Los Alamos NATIONAL LABORATORY

DOE Bioenergy Technologies Office (BETO) 2021 Project Peer Review

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

March 2021
Advanced Algal Systems

Alina Corcoran, New Mexico Consortium (NMC)
Shawn Starkenburg, Los Alamos National Laboratory (LANL)

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Background and Project Overview

Project Goal: to increase stability/yield of open, outdoor ponds via (rational) eco-engineering of consortia

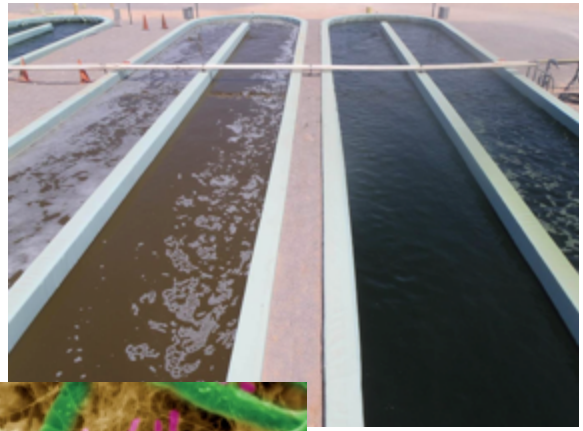
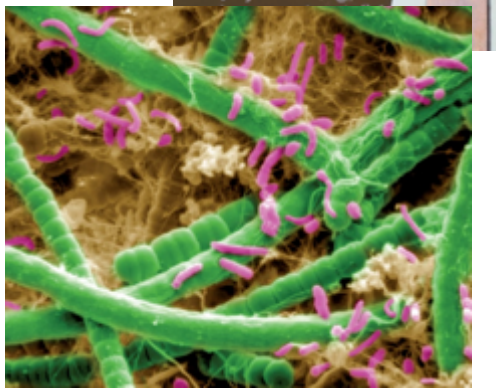


photo of "dead" and live ponds
photo credit: Sapphire Energy, Inc.

Motivation: Open pond systems challenged by low productivity and stability due to environmental stressors

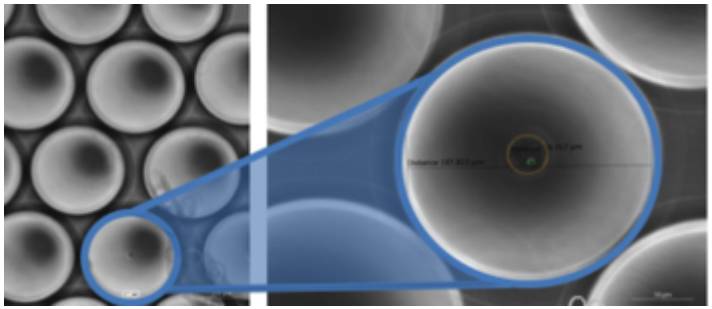
- fluctuations in abiotic factors such as temperature
- invading organisms (competitors, predators, parasites

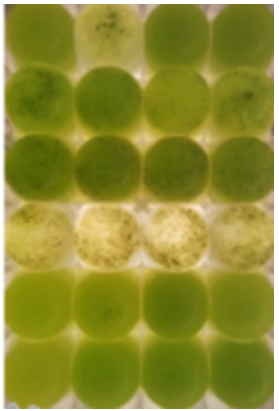


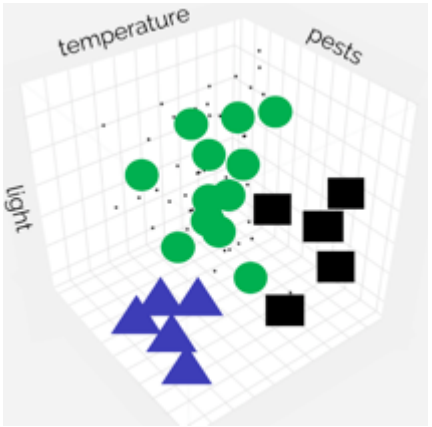
Learning from nature: microbial consortia
photo credit: A. Dohnalkova/ PNNL

Background and Project Overview

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



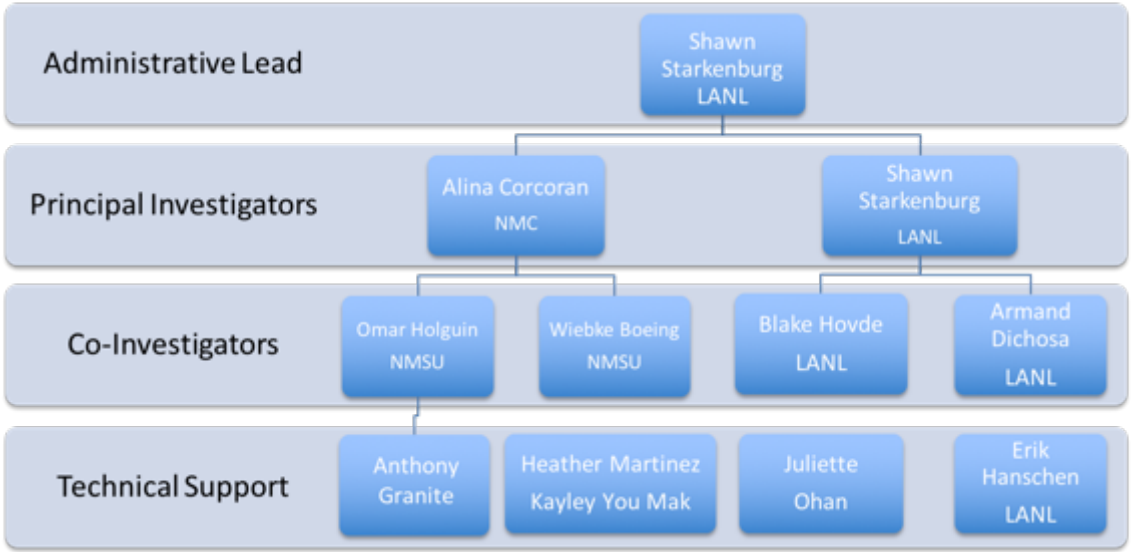




Management

Co-PI Leadership Structure

Weekly PI meetings
Weekly Full Team Meetings
Quarterly Reporting
6 week update calls with BETO PM



