DOE Bioengery Technologies Office (BETO) 2019 Project Peer Review

Success through Synergy: Increasing Cultivation Yield and Stability with Rationally Designed Consortia

March 6, 2019
Advanced Algal Systems

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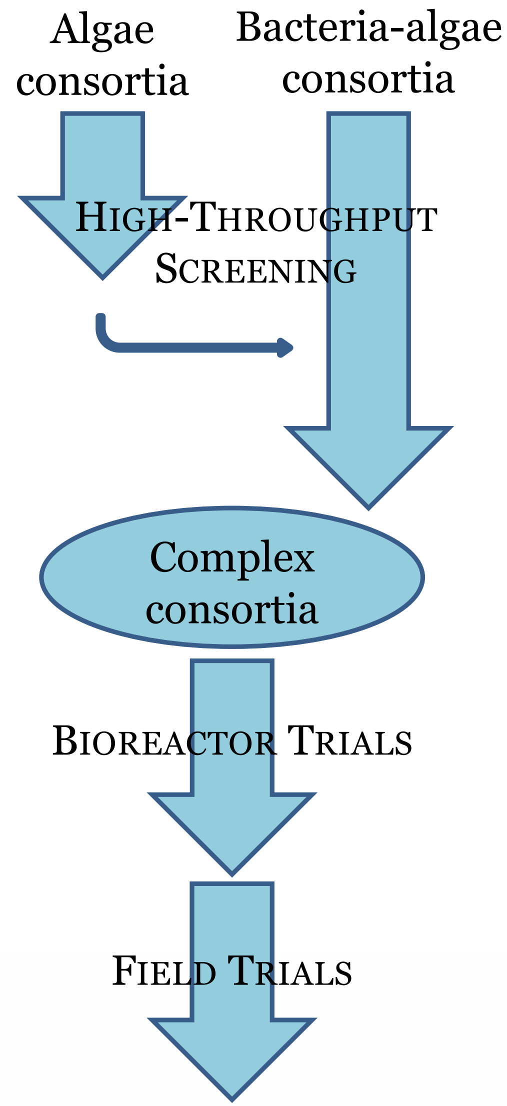
Goal Statement

Project Goal: to increase productivity of open, outdoor *Nannochloropsis* cultures from 7 g/m^2/d to >14 g/m^2/d (doubling of fall State of Technology value) via **development of (1) rationally-designed intrageneric *Nannochloropsis* consortia & (2) *Nannochloropsis*-bacteria consortia**

Outputs: molecular toolkits, pipelines, consortia

Outcomes: increased productivity & stability

Industry Relevance: increased yield, fewer crop losses, & enhanced economic feasibility



Quad Chart

Timeline

- start date -- February 1, 2018
- end date -- January 31, 2021
- 30% complete (with verification)

	FY 17 Costs	FY 18 Costs	Total Planned Funding (FY 19-21)
DOE Funded	\$0	LANL: \$351 K NMC: \$173 K	LANL: \$1.19 M NMC: \$1.29 M
Project Cost Share	\$0	LANL: \$0 NMC: \$41 K	LANL: \$0 NMC: \$276 K

Barriers Addressed

- Biomass Genetics & Development (Aft-C); to improve the productivity and robustness of algae strains against perturbations

Objective

- to rationally design intrageneric *Nannochloropsis* consortia & *Nannochloropsis*-bacteria consortia to increase productivity, stability and yield of open, outdoor cultures

End of Project Goal

- to reach a productivity target >14 $g/m^2/d$ with consistent biomass composition

1. Project Overview -- *Context and Project History*

- industrial economics of production systems limited by low crop productivity and stability
- consortia of microbial assemblages may address this limitation
  - ➢ combining algal strains with complementary traits increases yield and stability (Shurin 2014, Corcoran & Boeing 2012, Stockenreiter et al. 2011)
  - ➢ addition of bacteria enhances productivity and reduces crashes (Sapp et al. 2007, Croft et al. 2005, LLNL work)

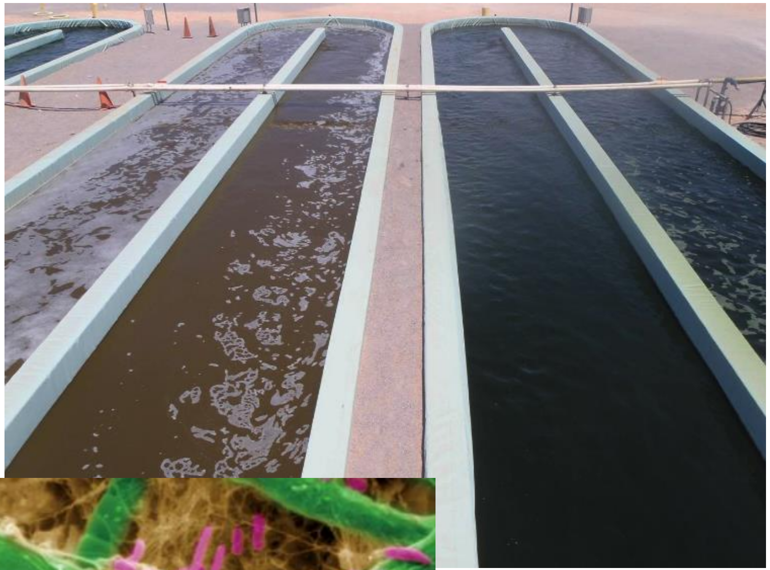
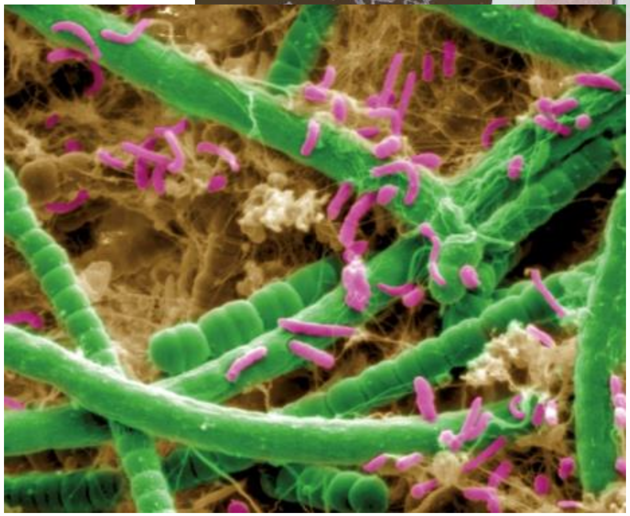


photo of “dead” and live ponds, photo credit: Sapphire Energy, Inc.



learning from nature: microbial consortia photo credit: A. Dohnalkova/ PNNL

1. Project Overview -- *Context and Project History*

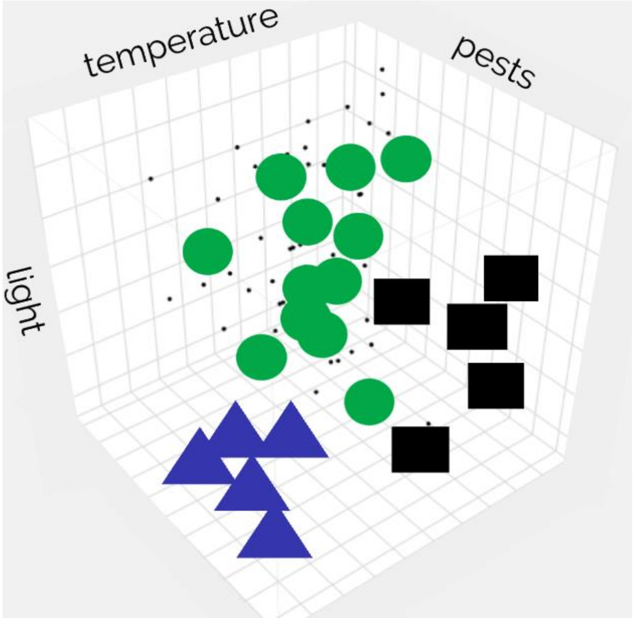
- despite strong theoretical and empirical underpinnings, consortia are not commonly cultivated at scale
- we aim to develop **intrageneric algal consortia** as well as **algal-bacteria consortia** (important for consistent biomass composition, downstream processing)
- novel approaches
  - *rational design*
  - *high-throughput screening*

