DISCOVR

Development of Integrated Screening, Cultivar Optimization, and Verification Research

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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 Goal

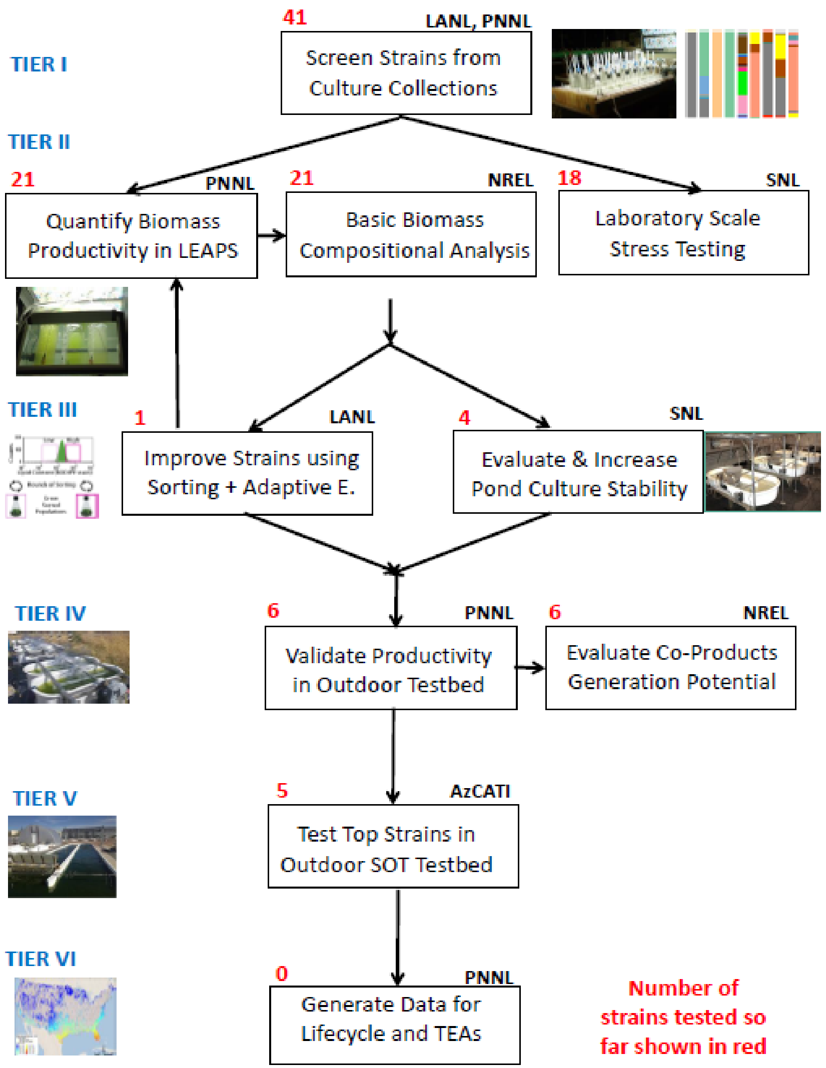
- **Reduce total microalgae biofuels production costs** by developing an **integrated screening platform** for the **identification of high productivity strains with cellular composition suitable for biofuels and bioproducts for resilient, year-round outdoor cultivation**.

Outcomes

- **Standardized identification, deep characterization, and delivery of robust, high productivity microalgae strains** to the bioenergy and bioproducts communities, such as **industry** and **BETO funded projects**.
- **Improved productivity and reduced costs** *via* a streamlined approach to strain characterization and implementation in **outdoor trials.**

DISCOVR Project Overview and Work Flow

Strains are tested and down-selected in pipeline consisting of 6 TIERs



Objectives & Outcomes

- **Standardized testing conditions** for strain comparison
- **Climate-simulated culturing** to quantify winter and summer season biomass productivities
- Information on **carbon storage and co-product** potential
- **Improvement** in salinity tolerance and lipid/biomass accumulation
- Data on **pest tolerance**
- **Outdoor validation** and streamlined funneling of strains into the **SOT**

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Approach: Overview

DISCOVR pipeline accelerates identification of top producing strains

Challenges

- **Demonstrate high seasonal biomass productivities in new and/or improved strains**
- **Optimize value of biomass via identifying best strains and culture conditions**
- **Prevent crop failures by deleterious agents via preventative and predictive methods**
- **Demonstrate at least 10% per year increase in SOT annual areal biomass productivity**

Critical Success Factors

- **Unique state-of-the-art** technical **capabilities** are employed at each TIER.
- **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.

Approach: TIER I Strain Characterization

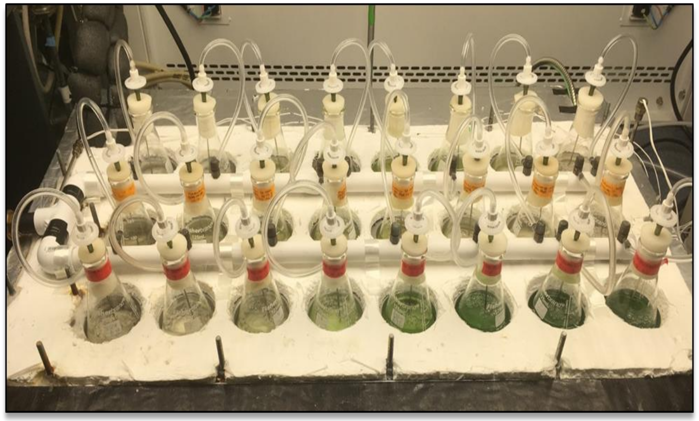
Temperature and salinity tolerance is measured in gradient incubators

Objectives

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

Approach

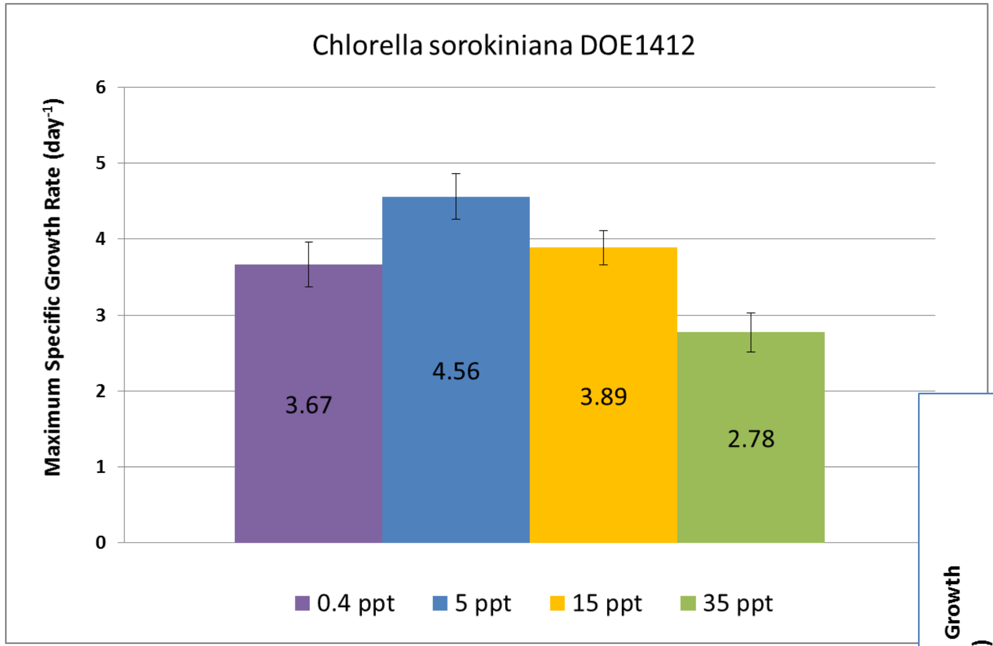
- PNNL **Thermal Gradient Incubator (TGI)**
  - Measure maximum specific growth rates at saturating light intensities
  - Temperature range from ~4 to 45 ˚C
- PNNL **Salinity Gradient Incubator (SGI)**
  - Abbreviated salinity screen at 25 ˚C
  - 5, 15, 35 parts per thousand (ppt)

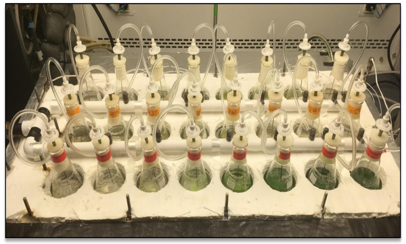


Results: Typical TIER I Strain Characterization Data

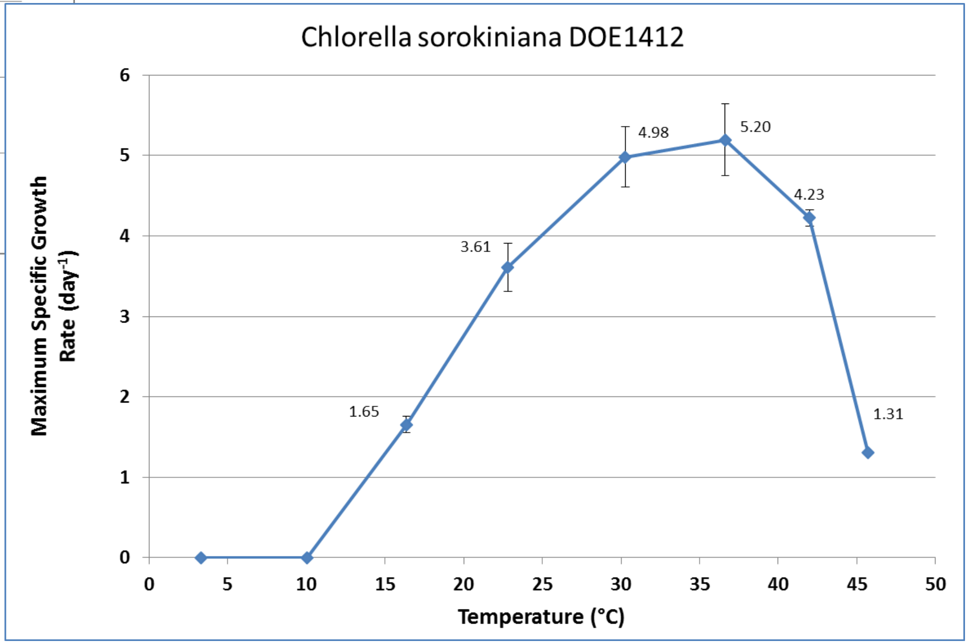
Each strain has a unique temperature and salinity tolerance range

Salinity Tolerance Profile





Temperature Tolerance Profile



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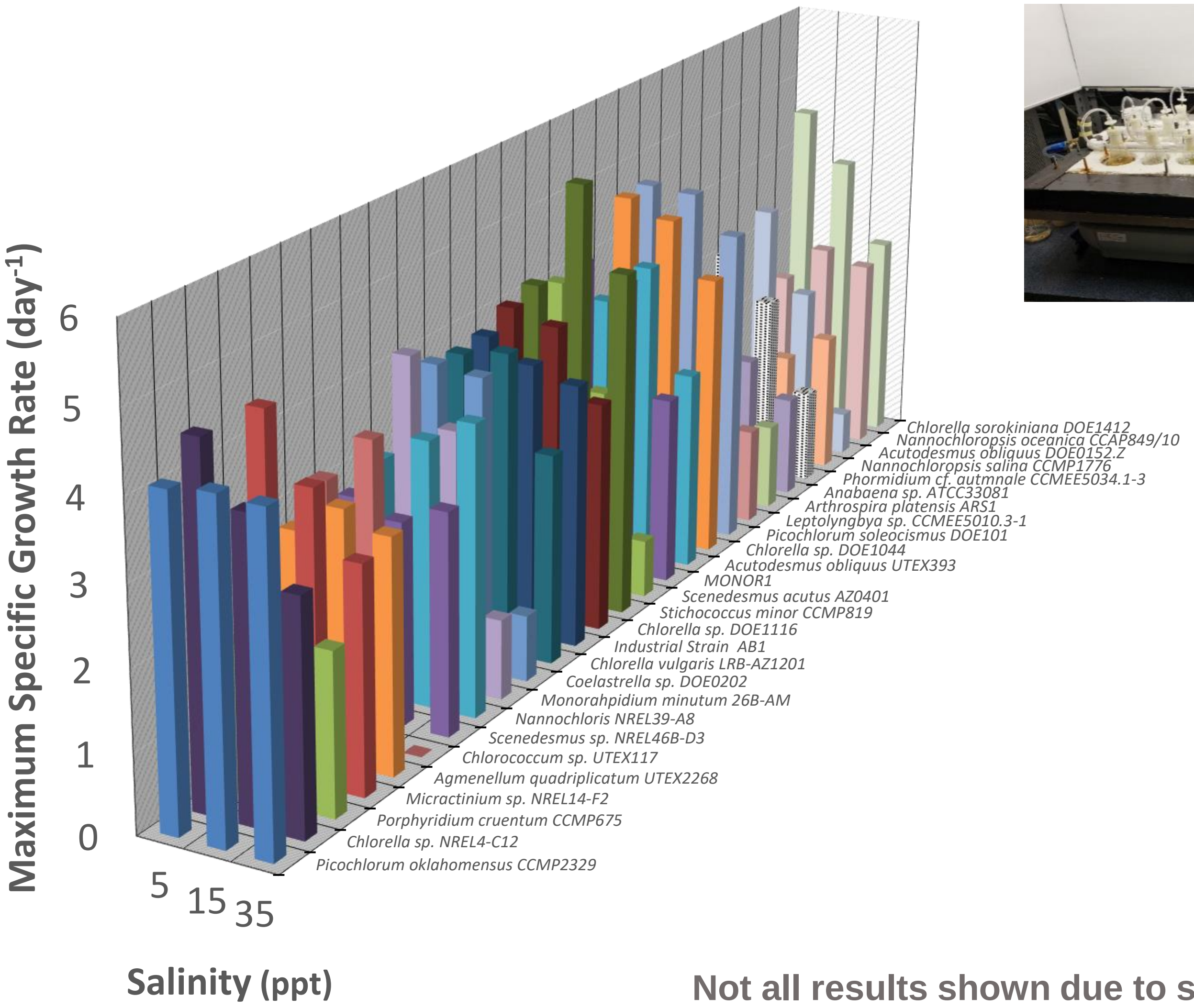
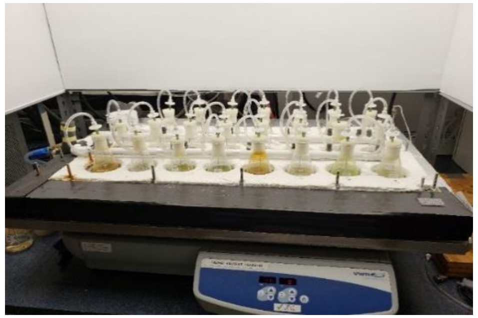
Results: Salinity Tolerance of 41 TIER I Strains

Optimum salinity determines choice of medium (brackish/seawater)