Risk Mitigation

- Managed through advanced planning (added mitigation tasks in the SOPO)
- Cross Training/overlapping capability at partner institutions to fill gaps when needed
- Frequent cross-site visits (enabled by proximity of partners)
- Production strains maintained/stored at 2 locations
- 2 “Beta” Field Trial Campaigns prior to PEAK Cultivation Challenge

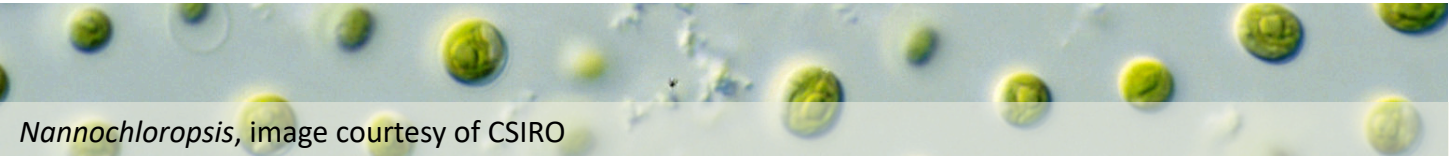
Management – Task Structure & Leads

#	Task	Leads	Site	Key Team Members
1	Validation. (M4-9)	Starkenburg/ Corcoran	LANL/ NMC	All team members
2	Design of Nannochloropsis Consortia & Tracking Tools (M4-9)	Corcoran/ Starkenburg	LANL/ NMC	Nathan Garcia (post-doc) Shovon Mandal (post-doc) Erik Hanschen (post-doc)
3	Screening & Identification of Growth-Promoting Bacteria (M4-15)	Starkenburg/ Hovde	LANL	Juliette Ohan (post-masters student) Kayley You Mak (post-bac student
4	Verification & Optimization of Consortia Performance (M7-27)	Starkenburg/ Corcoran	LANL/NMC/ NMSU	Shovon Mandal (post-doc) Anthony Granite (grad. Student) Rakib (post-doc)
5	PEAK Challenge (M28-36)	Holguin/ Boeing	NMSU	Heather Martinez (technician)

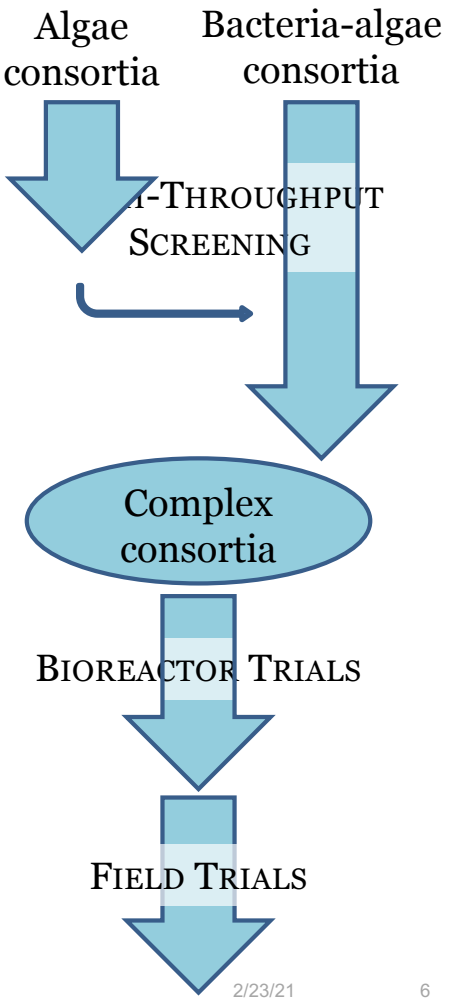
Approach

- Two pipelines:
  - rationally-designed algal consortia (combination of strains with complementary traits)
  - algal-bacteria consortia (high-throughput screening)
- species and strains within a single algal genus *Nannochloropsis* (Eustigmatophyceae)
  - constraint driven by economics of downstream processing
  - allows us to address questions of genetic and functional diversity at species and strain levels

- Winning consortia tested in bioreactor experiments and field trials



Nannochloropsis, image courtesy of CSIRO

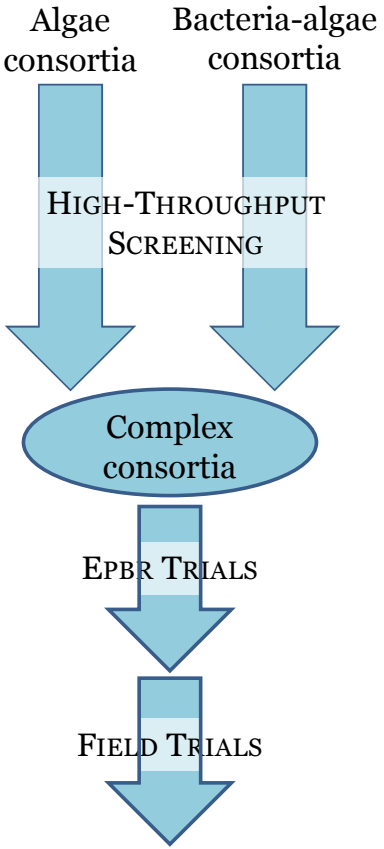


Impacts

- Project Outcomes:
  - Improve industrial economics of production systems by mitigating low crop productivity and stability due to environmental perturbations
  - (originally partnered with Sapphire Energy)

- 'Toolkits':
  - Microfluidics screening/culturing pipeline to identify probiotic bacteria
  - consortia libraries
  - genome sequences of novel *Nannochloropsis* strains
  - Diagnostic assay for multi-species/multistrain consortia

- Publications: Published (1), Submitted (1), In prep (2)
- Led to collaboration with Qualitas Health to address/test crop stability and pest management