Types of consortia

Intrageneric algal – group of algae containing strains from the same genus

Intergeneric algal – group of algae containing strains from different genera

Algal-bacteria – group of algae & bacteria



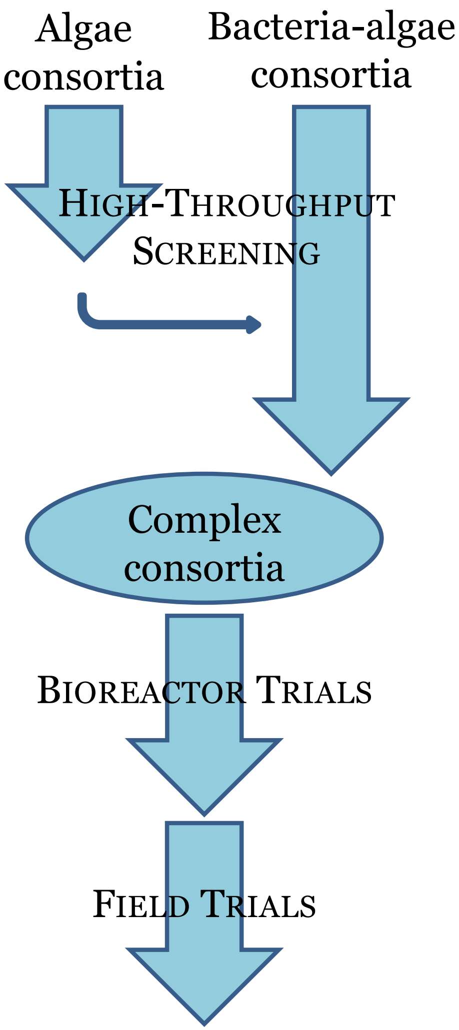
conceptual model of taxa represented by different colored shapes filling up niche space

2. Approach -- *Management*

- WBS, SOPO, and Gantt chart with Milestones and Go/No-Go Points serve as guiding documents for the team
- PIs enable communication and collaboration
  - weekly PI meetings
  - weekly team meetings to plan work, review progress towards milestones, and discuss technical barriers
- team members drive progress on specific tasks (see chart)

Task	Key Team Members
1. Verification	All
2. Design of *Nannochloropsis* Consortia & Tracking Tools	Garcia (Postdoc), Hanschen (Postdoc)
3. Screening & Identification of Growth-Promoting Bacteria	Hovde (Research Scientist), Ohan (Post-MS Student)
4. Verification & Optimization of Consortia Performance	Garcia (Postdoc), Hanschen (Postdoc)
5. PEAK Challenge	Holguin (Assistant Professor), Boeing (Professor) Rodriguz-Uribe (Postdoc)

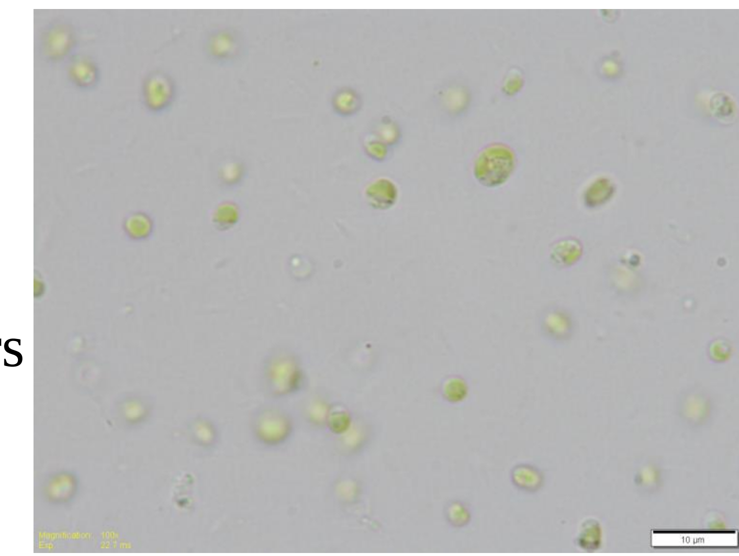
2. Approach -- *Technical*



<u>Task #2</u>: Design of Candidate *Nannochloropsis* Consortia & Tracking Tools

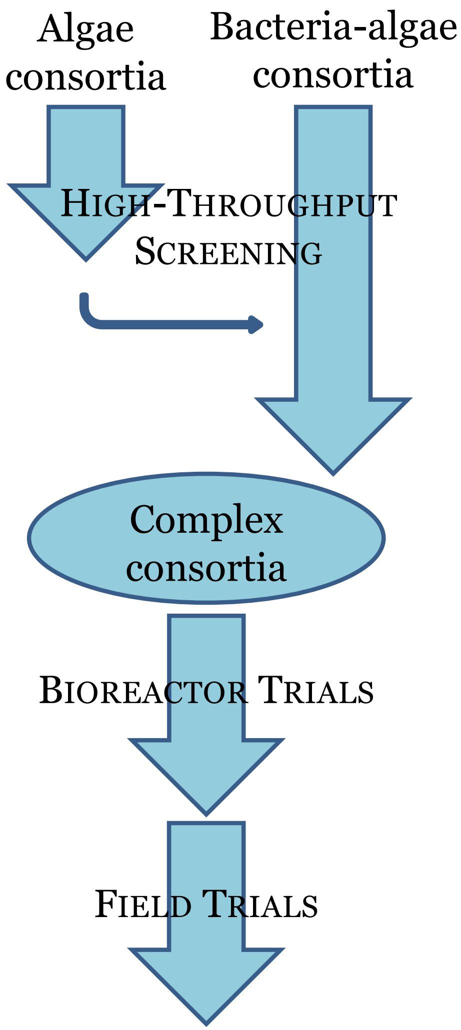
- rational design
  - ➢ match strains to outdoor regimes by combining taxa with different phenotypes
  - ➢ use Sapphire Energy, Inc.* data and strains
    *original award to Starkenburg at LANL and Corcoran at Sapphire

- molecular tracking of strains
  - ➢ needed to distinguish consortia members
  - ➢ cox1 amplicon sequencing



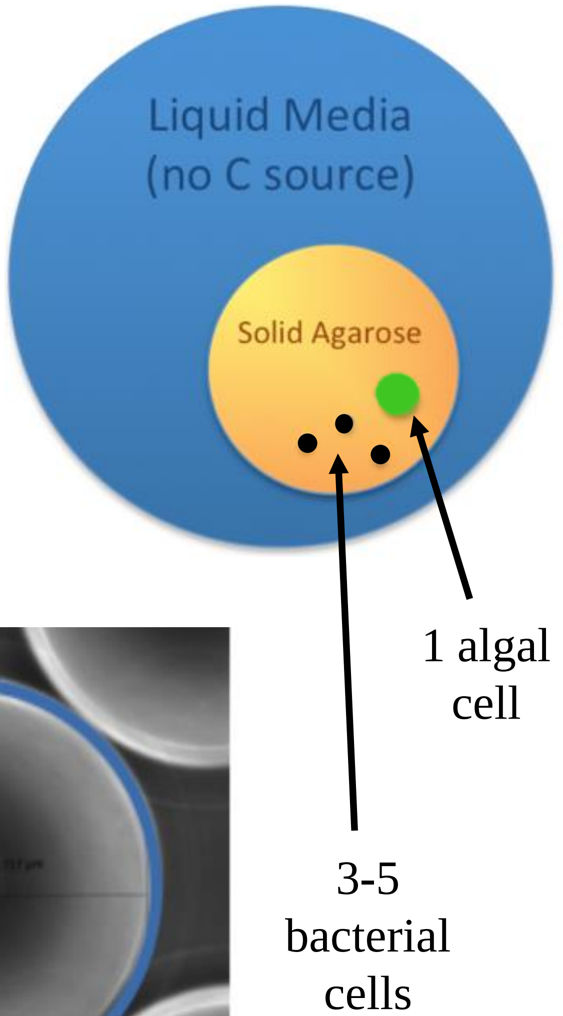
light micrograph of *Nannochloropsis* strain