Not all results shown due to space limitations

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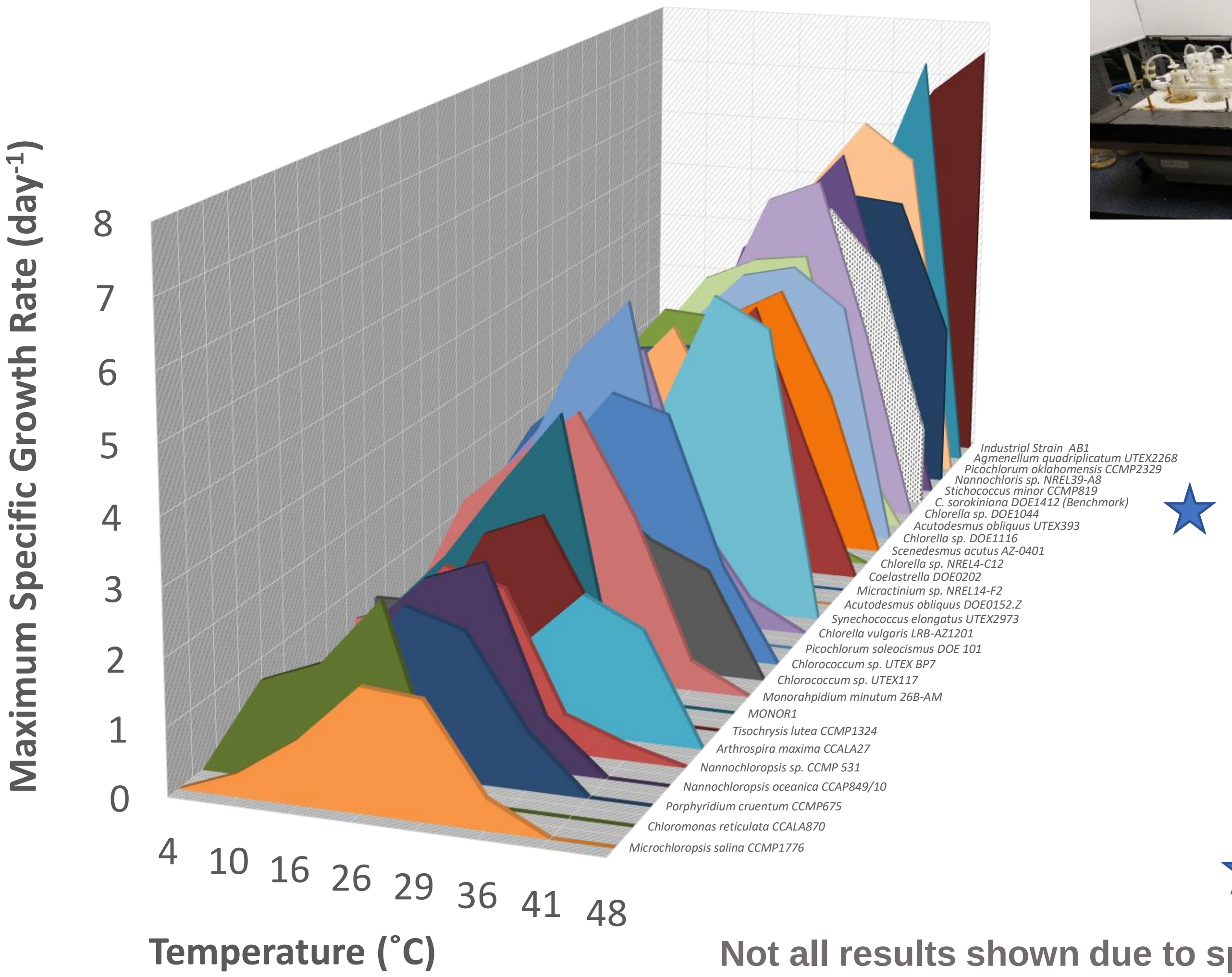
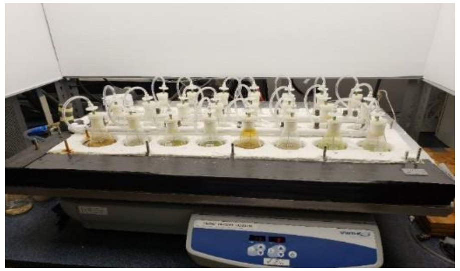
Results: Temperature Tolerance of 34 TIER I Strains

Temperature tolerance range determines choice of cultivation season



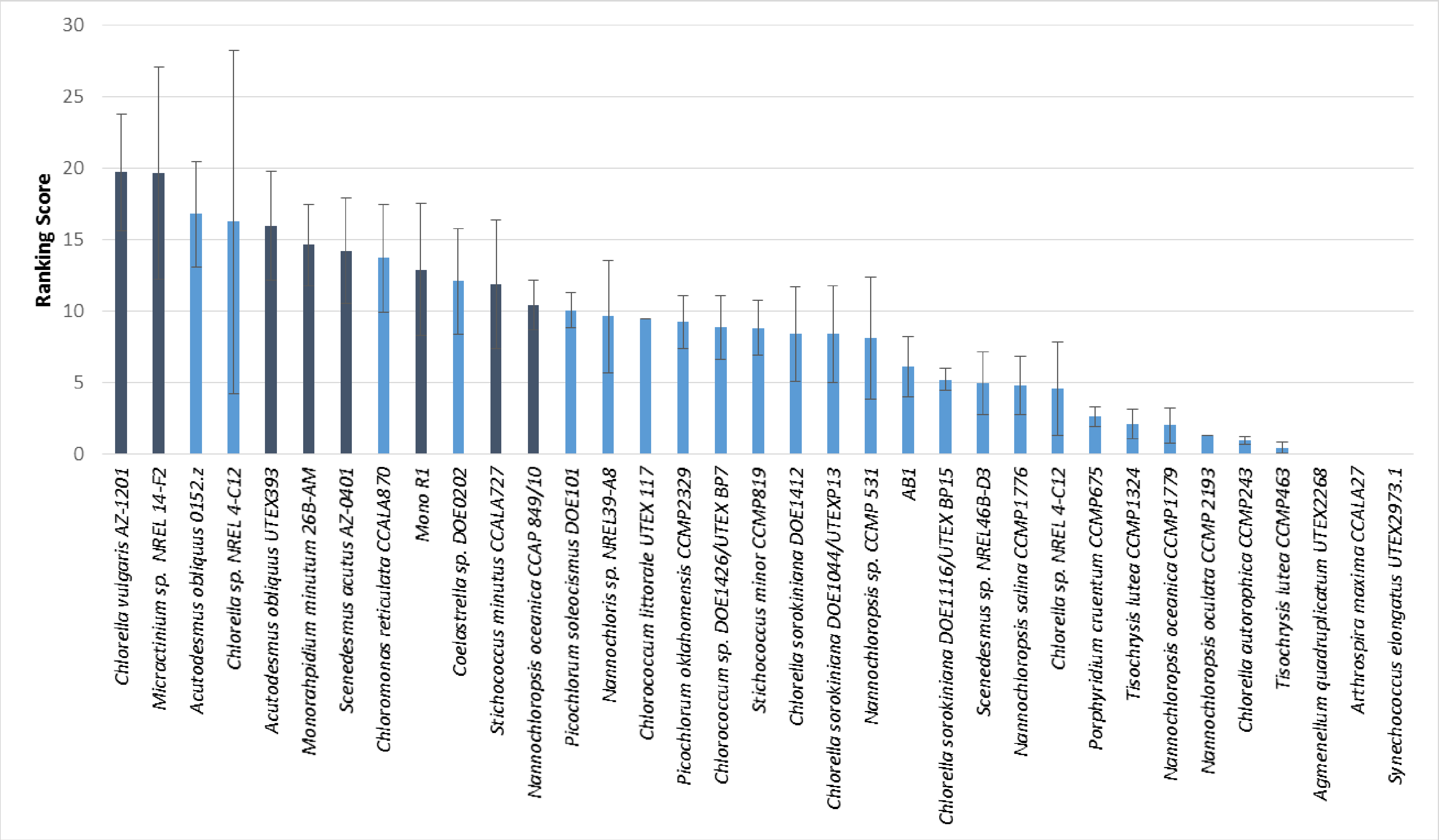


★ = Benchmarks

Not all results shown due to space limitations

Results: Ranking TIER I Strains in Winter Season

Top ranked TIER I strains are tested in LEAPS PBRs at TIER II



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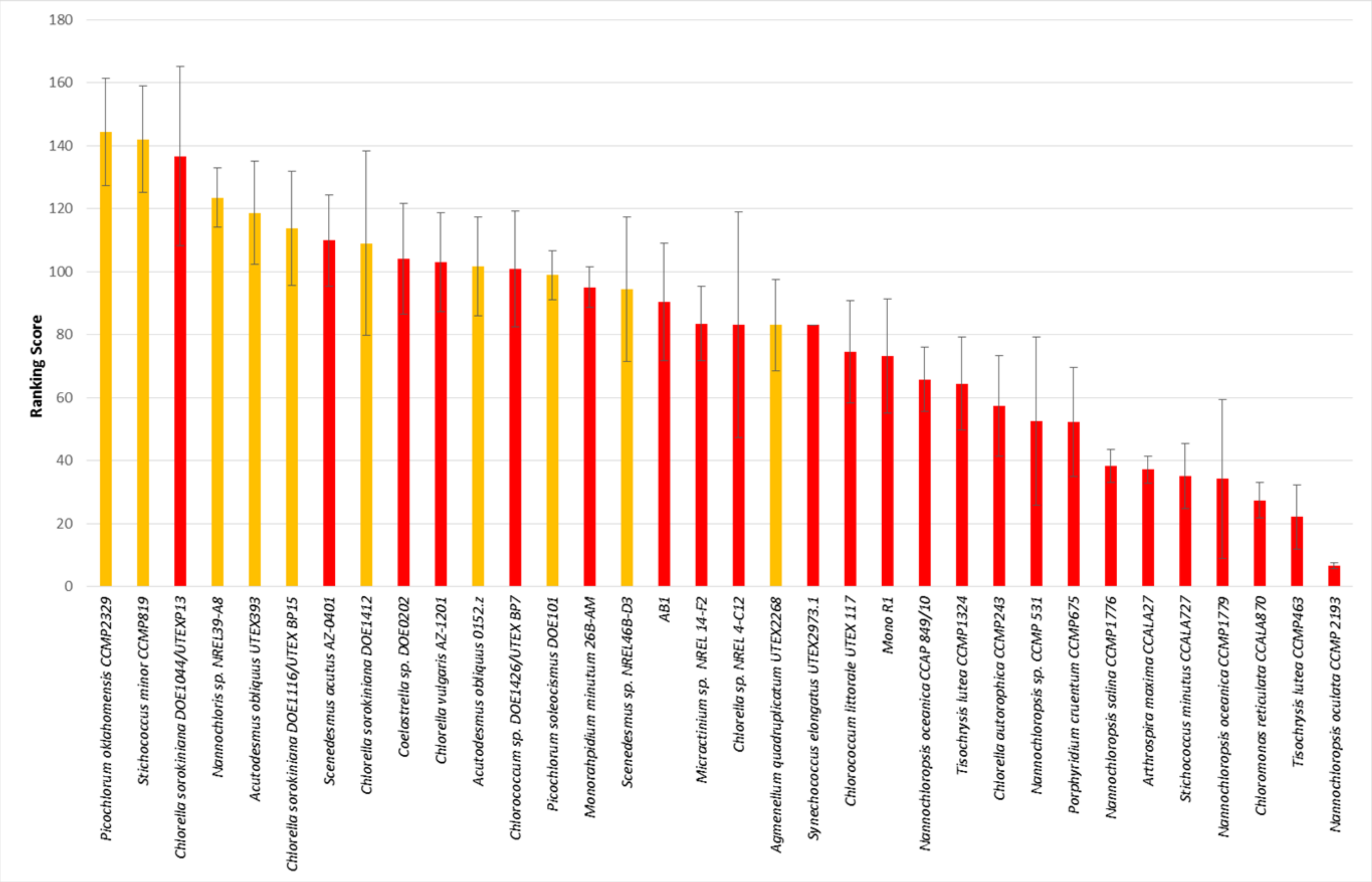
Results: Ranking TIER I Strains in Summer Season

Top ranked TIER I strains are tested in LEAPS PBRs at TIER II



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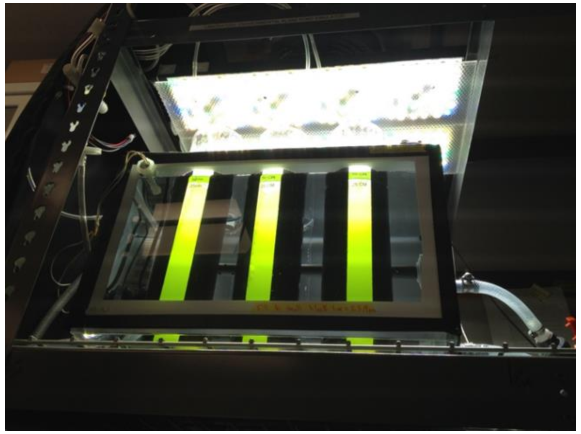
Approach: TIER II Strain Culturing in LEAPS

Use unique pond simulator PBR to measure productivity (21 strains)

Objective

Quantify Arizona winter and summer season **biomass productivity** under **identical climate-simulated culture conditions** and **identify best strains**

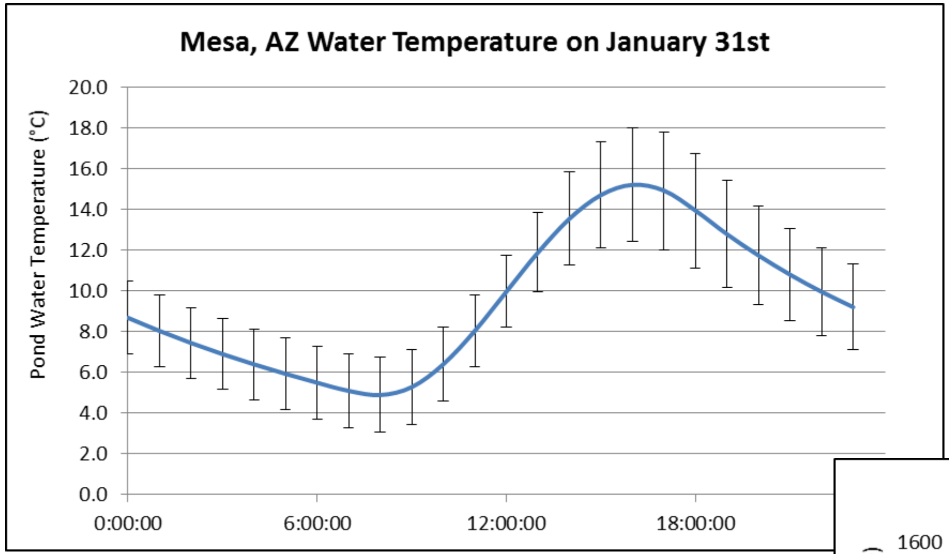
Approach

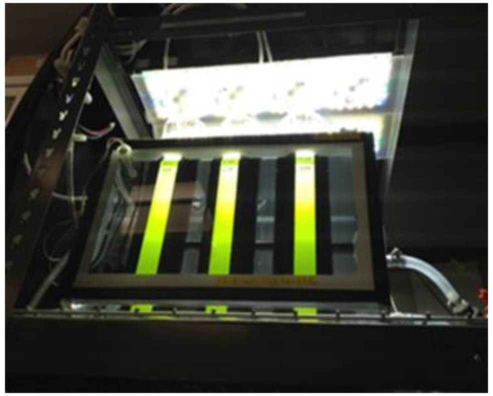


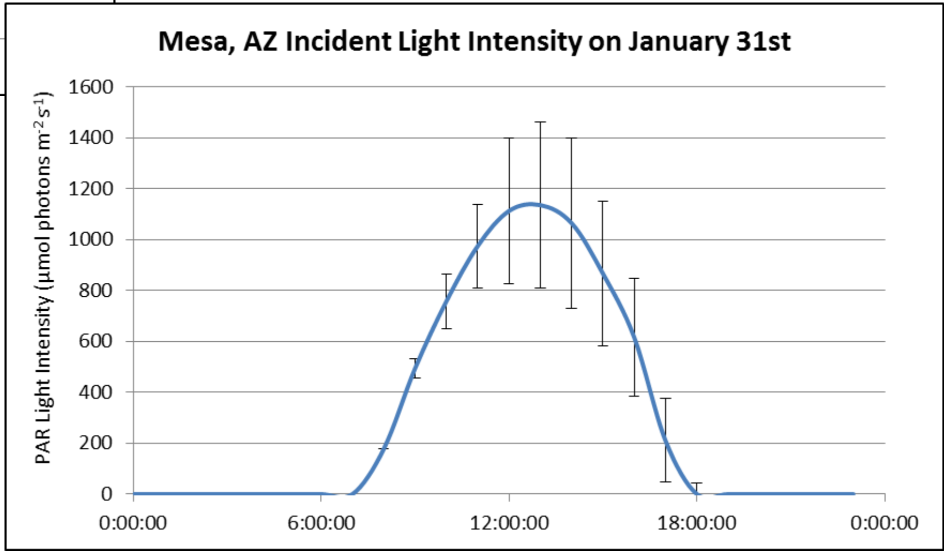
- The PNNL Laboratory Environmental Algae Pond Simulator **(LEAPS) accurately simulates microalgae growth in outdoor ponds.**
- The top winter and summer season TIER I strains were cultured in LEAPS using **January 31 and July 1** light & temperature scripts for Mesa, Arizona (AzCATI).
- LEAPS cultures were grown first under **nutrient-replete** conditions (DISCOVR medium, 20 cm), then under **nutrient-deplete** conditions.
- **Biomass composition** was quantified by NREL.

Approach: LEAPS Light/Temp Scripts

LEAPS photobioreactors simulate AzCATI ponds for January 31







PNNL Biomass Assessment Tool (BAT) generated light intensity and water temperature scripts for Mesa, AZ, January 31, error bars are for 30 year averages.

