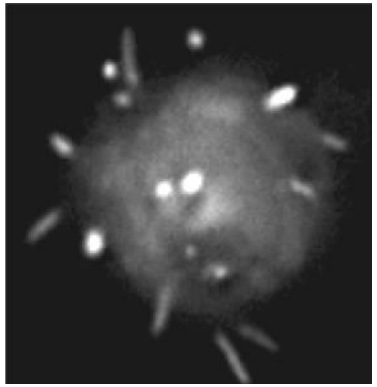
DOE BIOENERGY TECHNOLOGIES OFFICE (BETO)
2019 PROJECT PEER REVIEW

MICROBIOME ENGINEERING OF *DESMODESMUS* TO ALLEVIATE CARBON LIMITATION



March 5, 2019

Advanced Algal Systems

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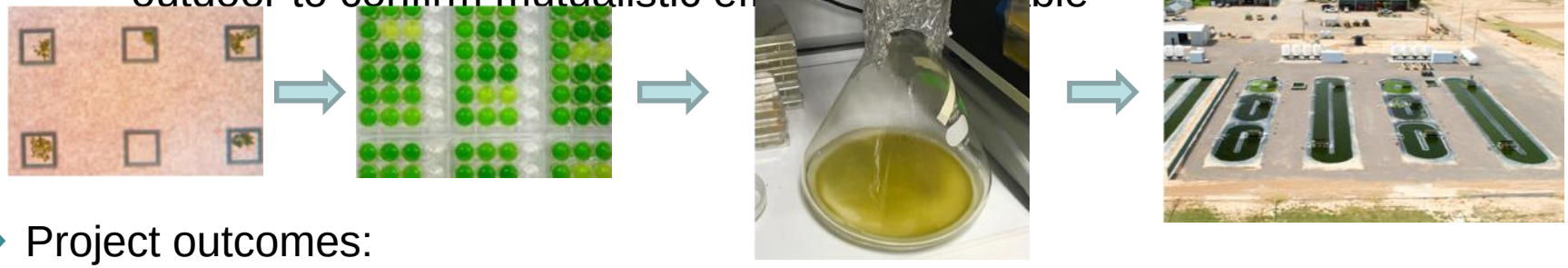
GOALS

- Reduce cost of algal biofuels by increasing production under summer (high light and high temperature) conditions
  - High-throughput cultivation of algal microbiomes in microfluidic chambers tested under summer Arizona conditions (mid-day peak 35℃, 2,000 µE m^{-2} s^{-1})
  - Scale up algal-microbiome combinations from 1 nL to 1,000 L, indoor to outdoor to confirm mutualistic eff ble



- Project outcomes:
  - Obtain <u>at least</u> 5 mutualistic microbiomes that alleviate temperature and light stress, test 1 in 1,000L outdoor raceways
  - Toolkit development for the research community
    1) microwell screening system for algal growth (GALT)
    2) 96-well plate method to screen for low dissolved organics (LLNL)

QUAD CHART OVERVIEW

Timeline

- Project start 1/1/2018
- Project end 3/31/2021
- Percent complete: 30%

Funding

	Pre FY17 Total Costs	FY17 Costs	FY18 Costs	FY19-Project End Date Total Planned Funding
DOE Funded			$203K	$1,249K
Project Cost Share			$60K	$112K

Partners:

- General Automation Lab Technologies (GALT)
- Arizona Center for Algae Technology and Innovation (AzCati)

Barriers Addressed

AFT-C. BIOMASS GENETICS & DEVELOPMENT:
The productivity and robustness of algae strains against such factors as temperature, seasonality, predation, and competition needs to be improved

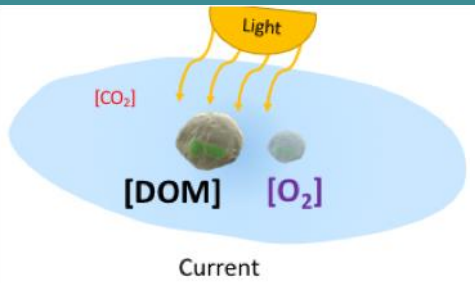
Objective: Using a high-throughput microfluidic screening system, identify mutualistic bacteria with high respiration metabolism and protective pigmentation that lead to increased *Desmodesmus* growth under summer conditions, test at different lab and outdoor scales