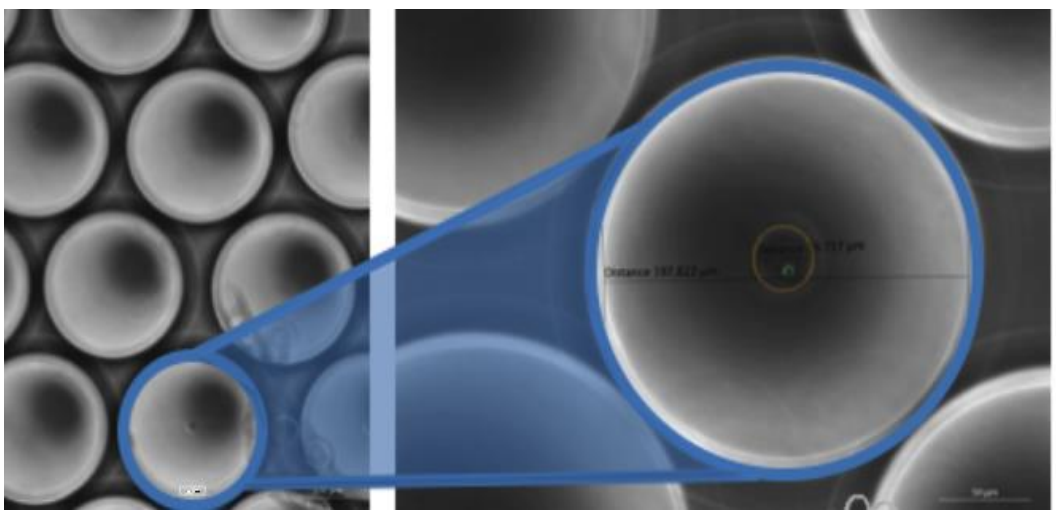
2. Approach -- *Technical*



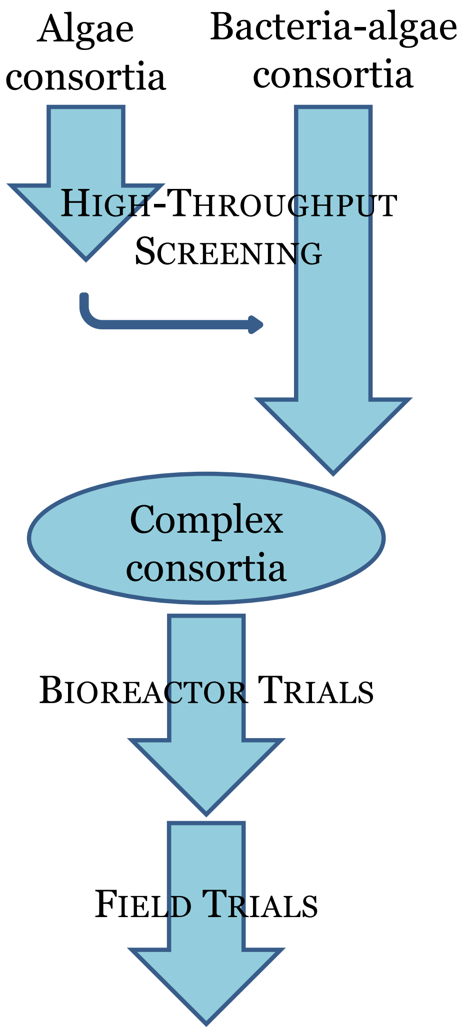
Task #3: Screening & Identification of Growth-Promoting Bacteria

- High-throughput Screening of Cell-to-cell Interactions (HiSCI) system
- sorting, scale-up, and ID of beneficial bacteria
- sourcing of environmental samples



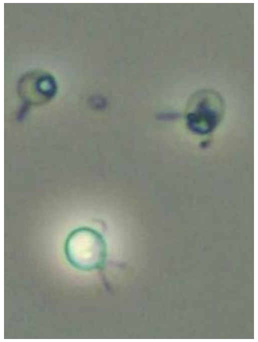


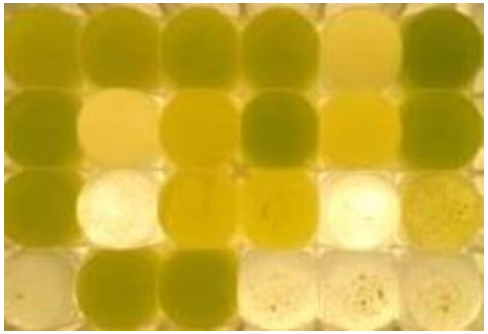
2. Approach -- *Technical*



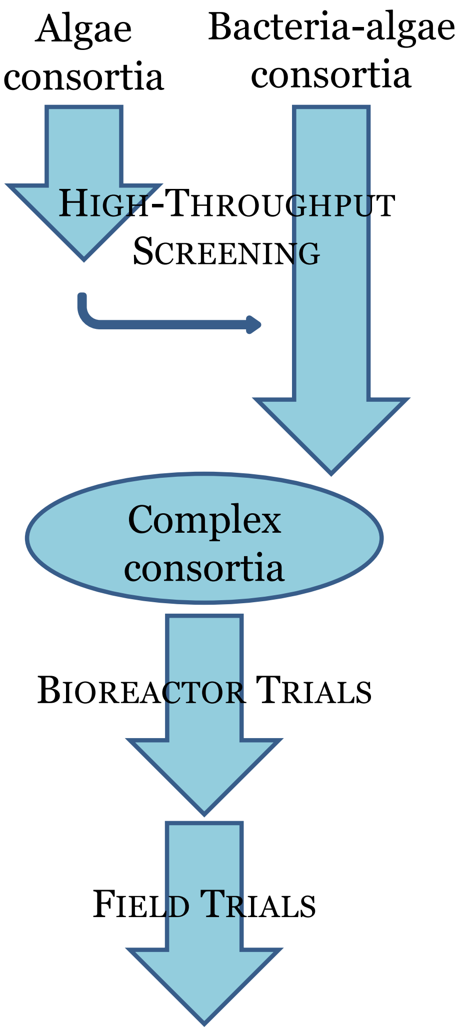
Task #4: Verification & Optimization of Consortia Performance

- individual/complex consortia tested at increasing scales
  - ➢ high-throughput screens to confirm co-existence (no stressors)
  - ➢ high-throughput screens with perturbations (temperature fluctuations, invasions)
  - ➢ bioreactor trials to better simulate field conditions
  - ➢ β-field trials in micro- (100 L) or mini- (300 L) ponds





2. Approach -- *Technical*



Task #5: PEAK Field Challenge

- best performing consortia validated through outdoor field trials
- 300-L miniponds located at the Fabian Garcia Center in Las Cruces, NM
- metrics of productivity (e.g., AFDW) and stability (e.g., temporal CV)

Aerial view (top left) of miniponds (top right, bottom) at the Fabian Garcia Center at NMSU

2. Approach -- *Technical*

Potential Challenges

- insufficient phenotypic variability across algal strains
- data gaps
- challenges in scaling
- variability in media due to sourcing
- environmental variability difficult to simulate in lab
- control failures

Critical Success Factors

- ➢ access to data/analysis methods
- ➢ creative design approaches
- ➢ access to bioreactors, ponds, infrastructure
- ➢ trained pond operators/technicians
- ➢ coordination across team members/sites