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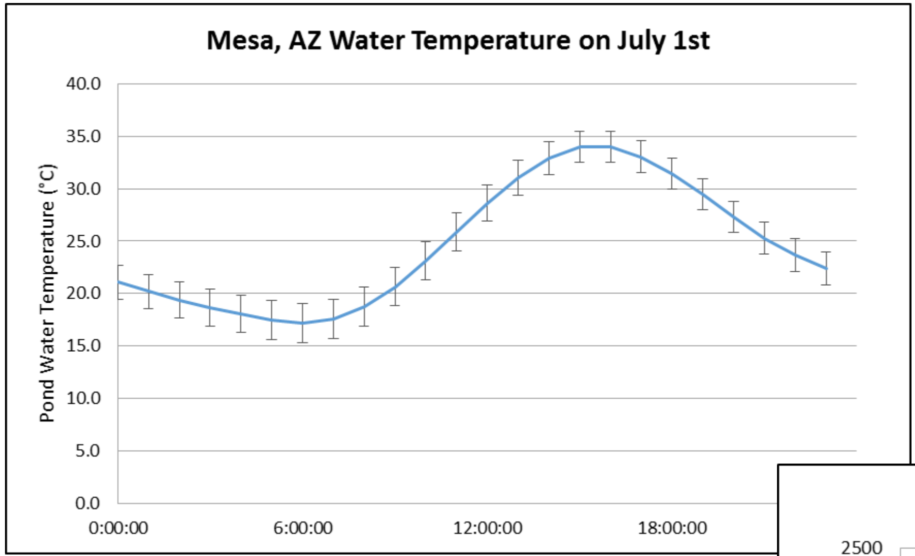
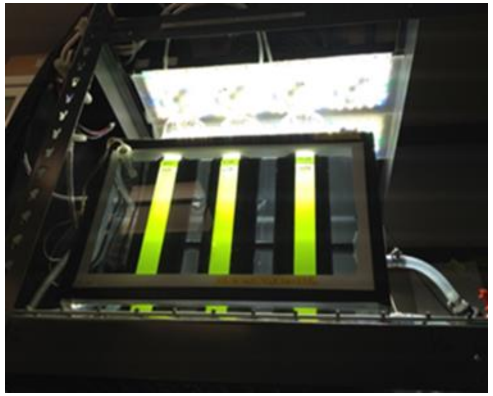
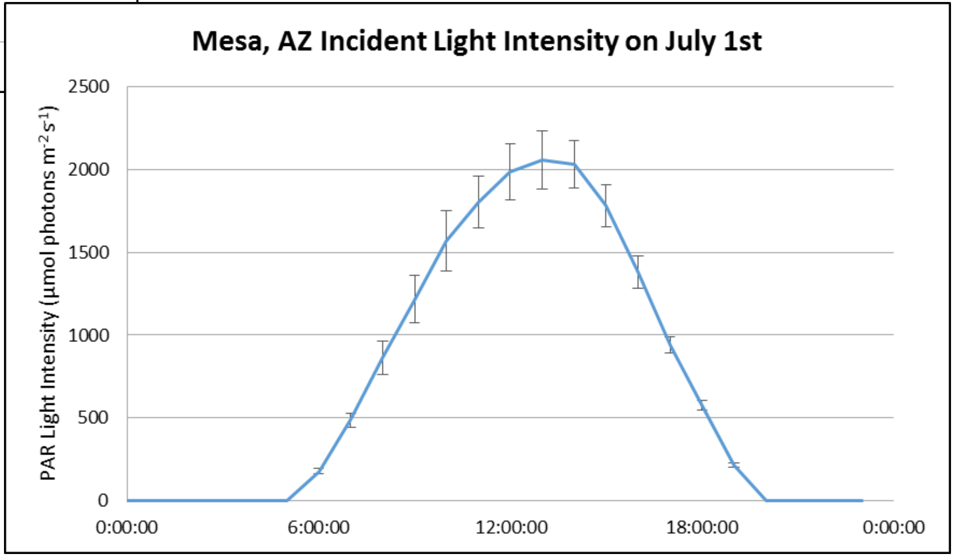
Approach: LEAPS Light/Temp Scripts

LEAPS photobioreactors simulate AzCATI ponds for July 1



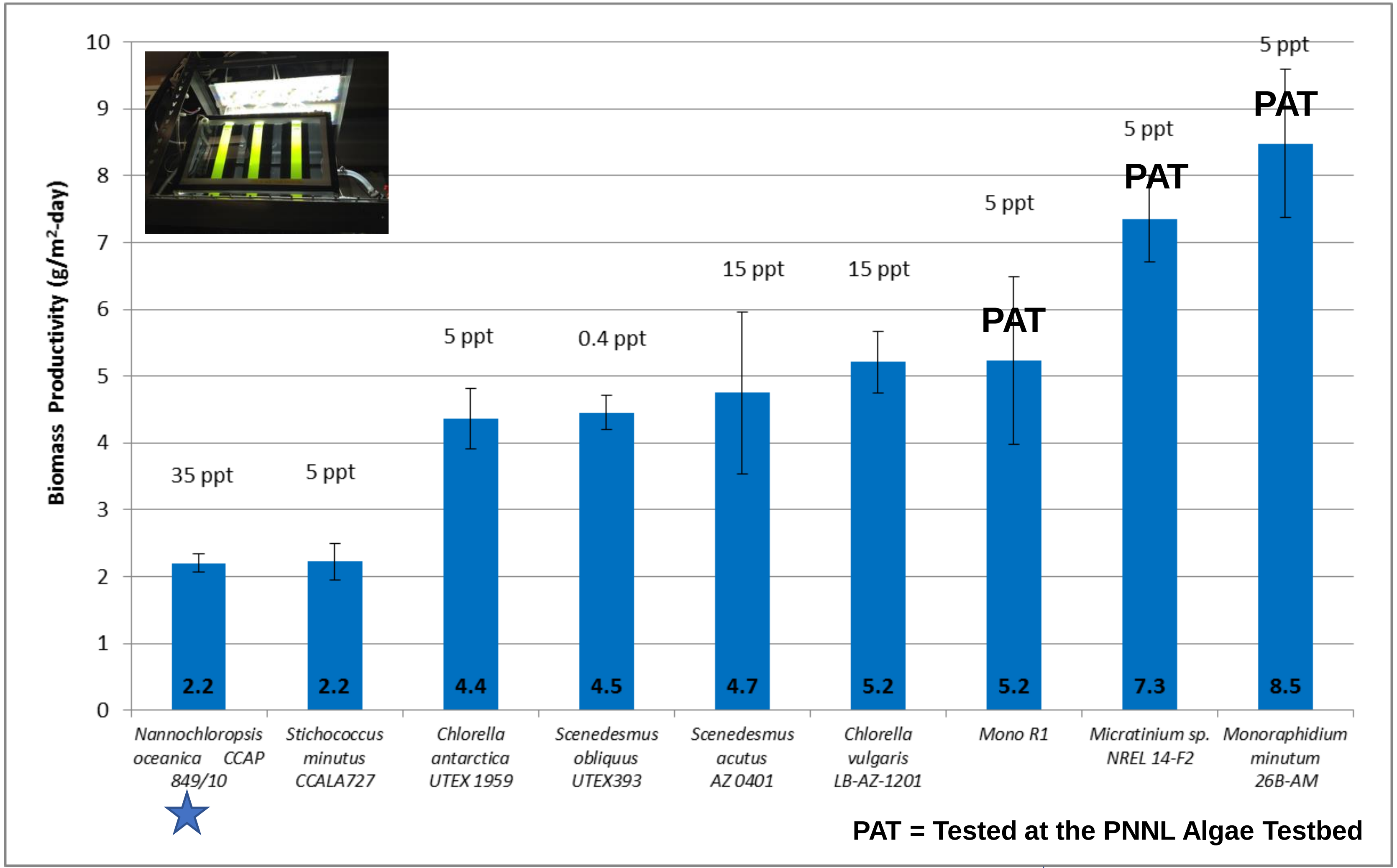




PNNL Biomass Assessment Tool (BAT) generated light intensity and water temperature scripts for Mesa, AZ, July 1, error bars are for 30 year averages.

Results: LEAPS Cultivation of Cold Season Strains

Two top TIER II strains: Monoraphidium minutum & Micractinium NREL



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

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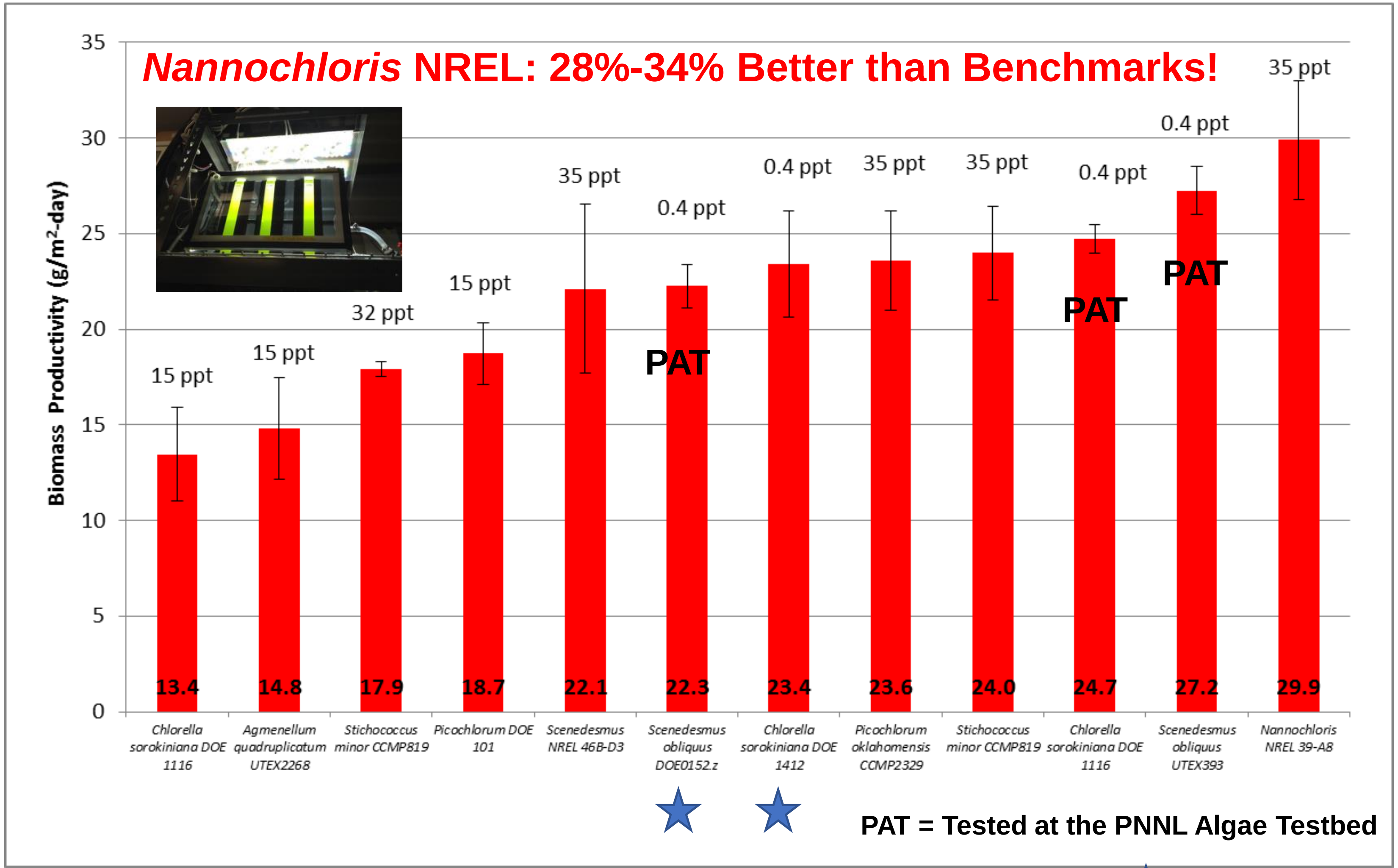
Results: LEAPS Cultivation of Warm Season Strains

Two top TIER II strains: Nannochloris NREL + Scenedesmus obliquus 393



Error bars are one stdev (n=4, with the exception of *Nannochloris*, n=20). ★ = Benchmarks.

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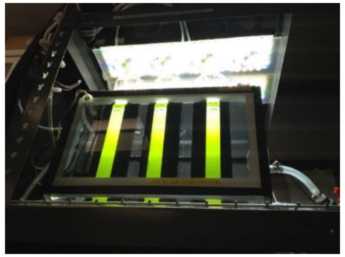
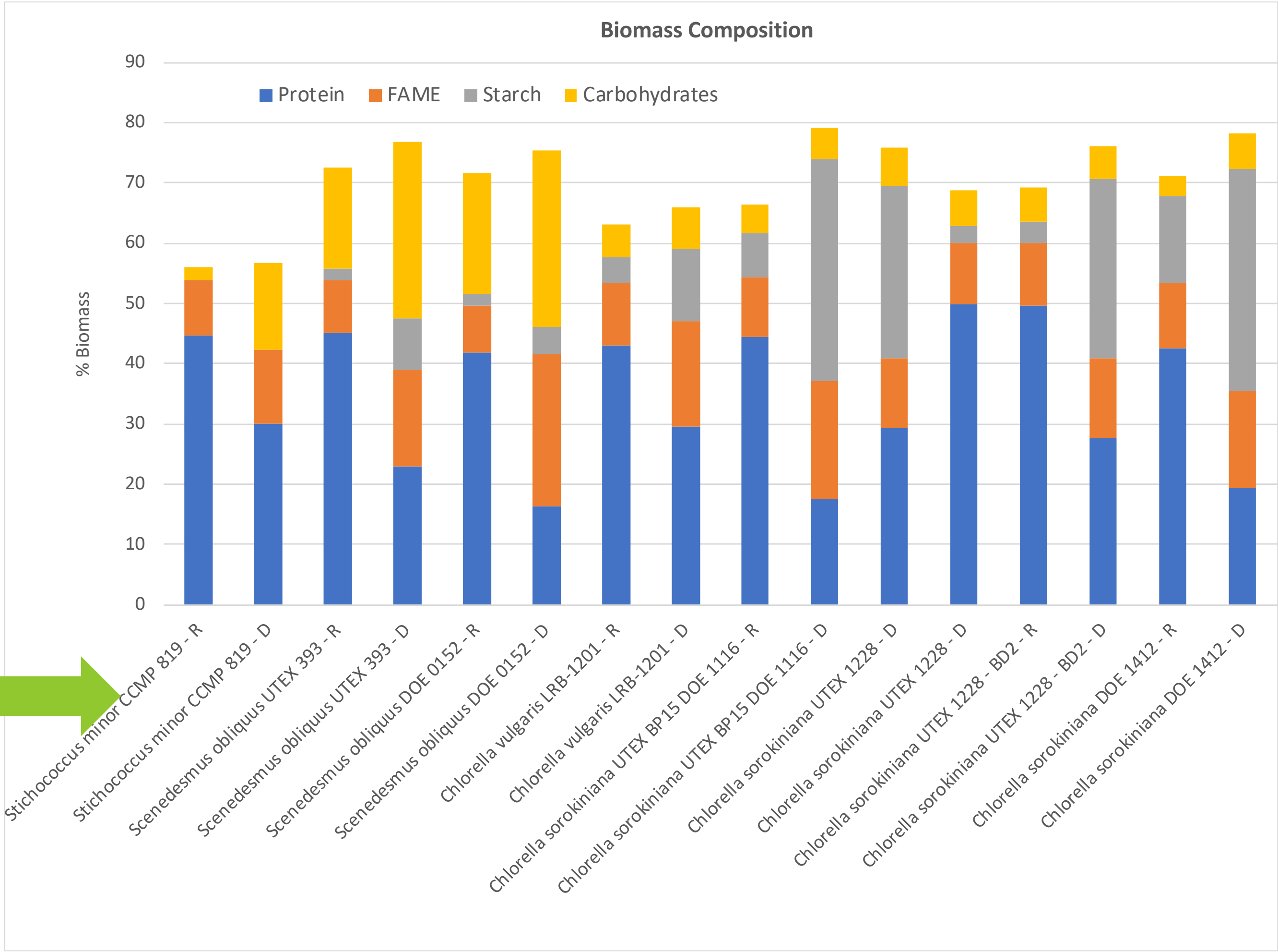
Results: TIER II Strains Show Strong Compositional Dynamics

Strain composition for LEAPS biomass measured & compared

Biomass collected from LEAPS experiments using winter/summer simulations and replete and deplete nutrients