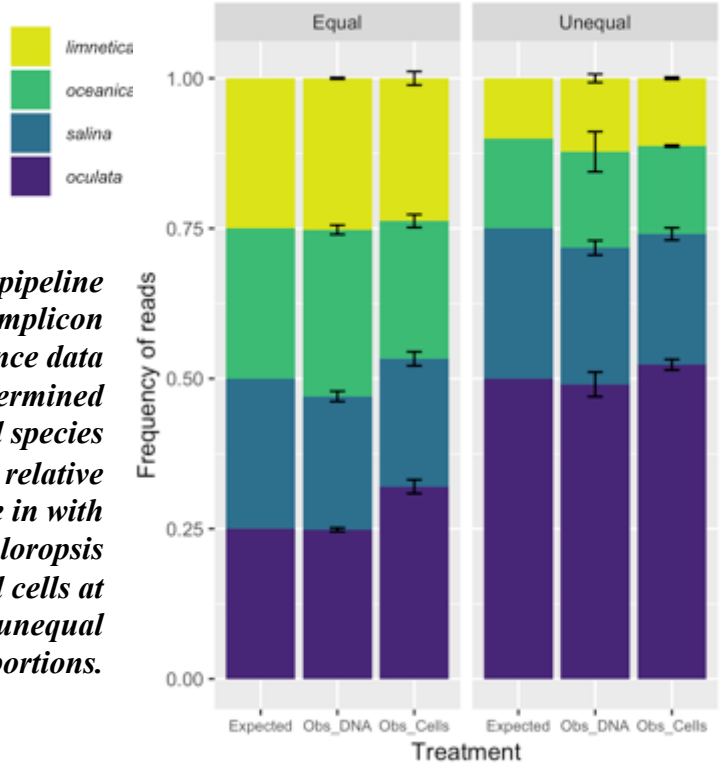


Progress and Outcomes: Intrageneric Consortia

Molecular tracking tool developed and validated

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*: australis, gaditana, salina, oculata, granulata, oceanica, limnetica,* and other species			

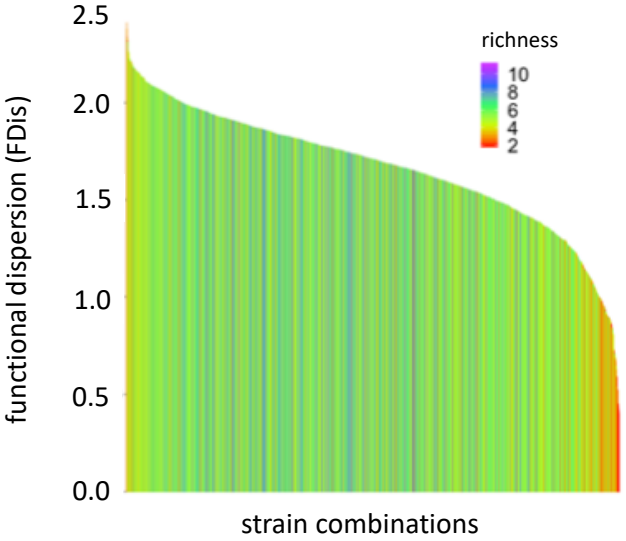
Evaluation of conserved genes to differentiate species and possibly strains revealed chloroplast ccsA gene a good candidate, compared to cox 1 with multiple copies in Nannochloropsis.

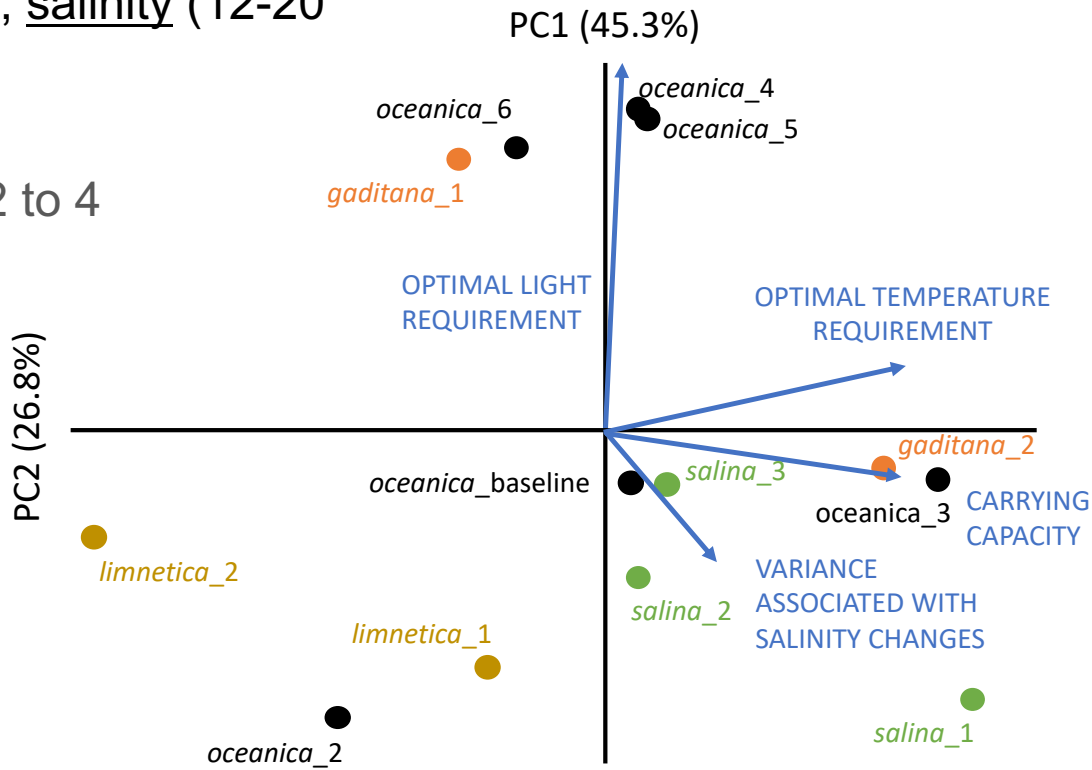


Custom pipeline with amplicon sequence data determined identified species and relative abundance in with Nannochloropsis DNA and cells at equal and unequal proportions.

