Continuous Biological Protection and Control of Algal Pond Productivity

U.S. Department of Energy (DOE)
Bioenergy Technologies Office (BETO)
2017 Project Peer Review

March 9, 2017

Advanced Algal Systems

PI: Michael Thelen, LLNL

Presenter: Rhona Stuart, Project Lead, LLNL

This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344

GOAL STATEMENT

- **Competitive award granted under Targeted Algal Biofuels and Bioproducts (TABB) FOA, directly addresses barriers from FOA:**
  - "Biological contamination presents one of the greatest challenges in cultivating robust, reliable algal cultures that meet target performance...novel, safe, and effective strategies need to be developed to control culture contamination events"
  - "...barrier exists in translating laboratory success to demonstrated, scalable, outdoor cultivation environments that capture all of the variables not present in laboratory systems"
- FOA sought "bench and process development scale applied R&D that focuses on: ...increased biomass productivity leading to higher yields by developing **crop protection"**

Improve the resilience of algal crops to predators and pathogens by using *probiotic* bacteria that will increase annual algae biomass yields by 5-30%.

QUAD CHART OVERVIEW

- Project start: 10/1/15
- Project end: 9/30/18
- 37% spent

	FY 15 Costs	FY 16 Costs (from 1/16)	Total Planned Funding (FY 17-project end date)
DOE Funded	--	$232K	$768K
Project Cost Share (Comp.)	--	$385k	$110k

- Barriers addressed:
  - TABB FOA barriers:
    - "high costs of producing algal biomass and low yields of target biofuel and bioproduct feedstocks produced from algae"
    - "Translating laboratory success to demonstrated, scalable, outdoor cultivation environments"
  - MYPP: Aft-A Biomass Availability and Cost

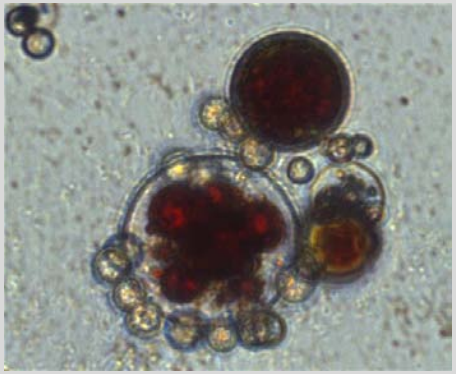
Partners	%
LLNL	34
Sandia	30
Heliae, LLC	28
UC Davis	8

Project Overview

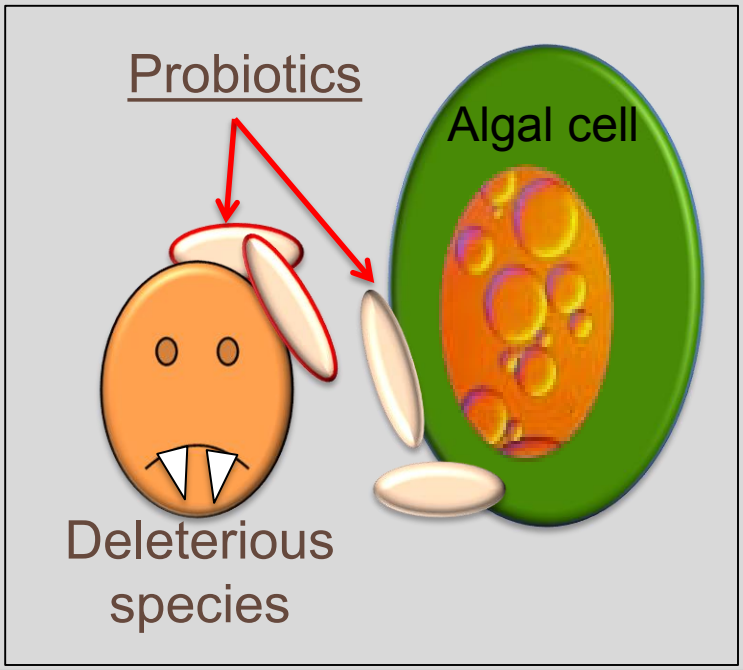
- Problem: Pond crashes lead to unpredictable losses to annual productivity
  - Currently, pests that cause crashes are treated with chemicals or extreme environments
  - Evolved resistance is a risk with these treatments
- We know the pond microbiome can be an indicator of pond health, AND that bacteria influence algal physiology
- We propose to identify and apply bacteria as a **tool for crop protection**
- We will develop a pipeline to identify and test novel probiotics and apply probiotics at increasing scale to improve algal resistance to pests (rotifers and chytrids)

Common culprits of pond instability:

