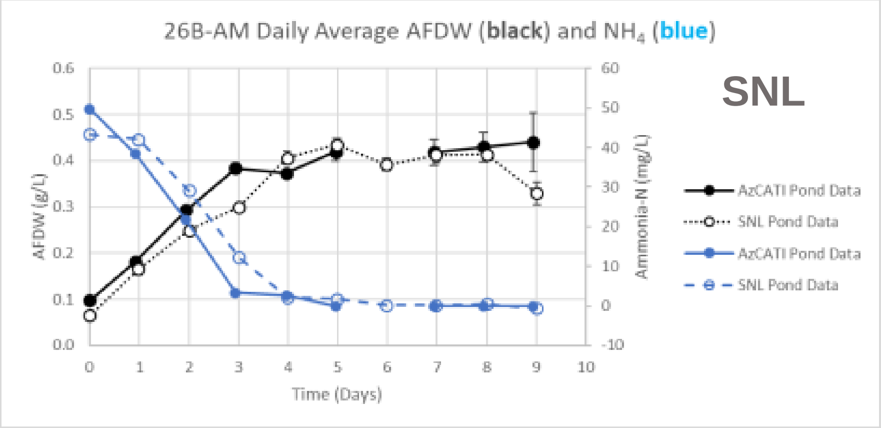
Comparative kinetics of biomass growth and NH_4 depletion for Monoraphidium minutum 26B-AM

- **Linear-phase productivities were similar (<20% error) but final yields were higher compared to AzCATI**









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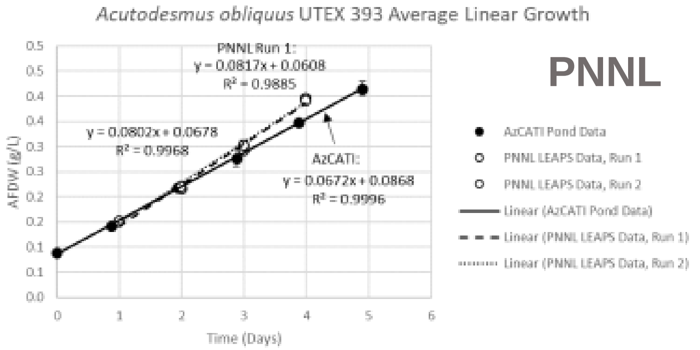
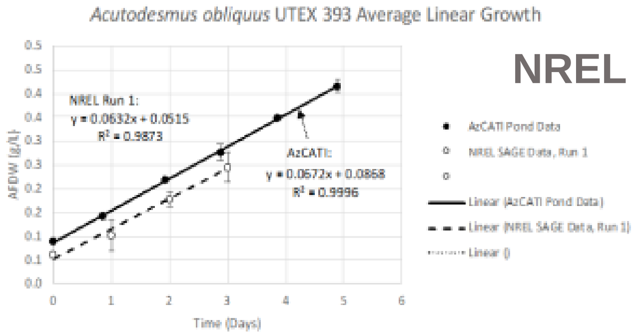
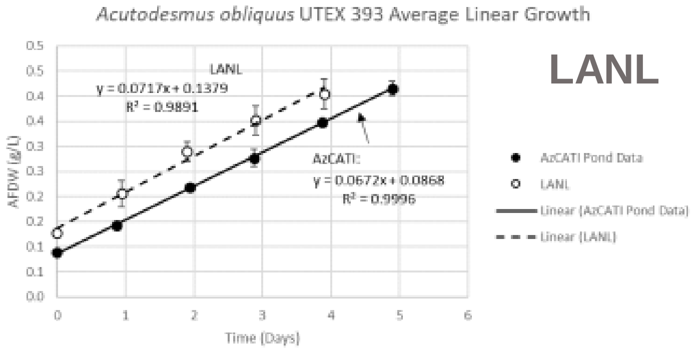
Supplemental Outcomes: Climate-simulation Culturing of AzCATI Ponds

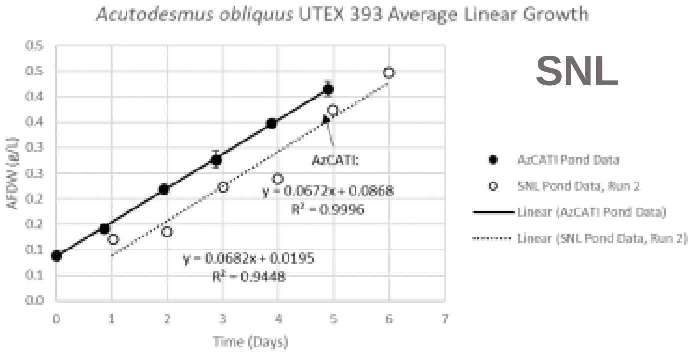
Linear regression slopes of AFDW vs. time for Acutodesmus obliquus UTEX393 **Pipeline**

- **Linear-regression slopes were used to calculate linear-phase biomass productivities** (see next slide)









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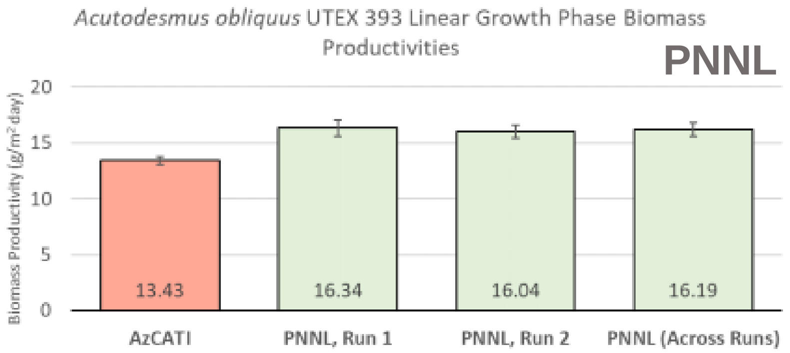
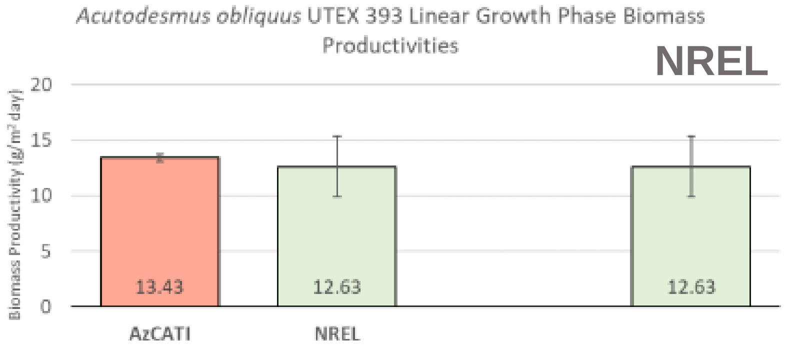
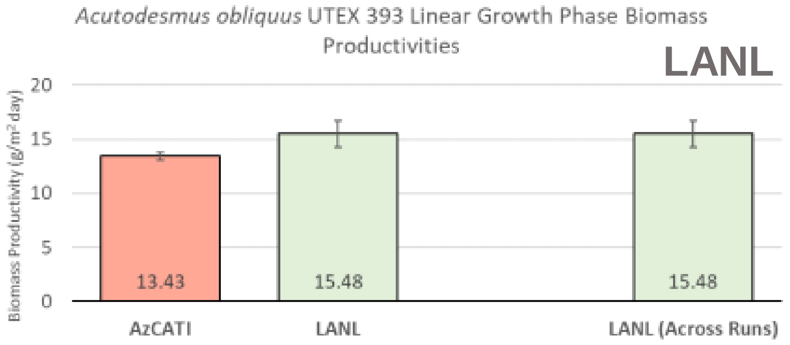
Supplemental Outcomes: Climate-simulation Culturing of AzCATI Ponds

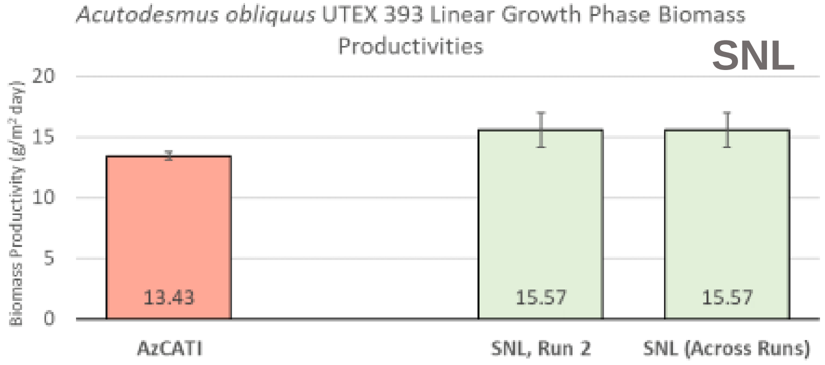
Comparative linear-phase biomass productivities for Acutodesmus obliquus UTEX393 **Pipeline**