Project Goals

- Target = 26 g m^{-2} d^{-1} biomass production under high light and high temperature stress (1,000L scale)
- Toolkit #1: microwell screening approach to identify microbiome-enhanced algal growth
- Toolkit #2: 96-well screen for high bacterial respiration

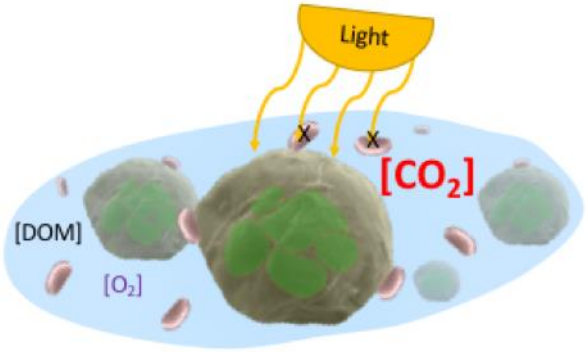
1 – PROJECT OVERVIEW

- Early focus on baseline characterization
  - Impact of non-optimized bacteria on biomass production
  - Development of toolkits for microbiome screening



Current

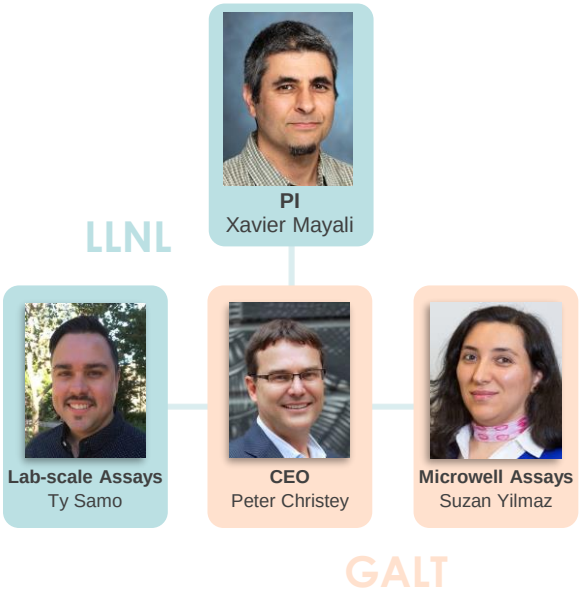
- Task 2 (Q2-Q6) Obtain microbiomes that increase growth under summer conditions
  - Bulk analyses of algal cultures with and without bacteria
  - Optimize microwell screening (Toolkit 1)
  - Optimize DOM/oxygen assay in well plates (Toolkit 2)
  - Identify consortia that increase growth
  - Randomize consortia in microwells
  - Test productivity of combined microbiomes at larger scale



Engineered microbiome

- Task 3 (Q7-Q12): Scale increased productivity from laboratory to outdoors
  - Scale up to outdoor mesocosms.
  - Test subset of consortia with isotope tracing
  - Quantify the interaction between algal growth and DOC disappearance.
  - Test at outdoor scale
  - Data synthesis

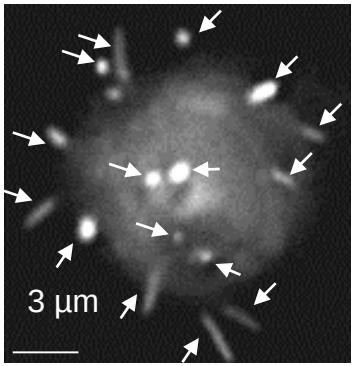
2 – APPROACH (MANAGEMENT)