Rotifers. (Photo credit: Microscopy UK)



Chytrids infecting *Haematoccocus* algae (red)



A UNIQUE TEAM

- We have the combined expertise to tackle this multidisciplinary challenge:
- **LLNL** leverages our expertise in complex microbial community analyses
- **Sandia** provide expertise in monitoring and mitigating algal pond contaminants
- Our industrial partner, **Heliae, LLC** provides years of industrial algal culturing experience
- Our partners at **UC Davis** provide modeling expertise

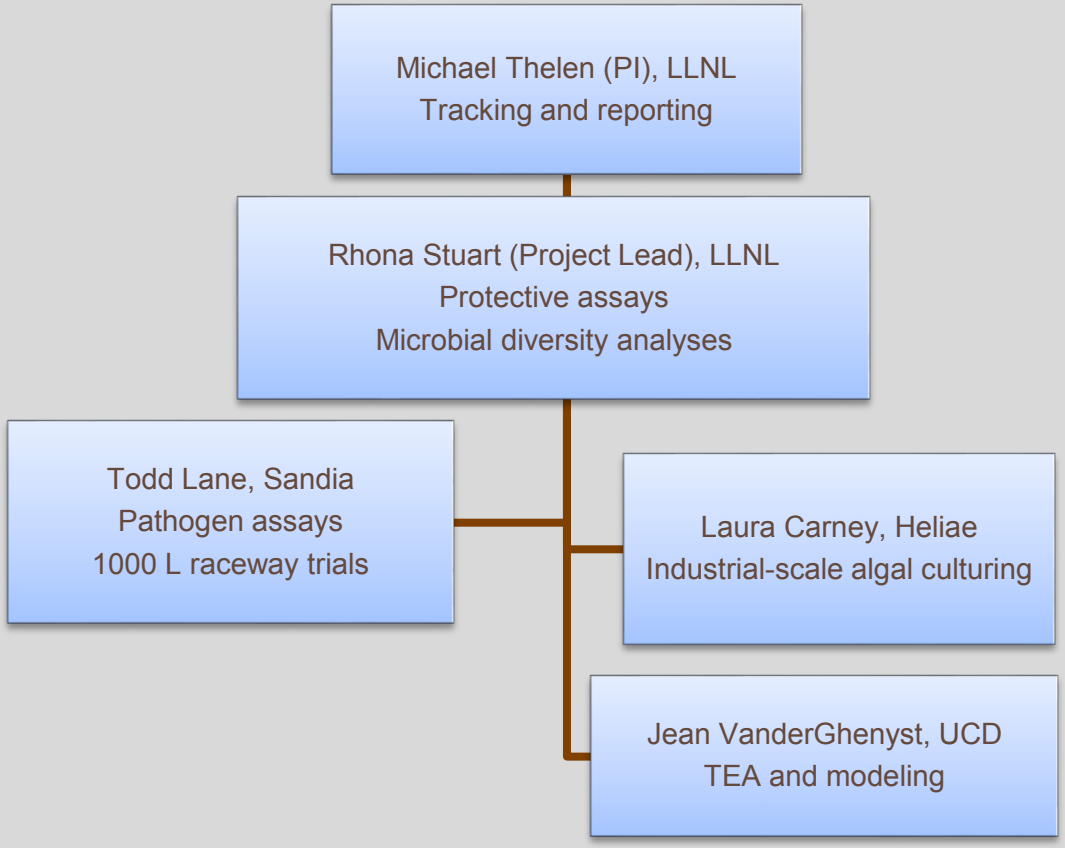
Lawrence Livermore National Laboratory