* Note lack of full mass balance accounting will be addressed in FY19-FY20

Results: Downselection based on Biomass Composition

Preliminary valorization algorithm based on TEA being developed

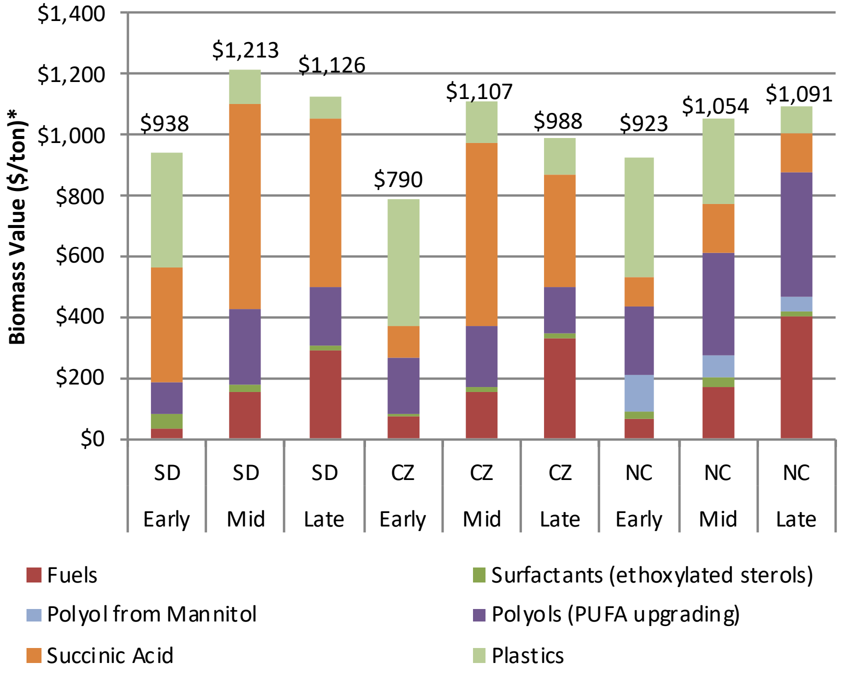
If after full TEA, cumulative "value" exceeds MBSP → profitable

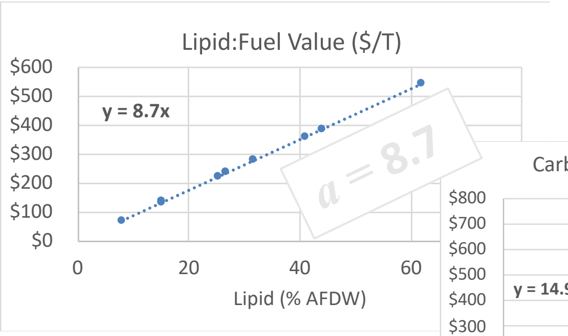
$$\text{Biomass Intrinsic Value} = \sum_{i=0} \text{Component Intrinsic Value}_i \quad (Eq. 1)$$

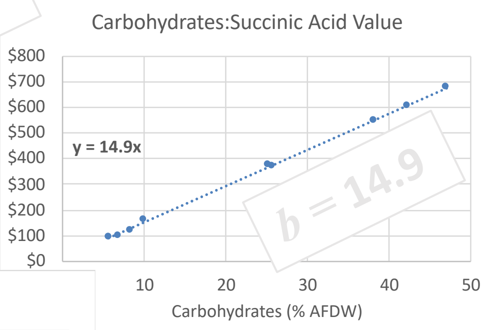
$$\text{Component Intrinsic Value} = \text{Product Revenue} - \text{Consumed Chemicals Operating Costs} \quad (Eq. 2)$$

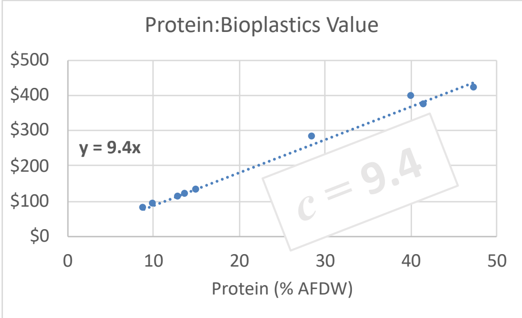
$$\text{Component Intrinsic Value} = (S_c)(Y_p)(P_p) - \sum_{i=2}^{n} (S_c)(R_{r,i})(P_{r,i}) \quad (Eq. 2.1)$$

Example from previous work at NREL:









For demonstration of concept of biomass valorization only – preliminary analysis

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

Results: Downselection based on Biomass Composition

Early application of valorization algorithm allows for TIER II strain ranking

Tradeoff between productivity (from summer simulation experiments) and biomass value – across nutrient deplete and replete conditions for 11 species indicates inverse correlation

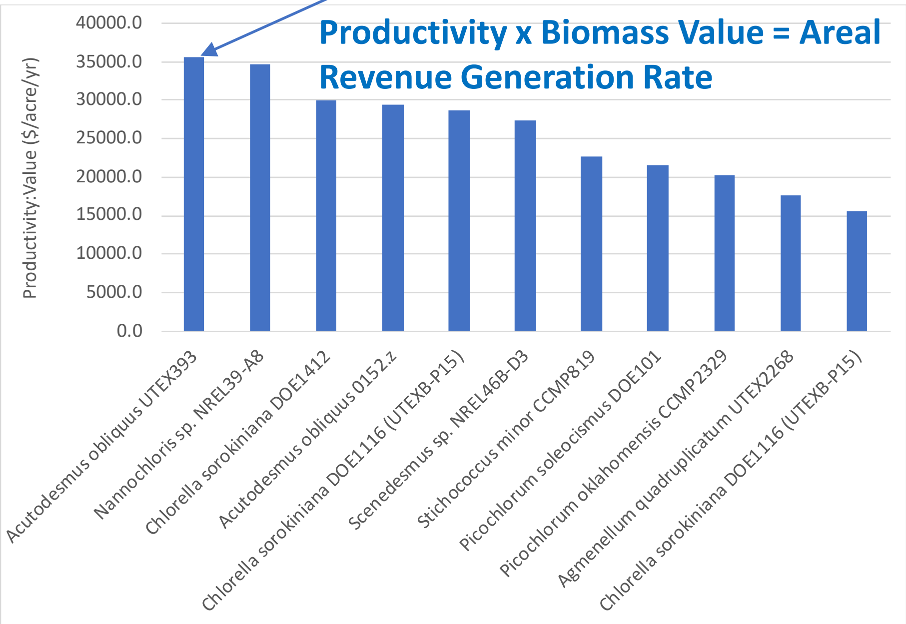
Chlorella sorokiniana DOE1412 (deplete conditions)

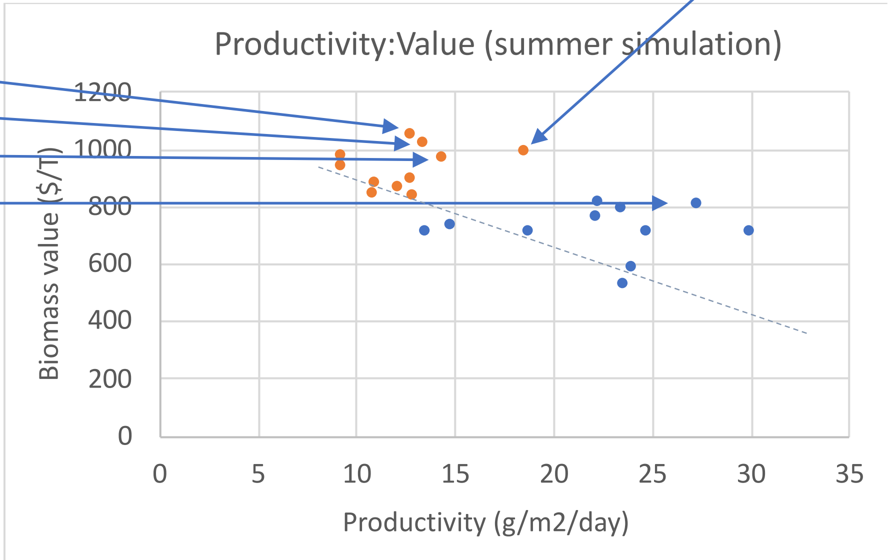
Picochlorum oklahomensis CCMP2329

Chlorella sorokiniana DOE1116 (UTEXB-P15)

Acutodesmus obliquus UTEX393 (deplete conditions)

Acutodesmus obliquus UTEX393 (replete conditions)





Preliminary application for demonstration only shows potential for **ranking strains based on areal revenue generation rate,** but highly dependent on component valorization and cultivation environment

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Approach: TIER II &III Culture Resilience Testing

Resistance of DISCOVR strains to grazers is tested at lab- and pond-scale

Objective

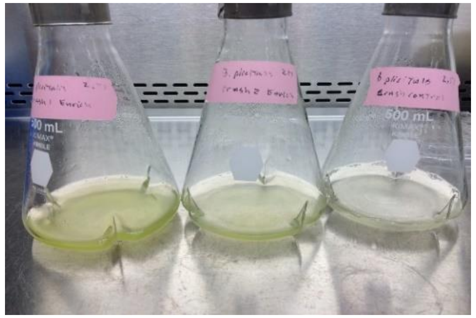
- Screen DISCOVR strains for **resistance to grazers** and other **deleterious species** and **identify most resilient strains, to maintain long term culture stability and thus highest seasonal yield.**

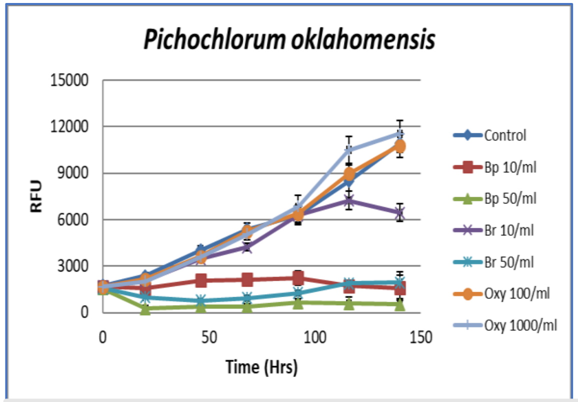
Approach

- The **SNL Crash Lab creates pond crashes on demand** at laboratory scale and in biocontained 100L and 1000L (climate controlled) raceways.
- Established a **panel of algal grazers and other deleterious species** (currently ~20) that represents the widest possible taxonomic breadth.
- Evolve the panel to include deleterious species isolated from production sites. **Leverage other BETO project** (PEAK) to isolate additional deleterious species
- **Test crashes under standard conditions** to eliminate other influences and determine **innate resistance**.

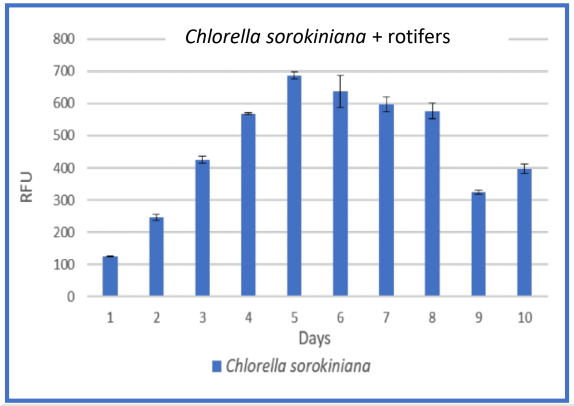
Results: Laboratory and Pond Scale Grazer Assays

Identifying strains with highest potential for stable outdoor cultivation





Lab-scale crash test of *P. oklahomensis* vs. *Brachionus plicitalis* (Bp), *B. rotundiformis* (Br) & *Oxyrrhis marina* (Oxy). Biomass is measured in relative fluorescence units (RFU).



1000L pond- scale crash test of *Chlorella sorokiniana* and *Brachionus plicitalis.* Algal biomass is measured in relative fluorescence units (RFU).

- Established a panel of deleterious species (~ 20) that represent the widest possible taxonomic breadth and known to infect ponds (ATP^3 data)
- Generated reproducible and quantitative standard crash assays at laboratory and pilot scale
- 18 algae species tested at lab scale
- Tested 4 selected species at 1000L scale
- **Identified strains with highest resilience and therefore potential for high seasonal productivity**

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Approach: TIER IV Strain Outdoor Pond Culturing

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

Objectives

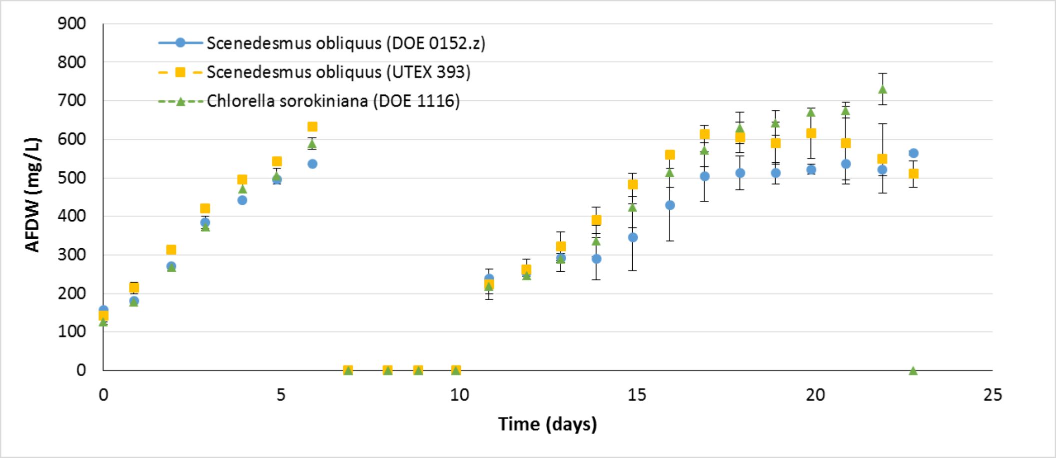
- Quantify **areal biomass productivities** of top DISCOVR winter and summer strains in PNNL's outdoor raceway ponds in Arizona (PAT).
- Demonstrate **sustainable and stable culture performance**, i.e., determine susceptibility to invaders and predators.
- Evaluate **harvestability** by centrifugation.
- Provide sufficient **biomass for NREL analyses** for proximate composition and co-products.

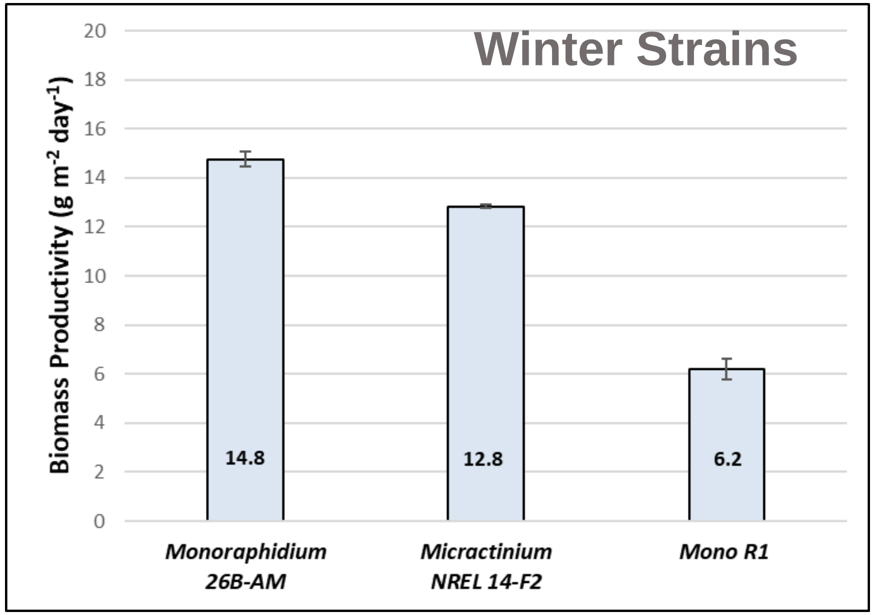
Approach

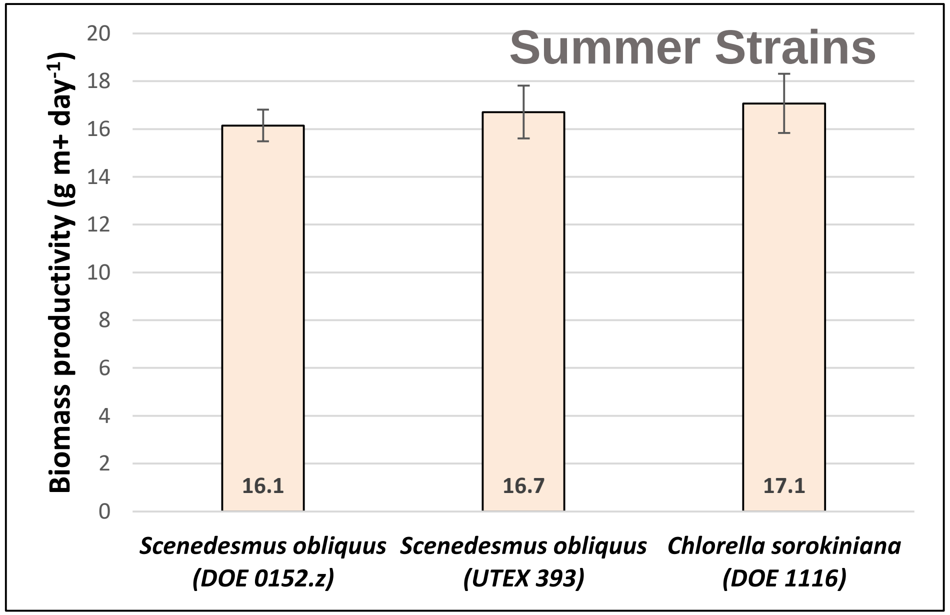
- For each season, the **three top DISCOVR strains** were cultured in duplicate raceways.
- Pond cultures were grown first under **nutrient-replete** conditions (DISCOVR medium, 20 cm), then under **nutrient-deplete** conditions.
- Culture health was examined via periodic **microscopic inspections**.
- Biomass was **harvested via centrifuge** and shipped to NREL.