- **Linear-phase biomass productivities were slightly higher than at AzCATI**









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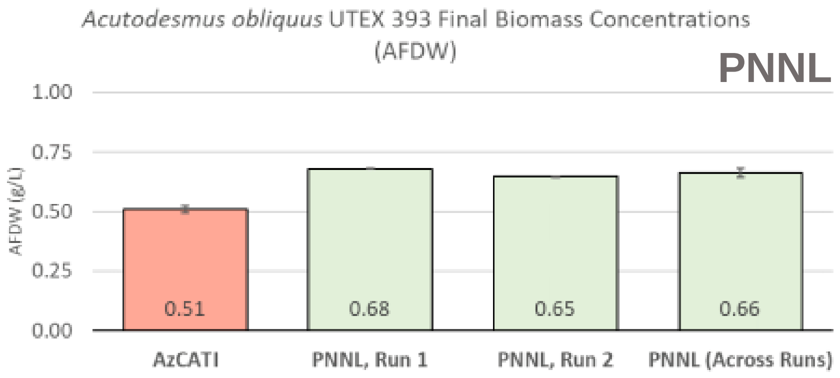
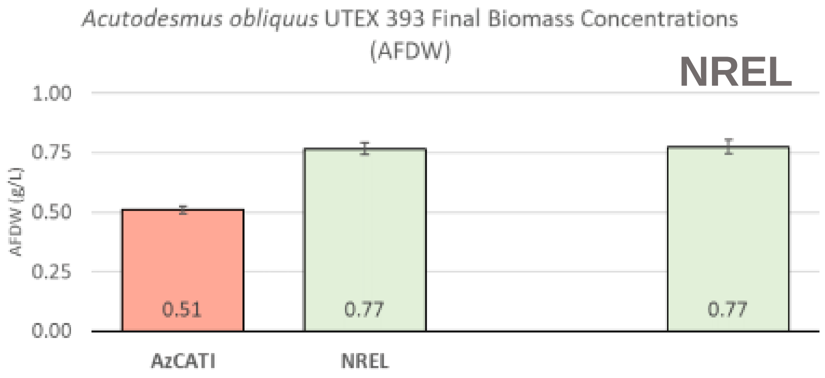
Supplemental Outcomes: Climate-simulation Culturing of AzCATI Ponds

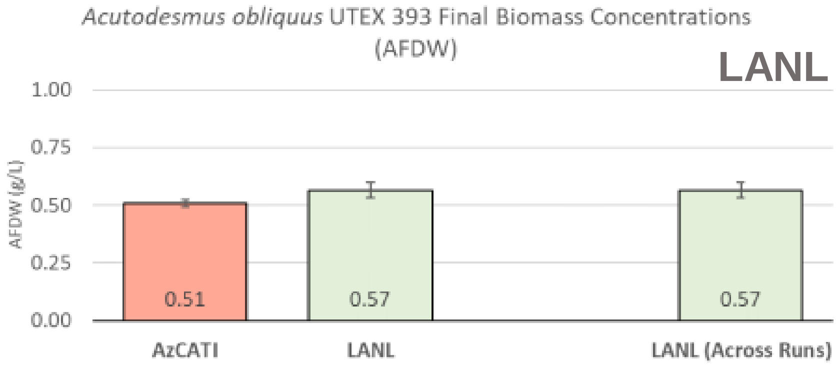
Comparative final biomass concentrations for Acutodesmus obliquus UTEX393

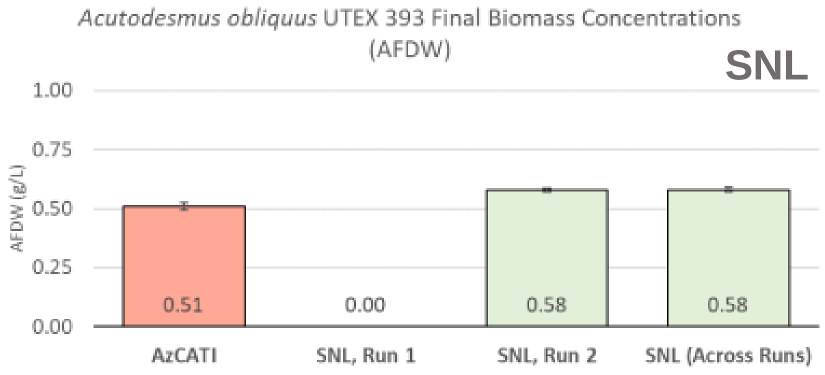
Pipeline

- Compared to AzCATI, final biomass concentrations were slightly higher in all cases.







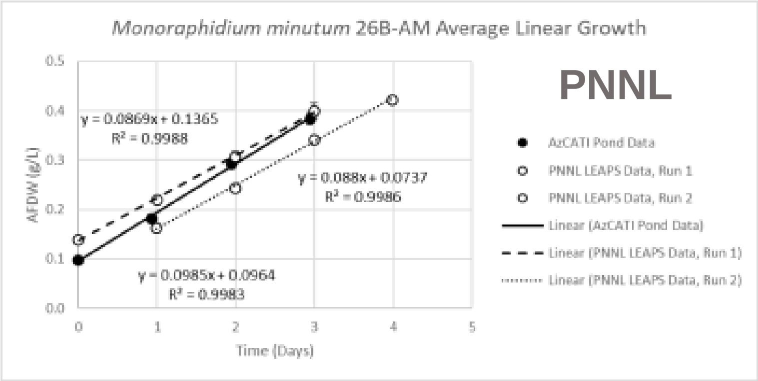


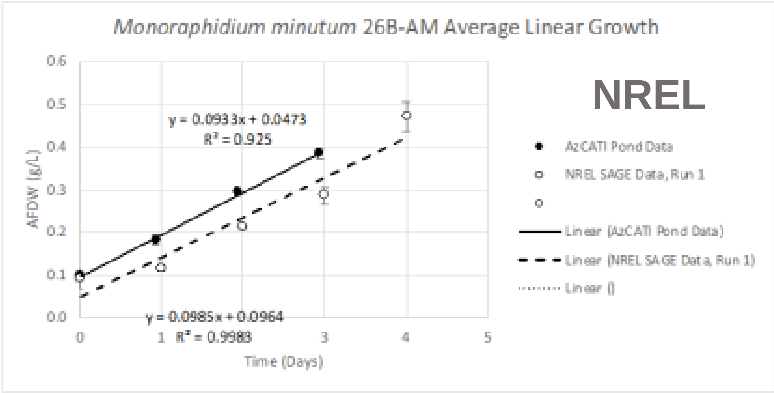
Supplemental Outcomes: Climate-simulation Culturing of AzCATI Ponds

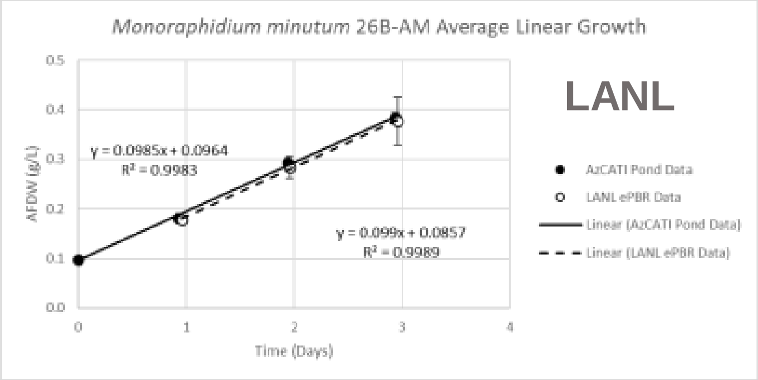
Linear regression slopes of AFDW vs. time for Monoraphidium minutum 26B-AM

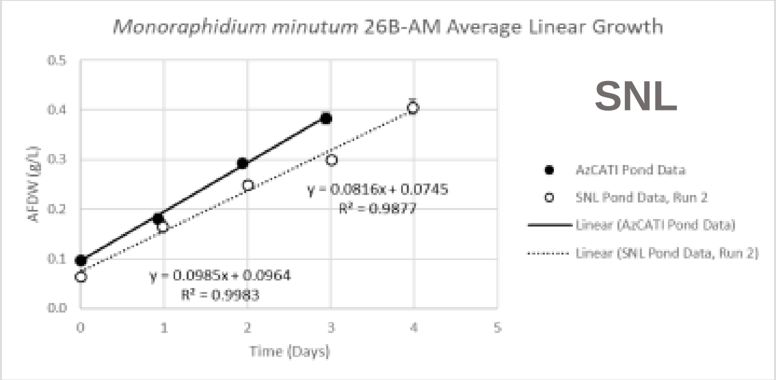
Pipeline

- **Linear-regression slopes were used to calculate linear-phase biomass productivities** (see next slide)









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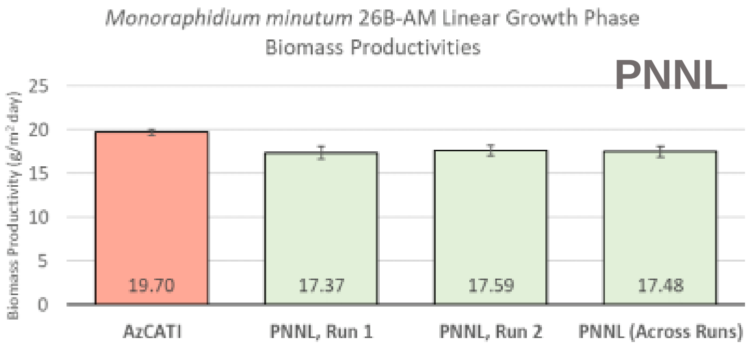
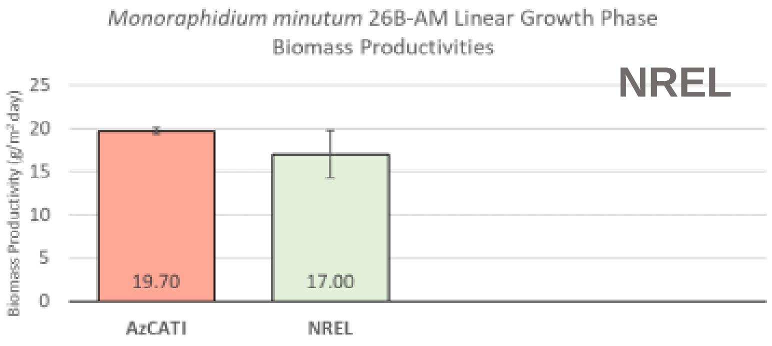
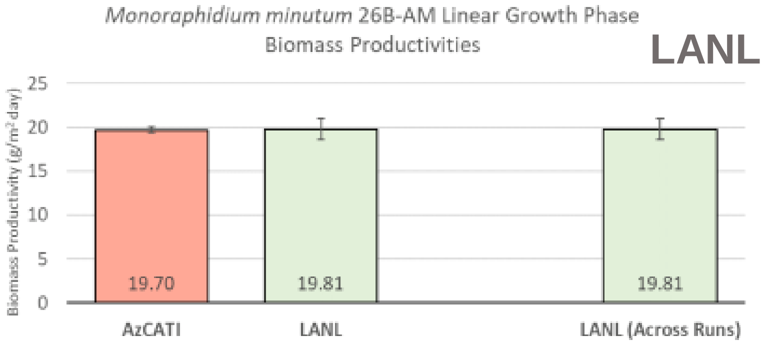
Supplemental Outcomes: Climate-simulation Culturing of AzCATI Ponds