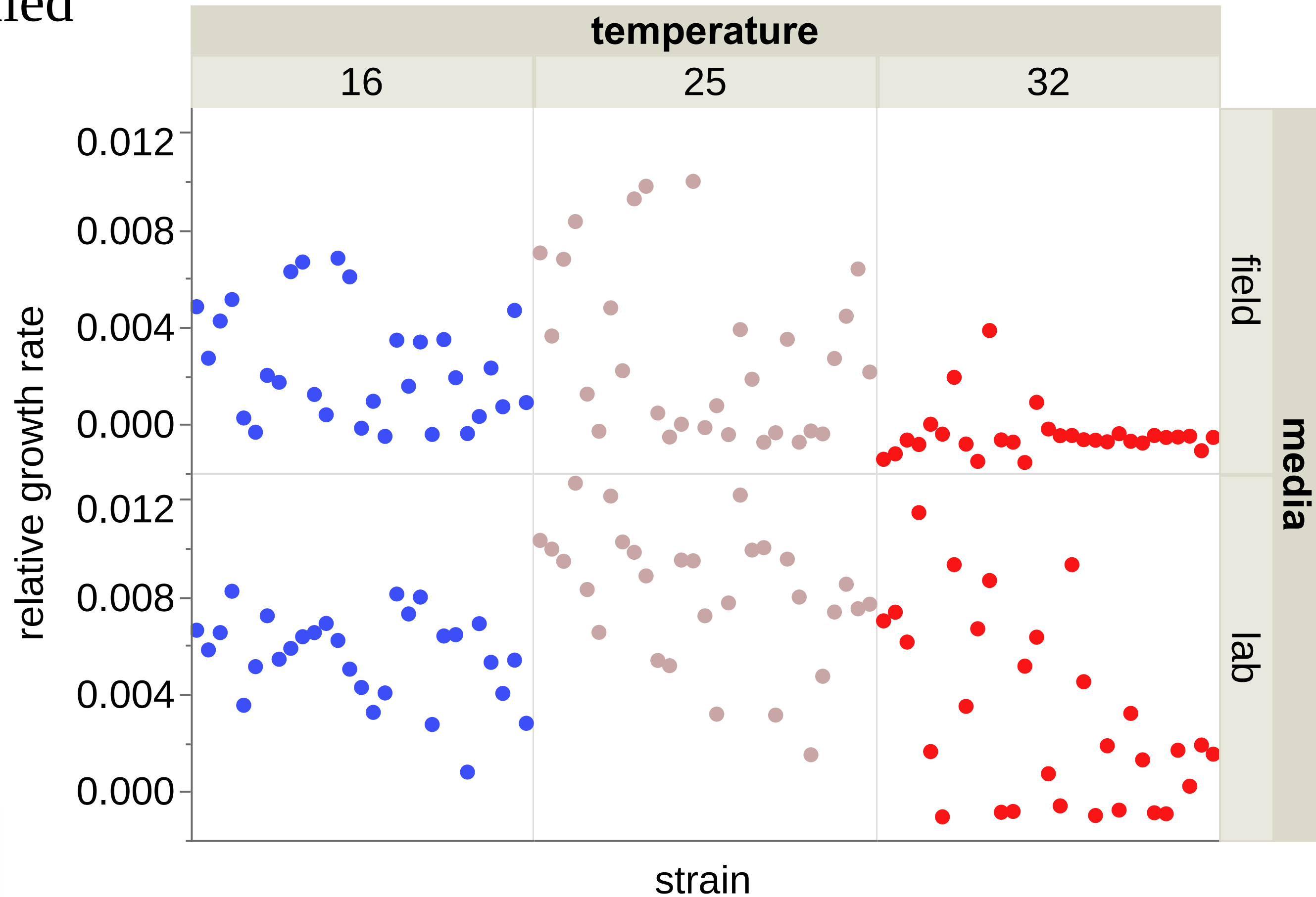
The success of this project hinges on consortia performance exceeding the baseline -- *this is the critical success factor for commercial viability*.

3. Accomplishments/Progress/Results

Task 2 (Design of Consortia & Tracking Tools)

1) strain data compiled

Growth rate bioassay data from Sapphire Energy, Inc. for ~30 Nannochloropsis strains (along x-axis) in different media types and temperatures were compiled. Strains grew better in lab media at low or intermediate temperatures.

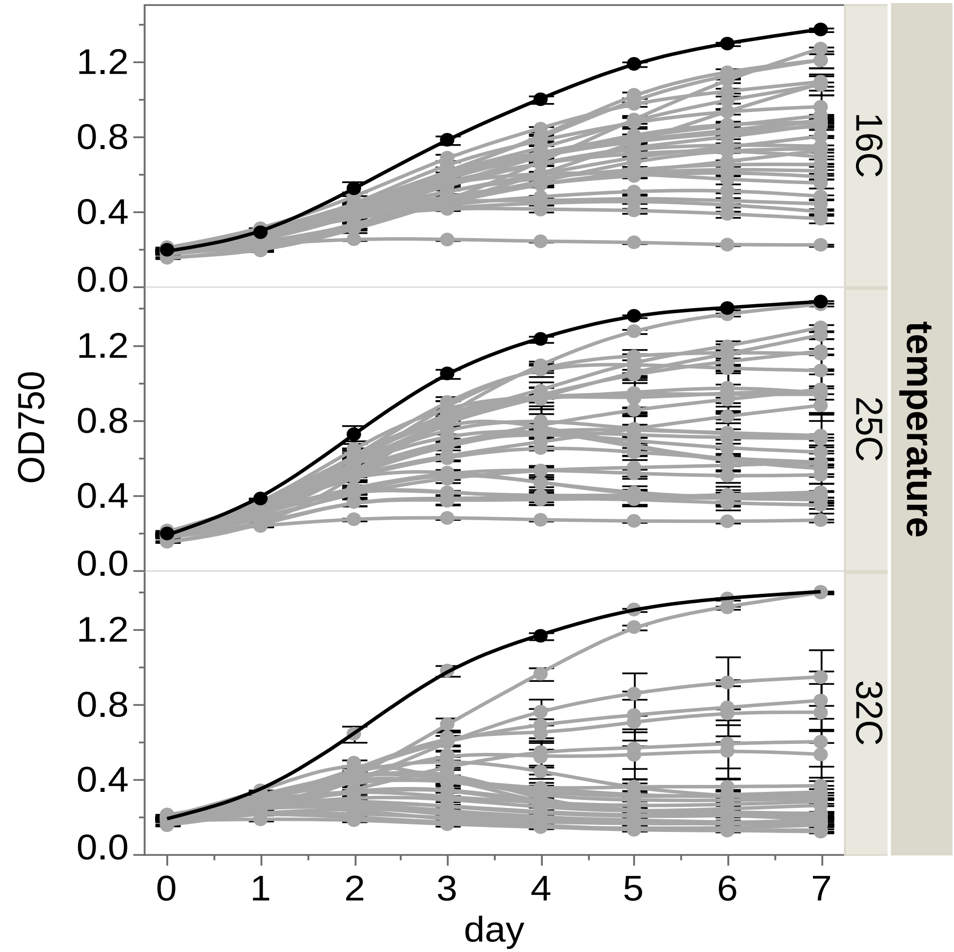


3. Accomplishments/Progress/Results

Task 2 (Design of Consortia & Tracking Tools)