Calculated functional dispersion of strains across gradients of temperature (10-35°C), salinity (12-20 PSU), irradiance

- Screened 50 consortia
- Top 5 consortia had richness of 2 to 4

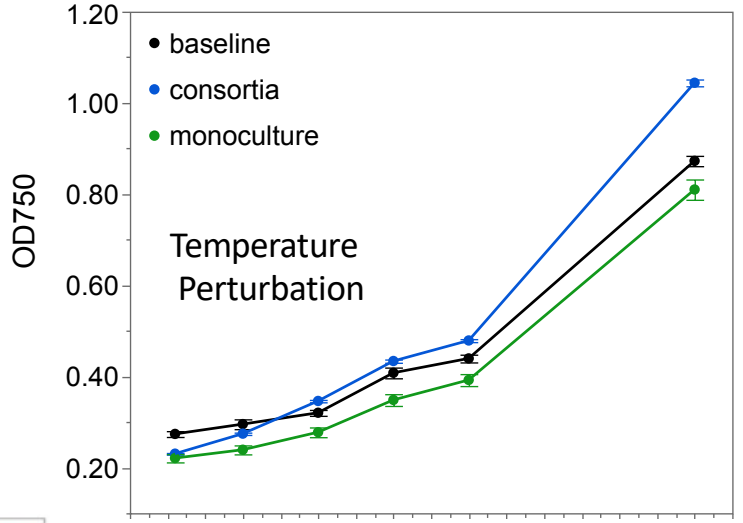


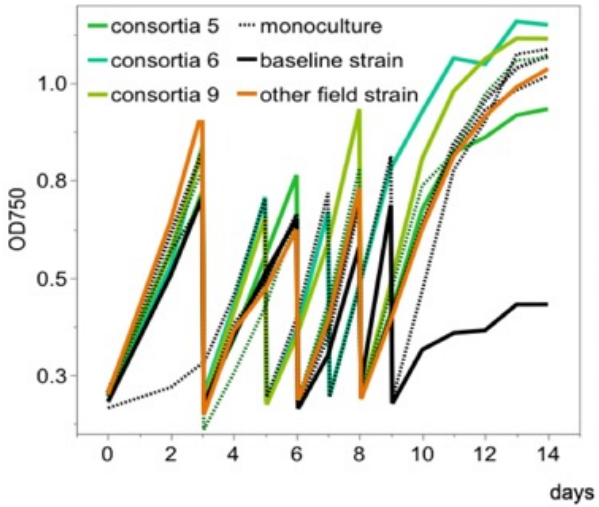


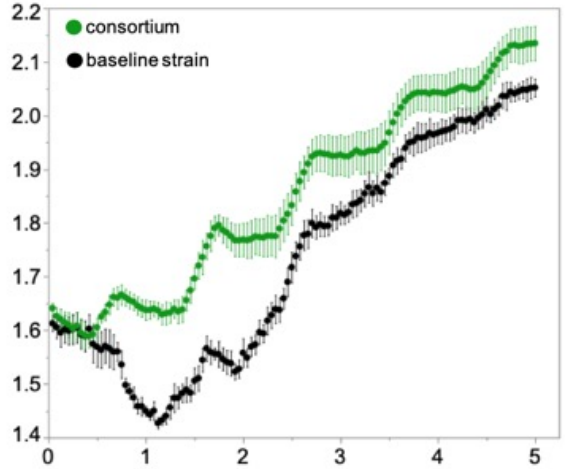
Shovon and Corcoran, 2021, submitted.

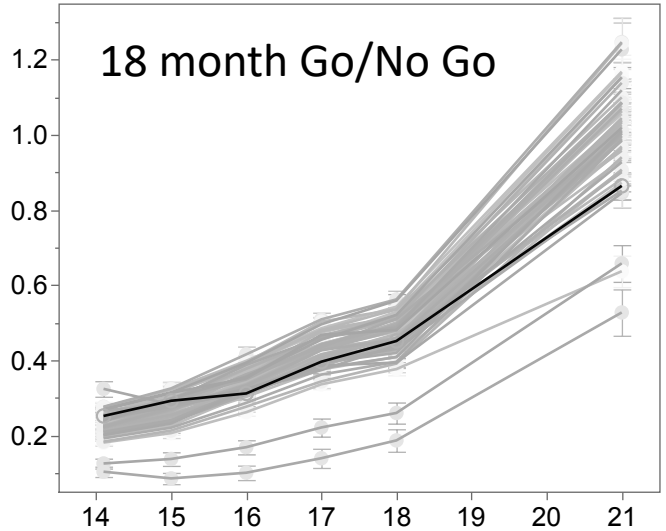
Consortia outperformed baseline strain in both well-plate and reactor formats

Consortia effects found under sub-optimal conditions (e.g., sick baseline strain, media issues, low temperature perturbation)





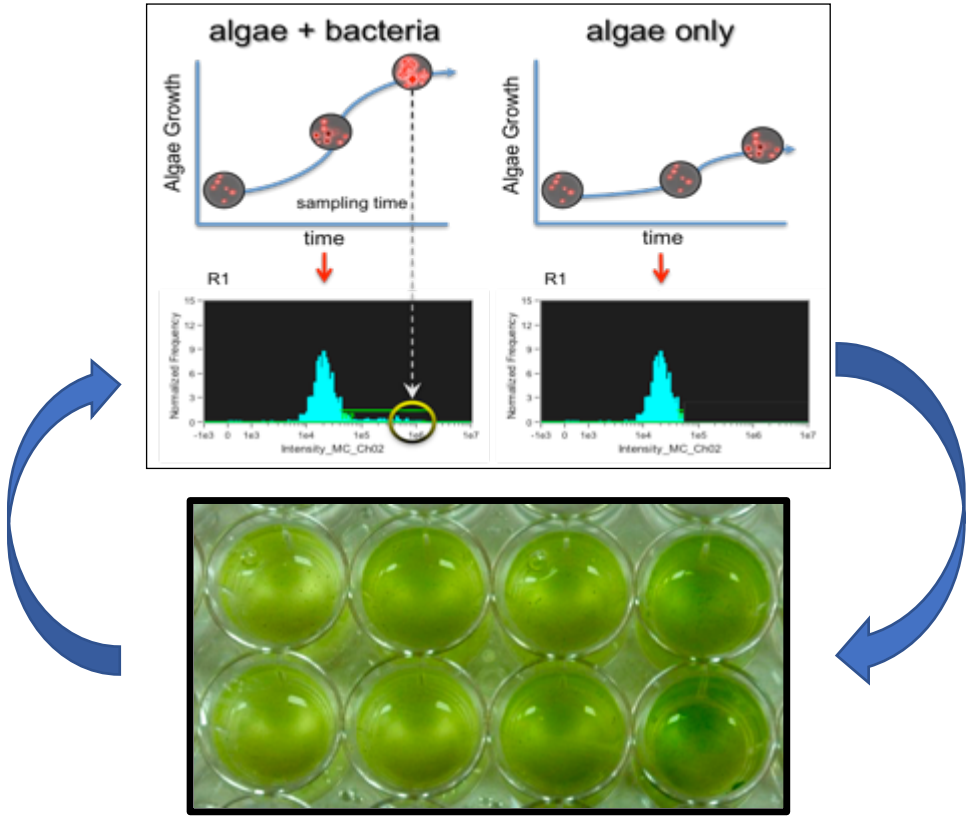




Progress and Outcomes: Algal-Bacterial Consortia

High Throughput Screening of Cell-to-Cell Interactions (HiSCI)