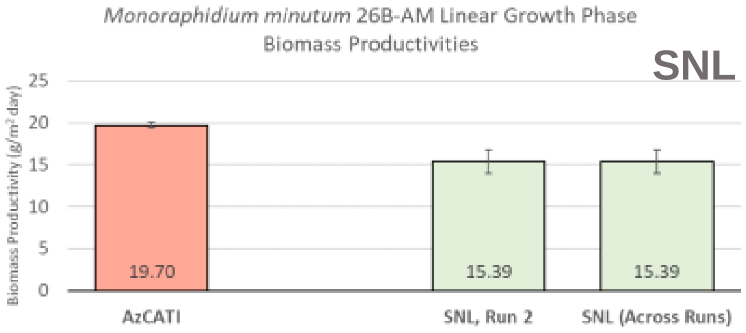
Comparative linear-phase biomass productivities for Monoraphidium minutum 26B-AM **Pipeline**

- **Linear-phase biomass productivities were similar or slightly lower than at AzCATI**









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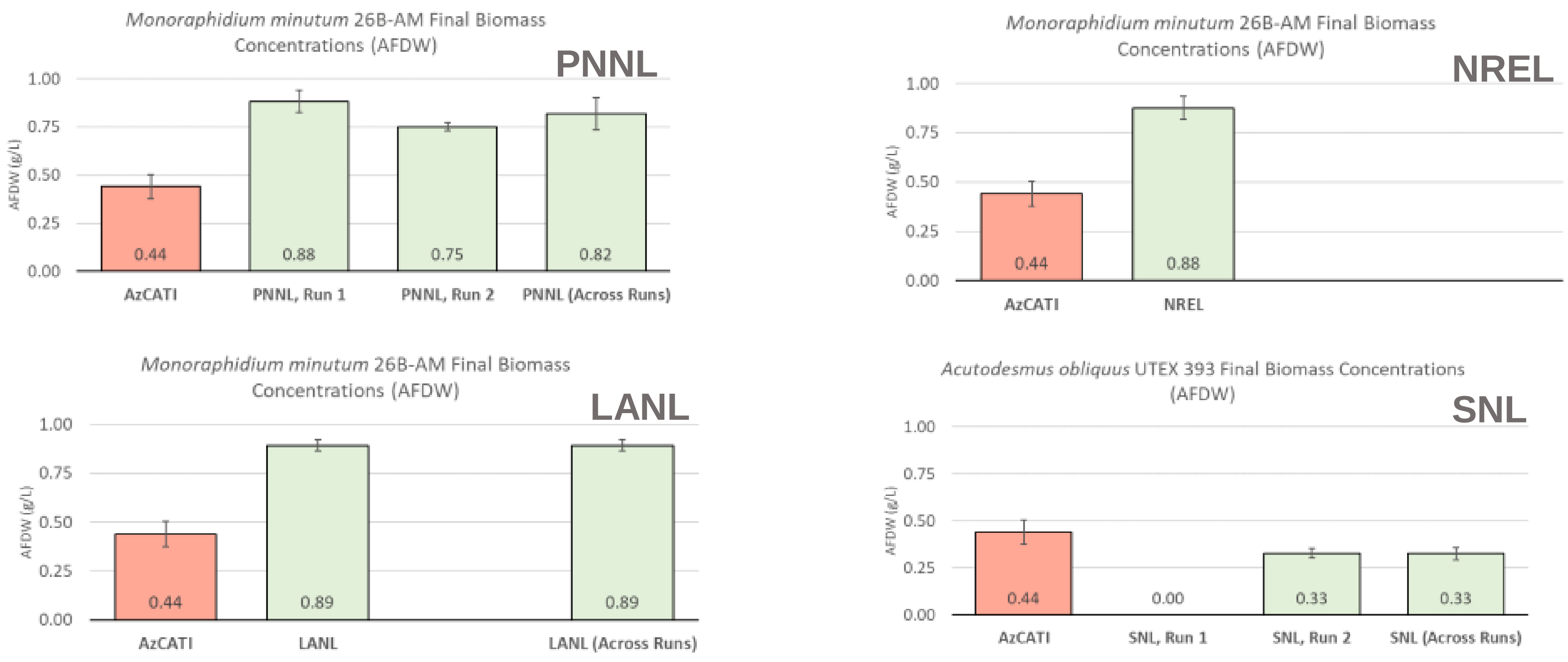
Supplemental Outcomes: Climate-simulation Culturing of AzCATI Ponds

Comparative final biomass concentrations for Monoraphidium minutum 26B-AM

Pipeline

- Compared to AzCATI, final biomass concentrations were higher in the PNNL, NREL and LANL climate-simulation PBRs but lower in the SNL indoor crash ponds



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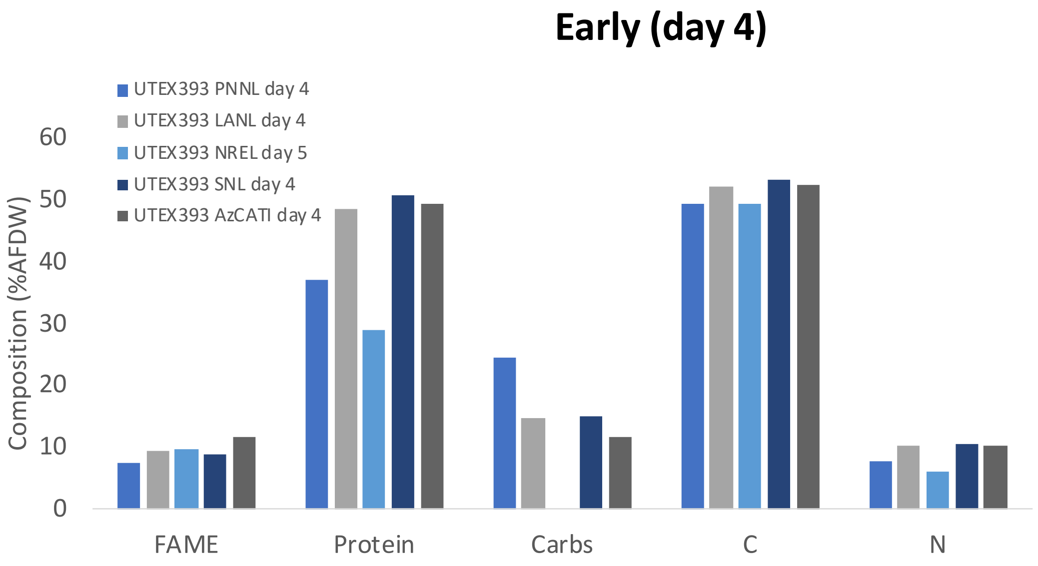
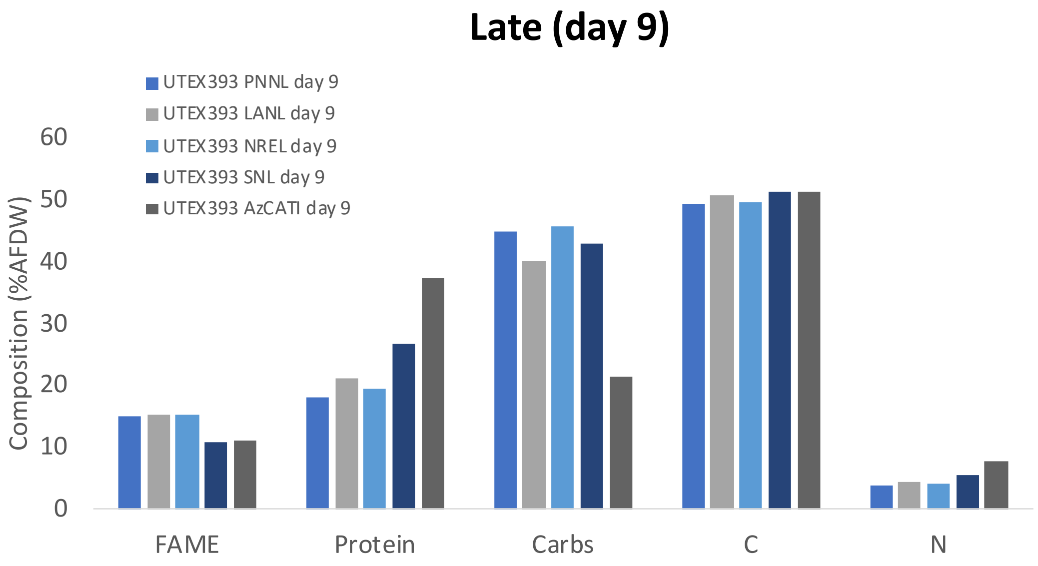
Supplemental Outcomes: Climate-simulation Culturing of AzCATI Ponds