1) strain data compiled

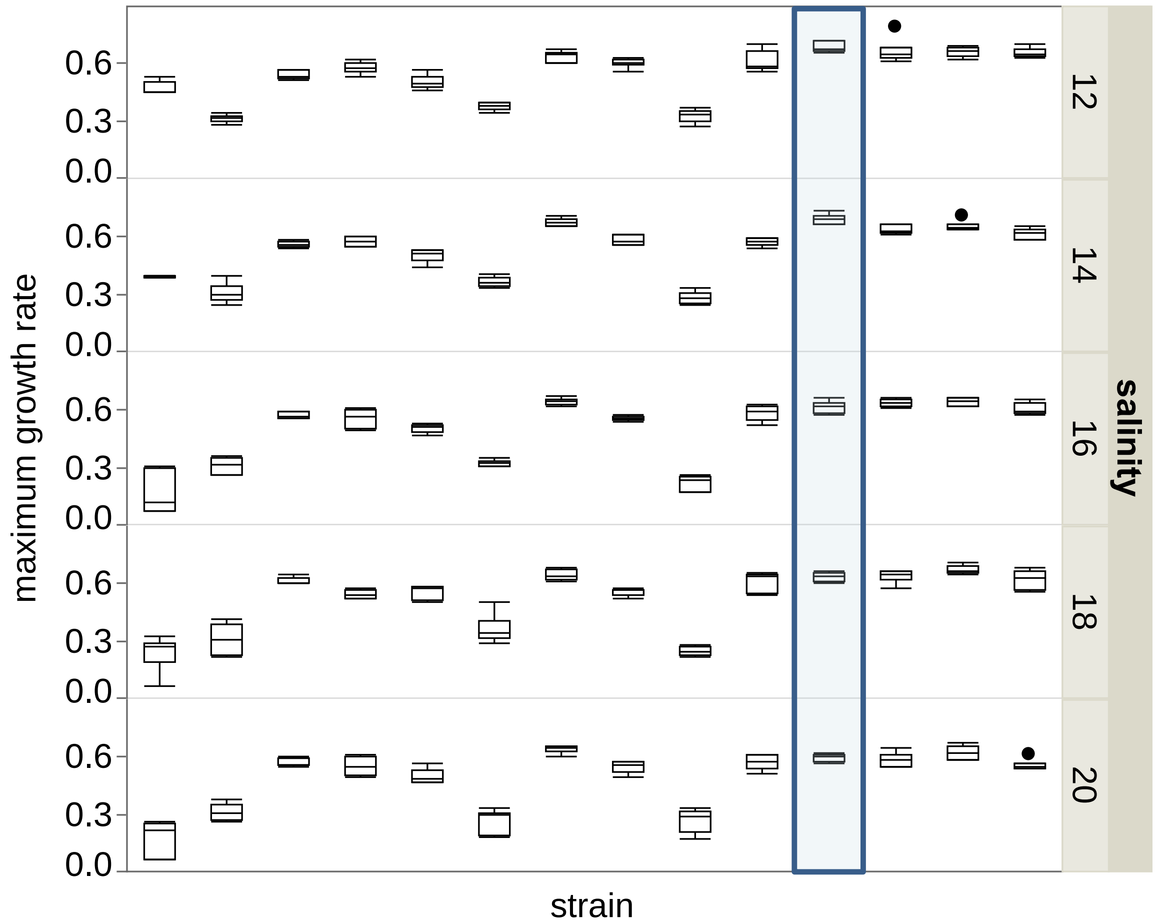
Within the bioassay data, a single top performer (black line) was revealed. This strain was a production strain at Sapphire Energy and is our baseline strain for consortia comparisons.



3. Accomplishments/Progress/Results

Task 2 (Design of Consortia & Tracking Tools)

1) strain data compiled
2) screenings to enhance database *(added assays to overcome data gaps)*
   - salinity

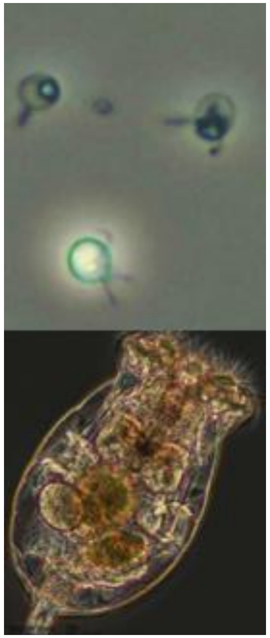


Salinity screenings showed some differential tolerances by some strains. Baseline strain (indicated by box) grew well under all salinities.

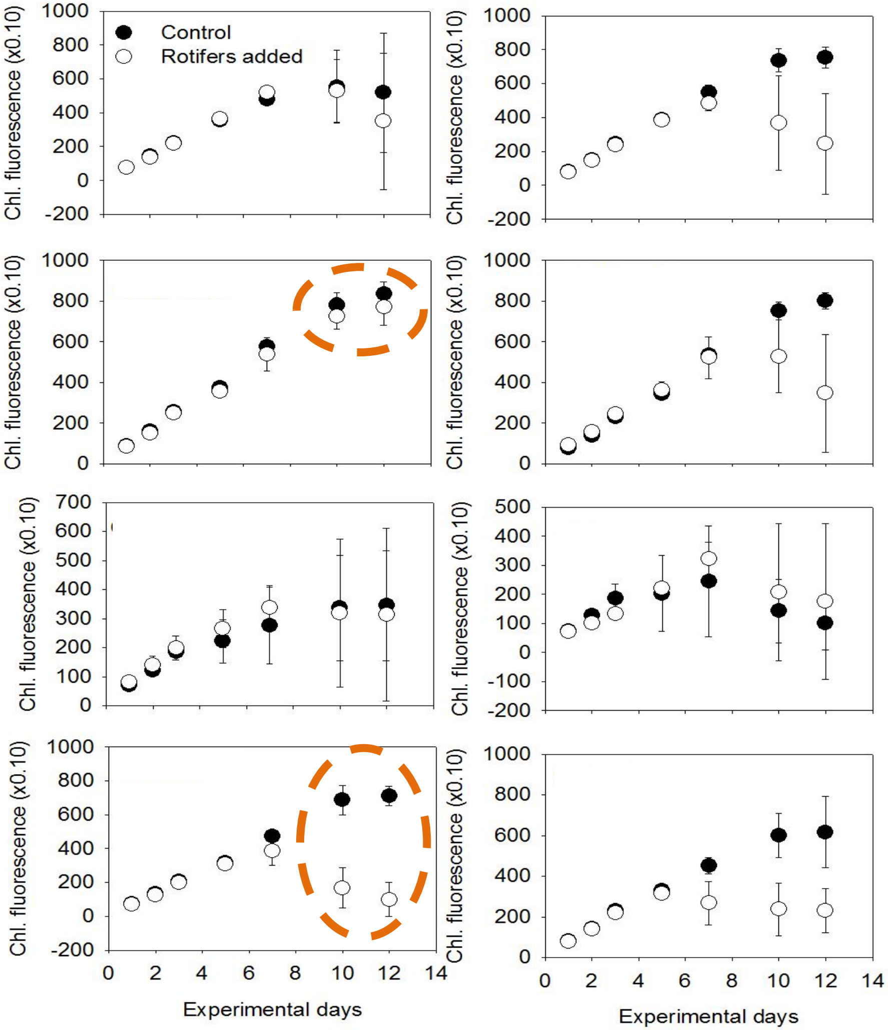
3. Accomplishments/Progress/Results

Task 2 (Design of Consortia & Tracking Tools)

1) strain data compiled
2) screenings to enhance database *(added assays to overcome data gaps)*
   - salinity
   - pests



Pest challenges showed differential tolerances (i.e., crash rates).