Sandia National Laboratories

heliae

UCDAVIS UNIVERSITY OF CALIFORNIA

MANAGEMENT STRUCTURE REFLECTS TASK STRUCTURE



Meetings

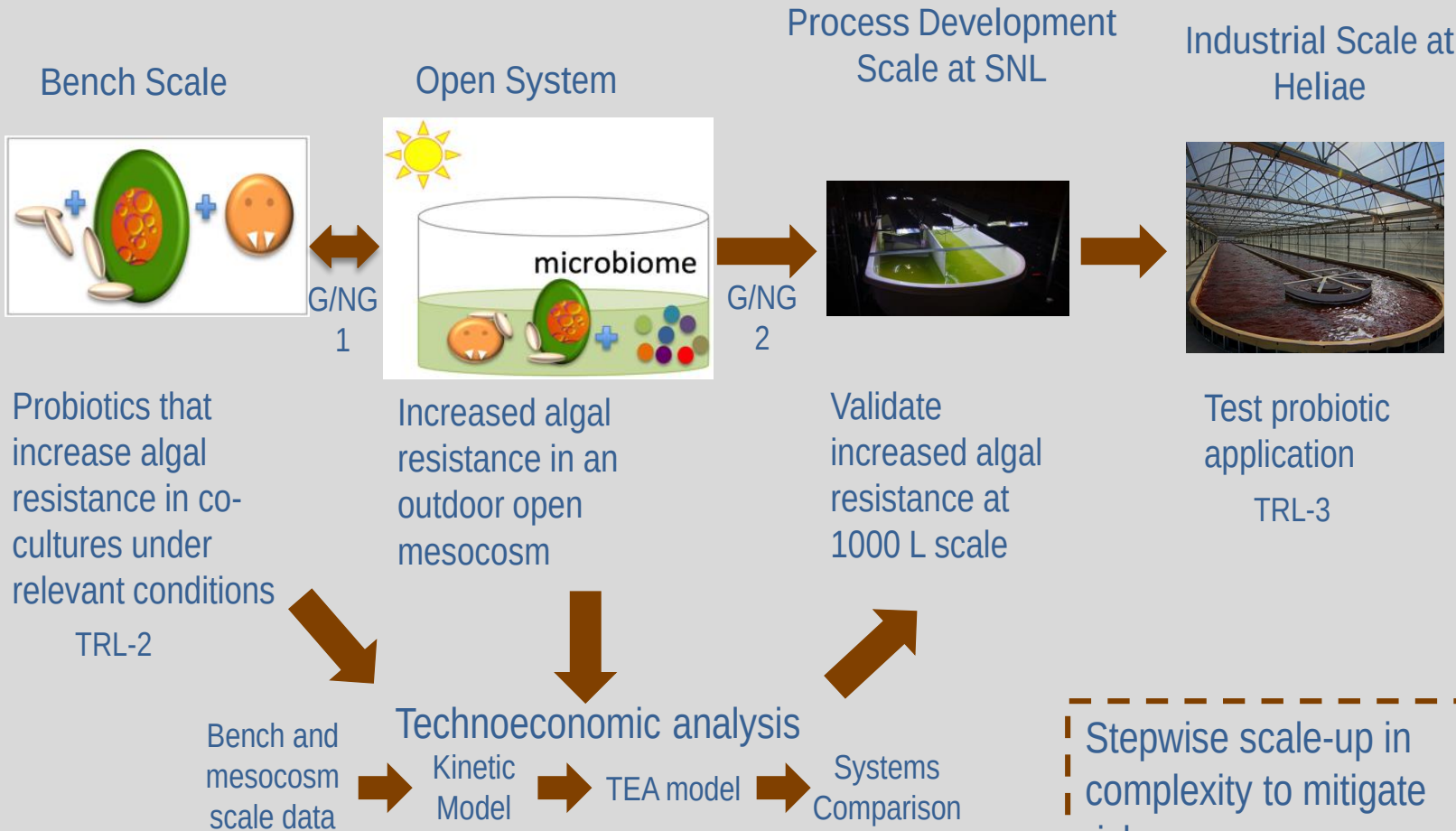
- Monthly telecon with team members
- Annual face to face meetings
- Other meetings as needed

PI Responsibilities

- Tracks milestones & data
- Generates quarterly reports
- Synthesizes results into publications and solutions

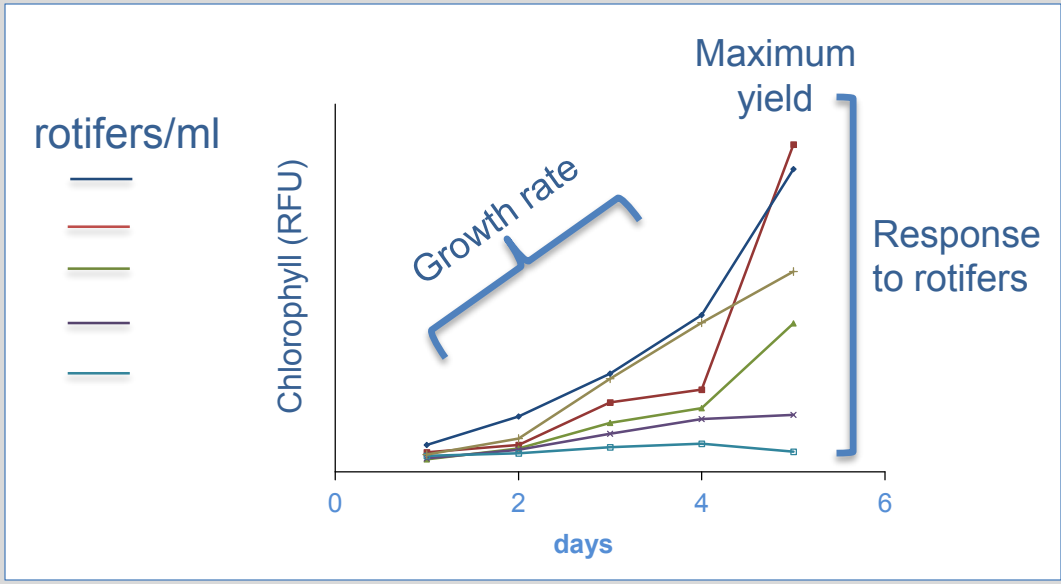
- Decision making through consensus
- Team leads responsible for achieving task milestones
- PI retains ultimate decision-making authority