Comparative shift in biomass composition for A. obliquus UTEX393 **Pipeline**

- Biomass composition varies between each climate-simulating photobioreactor
- Early time point shows reactor conditions diverge from outdoor AzCATI actual biomass composition
- Final timepoint diverges up to 2-fold with all small-scale indoor reactors more rapidly shifting to high carbohydrate content





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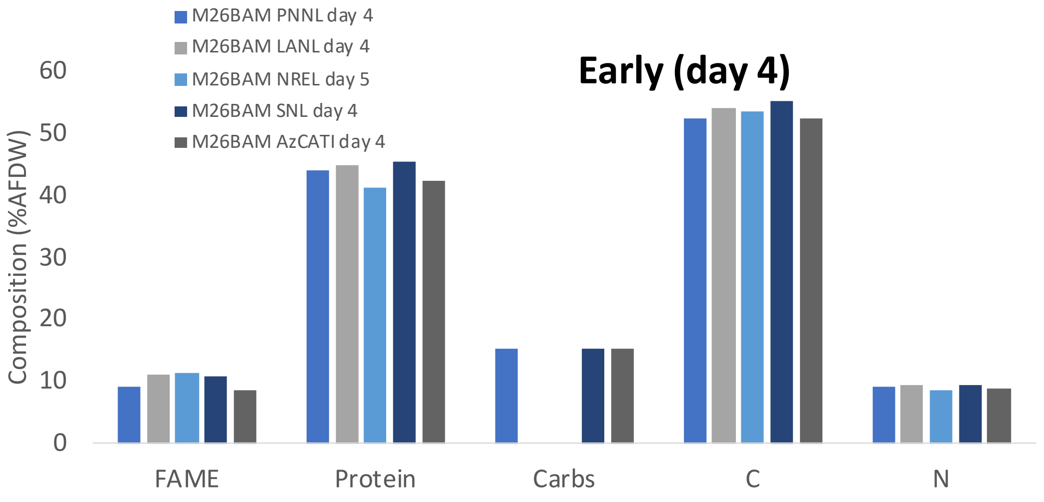
Supplemental Outcomes: Climate-simulation Culturing of AzCATI Ponds

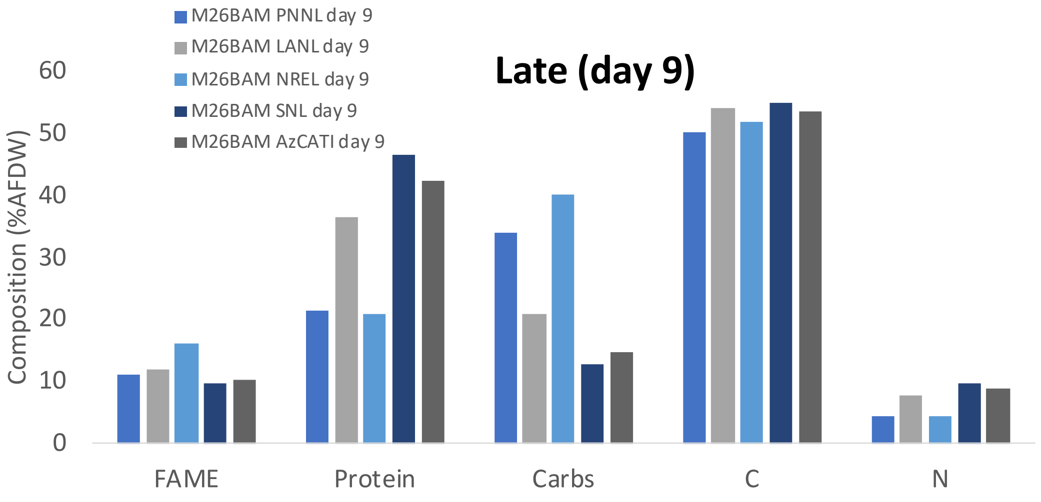
Comparative shift in biomass composition for M. minutum 26B-AM

Pipeline

- Biomass composition varies between each climate-simulating photobioreactor and between species
- Early time point agrees within 10% for each component with outdoor AzCATI actual biomass composition
- Final timepoint diverges up to 2-fold with two of the small-scale indoor reactors more rapidly shifting to high carbohydrate content while the outdoor/pond cultivation did not shift the compositional profile

- Light, nutrient and temperature are not the sole drivers of compositional responses





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Suppl. Approach: Mixotrophic Growth on Corn Stover Hydrolysate

New Concepts

Increasing biomass productivity by adding corn stover hydrolysate

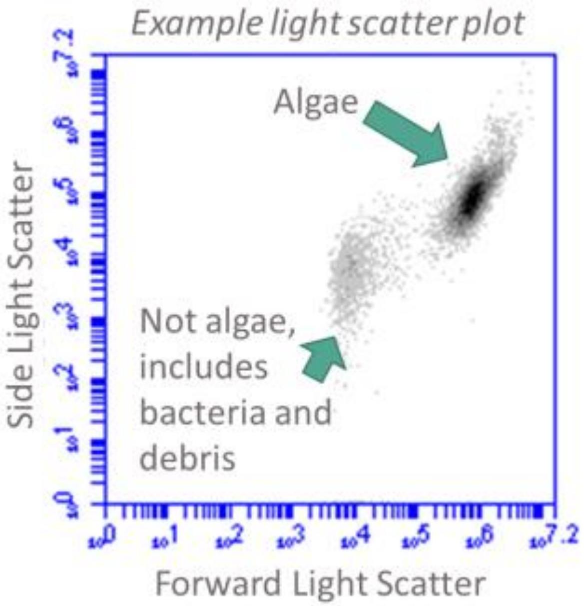
- **Background/history:** Mixotrophy is of ongoing interest for increasing algae productivity
  - *Corn stover hydrolysate is a source of sugars that can be used as a C source for microbial growth*
    - *Used with bacteria/fungi in other BETO programs as a potential path to converting biomass waste into biofuels and bioproducts*
- **Objective:** Examine growth of DISCOVR algae strains in presence of corn stover hydrolysate
- **Challenges:**
  1. Effect of hydrolysate on growth is masked in CO_2 incubator
     - *Conducted experiments in ambient* CO_2
  2. OD values may also reflect bacterial growth due to presence of hydrolysate
     - *Used flow cytometry to specifically measure algae and bacterial cell counts*
- **Economic/Technical Metrics:** Aim to increase biomass productivity by at least 20%.

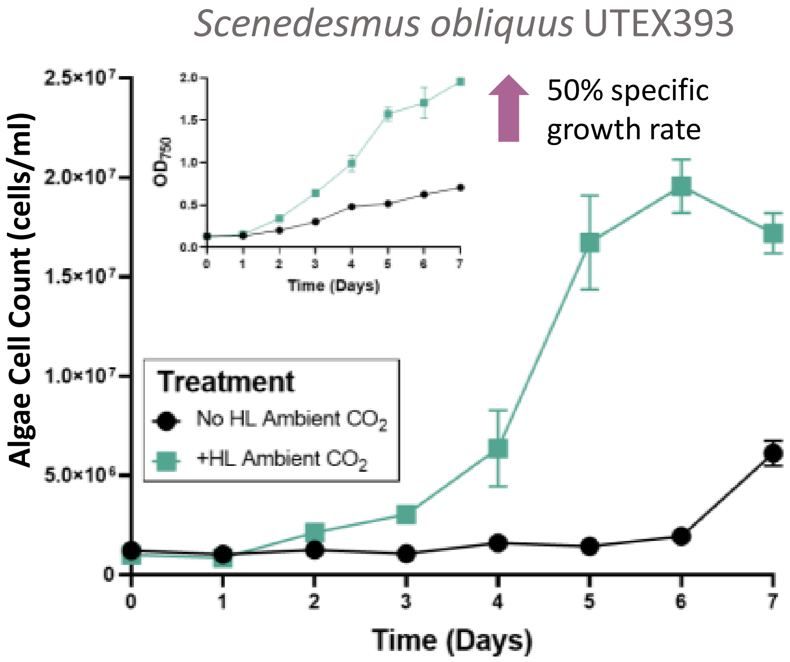
Suppl. Outcomes: Mixotrophic Growth, Corn Stover Hydrolysate

5 of 6 Strains (including 2 SOT strains) showed a boost in growth by OD and cell count

New Concepts

- Corn stover hydrolysate (HL) provided by NREL
- 16:8 light:dark, 25°C, +/- 1.48 g/L HL, ambient CO_2
- OD values and algal cell counts measured independently; significant growth boost seen with HL treatment in most strains, including UTEX393 (50%) and 26BAM (120%) SOT strains.
- Light scatter plots and cell counts from flow cytometry showed that OD changes can be attributed to changes in algae growth and not bacteria.