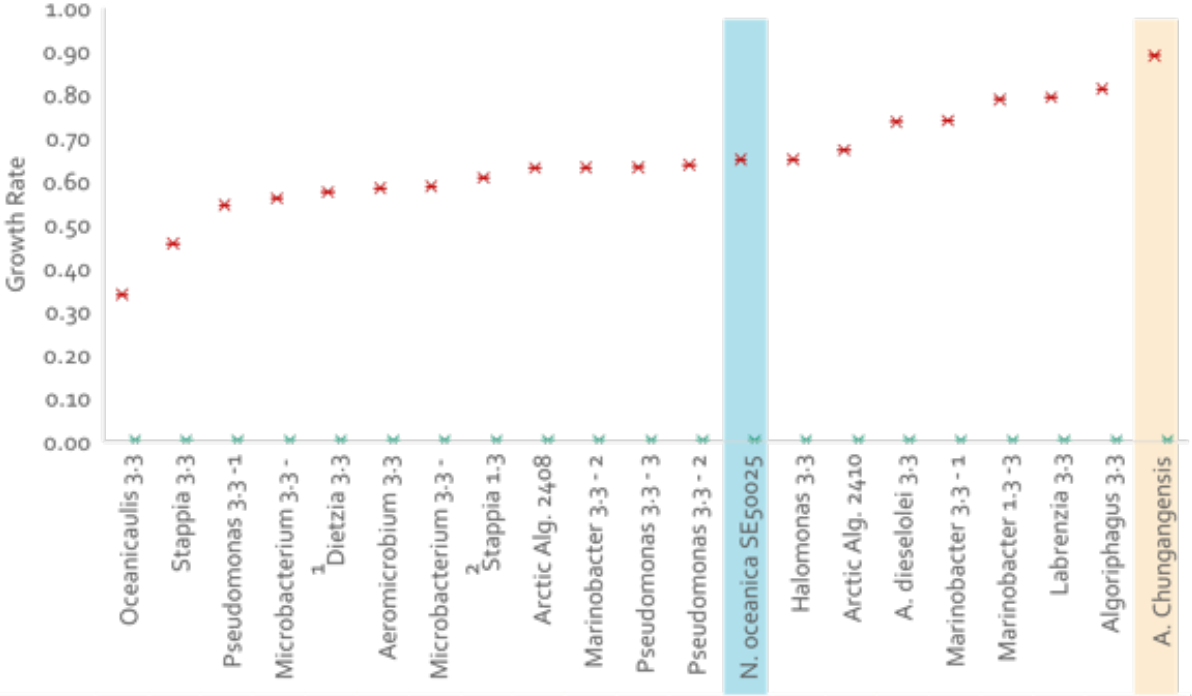
- Deployed microfluidics approach to screen thousands of artificially-created microbial communities
  - ➢ sourcing of environmental samples
  - ➢ encapsulation of bacteria with the baseline algal strain
  - ➢ sort to select improved phenotypes
  - ➢ rounds of iteration/scale-up



J. Ohan, et al 2019. *Biotechniques*

Algal-Bacterial Consortia Validation Tests

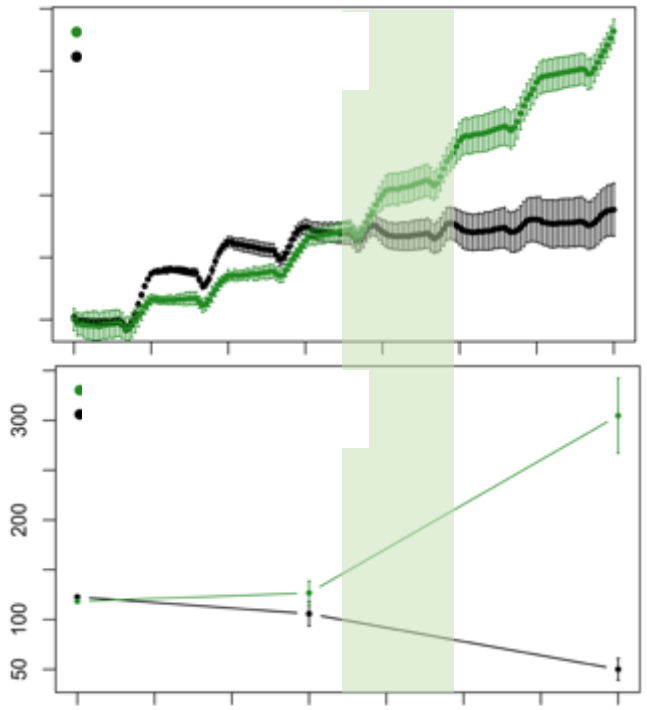
- Addition of bacterial Isolates outperformed algal monoculture baseline strain
- Effects of growth enhancements were temperature dependent



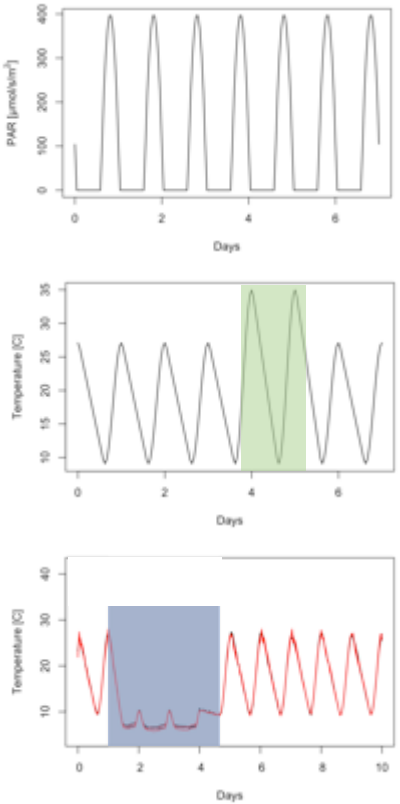
% change																				
hr. 20-116, 25C	-48	-30	-16	-14	-12	-10	-10	-6	-3	-3	-3	-2	0	0	3	13	14	21	22	25	37
+/-	6	4	7	7	6	7	7	4	7	8	6	8	15	5	6	9	7	6	7	8	4
hr. 165-211, 14C	-19	-1	21	-16	-3	22	22	7	15	-7	2	-2	0	18	4	22	17	-12	6	-9	22
+/-	10	8	4	21	6	9	9	6	9	24	19	7	19	6	11	10	8	13	10	7	12