Results: TIER IV Strain Outdoor Pond Culturing

3 winter + 3 summer strains grown under N-replete/deplete conditions







Results: TIER V Strain Culturing at SOT Testbed

6 strains were tested in FY19 resulting in 35.6% improvement over FY18 SOT

- *ATP^3 SOT framework successfully transitioned and implemented in DISCOVR Summer 2018.*
- *First full year of cultivation under DISCOVR complete Summer 2019*
- *Fall and Winter trials included three different cultivars: Desmodesmus sp. (CO46), Acutodesmus obliquus* (UTEX393) *and Monoraphidium minutum* (26BAM)
- *Spring and Summer trails included Desmodesmus sp. (CO46), Acutodesmus obliquus* (UTEX393) *and Monoraphidium minutum* (26BAM), *Desmodesmus Armatus, Picochlorum celeri, Picochlorum* sp. (NREL 39A8).
- *Six strains total tested in 2019 under DISCOVR SOT at AzCATI*
- **FY19 SOT results yielded 35.6% improvement over FY18 SOT** (Target is ≥ 10% improvement per year)

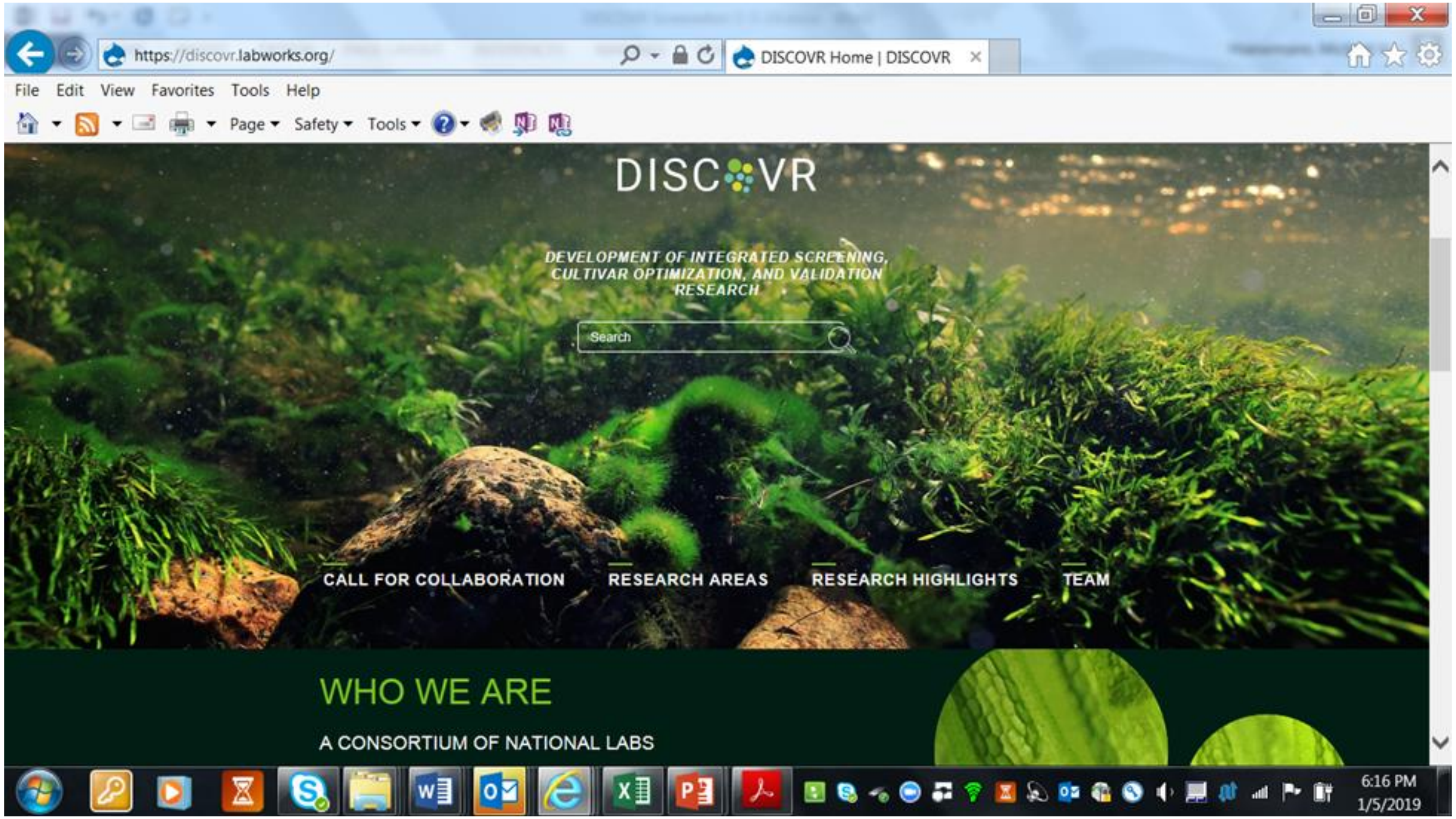
	FY2018				FY2019			
Season	**Prod. g/m2-day**	**Strain**	**Days**	**operation conditions**	**Prod. g/m2-day**	**Strain**	**Days**	**operation conditions**
Summer	15.4	Desmo sp.	51.0	20 cm - Semi	27.1	UTEX 393	85.0	20 cm - Semi
Spring	15.2	26BAM	80.0	10 cm - Semi	18.6	26BAM/UTEX393	84.0	10/20 cm (26BAM/393)- Semi
Winter	7.7	26BAM	46.0	10 cm - Batch	6.4	26BAM	91.0	10 cm - Semi
Fall	8.5	Nanno ('16)	42.0	25 cm - Batch	11.4	C046/26BAM	66.0	20/10 cm (Sep-Oct/Nov) - Semi
Average	**11.7**		**54.8**		**15.9**		**81.5**	
Year over year (YOY) Improvement	n/a		Total days	219.0	35.6%		Total days	326.0

*Details of SOT cultivation results – Thursday 8:30 am, Salon 3 (J. McGowen)

DISCOVR Website

Research areas, highlights, and Call for Collaboration publicly available

https://discovr.labworks.org



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Acknowlegements (Key Staff)

DISCOVR is a highly collaborative effort with many contributors

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  - Song Gao
  - Andrew Gutknecht
  - Mattias Greer
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  - Todd Lane
  - Pamela Lane
  - Jeri Timlin
  - Tom Reichardt
  - Kunal Poorey
- **AzCATI**
  - John McGowen

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Conclusions: Success Highlights

2018 SOT productivity increased by 13.6%, biomass price reduced by 10%

Strain Characterization

- Determined optimal temperature and salinity range for **>34 strains**
- Identified top TIER I strains using validated **down-selection algorithm**

Environmental Simulation

- Tested 21 strains and identified strains with up to **34% greater** areal productivity relative to benchmarks.

Grazer Resistance Testing

- Tested **18 strains** for **resistance** to diverse panel of **grazers** and identified most **resilient strains** for **downselection**

Biochemical Characterization

- Characterized **biomass composition** for > 20 TIER II strains
- Developed **biomass value down-selection algorithm** for TIER II strains

Non-GM Strain Improvement

- Increased **salinity tolerance** and **lipid** accumulation in *A. obliquus* by **~30%**

Outdoor Testbeds

- Achieved **>13% improvement** in **2018 SOT productivity** relative to 2017, **reducing biomass selling price by 10%**

- ***Strains from industrial partners*** *(Algenol, ExxonMobil, Micro-BioEngineering, Inc, Botryonyx LLC)*

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Supplemental Slides Section

Relevance of DISCOVR to Bioenergy Industry

Interaction with industry enhances overall impact

- Introduction of **industrial strains (***Algenol, ExxonMobil, Micro-BioEngineering, Inc, Botryonyx LLC)* into evaluation pipeline relates **productivity metrics for both BETO and non-BETO stakeholders.**
- **High productivity strains** identified by DISCOVR can be **transferred to industry for scale up and production**.
- **Call for Collaboration** provides facile **pathway for strains and technologies developed outside DISCOVR** to be **incorporated into pipeline** for rapid validation.
- **Technical Advisory Board** made up of algal community thought leaders provides **oversight to maximize DISCOVR relevance** as well as mechanism for **data dissemination.**
- **DISCOVR website** enables **impactful communication** of **research findings** to **algal community**.

ExxonMobil

ALGENOL BIOFUELS

μBIO MicroBio Engineering

Botryonyx LLC

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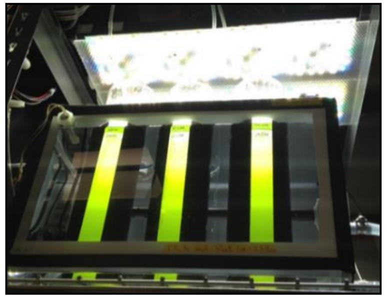
DISCOVR Project Overview – History and Context

Integrates BETO core capabilities to standardize strain characterization

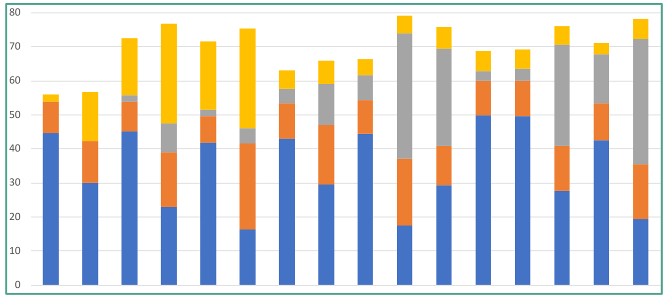
Environmental Simulation & Strain Characterization



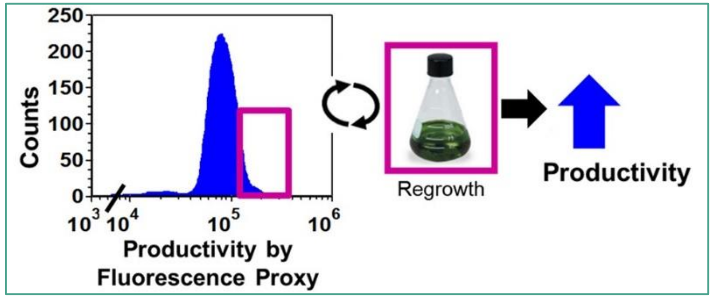
Indoor Crash Test Ponds

Outdoor Testbeds

Biochemical Characterization



Non-GM Strain Improvement



- *Capability development is/was funded in other BETO projects*
- *DISCOVR applies these capabilities in a single pipeline, offering collaborative synergies to accelerate "flask to farm"*

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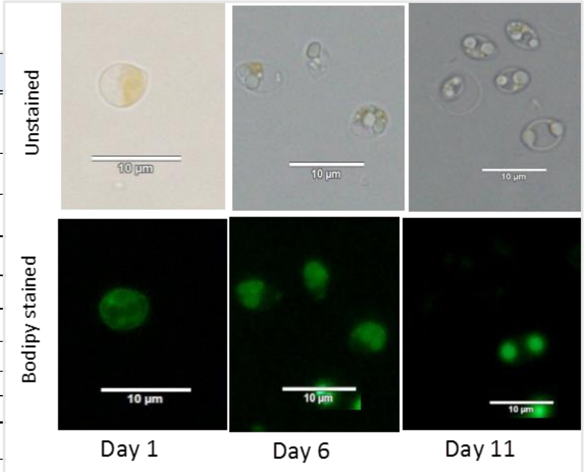
Approach: Tier I Strain Revival and Confirmation

Completed initial assessment of culture collection strains

- **Objective: Revive** strains, evaluate **bacterial load, confirm strain** identity**, adapt to DISCOVR media, and deliver to PNNL**

- Identify, order, and revive (**n = 23**)
- Initial growth curves & morphology
- Adapt to media (**18**)
- 16S and 18S sequencing (**14**)
- Clean-up cultures as needed
- Deliver to PNNL (**14**)

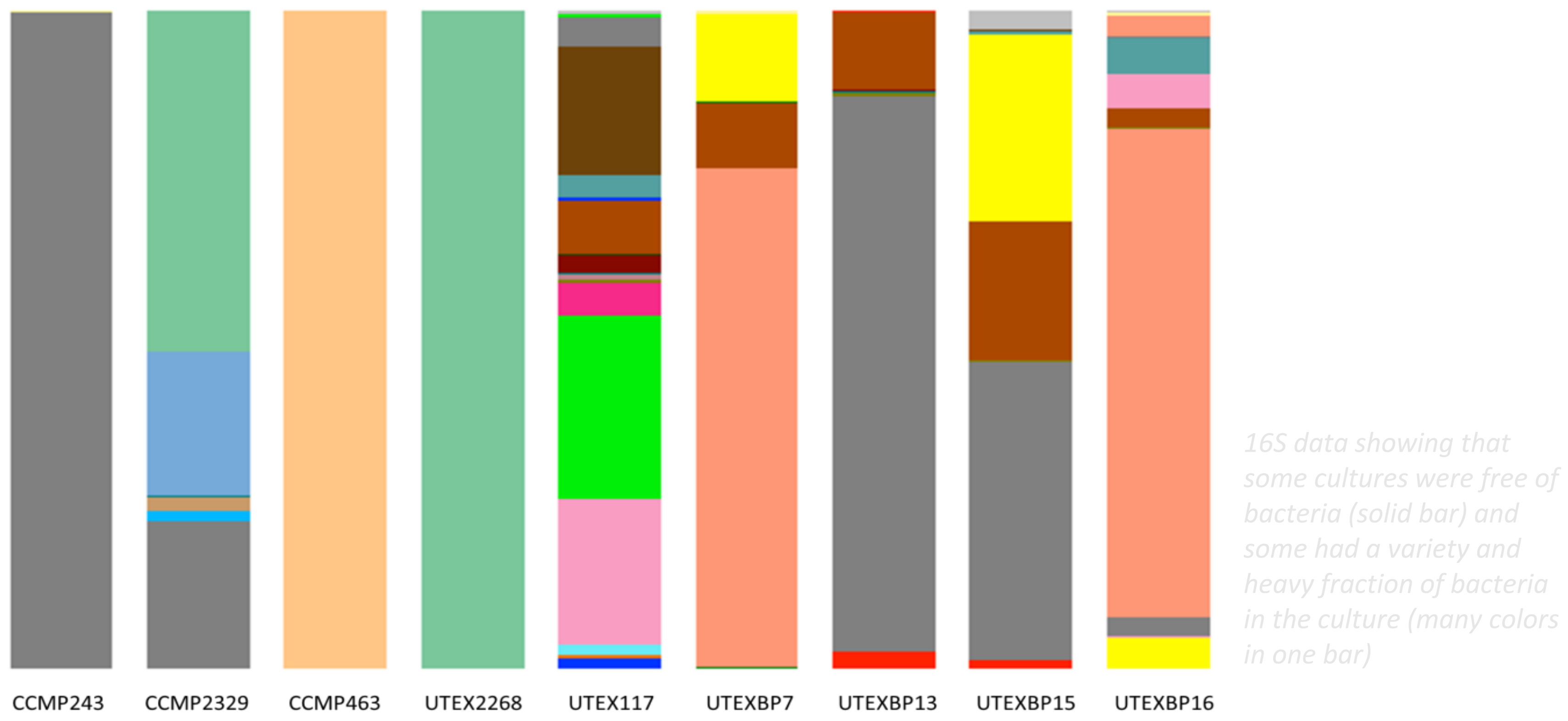
Culture Collection Name	UTEX BP13
Proposed species	*Chlorella sorokiniana* - DOE1044 (a green algae)
Species Identification by 18S	*Chlorella sorokiniana*
Sent to PNNL	Yes
PNNL Screening Status	In progress
Tier I	Yes
Tier II	TBD
Tier III-V	TBD
Media	Grows well in BG11 and DISCOVR media
Microscopy	Complete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



Example of a strain tracking sheet

Results: Tier I Strain Revival and Confirmation

Revived/evaluated 23 strains and delivered 14 to PNNL for screening



- **Strains** found to **vary** in **bacterial load**, we only 'cleaned up' heavily contaminated cultures
- **Most strains matched expected algae identity**, but not all –strains that could not be made uni-algal did not move forward