Suppl. Outcomes: Mixotrophic Growth, Corn Stover Hydrolysate

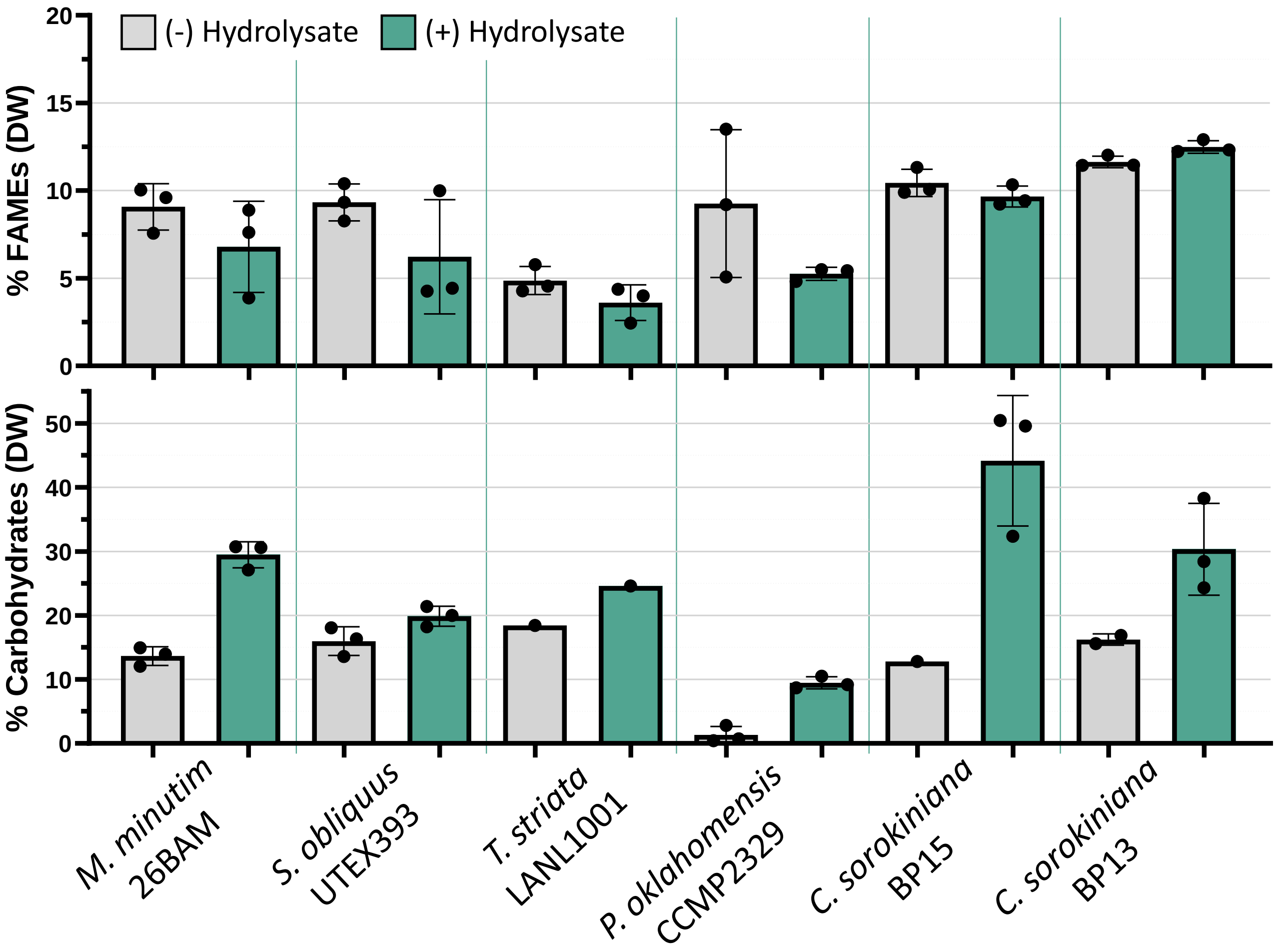
New Concepts

At least 3 strains showed a boost in carbohydrate storage with HL treatment

- End point samples were analyzed for FAME and carbohydrate content
- Increases in FAMEs were not observed, but at least 3 strains showed an increase in total carbohydrates.

% Carbohydrates (DW basis)

Strain	-HL	+HL
26BAM	14	25
UTEX393	16	20
T. striata	18	25
P. oklah.	1	9.5
BP15	13	44
BP13	16	30



Suppl. Impact: Using Mixotrophic Growth to Boost Productivity

New Concepts

Mixotrophic growth has potential for improving productivity, next test raw biomass waste

New approaches/ideas need to be tested to determine feasibility of application in outdoor ponds

- Mixotrophic growth being studied, but BETO corn stover hydrolysate previously untested for algal growth

Impact

If successful hydrolysate has the potential to:

- **Increase algae productivity**
- **Increase algae carbon storage (lipids, carbohydrates)**

Promising results could be further tested under more outdoor relevant conditions

However, opportunity to work with LEAF project (see separate slides) seemed a better fit (uses even less costly biomass by using plant waste instead of corn stover processed into hydrolysate)

Suppl. Approach: Mixotrophic Growth on Plant Substrates (LEAF)

New Concepts

Improving biomass productivity

- **Background/history: Preliminary work from LANL LDRD investment and the Leveraging Algae Traits for Fuels (LEAF) AOP project (presentation on 3/24):**
  - *A. protothecoides* directly degrades and utilizes non-food plant substrates for cell growth (Vogler et al. 2018).
  - *Nannochloropsis* sp. increases in biomass/lipid content (30%) with plant substrates (Schambach et al. 2020).
  - Raw plant substrates offer potentially cheap carbon source (ie. Lawn clippings)
  - Observe protection from crash in lab experiments
  - Multiple strains being examined at multiple scales shows trait is more widespread than previously thought.
- **Objective:** Improve biomass productivity of top DISCOVR strains with mixotrophic growth on plant substrates
- **Challenges:**
  1. **Flask data does not always translate to outdoor cultivation**
     - *CO_2 delivery to mixotrophic cultures decreases productivity impact*
     - *Scale-up to 100 L ponds shows smaller differences in productivity improvements*
  2. **Plant substrate selection for each strain is important**
     - *Plant substrates are not created equal → different strains prefer different substrates*
     - *Optimal concentration of plant substrate varies per strain*
- **Economic/Technical Metrics:** Improved growth rate/biomass productivity.

Suppl. Progress: Mixotrophic Growth on Plant Substrates (LEAF)

Evaluate biomass growth with plant substrate addition

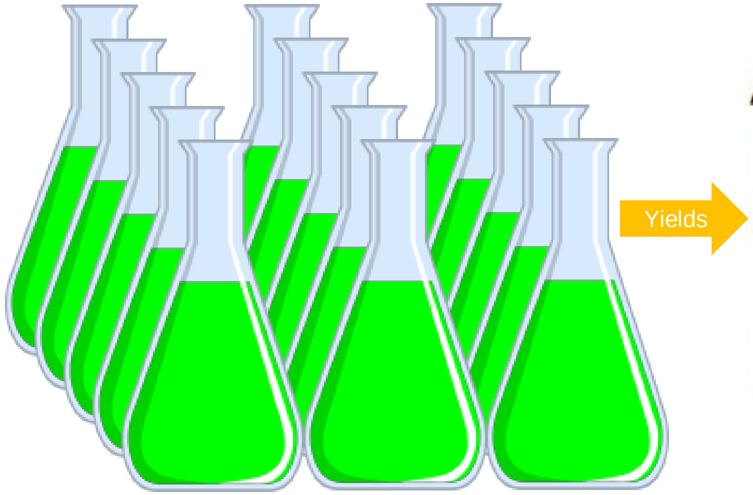
New Concepts

Experimental Approach for FY21 (Future Work)

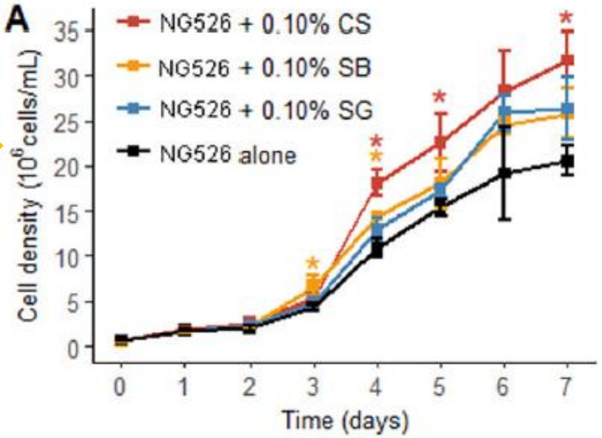
Examination of mixotrophic growth on plant substrates for top performing DISCOVR strains

- Test plant substrates (switchgrass, corn stover, and sugarcane bagasse obtained from INL Feedstock Library) with top performing target strains at flask scale initially without CO_2 to determine potential for mixotrophy on these substrates.
- Algae cells are counted with a hemacytometer

Algal Cultures +/- Plant Substrate



Growth analysis by cell count



FY21 Q3 Milestone:

Screen at least 5 strains for improved biomass productivity, as measured by specific growth rate, with plant substrate addition at flask scale in triplicate with controls.