3. Accomplishments/Progress/Results

Task 2 (Design of Consortia & Tracking Tools)

1) strain data compiled
2) screenings to enhance database *(added assays to overcome data gaps)*
   - salinity
   - pests
3) 9 consortia designed

#	Rationale
1	top performing strains from each salinity
2	top performing strains from each salinity plus baseline strain
3	top performing strains from temperature screenings
4 & 5	top performing strains from temperature and salinity screenings (two species compositions)
6	consortia with new field isolates
7	kitchen sink (non-rational design for comparison)
8	strains with different intrinsic growth rates
9	strains with known genetic diversity

3. Accomplishments/Progress/Results

Task 2 (Design of Consortia & Tracking Tools)

1) strain data compiled
2) screenings to enhance database *(added assays to overcome data gaps)*
    - salinity
    - pests
3) 9 consortia designed
4) molecular tracking tool developed and validated *(changed target gene to overcome technical barrier)*

Gene/ loci	Amplicon size	Species SNPs	Strain SNPs
cox1	322	5-40	0
rbcL	990	4-76	0
ITS	324	6-50	0-6
ccsA	**415**	**6-50**	***N. salina*** ***N. oceanica***
40 *Nannochloropsis* strains including the following species*: australis, gaditana, salina, oculata, granulata, oceanica, limnetica*			

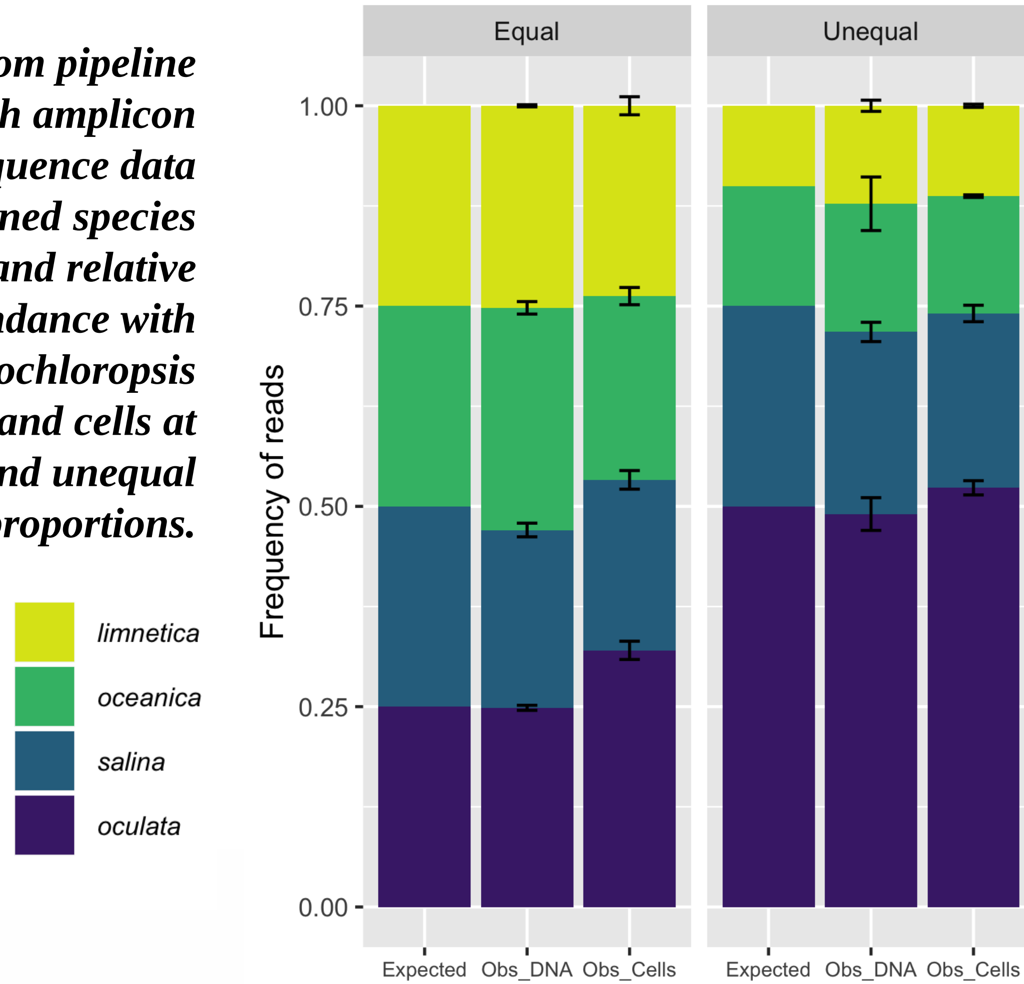
Evaluation of conserved genes to differentiate species and strains revealed chloroplast ccsA gene a good candidate, compared to cox1 with multiple copies in Nannochloropsis.

3. Accomplishments/Progress/Results

Task 2 (Design of Consortia & Tracking Tools)

1) strain data compiled
2) screenings to enhance database *(added assays to overcome data gaps)*
   - salinity
   - pests
3) 9 consortia designed
4) molecular tracking tool developed and validated *(changed target gene to overcome technical barrier)*

Custom pipeline with amplicon sequence data determined species and relative abundance with Nannochloropsis DNA and cells at equal and unequal proportions.

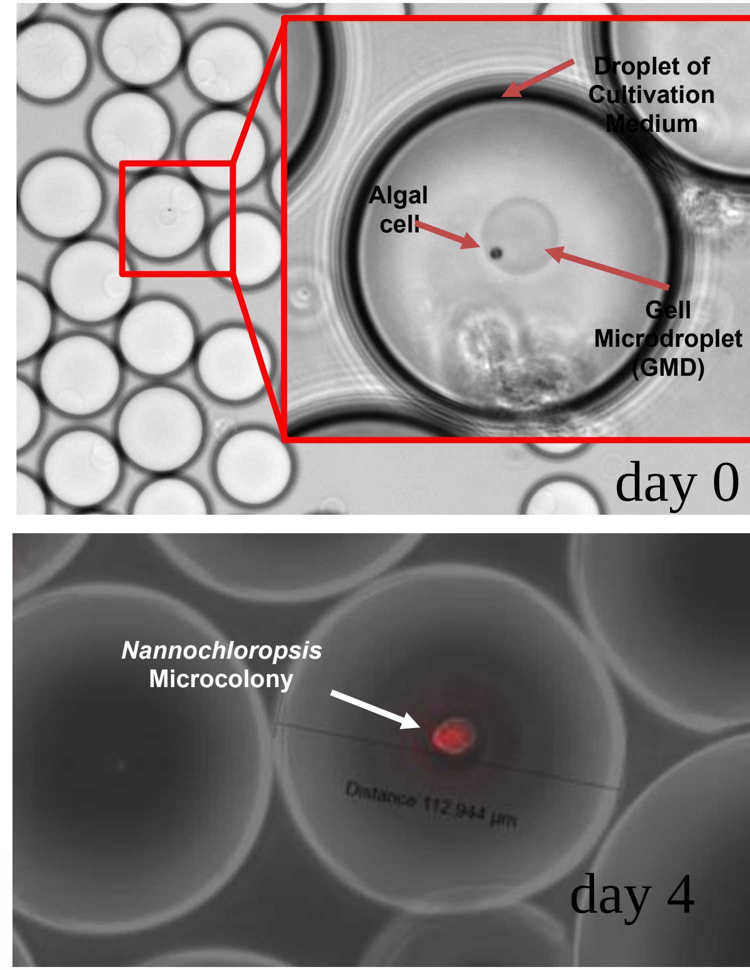


3. Accomplishments/Progress/Results

Task 3 (Screening & ID of Growth-Promoting Bacteria)

1) HiSCI system optimized for *Nannochloropsis*

Novel platform HiSCI (High-throughput screening of cell-to-cell interactions), previously used for larger cells, optimized for Nannochloropsis cells and used with field medium.



3. Accomplishments/Progress/Results

Task 3 (Screening & ID of Growth-Promoting Bacteria)