Results: TIER I Strain Ranking (Scoring) Algorithm

Score is integral of light- and temperature-weighted spec. growth rate

$$Ranking\ score = \int_{sunrise}^{sunset} I_t \mu_{T_t} d_t$$

Algorithm:

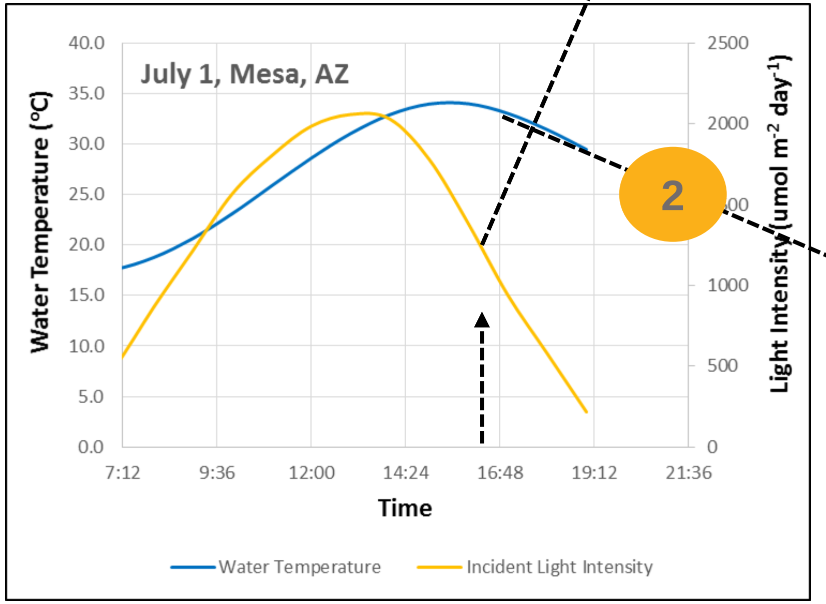
- **1. *I(t)* taken directly from script**
- **2. *T(t)* taken directly from script**
- **3. For each *T(t)* value, the corresponding *μ(t)* is obtained from the μ-vs-*T* curve**
- **4. Repeat for each *dt***
- **5. Integrate for entire light period**

1

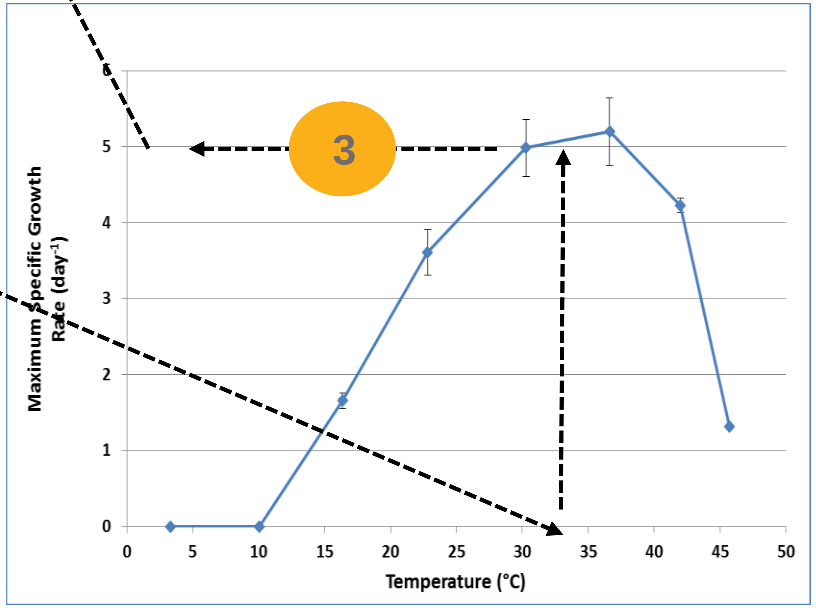
2

3

Light + Temperature Script



μ versus T Curve (Strain-specific)



Project Overview: Relation to BETO Project 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
- Biomass Characterization
- Strain Improvement
- Pond Crashes & Signatures
- 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

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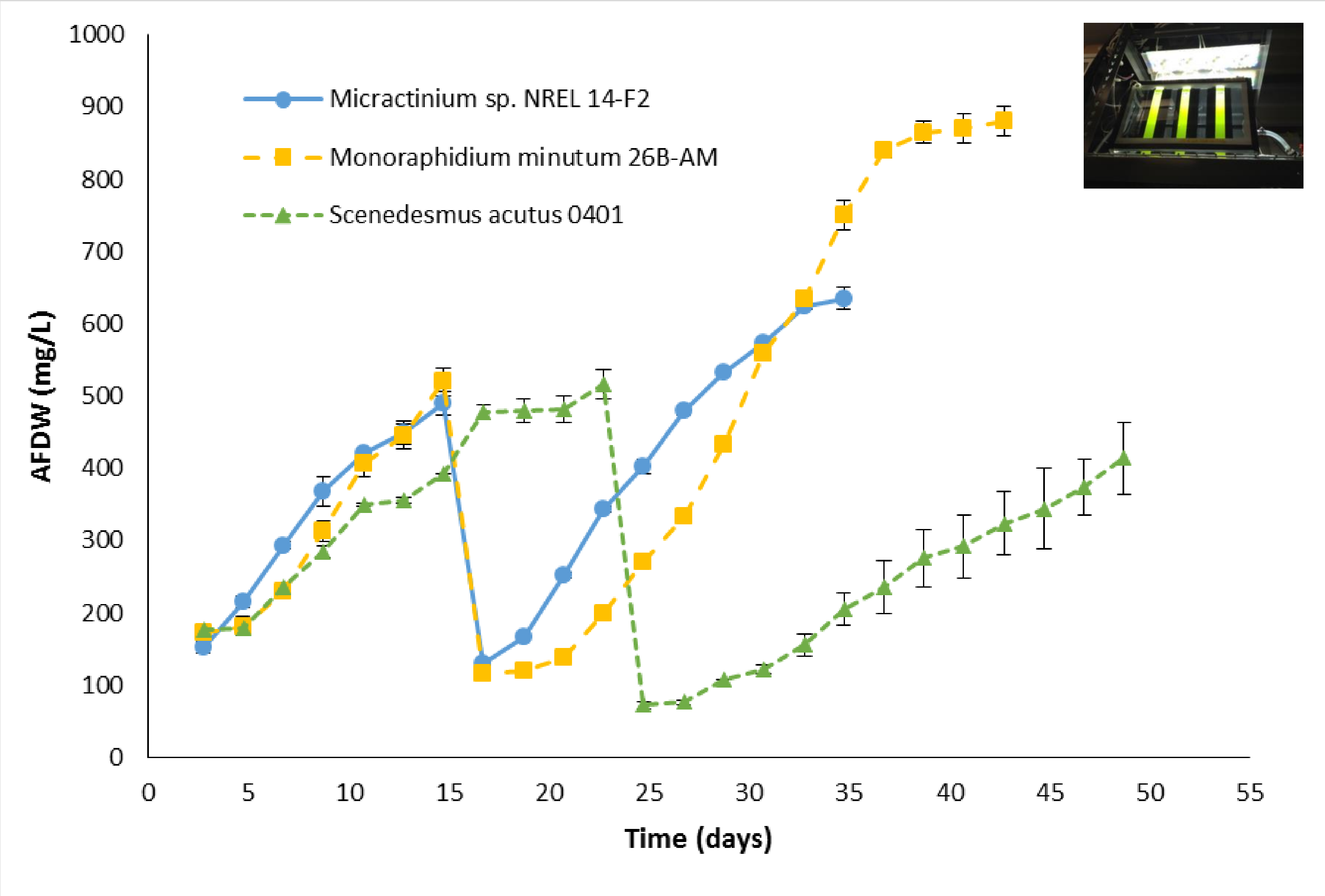
Results: Typical LEAPS Experiment

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



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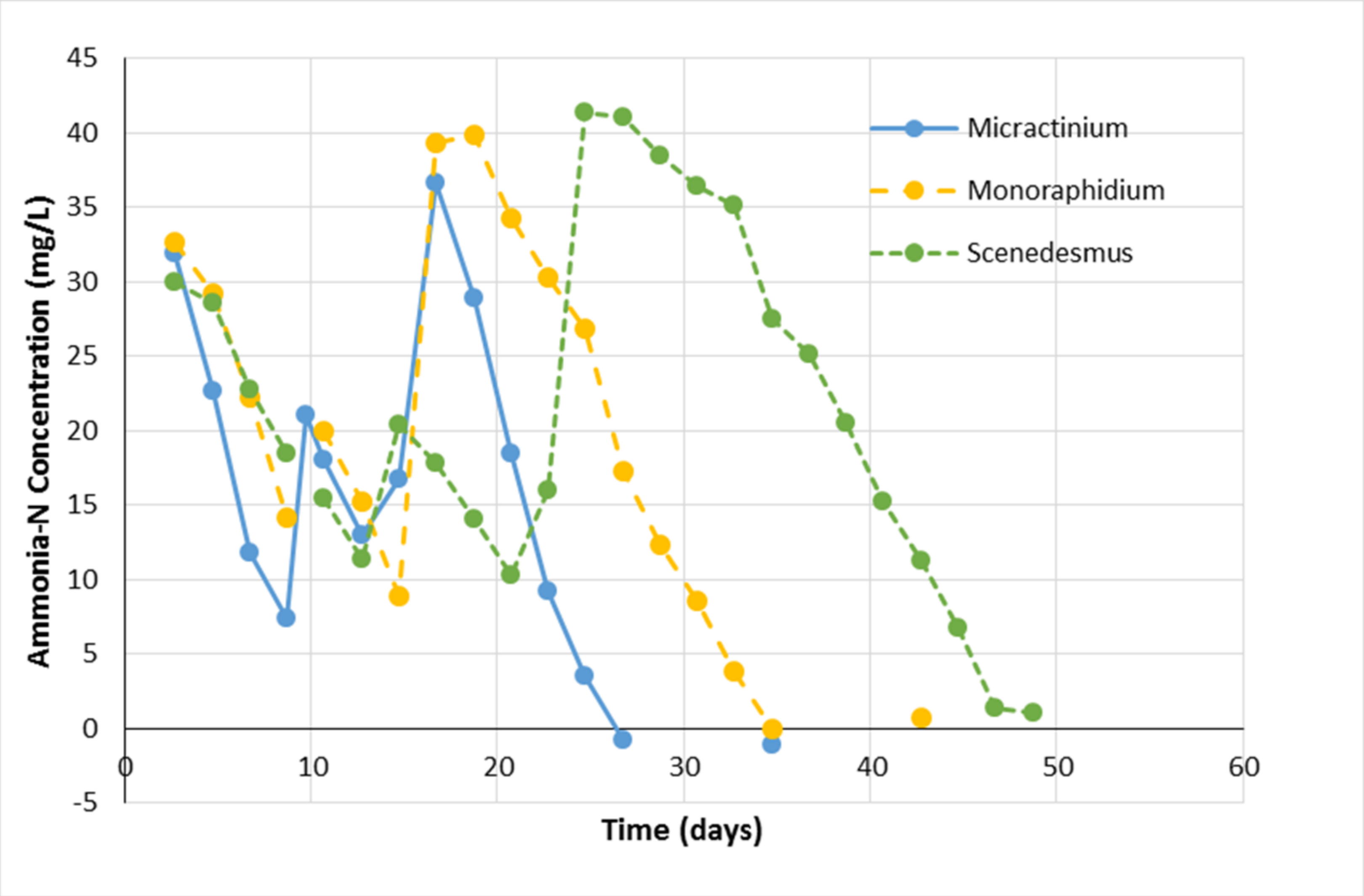
Results: Typical LEAPS Experiment

NH_3-N vs. time for nutrient-replete and nutrient-deplete growth phases



Approach: Compositional Analysis of Biomass

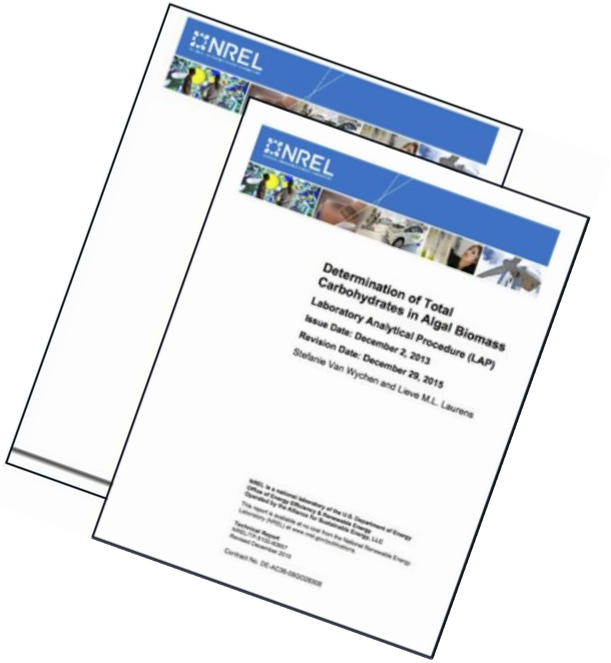
Determine fuel and bioproduct potential and value of biomass (Tier II + IV)

Objective

- To develop technologies to both **characterize and valorize algal biomass composition** for novel species identified and deployed.
- To measure **biomass compositional dynamics based on physiological and environmental inputs,** in order to be in a position to tailor the quality of biomass materials supplied to **maximize the output from a conversion process**

Approach

- **Compositional analysis** follows an NREL developed process for **standardized analysis** using reference procedures
  www.nrel.gov/bioenergy/microalgae-analysis.html
- Identify **high-value products** to feed the cost-value framework established by NREL's ABC project
- **Pretreatment susceptibility** testing using small scale experimental design response surface analysis of **lipid extractability** and **solubilization of sugars** for the CAP (Combined Algal Processing) pathway



Down-selection based on Grazer Resistance:

Aggregate growth rates 2 fold in excess of average across grazer panel

- Determined the **specific growth rates** in the **presence** and **absence** of **grazer species** at laboratory scale. Created **heatmap** to visually represent relative resilience.
- **Identified the most resilient freshwater and marine species**
- **Identified the most significant grazer species**

	Control	*Brachionus plicatilis* 10/ml	*Brachionus plicatilis* 50/ml	*Brachionus rotundaformis* 10/ml	*Brachionus rotundaformis* 50/ml	*Oxyrrhis marina* 100/ml	*Oxyrrhis marina* 1000/ml	*Euplotes* 40/ml	*Euplotes* 400/ml	Individual Average	Group Average
Salt Water											
Micractinium sp. 14-F2	1	0.782	-0.071	1.018	1.124	0.971	0.629	NA	NA	0.742	0.505
Nannochloris sp. 39-A8	1	-1.360	-0.264	0.872	0.656	0.904	0.936	NA	NA	0.291	
Nannochloropsis gaditana 1894	1	0.702	-0.041	0.810	0.860	0.835	0.917	NA	NA	0.680	
Scenedesmus sp. 46B-D3	1	0.871	0.138	0.828	0.638	0.940	0.638	NA	NA	0.675	
Nannochloropsis oceanica 1779	1	0.796	0.041	1.068	0.116	0.993	0.946	NA	NA	0.660	
Pichochlorum oklahomensis	1	-0.290	0.039	0.728	0.291	1.117	1.243	NA	NA	0.521	
Chlorella 4-C12	1	0.809	0.001	0.757	0.662	0.978	0.676	NA	NA	0.647	
Microchloropsis salina	1	1.033	-5.400	1.049	-0.082	1.180	1.148	NA	NA	-0.179	
Stichococcus minor	1	0.969	-0.814	0.837	-0.109	1.054	1.124	NA	NA	0.510	
Fresh Water											
Chlorella sorokiniana 1116	1	0.923	0.765	0.755	0.558	0.645	0.571	1.016	1.016	0.781	0.908
Monoraphidium 26B-AM	1	1.045	1.097	1.097	1.026	0.832	0.748	0.891	0.957	0.962	
MONOR1	1	0.791	0.755	0.791	0.827	0.718	0.218	0.957	1.034	0.761	
Acutodesmus obliquus UTEX393	1	1.160	1.180	1.220	1.280	1.150	0.480	1.068	1.102	1.080	
Chlorella sorokiniana 1044	1	1.056	0.990	0.990	1.080	0.973	0.8	1.053	1.047	0.999	
Chlorella vulgaris LRB 1201	1	0.748	0.855	0.828	0.807	1.0256	0.938	0.872	0.862	0.867	
Stichococcus minutus	1	0.917	1.076	1.057	1.051	0.922	1.080	0.758	0.076	0.867	
Scenedesmus DOE 0152z	1	1.013	0.927	1.020	0.993	0.855	0.827	0.960	0.974	0.946	