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Supplemental Impact: Mixotrophic Growth on Plant (LEAF)

New Concepts

Improving biomass productivity

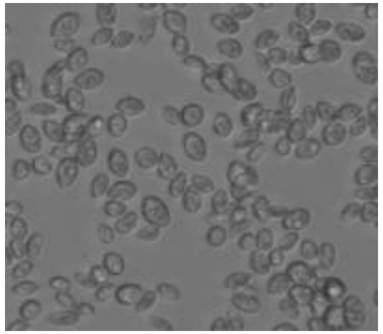
Current biomass growth completely phototrophic:
- Organic carbon (sugars) addition can be costly
- Dependent on CO_2 for biomass growth acceleration
- Lipid improvement relies on nutrient starvation

Impact

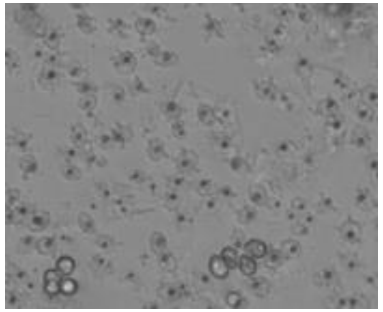
Top performing DISCOVR strains capable of utilizing raw plant substrates as a carbon source will potentially:
- **Gain at least 10% improvement in biomass/lipid productivity**
- **Shift lipid composition profile to valuable coproducts (see omega-3 shift in *Nannochloropsis*)**
- **Be less reliant on CO_2 for growth acceleration with potential growth during the dark**
- **Have greater culture resilience (longer time to pond crash)**
- **Impact FY21 SOT**
- **Have direct impact on industrial cultivation (already testing strains at industrial scale in LEAF)**

Suppl. Outcomes: Isolation & identification of seed train contaminant

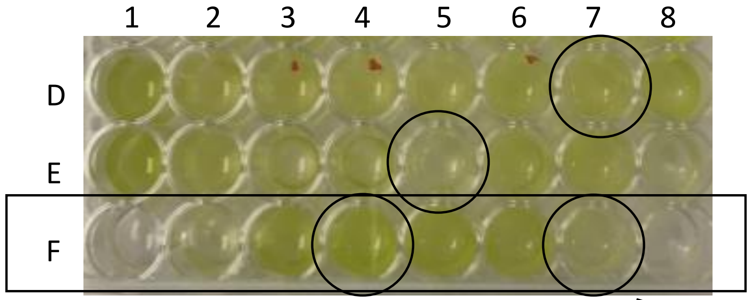
New Concepts



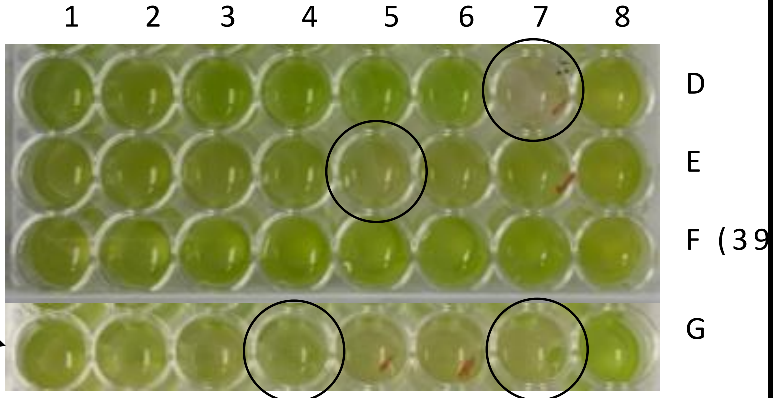
393 Control



393 + Isolate D7



Original Isolates



Isolates + 393

Row “G” here is Row F on the plate to the left

18S Sequencing on 4 Isolates; Top Hits

393 Seed (2/2020) Plate Isolate	Top Hit	
Isolate D7	Flamella;Flamella arnhemensis	1323
Isolate E5	Flamella;Flamella arnhemensis	10891
Isolate F4	Amoeboaphelidium;Amoeboaphelidium protococcarum	714
Isolate F7	Amoeboaphelidium;Amoeboaphelidium protococcarum	1517
	Flamella;Flamella arnhemensis	1495

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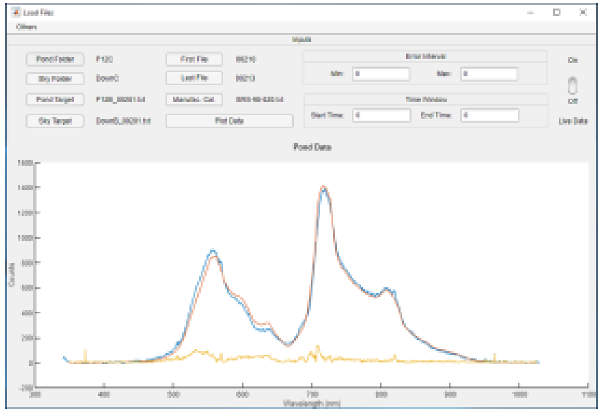
Supplemental Outcomes: Spectroradiometric Monitoring

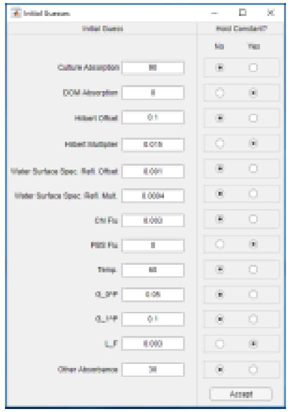
Modernized analysis code for increased flexibility and automation

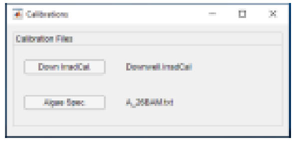
New Concepts

- **Key milestone (FY20, Q3, completed): Developed prototype GUI application for automated detection of pest presence**
  - Modernized/modularized code to enable rapid adaption to future needs
  - Implemented real-time analysis in addition to off-line analysis
  - *Impact: Rapid incorporation of different organisms, newly-encountered pests when signatures are characterized, improved user interface*

- **Key milestone (FY21, Q1, in progress):**
  - Implementing automated change detection algorithm into real-time analysis software
  - Statistical assessment of pest signal to identify initial and sustained pest presence was developed for publication.
  - *Impact: Real-time analysis by non-experts.*







File No.	C_a	C_b	C_H	C_Sun	C_f	T
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211	37.27840181	0.279340028	0.003246452	1.10377E-15	0.010097902	16.39072882
212	35.99178181	0.299404715	0.00211527	1.36027E-12	0.014797103	14.75726012
213	35.8715343	0.293519786	0.00217157	4.44581E-14	0.014451863	13.82339446

ASHARP Analysis software. Clockwise from left: Main window showing fit of individual spectrum, initial guess gui, calibration file selection window, example of excel output with results.

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Supplemental Approach: Machine Learning

Identify pond crash signatures for optimization of operational strategies

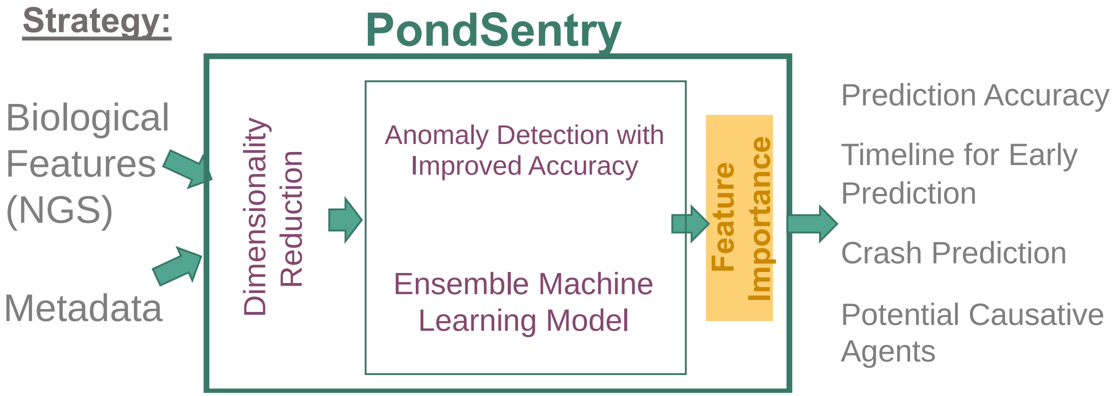
New Concepts

- **Background/history:** To minimize loss of biomass due to biological contamination we utilize data driven methods for early detection of an eminent crash and plausible crash agents.

- **Objectives:** To increase annualized productivity by early crash prediction to allow intervention.
  - **Identify pond crash signature** using **data-driving approaches**
  - Build and apply **algorithm** for early-detection of anomaly for SOT run

- Develop effective anomaly detection method for early detection of **Pond Crash (PondSentry)**
  - Improve algorithm for anomaly detection with tensor decomposition
  - Application on the SOT trials

Strategy:



We have developed an Anomaly detection tool based on tensor-decomposition of multiple features to detect anomalous events in timeline of algal cultivation. This method delivers predictions and important feature selection with improved accuracies that previous methods

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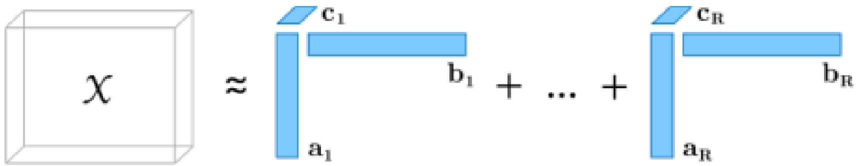
Suppl. Outcomes: Machine Learning and Data Driven Strategies

Machine learning and data driven for optimization of pond operational strategies

New Concepts

- Tensor Decomposition based anomaly detection method “Pond Sentry” we improved the early detection accuracy by 50%

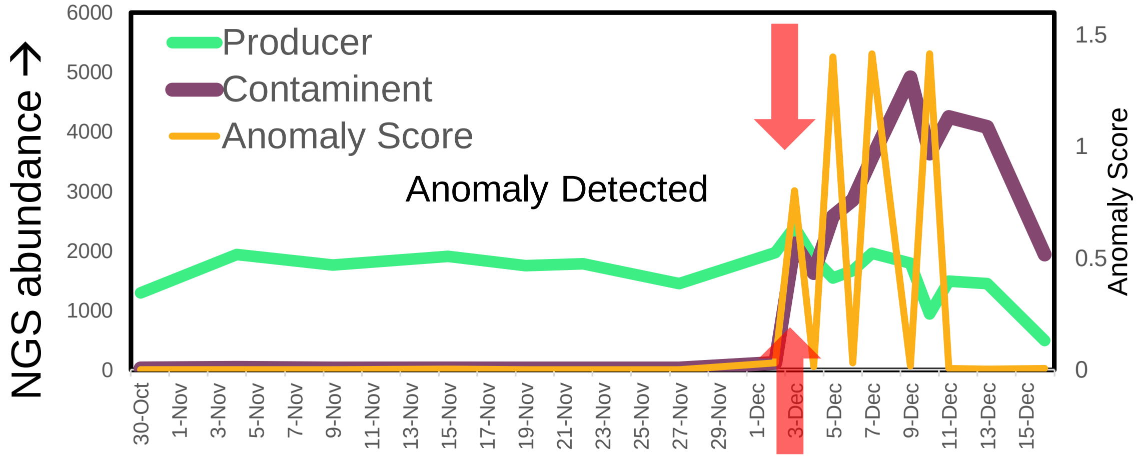


Tensor Decomposition

- Application of **PondSenry detected** FD01 infections 2019 AzCATI cultivation run which matched microscopy observations
  - **Current status:** 100% accuracy with >72hrs prediction warning.