- Meetings
  - Monthly WebEx meeting for data sharing
  - Quarterly PI meeting with BETO technology manager
  - Annual face-to-face meeting
- PI Responsibilities
  - Keep milestones on track
  - Generate quarterly reports
  - Track financial data
  - Synthesize data among LLNL/GALT
  - Track troubleshooting solutions
- Decision-making is through consensus but PI retains ultimate decision-making authority
- Interface with other projects (shared equipment, meetings)
  - DOE/BER-funded fundamental Science Focus Area (SFA) project on algal-bacterial interactions “A Systems Biology Approach to Interactions and Resource Allocation in Bioenergy-Relevant Microbial Communities” (bio-sfa.llnl.gov)
  - BETO-AOP to LLNL on anti-grazing probiotic bacteria (Thursday 10:30 presentation)

2 – APPROACH (TECHNICAL)

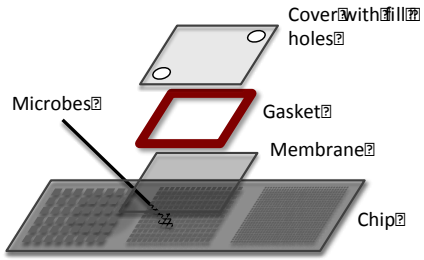
- Study organism: *Desmodesmus sp.* strain C046
  - Isolated through BETO funding
  - Model saltwater strain (13.3 g/m^2/day AFDW)



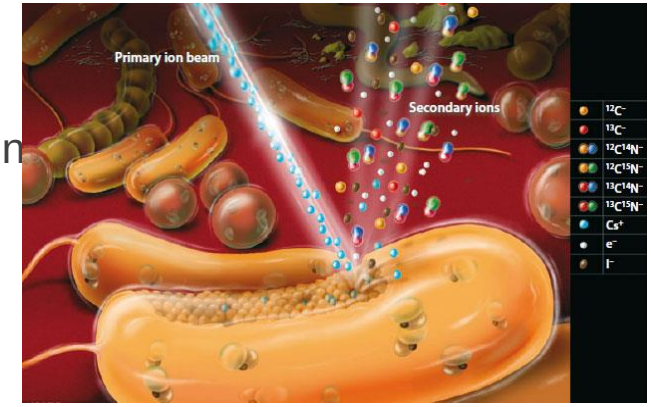
One *Desmodesmus* cell with 16 attached bacteria (DAPI staining)

- Microbial community analysis
  - Cultivation independent
  - PCR amplification of 16S rRNA gene

- GALT microwell system for high-throughput cultivation
  - High throughput, low volume
  - Allows testing of thousands of combinations



- Isotope tracing and NanoSIMS to quantify single cell C fluxes
  - State of the art isotope enabled technology developed with DOE Office of Science fundin
  - Quantitative tracing of metabolism
  - Single-cell resolution



2 – APPROACH (TECHNICAL)

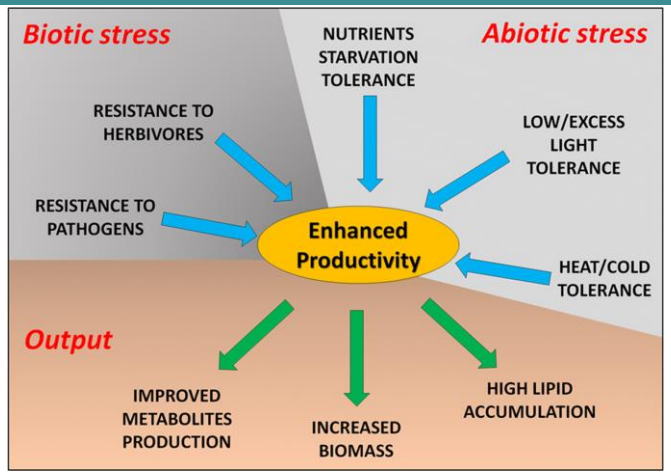
The General Automation Technologies (GALT) microwell cultivation system

https://www.galt-inc.com/

2 – APPROACH (TECHNICAL)

Critical Success Factors

- **Technical**: experimental demonstration that the *Desmodesmus* microbiome can confer increased growth under stress conditions
- **Economic**: achieve a decrease in cost at outdoor scale to achieve 80 GGE/AFDW ton biomass (BETO 2021 multi-year plan)