Partial heatmap of relative growth rates in the presence of grazers

- **Downselected to the most resilient freshwater and marine strains:** those that display **highest average specific growth rates** across the grazer panel.
- Strains selected for **pond scale analysis at SNL and field deployment (SOT):**
- ***Acutodesmus obliquus* 393**
- ***Scenedesmus* DOE 0152z**
- ***Micractinium sp* 14-F2**

Approach: Spectroradiometric Monitoring

An early warning pond-pest detector

Objective

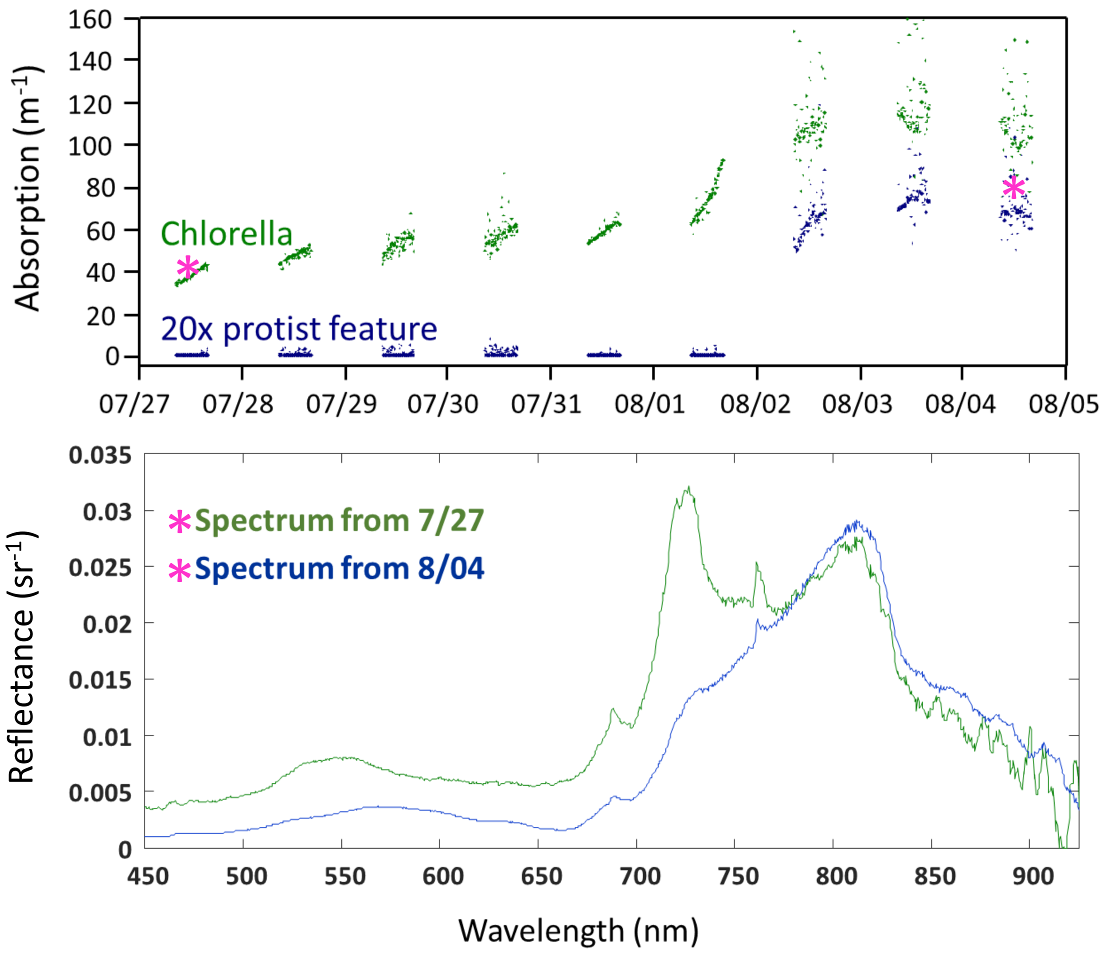
- Extend **spectroradiometric monitoring** capabilities to rapidly **detect a broad array** of **pond pests**

Approach

- Conducted **laboratory studies** to determine signatures for detecting ***Vampirovibrio chlorellavorus* infecting *Chlorella sorokiniana*** cultures.
- **Analyzed data from ATP^3 field trials** to identify **signatures** representative of a **diatom invasion** and ***Poteriochromonas* predation**, comparing results with microscopy and sequencing analysis
- Quantified the **sensitivity of detection** to multiple diatoms via the assessment of titrated mixtures

Results: Spectroradiometric Monitoring

Identifying strains with highest potential for stable outdoor cultivation



Upper panel: Absorption of Chlorella and a protist over a 10 day period as determined from spectroradiometric monitoring of a ATP3 pond.
Lower panel: Selected spectra from the time points highlighted with the pink asterisks in upper panel. Distinct spectral differences between healthy and protist contaminated ponds are visible in the 700 – 770 nm near-infrared region.

- Demonstrated that method can identify spectral signatures from two classes of algal pests in outdoor field trial data– **diatoms and grazers**
- Demonstrated detection is sensitive to only ~1% absorption by the diatom *Thalassiosira pseudonana*
- Expanded knowledge of host range of *V. chlorellavorus* to include susceptibility of two marine strains of chlorella (DOE1044 & 1116)

Approach: Machine Learning

Identify pond crash signatures for optimization of operational strategies

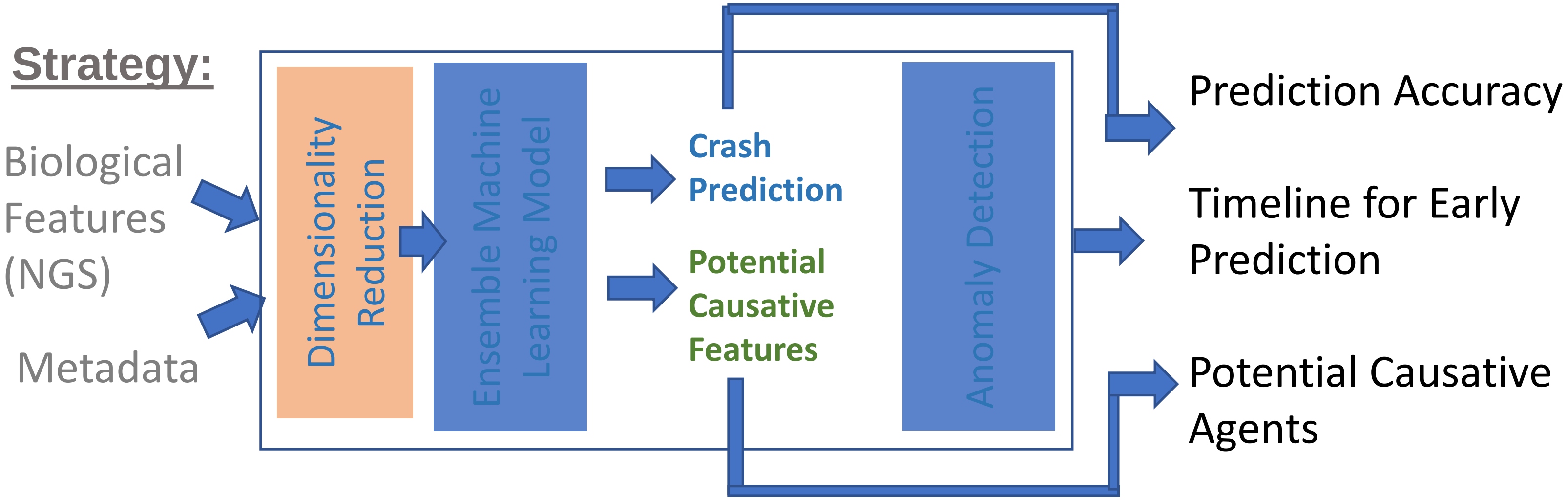
Objectives:

- To increase annualized productivity by early crash prediction to allow intervention.
  - **Identify pond crash signature** using **machine learning**
  - Build an **algorithm** for early detection of anomaly for each cultivation run

Approach:

- Built a predictive machine learning models using ensemble models **identifying Pond Crash Signatures**
- Derived anomaly detection strategy based on species diversity index for early detection.

Strategy:



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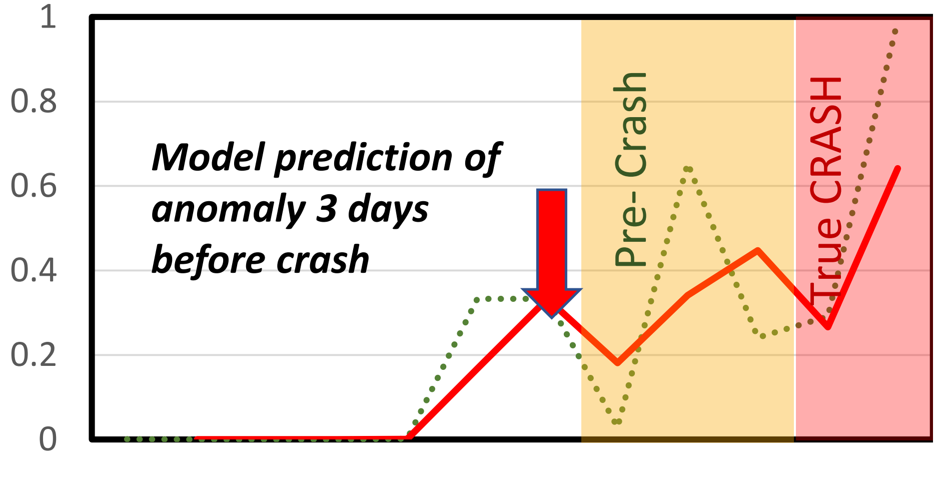
Results: Machine Learning

Machine learning for optimization of pond operational strategies

- Successfully build a predictive model for classification of healthy and crash samples from a cultivation run.
  - **Accuracy of Predictive Machine Learning Model for Crash Prediction >87%** (Completed FY17)
- **Identified potential causative agents** for crashes from model feature importance metrics. (completed FY18)
- Preliminary result for **early anomaly detection** results for summer 2014 AzCATI cultivation run. **Median prediction – 3 days before the crash** (FY19 and beyond)

Prediction of anomaly in AzCATI summer data



Summary for all raceways experiments:

Raceway #	Early prediction before crash	Major contaminant
Pond 9	3 days	Diatoms *(Amphora)* 
Pond 10	3 days	
Pond 11	2 days	
Pond 12	1 day	
Pond 13	5 days	
Pond 14	3 days	

Approach: Tier III *Strain Improvement*

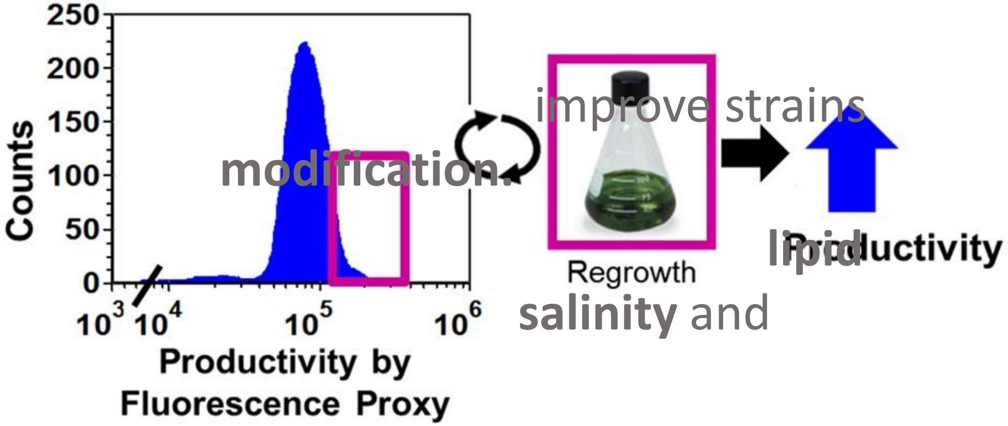
Use Non-GMO approaches to further improve promising strains

Objective

Increase productivity (biomass/lipids) and/or **environmental robustness** in a subset of Tier III strains, using **non-GMO approaches**, such as cell sorting and adaptive evolution strategies.

Approach

- Use tools developed at LANL to improve strains **without genetic modification.**
- Aim to increase **biomass** and/or **lipid improvements** in concert with **salinity** and **temperature tolerance**



- Resubmit strains to DISCOVR pipeline
- Risk: If strains prove recalcitrant, random mutagenesis will be used to increase genetic diversity and chance of improving phenotypes.

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Results: Tier III Strain Improvement - Adaptation

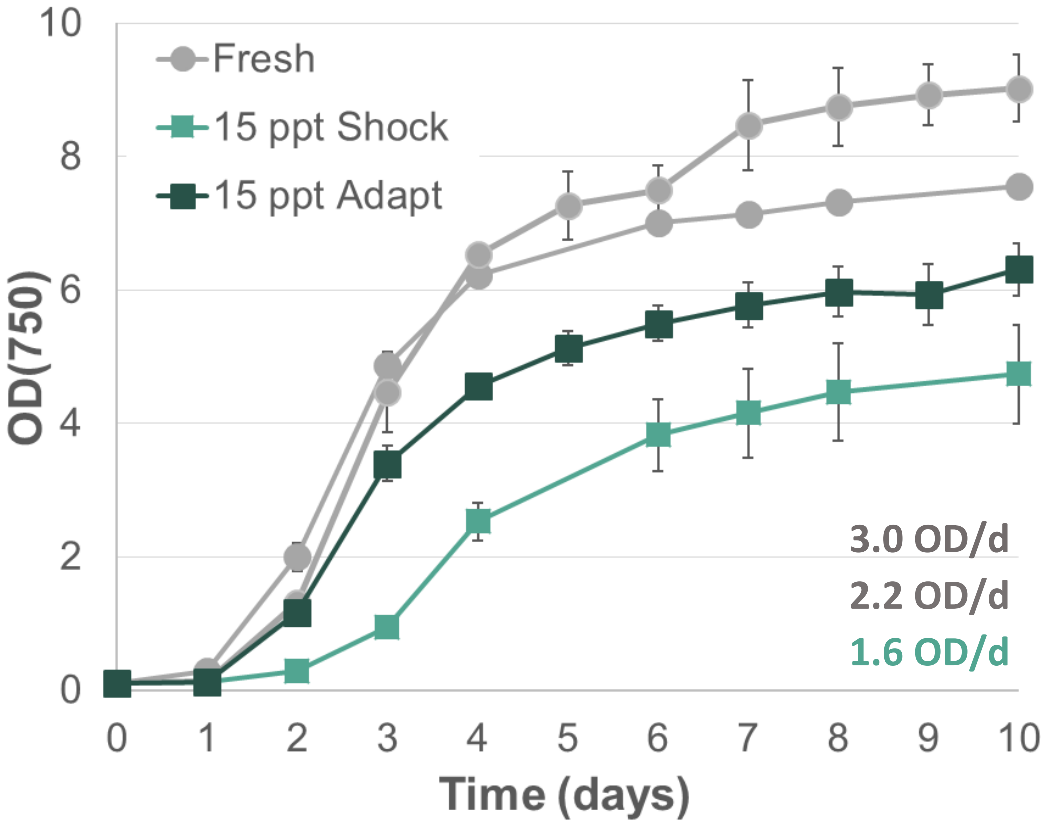
Growth rates of UTEX393 in 15 ppt salinity was improved >30%

- *Acutodesmus obliquus* UTEX393 was identified as a promising summer and winter strain, but demonstrated **poor salinity tolerance**
- Adapted for improved growth at 15 ppt for **increased environmental robustness** and closer linear growth rates to freshwater
- We also see **a ~30% increase in % FAMEs**:

	Fresh	15ppt
Day 6	17.0	22.2
Day 10	20.0	26.6

Nitrogen depleted on ~Day 3.