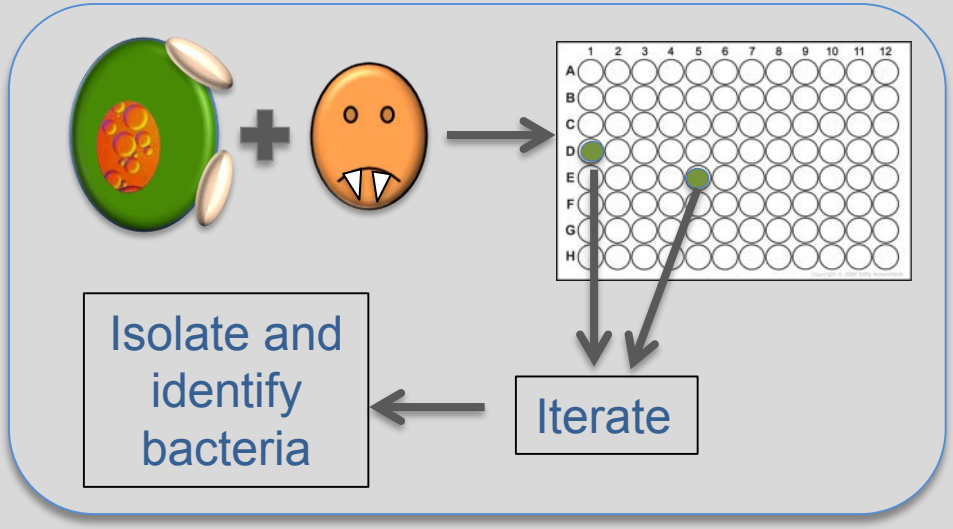
TECHNICAL APPROACH



Year 1 Tasks/Milestones

- Quantify baseline algal resistance to challenges
- Quantify protection of promising probiotics
- Demonstrate pipeline for isolation of probiotics
- Identify core microbiome at industrial scale
- G/N-G1: increase of algal protection when (1) under challenge, (2) within a relevant range of nitrate and phosphate, (3) in the presence of another bacterium