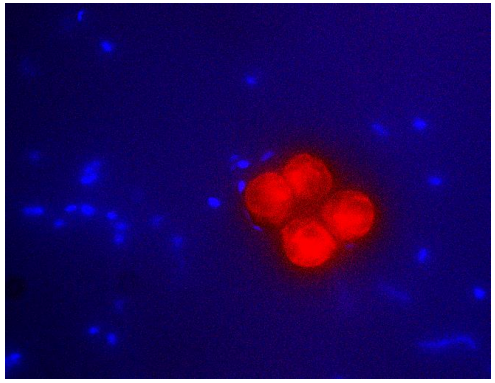
Benedetti et al. 2018 Microb Cell Fact

Challenges and Solutions

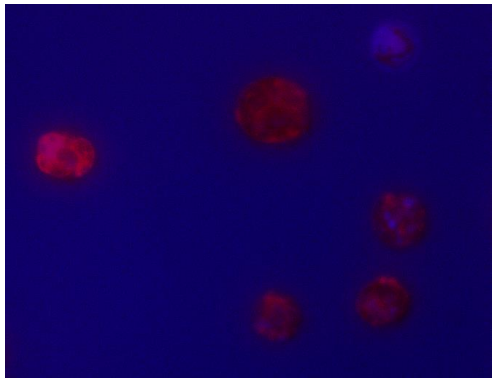
Challenges	Solutions
Results do not translate between small volumes in the laboratory and outdoor cultivation	Lab experiments (including GALT microwells) carried out under simulated outdoor conditions (sinusoidal light/temperature)
Microbiomes optimized in the lab will be altered in outdoor cultures due to competitors	Focus on algal-attached bacteria to minimize dilution effect and increase mutualism stability; microbial community tracked by sequencing
Cost of microbiome engineering will counter-act any biomass gains	No cost once microbiomes are identified (they are pre-loaded with the algal cells)
GALT system never tested with algae	Alternate materials for micro-cultivation chambers are being tested for optimal growth

3 – TECHNICAL ACCOMPLISHMENTS

- *Desmodesmus* "sterilized" via washing and plating vs. *Desmodesmus* with "native" microbiome