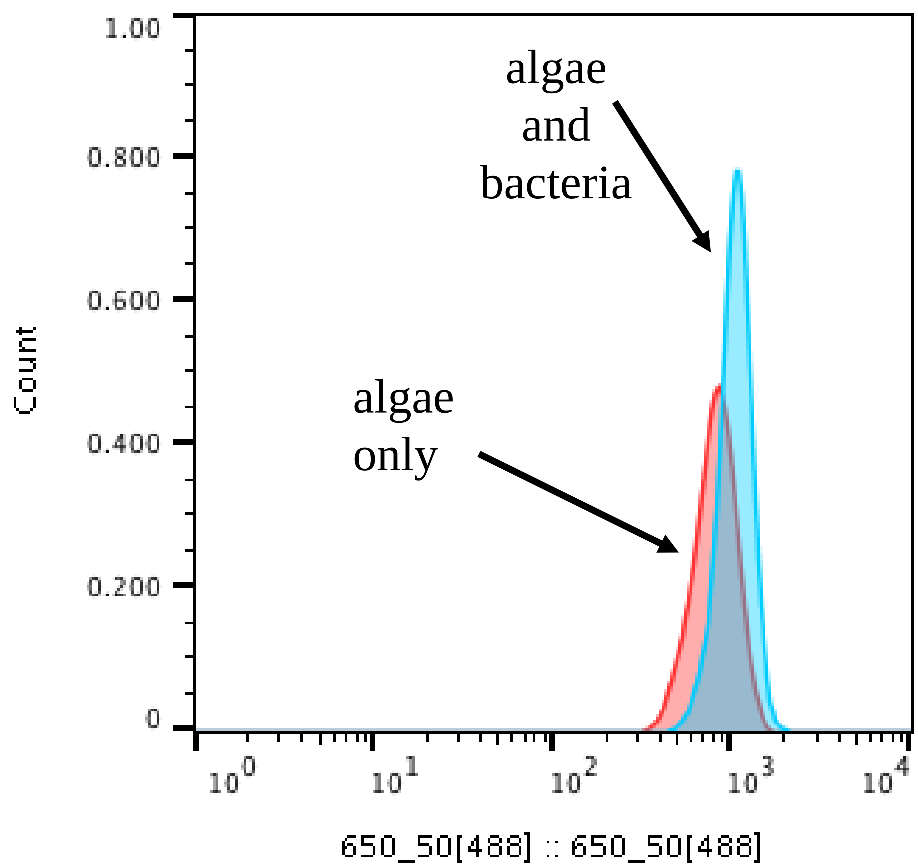
1) HiSCI system optimized for *Nannochloropsis*
2) environmental samples collected

Environmental samples collected from 30+ locations across NM, including Sapphire and Fabian Garcia ponds.

3. Accomplishments/Progress/Results

Task 3 (Screening & ID of Growth-Promoting Bacteria)

1) HiSCI system optimized for *Nannochloropsis*
2) environmental samples collected
3) screenings conducted
   - 3 iterations with environmentally-sourced bacteria
   - 3 isolates being scaled for further testing



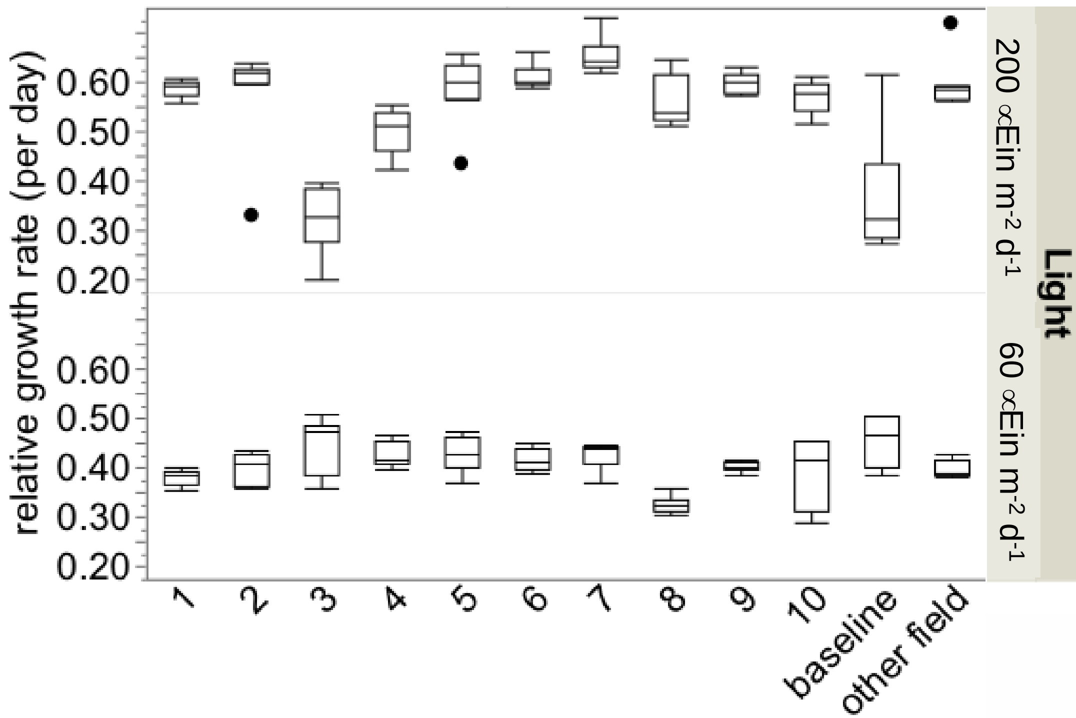
Initial data show increased algal productivity, verified by cell sorting and microscopy during each round of selection when compared to controls.

3. Accomplishments/Progress/Results

Task 4 (Verification & Optimization of Consortia Performance)

- intrageneric *Nannochloropsis* (algae only) consortia testing in progress
  1) high-throughput bioassays

High-throughput assays in field medium with 12/12 LD cycle and diel temperature changes (20 to 30°C) showed higher productivity by some of the consortia (labeled 1-10) than the baseline field strain under higher irradiance.

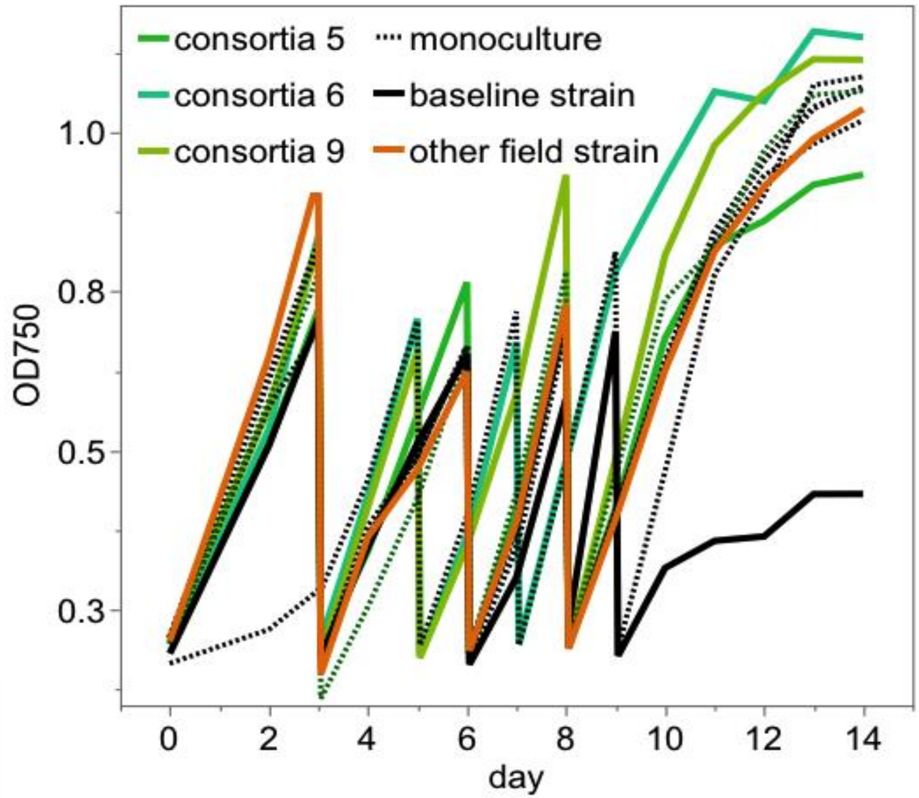


3. Accomplishments/Progress/Results

Task 4 (Verification & Optimization of Consortia Performance)

- intrageneric *Nannochloropsis* (algae only) consortia testing in progress
  1) high-throughput bioassays
  2) flask-scale bioassays