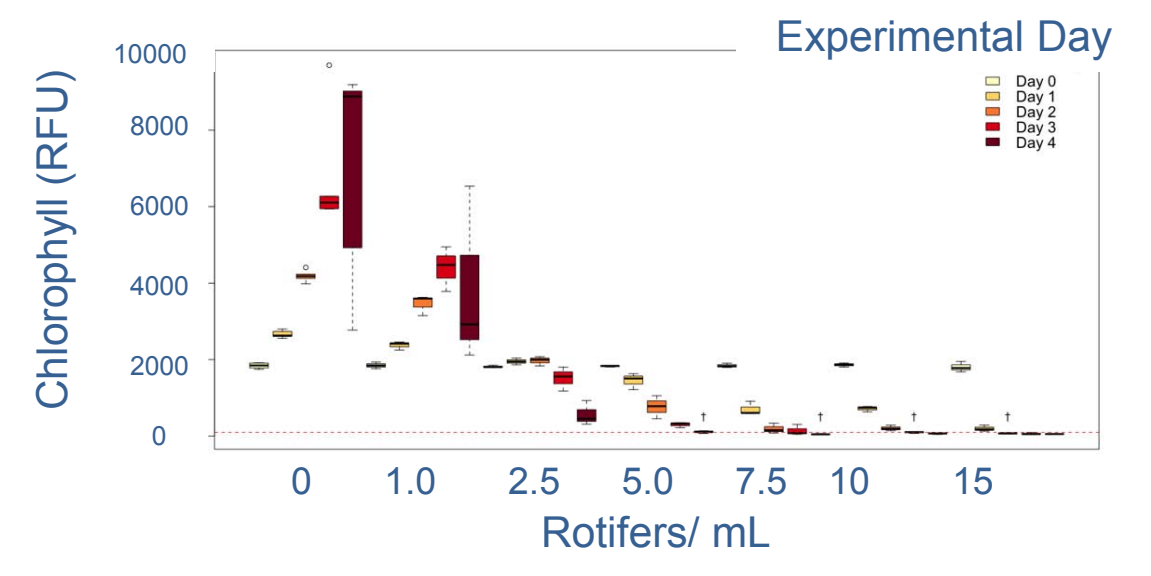
Potential Challenges

- *Protective bacteria may not persist long enough in open cultivation*
  - We will test multiple additions (both experimentally and in TEA modeling)
  - We will also test the efficacy of acclimating the probiotic and algae in inoculum stages
- *There may be unforeseen negative effects of additions on native microbiome, decreasing productivity*
  - Monitoring native microbiome to establish a clear baseline, which will allow for optimization of probiotic application.

Critical Success Factors

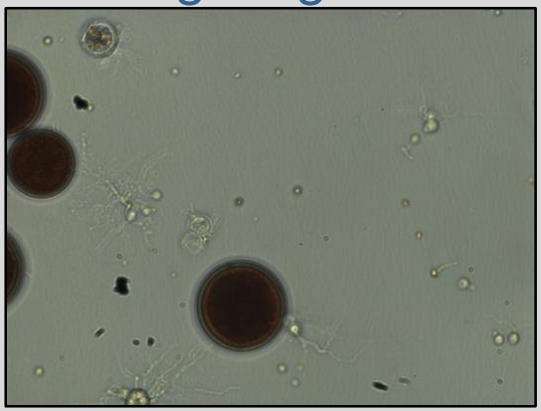
- Technical
  - Systems with protective bacteria must have increased algal biomass yield compared to unprotected systems in the presence of deleterious species pressures
  - Protective bacteria cannot decrease algal productivity in the absence of deleterious species
- Market/Business
  - Cost of probiotics application must be cost-effective as compared to:
    - costs of alternative management using chemical treatments
    - risks of pond crash when treatments fail
  - Application of probiotics must be realistic in the timeline of pond development

Assay development: Grazing and parasitism

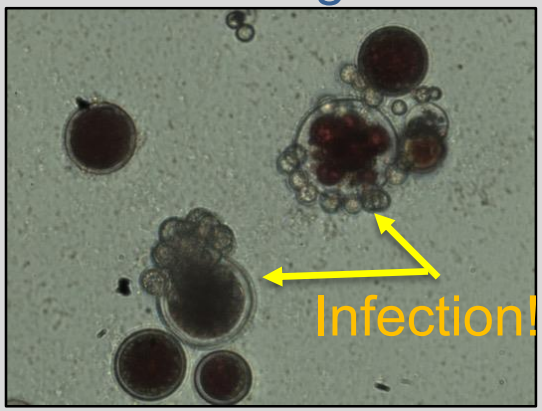


- 'High' throughput plate-based assays
- 'High' precision flask-based assays
- Correlated chlorophyll fluorescence to ash free dry weight (AFDW), fitted standard curve under controlled incubation conditions
- Infection assay, induced via low light

High Light:



Low Light:



We developed assays to determine rates of grazing and parasitism and quantified the baselines (Milestone 1)

CANDIDATE BACTERIUM PROTECTS ALGAE FROM ROTIFER GRAZING

We have identified a robust protective bacterium (Milestones 2 and 3)