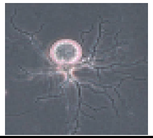
Anomaly Detection Method	Abundance Derived Anomaly Score	Tensor Decomposition Method **(Improved)**
Accuracy	67%	100%

Example of Prediction of Anomaly in Cultivation Run Timeline



Cultivation run timeline →

Application of Pond Sentry on SOT run

Raceway	Early Prediction Before Crash	Major Contaminants
Pond 1	>3 days	FD01  Hydra 
Pond 2	>3 days	
Pond 3	>2 days	

* **No anomaly was detected for the negative control cultivation runs**

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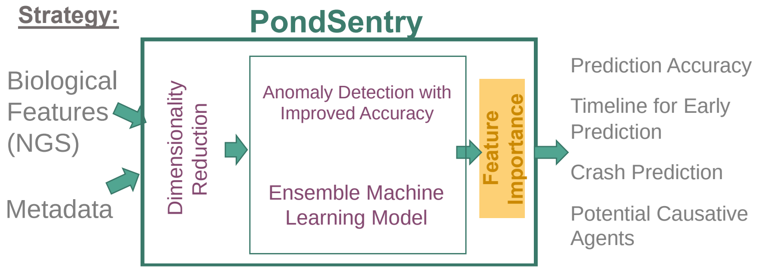
Supplemental Impact: Machine Learning

New Concepts

Understanding pond ecology & designing novel early detection for anomalies in cultivation

Research Strategy:

- SNL Carried out metagenomics analysis of SOT cultivation samples for top performing strains.
- Described pond ecology of the cultivation timeline and identified potential causative agents for crashes
- Improved anomaly detection method "PondSentry" algorithm improving early detection accuracy by 50%.



Impacts

- Development of state-of-the art application for future crop monitoring strategies with:
  - Greater accuracy of prediction and earlier identification
  - Identification of causative agents
- Understanding the features influencing yield and characteristics of the biomass
  - Anomalies, resulting in biomass loss
  - biomass characteristics (proteins, lipids content)

- FY20: complete metagenomics assay and analysis of SOT cultivation run samples for 2 strains (Q2 and Q4).
- **Current algorithm operating at: 100% accuracy with >72hrs prediction warning well above requirement for FY20 Q4 and FY21 Go-no-go requirement.**
- Two Conference presentations at Algae Biomass Summit 2018 and Chemical Biological Defense S&T Nov 2019 by DTRA
- One collaborative paper and one planned peer reviewed submission

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Supplemental Progress and Outcomes: TEA Modeling

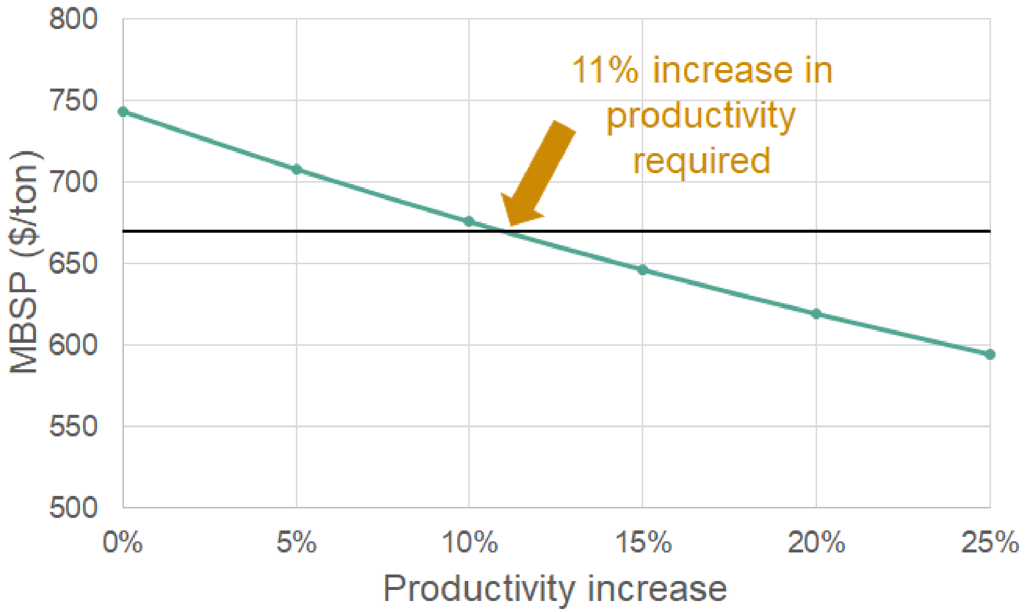
TEA

"What-If" sensitivity analysis for growth promoting molecules

Cost-benefit analysis to consider strategy for GPM use in large scale cultivations:

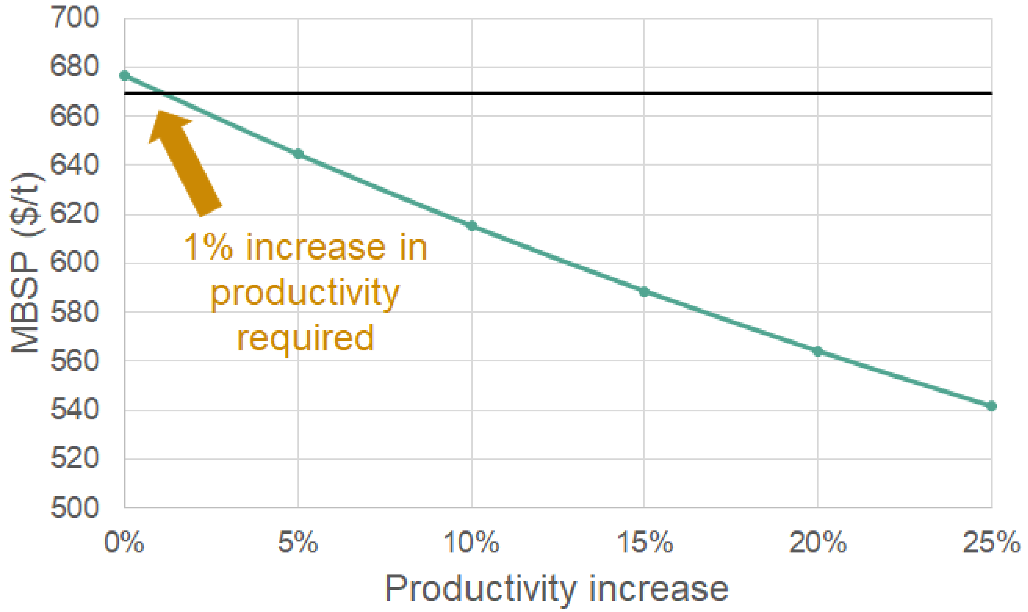
Gibberellic acid (GA3)
1 μM (0.34 mg/L)
$4.00/g

2.1 t/year



Gibberellic acid (GA3)
0.1 μM (0.034 mg/L)
$4.00/g

210 kg /year



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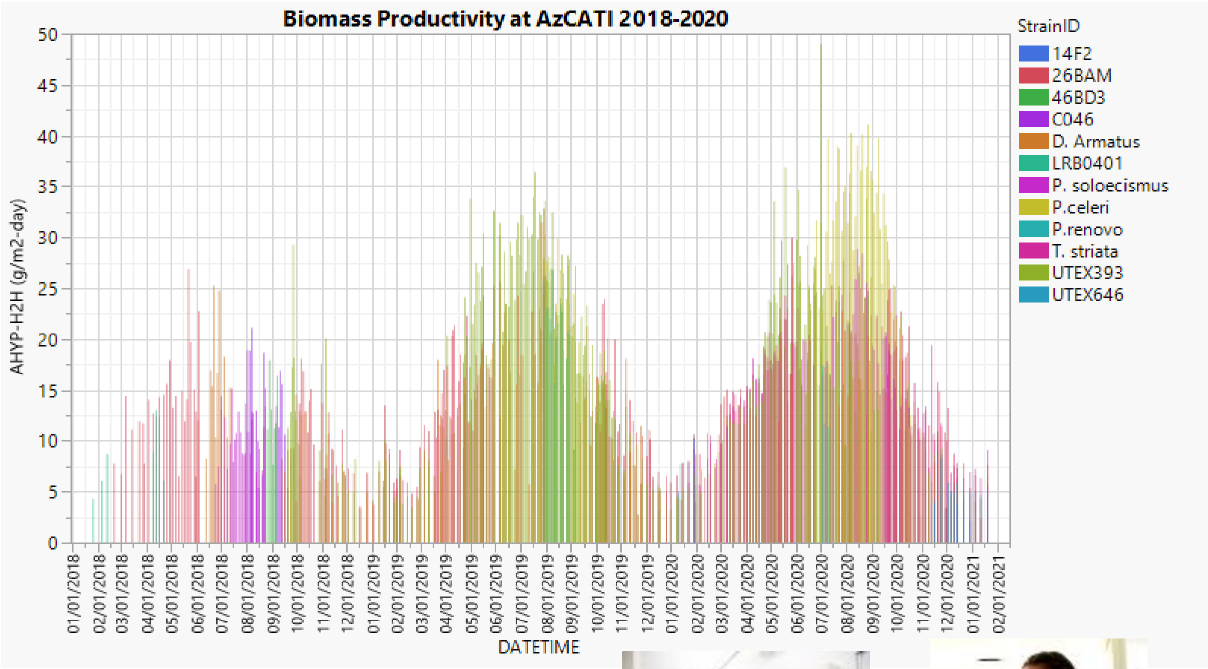
Supplemental Progress and Outcomes: State of Technology Trials