Perturbation studies demonstrate improved stability and recovery with added bacteria

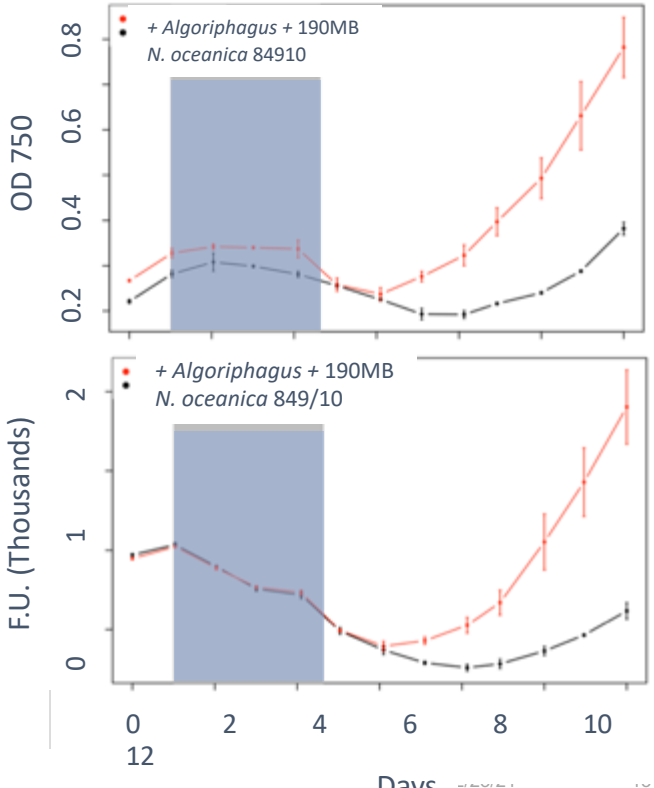
A. Suspect Media, Heat Shock



Parameters

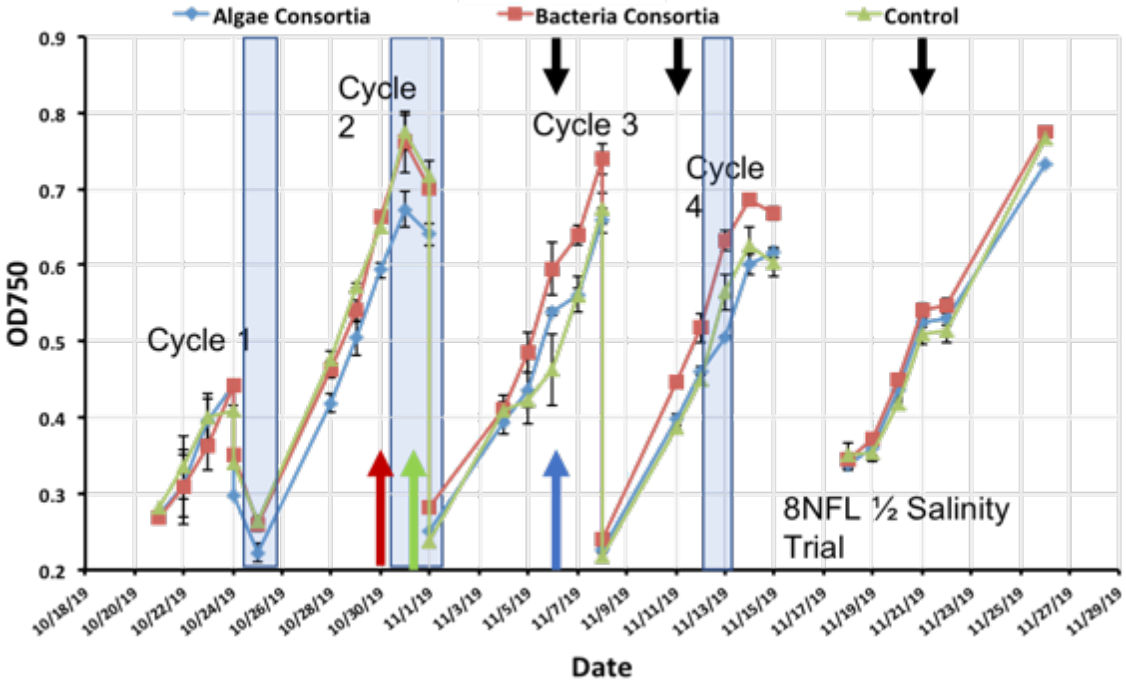


B. Cold Shock



‘Beta’ Field Trials

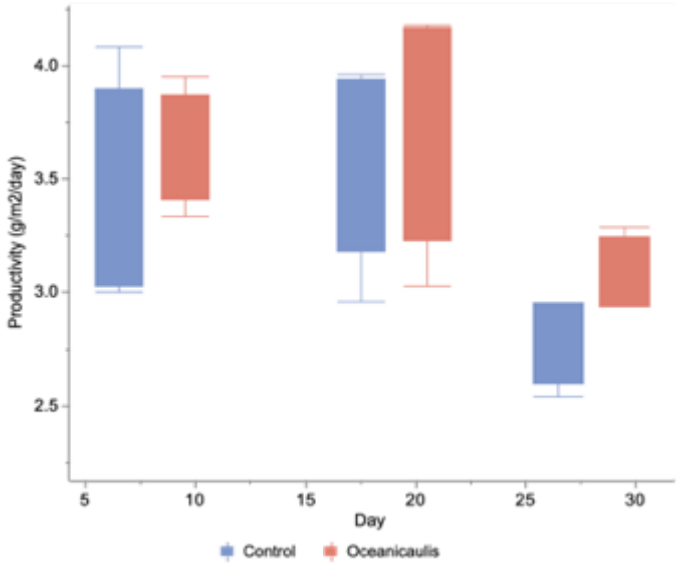
- Preliminary evaluation of consortia compared to the baseline strain for improvements in productivity and stability
- Results: Greater stability (lower variance in biomass) in consortia than in control cultures during third growth cycle