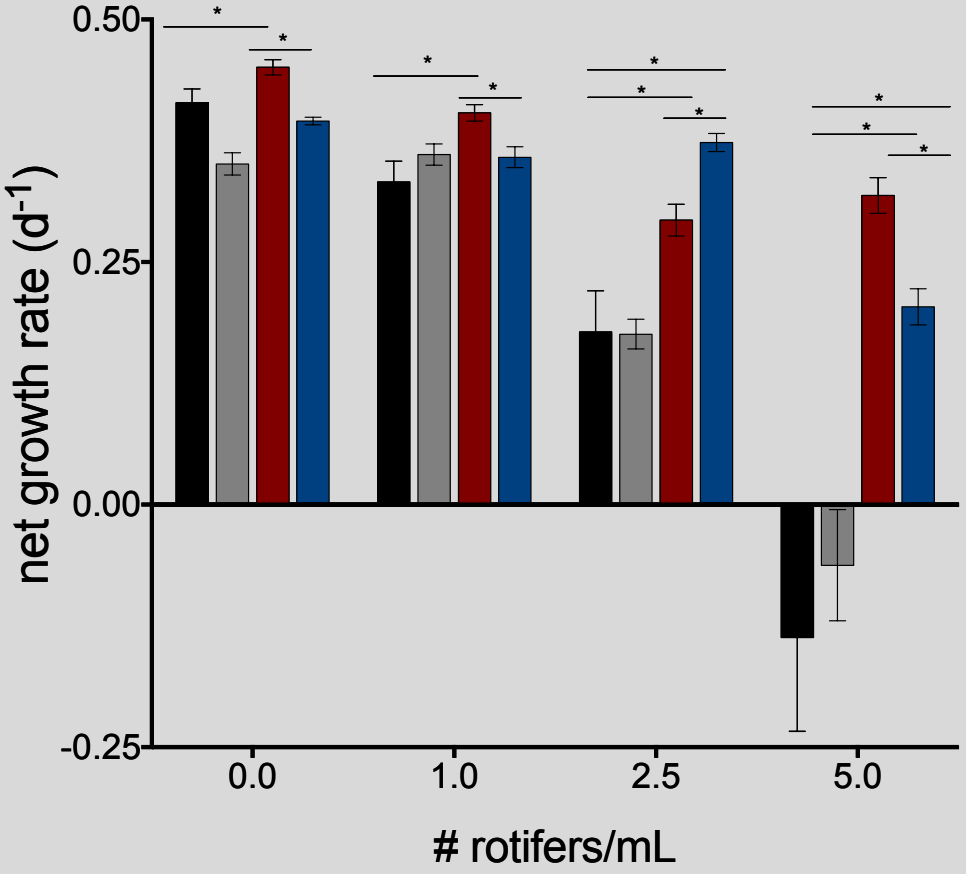
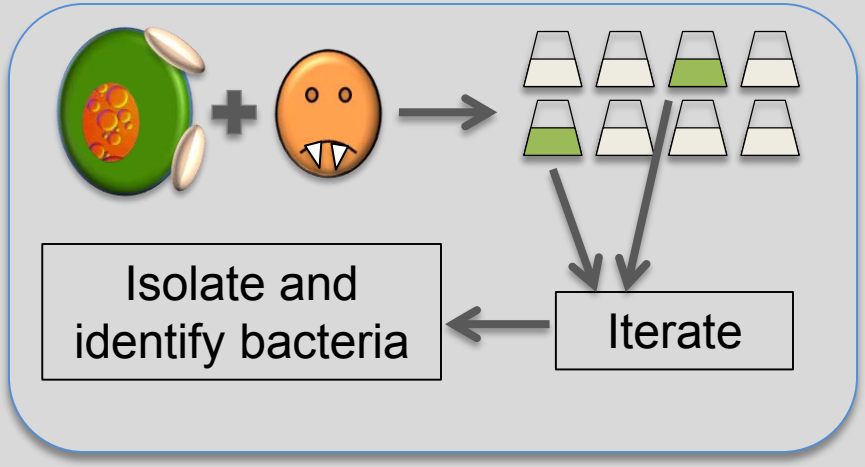
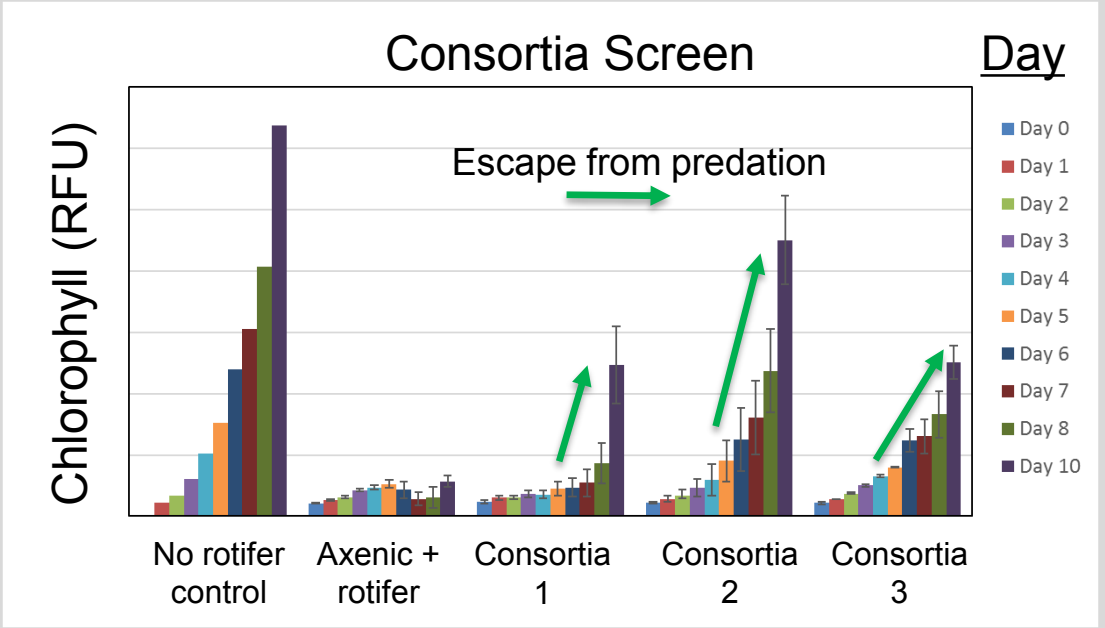