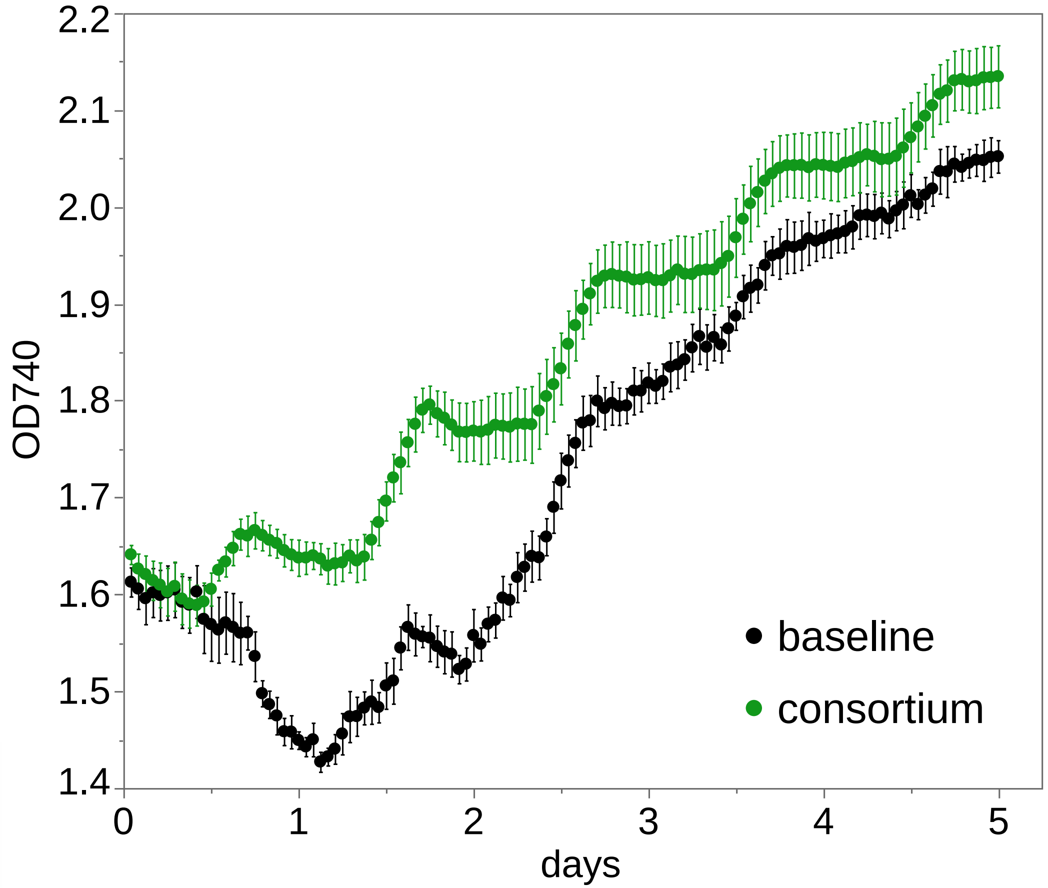
Flask-scale assay (50 mL) in field medium with 12/12 LD cycle and diel temperature changes (20 to 30 °C) showed higher productivity by consortia than baseline field strain (which crashed after final dilution).



3. Accomplishments/Progress/Results

Task 4 (Verification & Optimization of Consortia Performance)

- intrageneric *Nannochloropsis* (algae only) consortia testing in progress
  1) high-throughput bioassays
  2) flask-scale bioassays
  3) bioreactor trials



Bioreactor trial under field-relevant light and temperature profiles showed lag in growth by baseline strain upon inoculation compared to 4-strain consortium (n = 3).

3. Accomplishments/Progress/Results

Summary of Milestones

Measure	Description	Due Date	Status
GNG.1	Technical and Cultivation Readiness Validated	30-Apr-2018	✓
2.1. ML	DNA molecular tracking tool developed to differentiate at least 4 species of *Nannochloropsis*	31-Jul-2018	✓
2.2. ML	At least two *Nannochloropsis* consortia designed based on two key functional characteristics (e.g. growth rate, temperature, salinity, pest tolerance)	31-Oct-2018	✓
3.1.ML	HiSCI system modified to achieve one *Nannochloropsis* cell per microdroplet and viable recovery of cells in Las Cruces, NM fall light and temperature conditions	31-Jan-2019	✓
3.2. ML	Bacteria collected for screening from at least 20 different sources	31-Jan-2019	✓
3.3. ML	At least one bacterium identified that improves biomass production rates ($g/m^2/day$) >30% in fall cultivation simulations (Las Cruces temperature and light profiles in September, grown in field cultivation media) for one strain/species of *Nannochloropsis* over the monoculture baseline	30-Apr-2019	**In Progress**
4.1. ML	Intrageneric consortia demonstrating a 30% productivity improvement over a *Nannochloropsis* monoculture grown in triplicate under fall Las Cruces environmental conditions in commercial cultivation media	31-Jan-19	✓

4. Relevance

Project Goal:

- double productivity of open, outdoor *Nannochloropsis* cultures via through **intrageneric consortia & algae-bacteria consortia**
- important in its utilization of ecological and high throughput approaches

Industry Relevance:

- project has potential to advance the state of technology and enhance the economic viability of algal biofuels **by increasing yield & reducing losses in cultivation systems**
- project supports BETO goals by **advancing toolkits and technologies** to meet 2020 DOE PEAK targets of 18 $g/m^2/d$ and 80 GGE ton^{-1} biomass

Technology Transfer:

- consortia will be available for use in industry
- information will be available to broader community via publications

5. Future Work

Task 3 (Screening & ID of Growth-Promoting Bacteria)

- **Main Tasks**
  - scale-up to larger volumes in flasks to verify phenotype(s)
  - new HiSCI runs with field isolate and known growth-promoting taxa
- **Stretch Goal**
  - assays with taxa described in literature or acquired from collaborators (Sandia)
- **Milestones**
  - **3.3.ML –** At least one bacterium identified that improves biomass production rates >30% in fall cultivation simulations for one strain/species of *Nannochloropsis* over the monoculture baseline
  - **GNG.2 –** Bacteria-augmented *Nannochloropsis* cultures demonstrate a 30% productivity improvement over the monoculture baseline

mitigation: low-throughput screening with known beneficial taxa

5. Future Work

Task 4 (Verification/Optimization of Consortia Performance)

- **Main Tasks**
  - scale-up to larger volumes to verify phenotype(s)
  - continue perturbations
- **Stretch Goal**
  - testing of intergeneric consortia
- **Milestones**
  - **4.1.ML –** Intrageneric consortia demonstrating a 30% productivity improvement over a *Nannochloropsis* monoculture grown in triplicate under fall Las Cruces environmental conditions in commercial cultivation media
  - **GNG.3 –** Simulations or fall field trials demonstrating productivity improvements over the monoculture baseline to reach target of 14 $g/m^2/d$

mitigation: UV mutagenesis, intergeneric consortia

5. Future Work

Task 5 (PEAK Field Challenge)

- **Main Tasks (at NMSU)**
  - scale consortia members for field trials
  - grow consortia and baseline control in miniponds (25 cm depth, 300-L) semi-continuously for >2 growth cycles and sample for metrics of biomass
- **Stretch Goal**
  - repeat with pest challenges or intergeneric consortia
- **Milestones**
  - **5.1 ML –** Algae and bacteria inocula scaled to >200L for 300-L PEAK field challenge
  - **5.2.ML –** Field trials demonstrating >30% productivity and stability improvements over monoculture baseline for a target of at least 14 g/m^2/d in the fall growth season (Sept-October)

Summary

- Overview: project aimed to improve productivity and robustness of strains, resulting in a doubling of fall productivity
- Approach: development of **intrageneric algal consortia via rational design** and **algal-bacteria consortia via high throughput screening**
- Accomplishments/Progress/Results
  - Task 2: strain data compiled, additional screenings conducted, 9 consortia designed, molecular tracking tool developed and validated
  - Task 3: HiSCI system optimized, >30 environmental samples collected, 3 iterations conducted, additional screenings in progress
  - Task 4: high-throughput and flask-scale bioassays completed, additional trials in progress
- Future Work: continuation of Tasks 3 and 4 (consortia development and testing) and PEAK Field Challenge
- Relevance: **direct relevance to the bioenergy industry** because project addresses challenges associated with open ponds