- Sent to PNNL for LEAPs experiments



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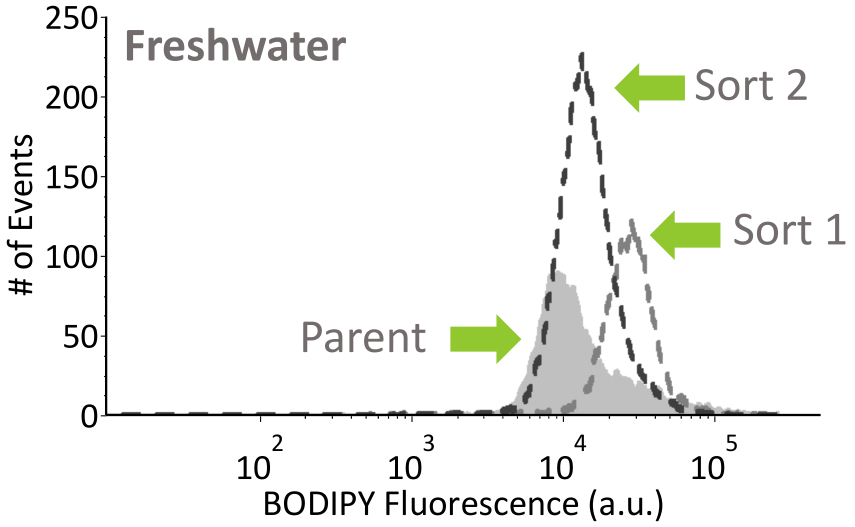
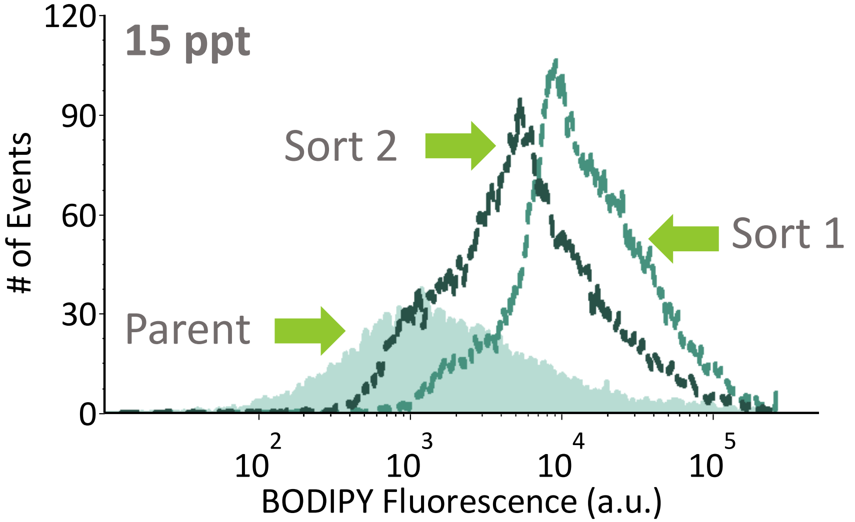
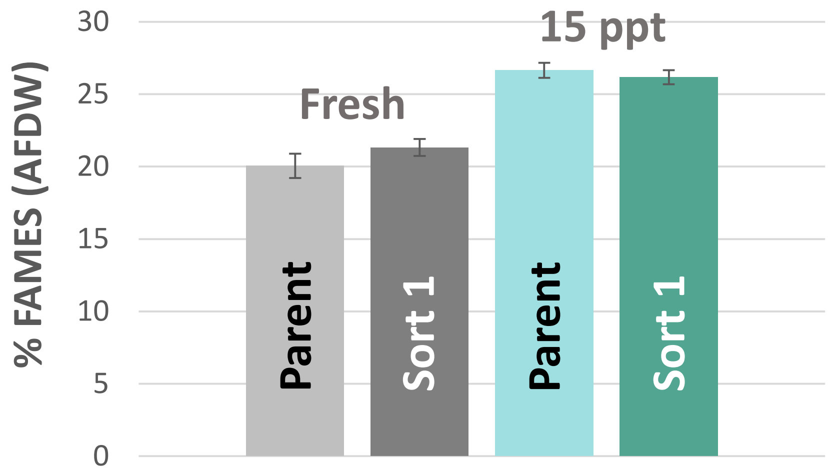
Results: Tier III Strain Improvement – Cell Sorting

Multiple rounds of sorting conducted for fresh & adapted cultivars







- Cell sorting has not yet resulted in increased FAMES in UTEX393
- Cells do not stain evenly; 15 ppt cells stain very broadly in spite of having more homogeneous morphology
- UV Mutagenesis for increased genetic diversity and improved success

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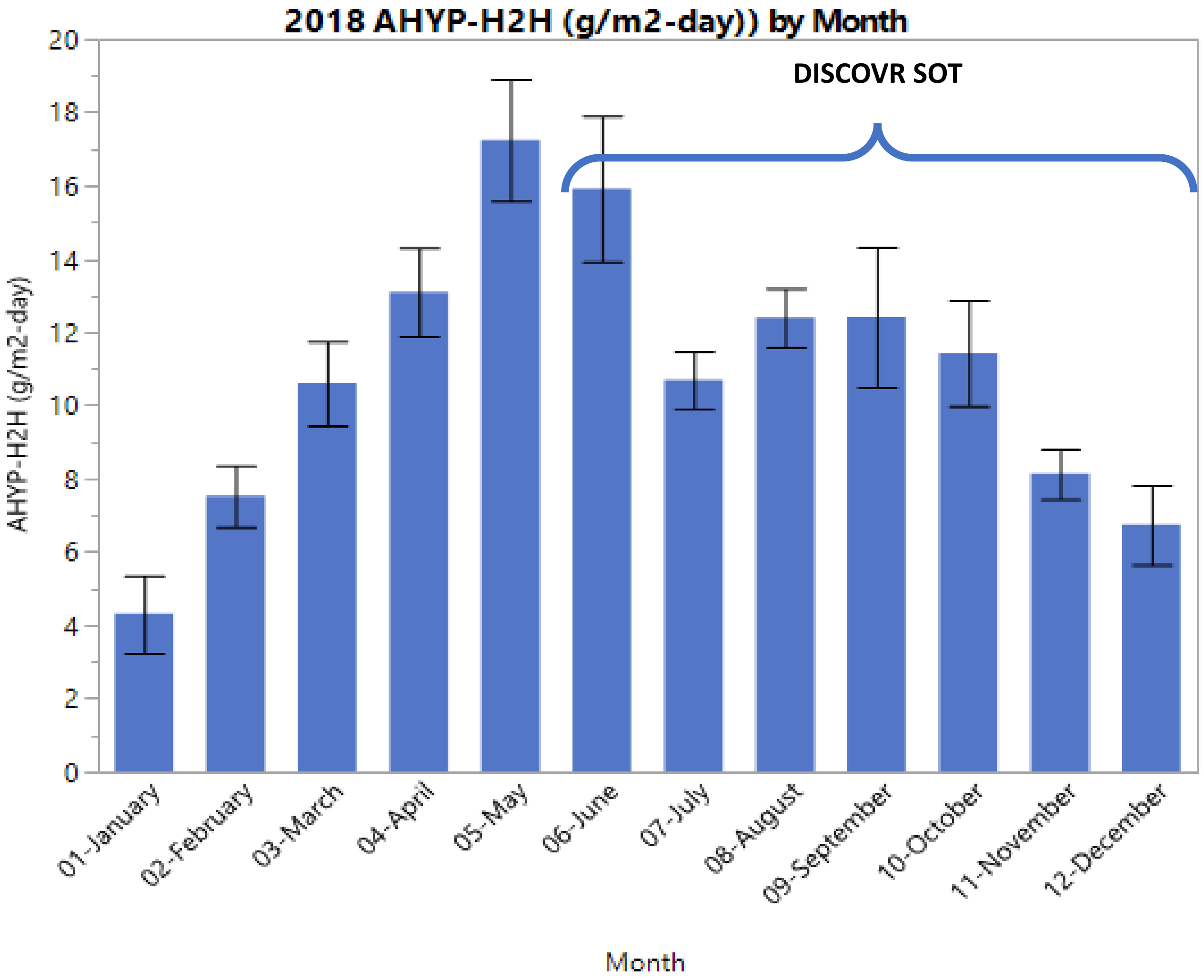
Approach: TIER V Strain Culturing at SOT Testbed

Top strains are performance tested in outdoor ponds at AzCATI

- **ATP^3's established framework for cultivation trials** to inform the **state of technology (SOT)** for algal based biofuels **transitioned fully under DISCOVR as of Summer 2018**
- Utilizes standard **mini-pond raceways** (4.2 m^2 ATP^3 raceway design) and existing infrastructure and expertise
- **Best performing cultivars and operational conditions** are identified and implemented in **seasonal trials** with **standardized protocols** for data collection, analysis and curation
- **Cultivation trials** run in **triplicate** with up to **four conditions** tested simultaneously lasting up to **10 weeks within a season** with flexibility to adjust experimental design as conditions warrant
- Biomass samples are collected for
  - **Productivity monitoring**
  - **Biomass composition**
  - **Storage stability**
  - **Pond ecology/pond crash forensics**
  - New strain isolation

Results: Cultivation at SOT Testbed

Dip in areal productivity during summer is caused by infection/predation



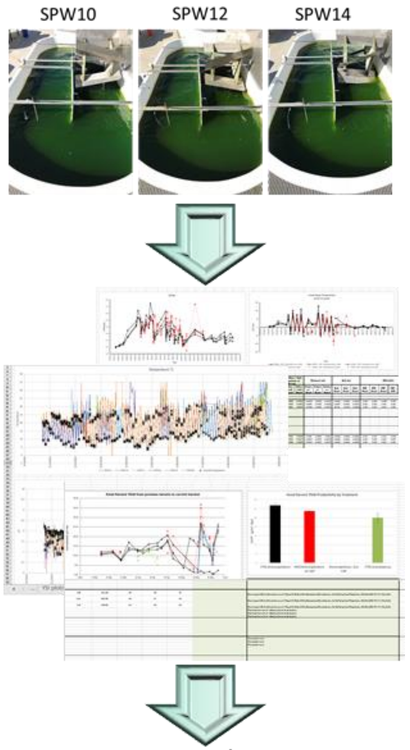
Areal Productivity (harvest to harvest) for all cultivation trials conducted in calendar year 2018 at AzCATI in Mesa, AZ

DISCOVR SOT specific trials indicated on graph

Results: SOT Data Management

Comprehensive centralized data enables analysis of current cultivation

- Strategy designed to provide immediate/ongoing data analysis, dissemination, and discussion to understand relevance/significance in moving the needle of the SOT
- Comprehensive spreadsheets collaboratively developed to capture critical metrics of algae cultivation
- Active graphing at the top of each sheet for rapid/easy data visualization
  - Includes measured metrics and up to date calculations
    - areal harvest yield productivity
    - ratios/correlations to understand cultivation
      - C:N ratio, AFDW/OD_{750} correlation, etc.
  - Includes checks on data quality
    - comparison between on-line YSI sensors and manual temperature/pH measurements
    - % RSD to verify quality triplicate measurements fall within acceptable ranges
  - 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 DropBox/SharePoint

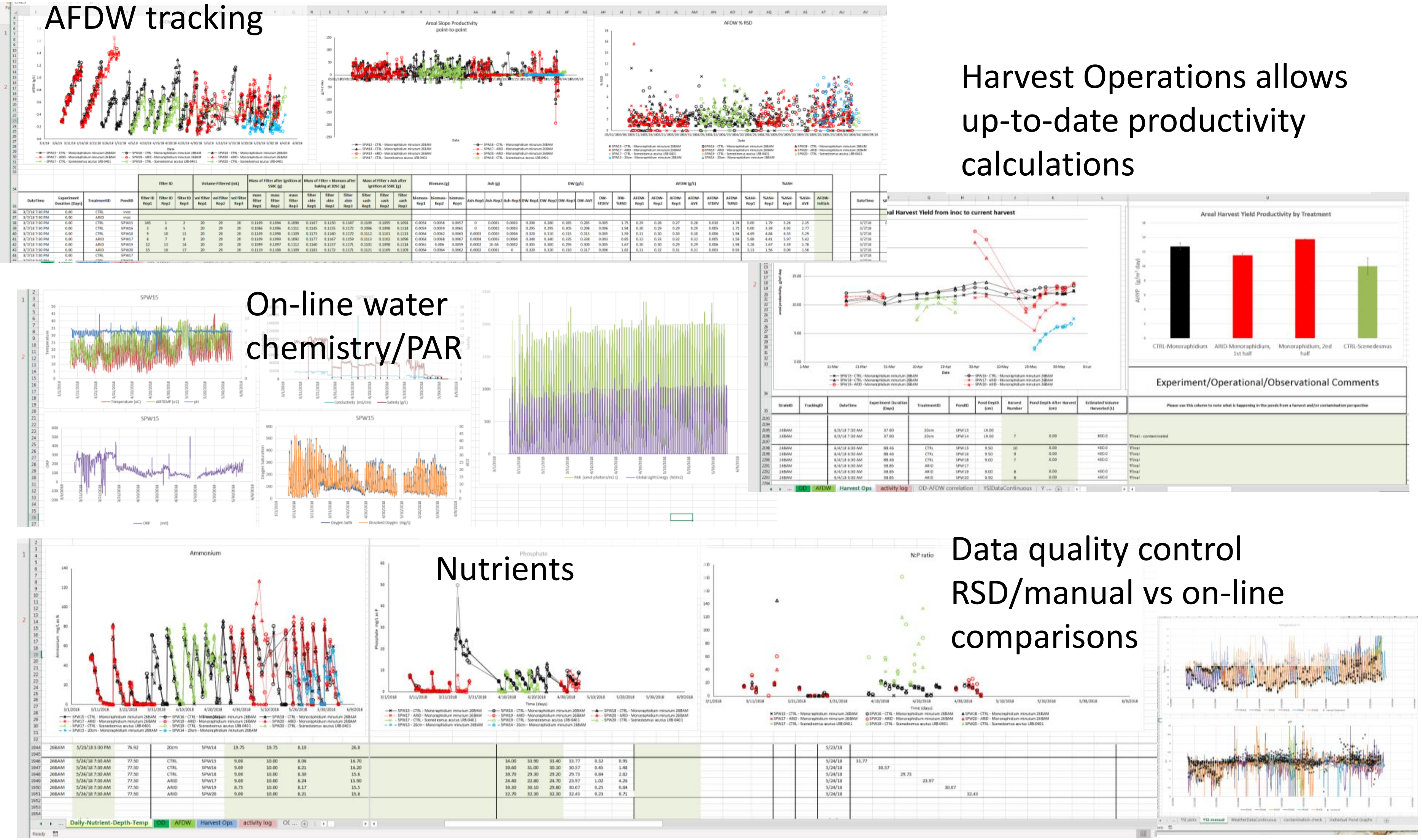


SOT cultivation data for ATP[3] calibration trials

	Productivity, g/m²/day	Harvest Density, g/L	Evaporation Rate, cm/day	Algae Strain	Harvests per week	Harvest volume, fraction of pond	Daily dil rate, fra of po
2015 SOT (Florida Algae)							
Spring 2014	11.4	0.26	0.14	Nanno	1x	0.75	0.11
Summer 2014	10.9	0.25	0.02	Nanno	1x	0.75	0.11
Fall 2014	6.8	0.22	0.01	Nanno	1x	0.75	0.11
Winter 2014	5.0	0.23	0.01	Nanno	1x	0.75	0.11
Average	8.5	0.27	0.04				
2016 SOT (Florida Algae)							
Spring 2015	11.1	0.28	0.14*	Nanno	2x	0.25	0.11
Summer 2015	13.3	0.32	0.02*	Desmo	2x	0.50	0.21
Fall 2015	7.0	0.20	0.01*	Desmo	2x	0.50	0.21
Winter 2014*	5.0	0.23	0.01*	Nanno	1x	0.75	0.11
Average	9.1	0.26	0.04				
2017 SOT (ASU)							
Spring 2016	13.2 (ARID)	0.74	0.9	Scened	5x	0.25	0.18
Summer 2015*	14.1	0.32	1.2	Desmo	2x	0.50	0.21
Fall 2016	8.5	0.30	0.7	Nanno	NA (batch mode, harvested every 1-2 we		
Winter 2016	5.5	0.36	0.2	Kirch	NA (batch mode, harvested every 2-3 we		
Average	10.3	0.43	0.7				
2018 SOT (ASU)							
Spring 2018	15.2	0.70	0.7	Monor	1-2x	0.83	0.17
Summer	15.4	0.25	1.2	Desmo	2x	0.55	0.2
Fall 2016	8.5	0.30	0.7	Nanno	NA (batch mode, harvested every 1-2 we		
Winter	7.7	0.69	0.2	Scened/Monor	NA (batch mode, harvested every 10-12		
Average	11.7	0.51					

Results: SOT Data Management

Examples of available data and visualizations



AFDW tracking

Harvest Operations allows up-to-date productivity calculations

On-line water chemistry/PAR

Nutrients

Data quality control RSD/manual vs on-line comparisons

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Technical Advisory Board

Thought leaders with range of expertise provide project oversight

- DISCOVR **quarterly reports** are distributed to TAB.
- DISCOVR team **presents technical updates** to TAB using WebEx on quarterly basis with BETO staff in attendance.
- Presentations are designed to **spark discussion and elicit dialog** on DISCOVR **critical path elements**.
- TAB members
  - Philip Pienkos, NREL, Chair
  - Rebecca White, Qualitas Health
  - Toby Ahrens, Larta Institute
  - Lou Brown, Synthetic Genomics
  - John Benemann, MicroBio Engineering
  - Valerie Harmon, Harmon Consulting
  - Juergen Pohle, Brooklyn College
  - Craig Behnke, Lumen Biosciences

Relevance: Call for Collaboration

Issued "Call for Collaboration" aimed at testing the "Best of the Best"

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, 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.