Photo credit: Microscopy UK

Pipeline Development: selection-based Identification

- Pond or environmental sample inoculated with algae (*N.salina)*
- Challenge with rotifers
- Reiterate and demonstrate the rotifer die-off was communicable.

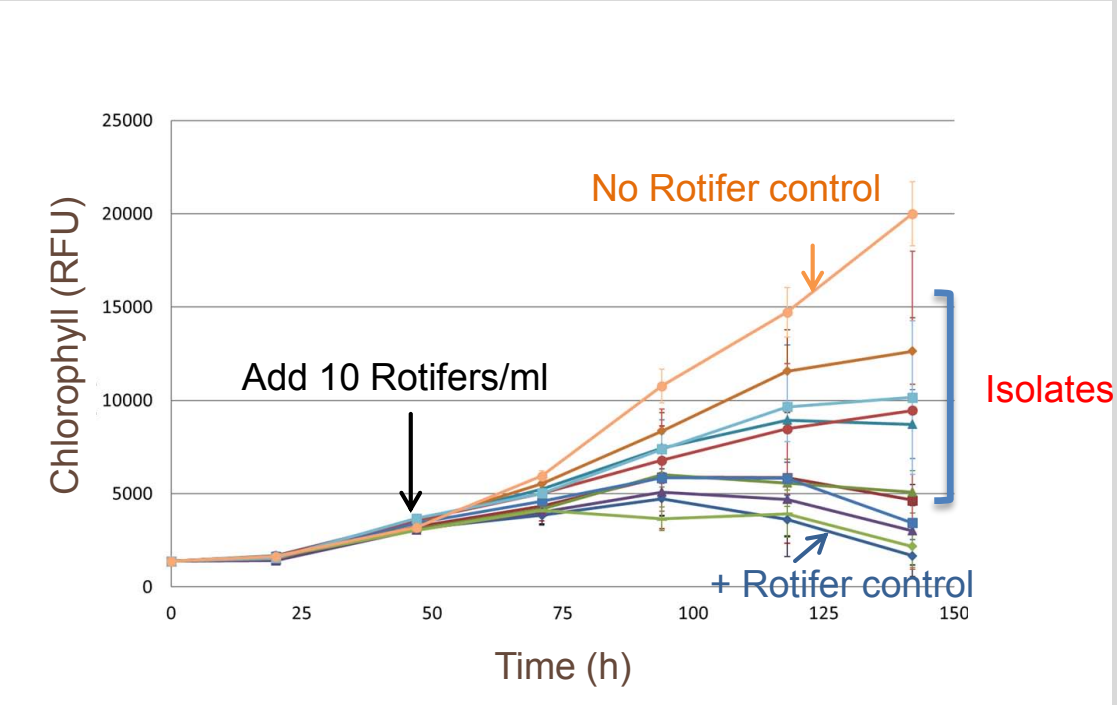




We have developed a pipeline to identify protective bacteria and consortia (Milestone 4)

SUCCESSFUL SELECTION-BASED IDENTIFICATION OF PROTECTIVE CONSORTIA

- Selection-based pipeline identified bacterial consortia with protective capabilities.
- Rotifers appeared moribund, lacking motility, live/dead assay in development
- Ongoing efforts at isolating members from the protective consortia are promising.