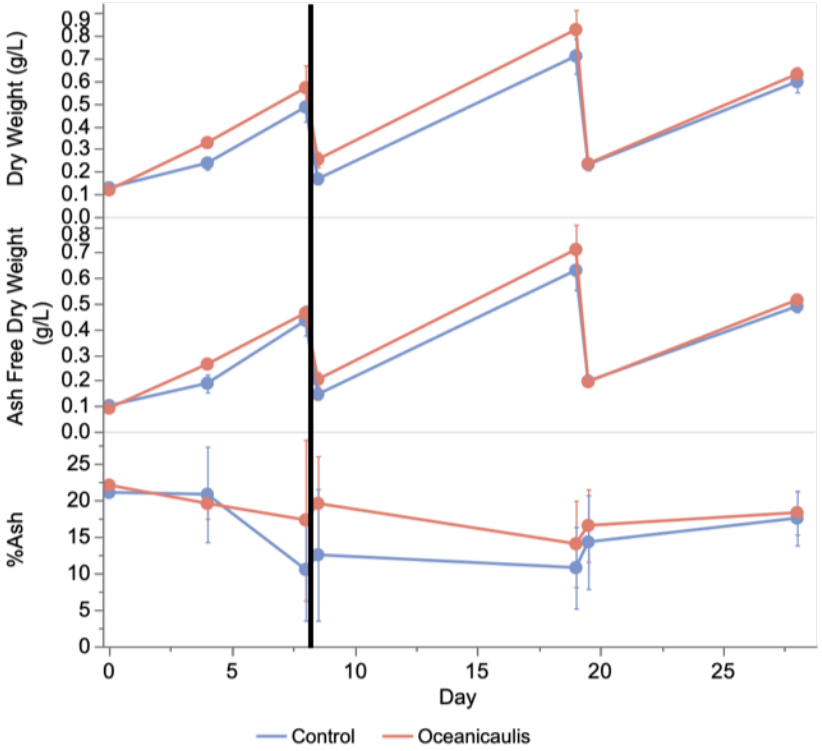


* Colored boxes represent freezing air temperatures
* Black arrows represent decreased solar irradiance

PEAK Field Challenge

Algal-Bacterial Consortia demonstrated modest/minor improvement in productivity over baseline strain





Black line = harvest event

Summary & Conclusions

- Functional diversity is present within *Nannochloropsis* species and strains, along axes of light, temperature, and salinity
- Average consortia productivity is greater than average monoculture productivity
- When cultivation conditions are not ideal, consortia are more stable and productive than the best performing strain
- Consortia are most likely to be advantageous over longer periods and under sub-optimal conditions
- Productivity improvements are condition specific (volume, physical perturbation (shaking), temperature) and possibly strain dependent/specific
- Critical to Implement “Straight-to-field testing” as a best practice
- Improvements with engineered bacteria consortia challenged by competition with natural pond bacteria.

Quad Chart Overview

Timeline

Start date -- February 1, 2018

End date -- January 31, 2021 (NCE June 2021)

	FY20 Costed	Total Award
DOE Funding	LANL: $430 K NMC: $480 K	LANL: $1.19 M NMC: $1.29 M
Project Cost Share	LANL: $0 NMC: $67 K	LANL: $0 NMC: $276 K

Project Partners

- New Mexico Consortium
- Los Alamos National Laboratory
- New Mexico State University

Project Goal

Improve the productivity and robustness of algae strains against environmental perturbations

End of Project Milestone

Annual Productivity and Yield Targets of >18 $g/m^2/d$ and >117 GGE ton^{-1}, respectively extrapolated from Fall Cultivation (14 $g/m^2/d$) with consistent Biomass Composition (35% protein, 13-15% FAME, 25% carbohydrate)

Funding Mechanism: 2017 DE-FOA-0001628

Productivity Enhanced Algae and Tool-Kits

Topic Area 2: Cultivation Biology Improvement

ADDITIONAL SUPPORTING SLIDES

Publications and Presentations

Corcoran A.A., E. Hanschen, S. Mandal, B. Hovde, J. Ohan, K.Y. Mak, N. Garcia, and S.R. Starkenburg. Use of synthetic ecological approaches and high throughput screening to generate microbial consortia for large-scale algal production systems. *Oral Presentation.* Algae Biomass Summit. September 2019. Orlando, FL.

Corcoran A.A., E. Hanschen, S. Mandal, B. Hovde, J. Ohan, N. Garcia, and S.R. Starkenburg. Use of synthetic ecological approaches and high throughput screening to generate microbial consortia for large-scale algal production systems (doi.org/10.7490/f1000research. 1117372.1). *Oral Presentation.* Ecological Society of America Annual Meeting. August 2019. Louisville, KY.

Ohan, J., C. Miller, A.A. Corcoran, B. Hovde, and S.R. Starkenburg. High-throughput screening of bacteria that improve the productivity of *Nannochloropsis oceanica*. 9th International Conference on Algal Biomass, Biofuels and Bioproducts. June 2019. Boulder, CO.

Starkenburg, S.R., A.A. Corcoran, B. Hovde, E. Hanschen, and N. Garcia. Increasing cultivation yield and stability with rationally designed consortia. *Oral Presentation*. Algal Biomass Summit. October 2018. Houston, TX.

Hovde, B., S. Steichen, K. Martinez, A.A. Corcoran, J. Brown, and S. Starkenburg. Genome assembly of three bacterial pathogens that target *Nannochloropsis* and *Chlorella* algal production strains. *Oral Presentation*. 8th International Conference on Algal Biomass, Biofuels and Bioproducts. June 2018. Seattle, WA.

Ohan J, Pelle B, Nath P, Huang JH, Hovde B, Vuyisich M, Dichosa AE, Starkenburg SR. High-throughput phenotyping of cell-to-cell interactions in gel microdroplet pico-cultures. Biotechniques. 2019 May;66(5):218-224. doi: 10.2144/btn-2018-0124. PMID: 31050307.