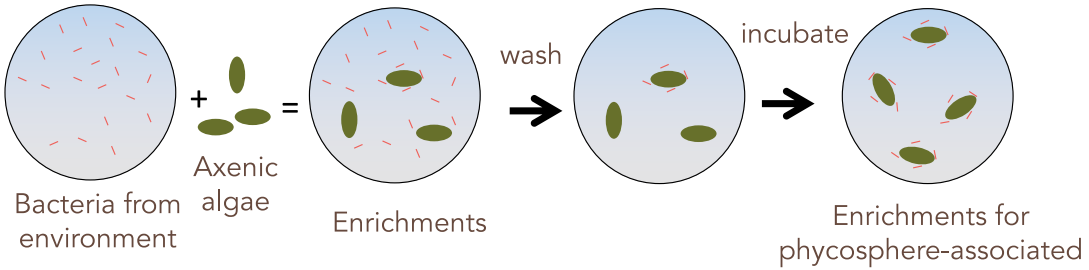


Pure culture from U. Hawaii stained with DAPI

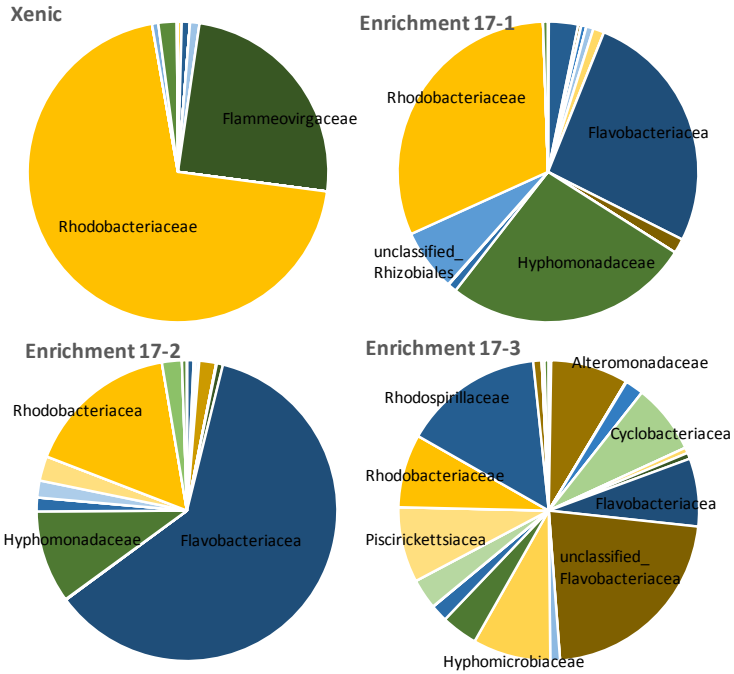


Culture streaked on F/2 agar and reinoculated into liquid F/2 (stained with DAPI)

- Procedure to obtain microbiome enrichments

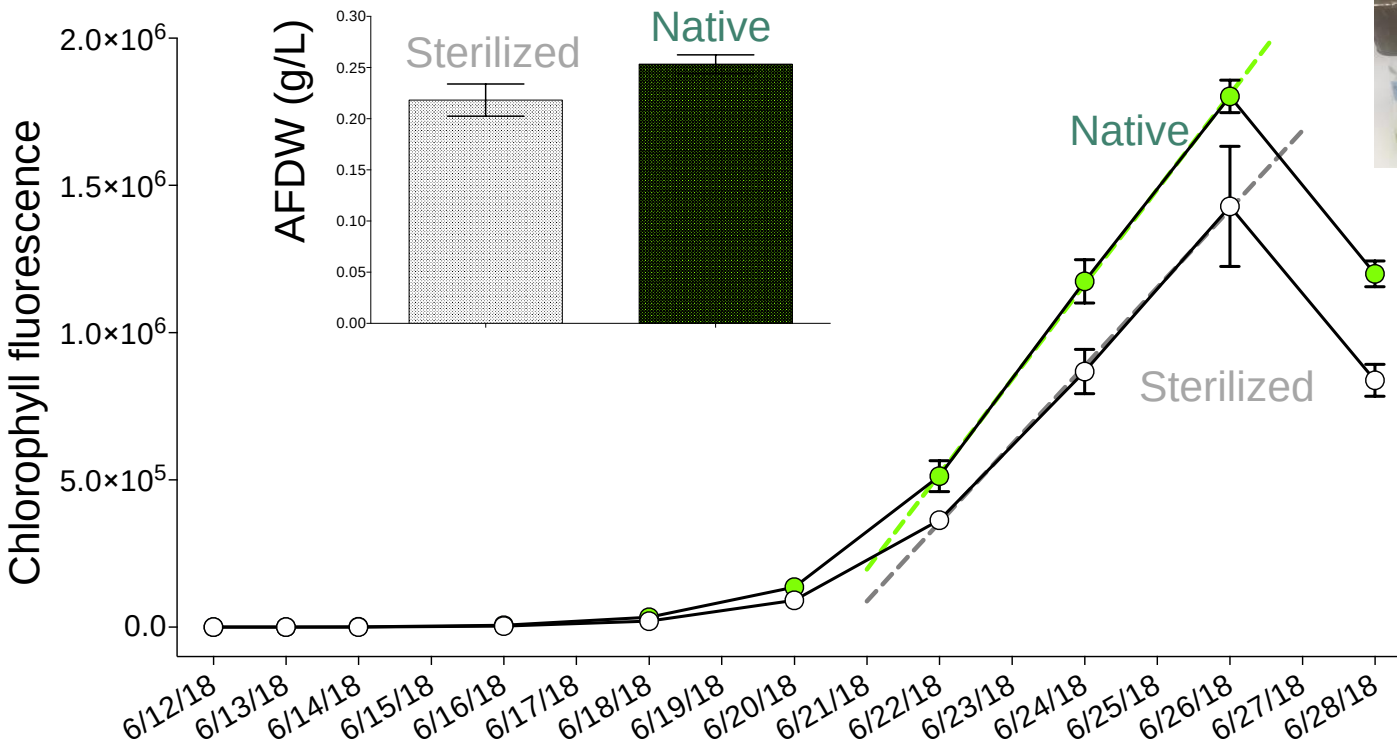


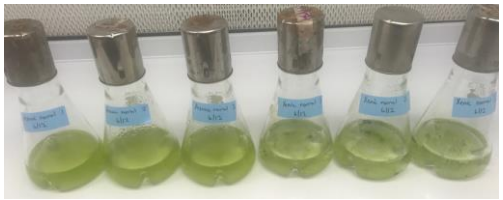
- *Desmodesmus* microbiomes from diverse origins have distinct bacterial community structure