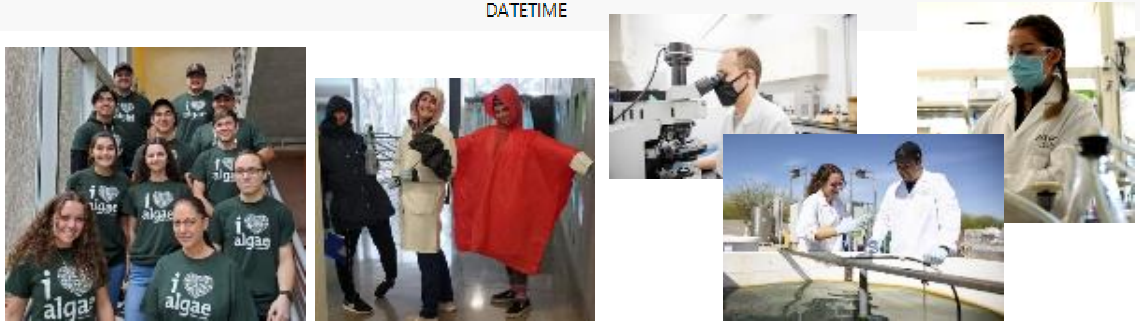
SOT Trials

Outdoor verification of best strains/approaches with primary focus on improving productivity



Between 2018 and 2020

- Cultivation experiments *365 days a year*
- *12 strains* tested outdoors at AzCATI
- *Over 3300* individual pond measurements of productivity
- *Over 17,000* individual grab samples:
  - AFDW, OD, nutrients, microscopy
- Biomass and pond samples supplied to DISCOVR and BETO AOP projects
  - *~380kg* of algae paste collected from >90 experiments
- Mature operations with experienced faculty, staff and student researchers
  - Over three dozen undergraduate students supported, with hands on training in lab and field work, data analysis, and teaming



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Supplemental Approach: State of Technology Data Management

SOT Trials

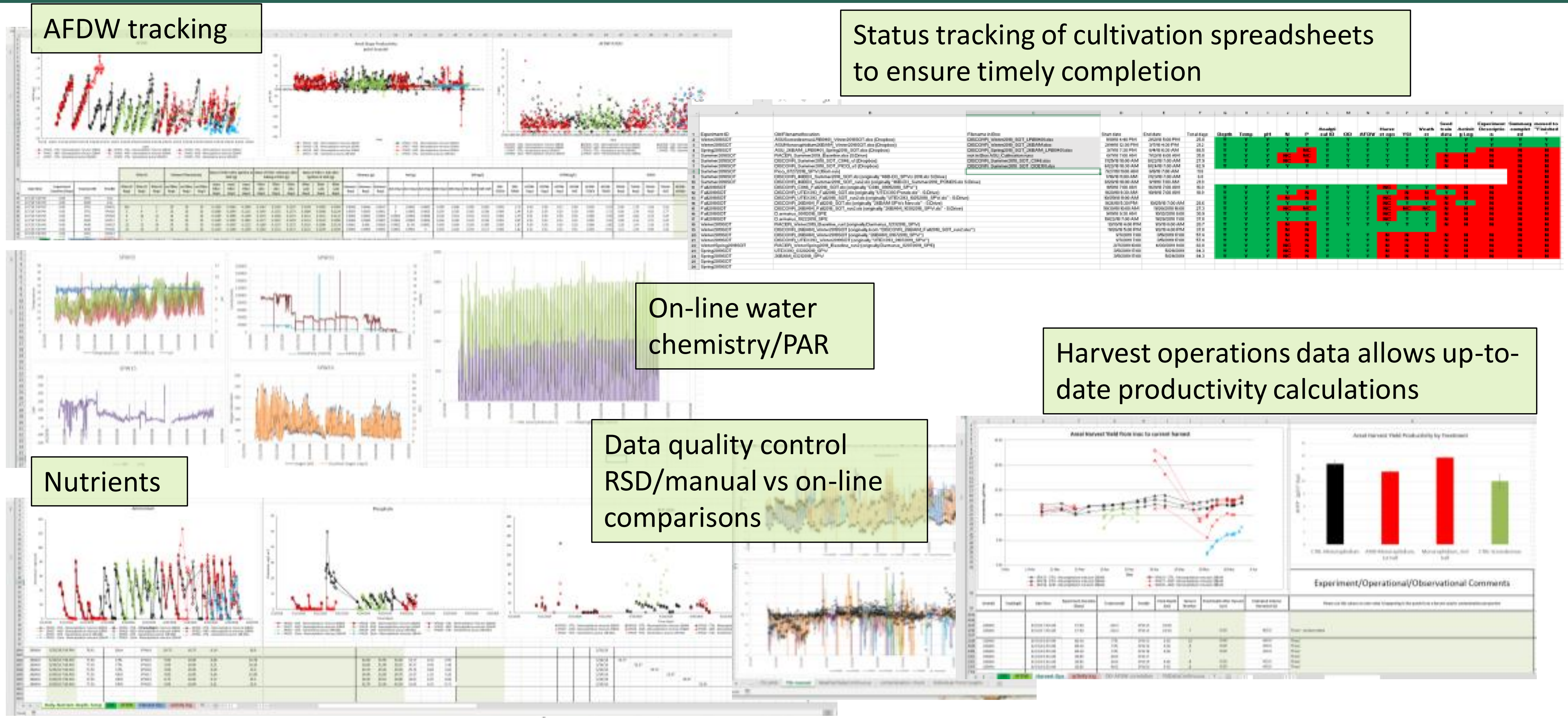
Centralized, accessible data storage to enable analysis, discussion, and planning

- **Background/history:** Expanded on previous data capture methods to collaboratively develop comprehensive data collection spreadsheets. Applied lessons-learned from previous data collection efforts under ATP[3].
- **Objective:** Capture cultivation and composition data from year-round, outdoor cultivation trials in a format that facilitates rapid analysis and allows up-to-date understanding of biomass productivity and enables planning for further improvements.
- **Challenges:** Tracking of multitude of experiments and delay between data generation and spreadsheet upload. Handled by experiment status tracking spreadsheet.
- **Metrics:** Currently data spreadsheets contain >8000 cultivation time points encompassing 53 variables and 5 species of algae. These data points provide the data to measure progress towards Program goals and facilitates the yearly SOT reporting milestone by providing a central repository for the underlying data showing 57% increase in biomass productivity over 2 years.

Supplemental Outcomes: State of Technology Data Management

SOT Trials

Integrated data visualization allows immediate understanding of results and data quality



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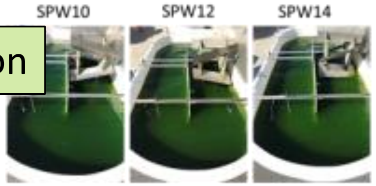
Supplemental Impact: State of Technology Data Management

SOT Trials

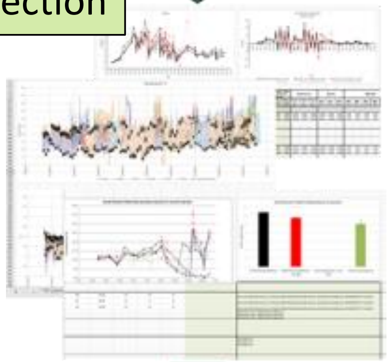
Data management facilitates current SOT position and allows for improvement planning

- Comprehensive spreadsheets collaboratively developed to capture critical metrics of algae cultivation
  - Active graphs at top of each tab for facile data visualization
  - Includes measured metrics and up-to-date calculations
  - Includes checks on data quality
  - Includes tab specifically for pond operator observations
    - Critical to understanding cultivation in the event of a pond failure
- In-progress and final spreadsheets are kept in a central depository on Box/SharePoint
- Public data dissemination website under development to be hosted on OpenEI (https://openei.org/apps/DISCOVR/)
- Allows easy visualization of underlying data leading to current improvements such as the 57% increase in productivity for 2021 over the last 2 years
- **Our Data management strategy facilitates data analysis and discussion of current experiments during biweekly meetings and facilitates discussion to develop favorable path forward**

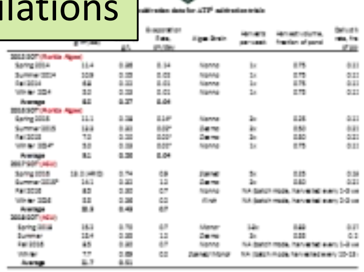
Cultivation



Data Collection



SOT Calculations



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