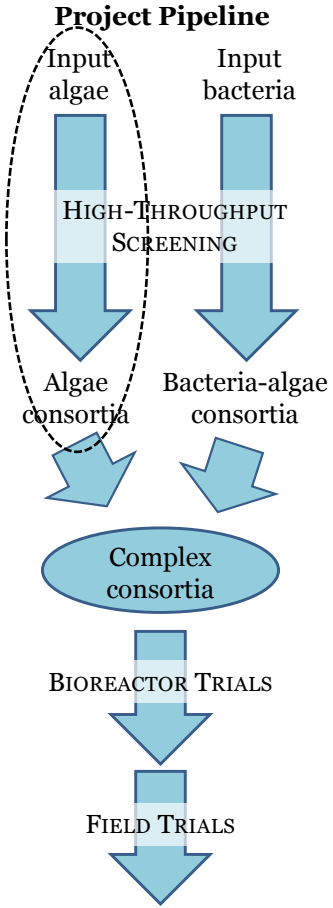
Shovon Mandal, AA. Corcoran. A novel approach to build algal consortia for sustainable biomass production. 2021. Algal Research. submitted.

Ohan, J. B. Hovde, A. Corcoran, S. Starkenburg. Identification and Validation of Bacteria that Modulate Growth of *Nannochloropsis*. 2021. In prep.

Approach Details

Task #2: Design of Candidate *Nannochloropsis* Consortia & Tracking Tools

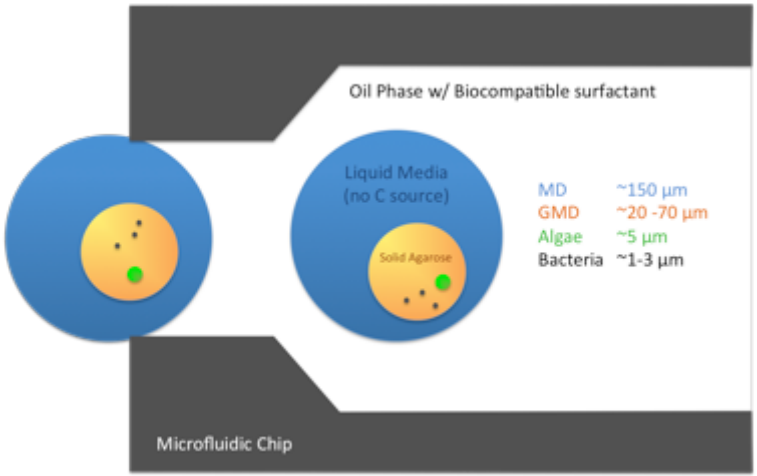
- rational design
  - match strains to outdoor regimes by combining unique strains that display different growth rates/stability under different conditions
  - use SEI, Inc. data and strains to generate consortia for testing (proposed)
- molecular tracking of strains
  - needed to distinguish consortia members
  - designed and deployed *ccsA* amplicon assay

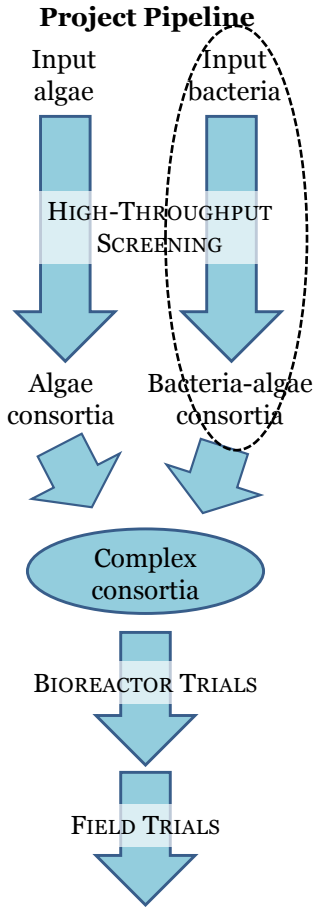


Approach

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

- modification of Hi-Sci system for *Nannochloropsis*
- collection of environmental samples
- sorting and scale-up of beneficial bacteria

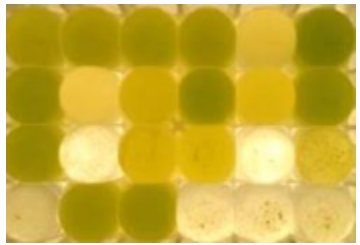


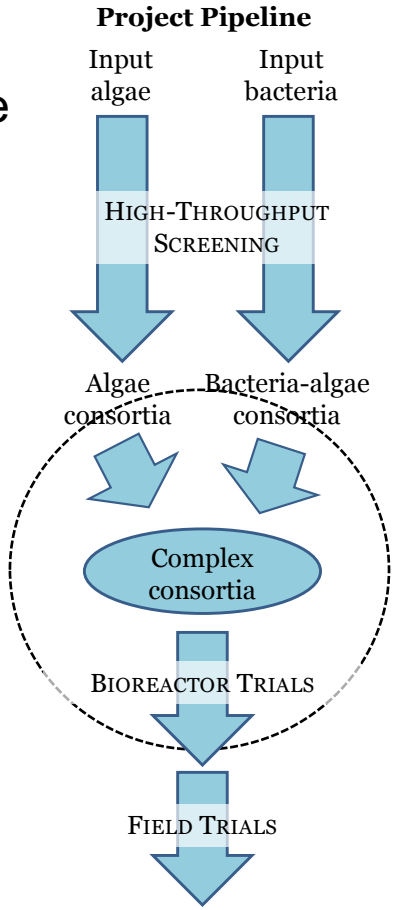


Approach

Task #4: Verification & Optimization of Consortia Performance

- individual/complex consortia tested at increasing scales
  - ➢ high-throughput screens to confirm co-existence and demonstrate proof of concept (no stressors)
  - ➢ high-throughput screens with environmental and biological perturbations (temperature fluctuations, invasions)
  - ➢ bioreactor trials to simulate Las Cruces, NM conditions
  - ➢ β-field trials in micro- (100 L) or mini- (300 L) ponds





Approach

Task #5: PEAK Field Trial

- best performing consortia validated through outdoor field trials
- 300-L miniponds (up to 12) located at the Fabian Garcia Center in Las Cruces, NM
- metrics in productivity (e.g., AFDW) and stability (e.g., temporal CV)