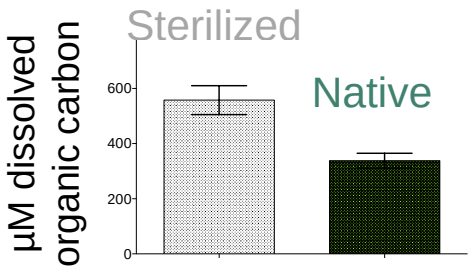


3 – TECHNICAL ACCOMPLISHMENTS

- Compared growth and yield of “sterilized” *Desmodesmus* and “native” microbiome under non-stress laboratory conditions (100 mL flasks, *F*/2 medium)



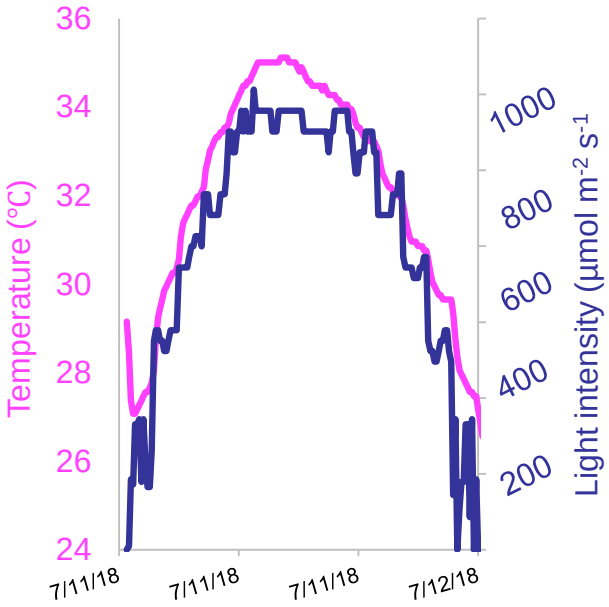


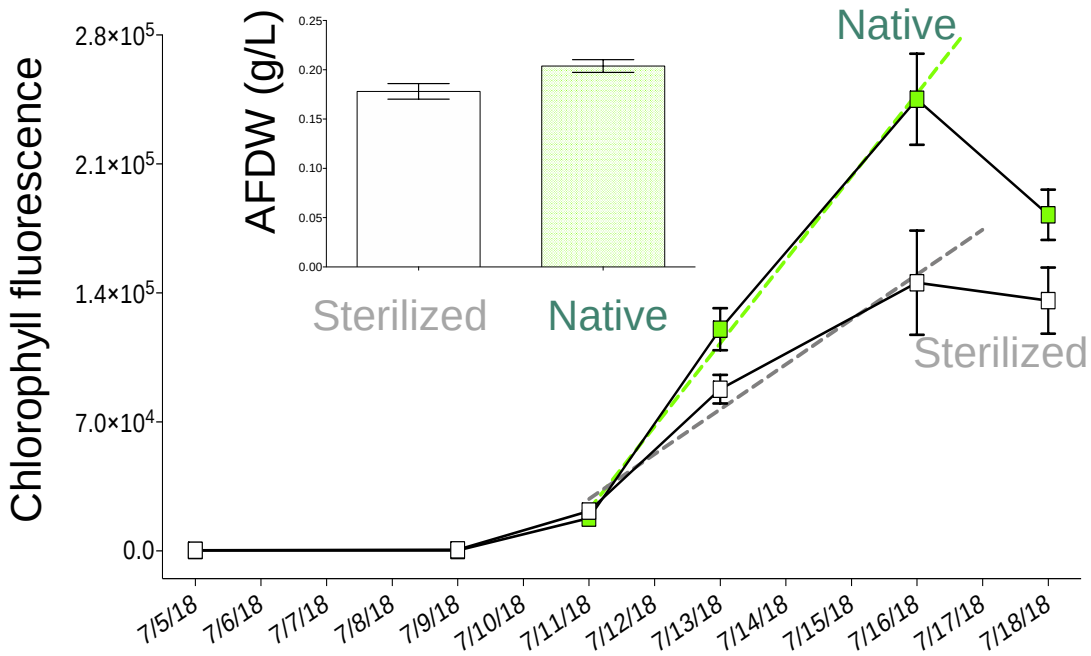


- *Desmodesmus* native microbiome = 15% increase in AFDW
  Microbiome consumes 36% of dissolved organic carbon

3 – TECHNICAL ACCOMPLISHMENTS

- Establish laboratory conditions to mimic summer Arizona conditions with Heliospectra RX30 light source
- Compared growth and yield of “sterilized” to “native” *Desmodesmus* under heat/light stress

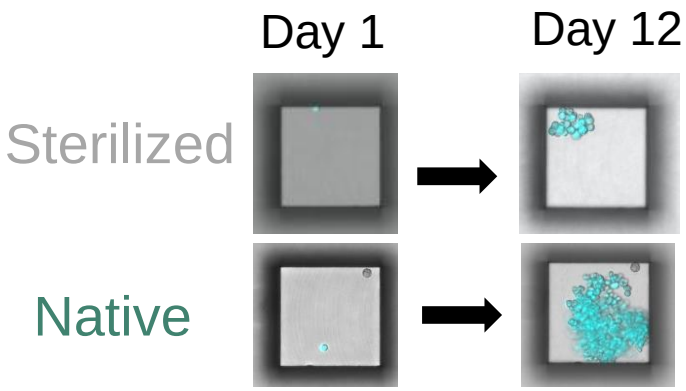


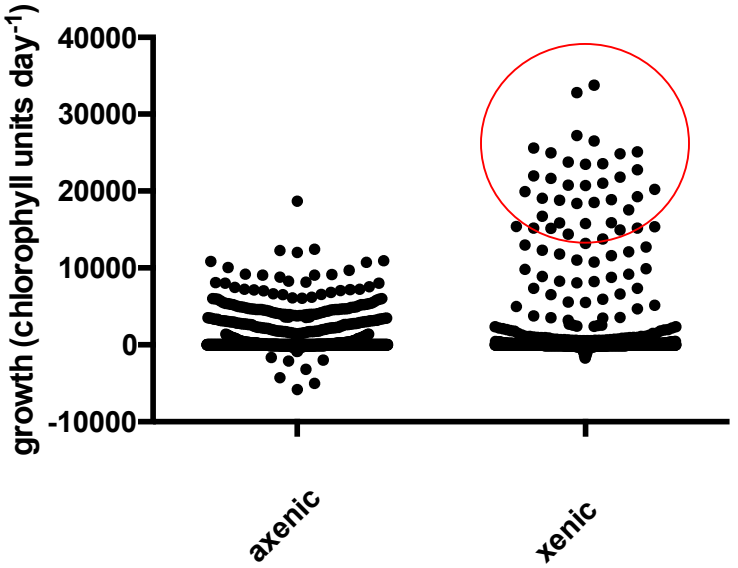


Desmodesmus native microbiome = 15% increase in AFDW under heat/light stress

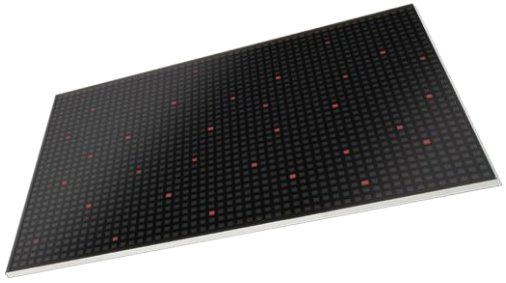
3 – TECHNICAL ACCOMPLISHMENTS

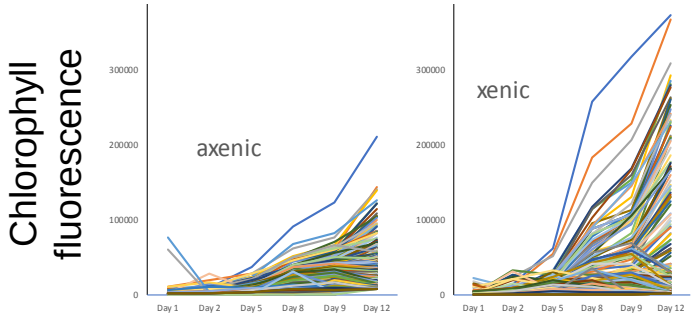
- Compared growth and yield of “sterilized” to “native” *Desmodesmus* under heat/light stress in GALT microwells (N =3000) starting from <u>one cell</u>





- Environment in microwells mimics laboratory-scale experiments (light/heat)
- Microwells with highest growth/yield to be targeted for future work



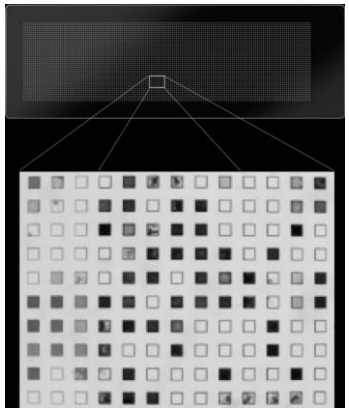


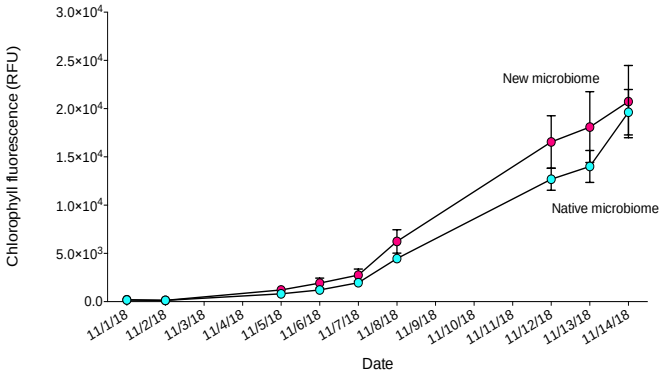
4 – RELEVANCE

- The microbiome plays a role in animal/plant health, but microalgae present a unique challenge (single cells, in liquid, open air): new approaches are needed
- 2021 Productivity-Enhanced Algae and Tookits (PEAK) goal: 80 GGE of advanced biofuel per ash-free dry weight ton of algae biomass
- Project supports BETO's goal to improve productivity and robustness of algae strains against such factors as temperature, seasonality, predation, and competition
- Once a microbiome is optimized for increased algal growth: no extra costs (can be maintained or frozen as any algal strain)
- Bioenergy industry will benefit from microbiome mutualism (as the health industry), will use our developed toolkits for their specific species and environmental conditions

5 – FUTURE WORK

- Apply the GALT high-throughput microwell growth assay to identify microbiome combinations that alleviate light/temperature stress (Task 2)
- Test microbiomes at large scales, including outdoors (Task 3)
- Characterize the microbial communities from mutualistic microbiomes via 16S rRNA sequencing
- Quantify C recycling with NanoSIMS-isotope tracing
- Optimize toolkits #1 (GALT microwell assay) and #2 (plate well dissolved organics assay) to enable approach in other species/environments





SUMMARY

- Goal: Improve temperature and light stress of *Desmodesmus*
  - Strain found from high-throughput screen and testing at scale
  - Algal microbiome should also be screened
- Approach: High-throughput, microscale screen of single-cell
  - Simulate outdoor summer conditions at the microscale
  - Scale up in volume and environmental complexity
- Results: with no optimization, already 15% biomass increase
  - Unstressed conditions: bacteria increase growth
  - Stress conditions: different communities have distinct effects
- Relevance: can be applied to others species and conditions