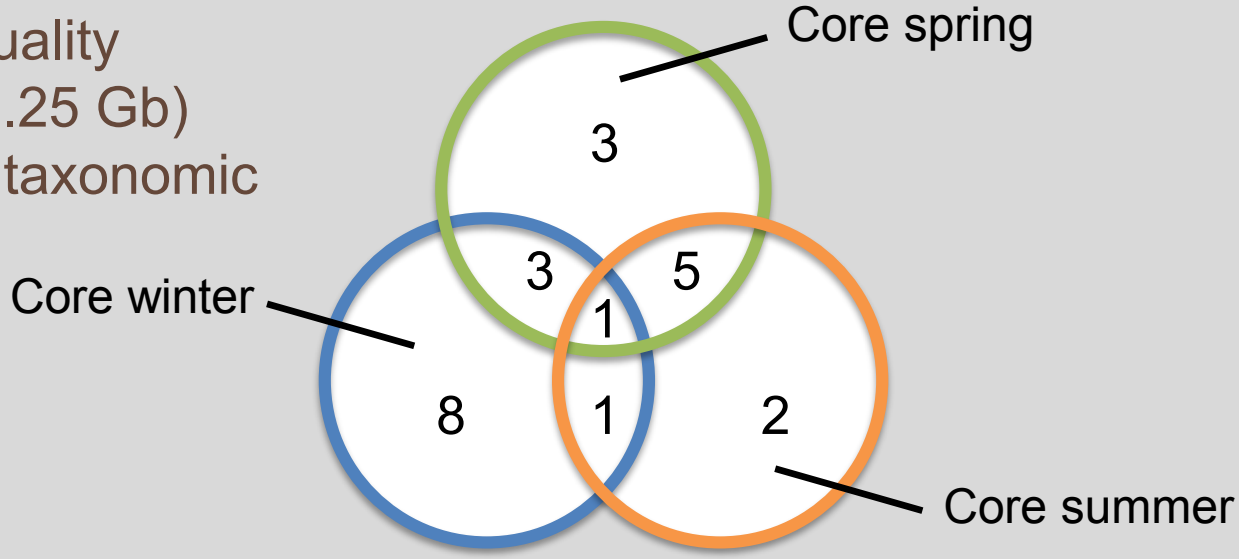


We identified a protective consortia, using our pipeline

IDENTIFYING THE CORE POND MICROBIOME

- 4 seasons with 2-3 sampling periods per season
- triplicate reactors
- >600 sequence libraries
- 13.5 million high quality sequence reads (7.25 Gb)
- >1000 operational taxonomic units (OTUs)

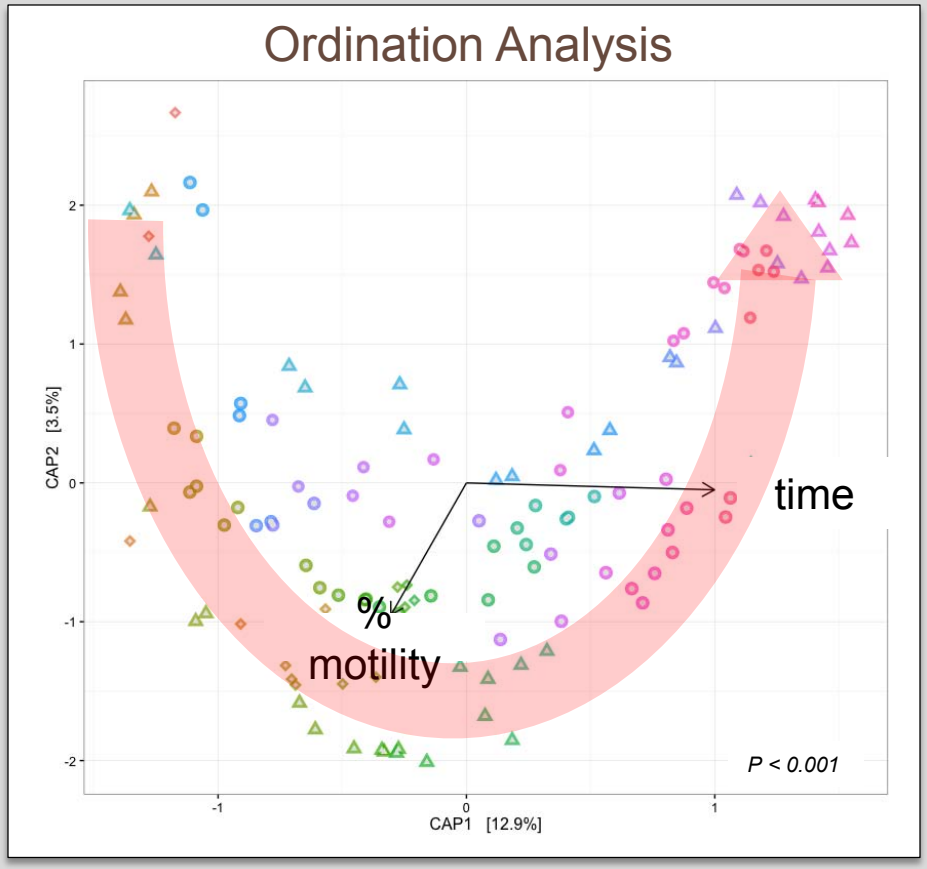
Heliae raceway

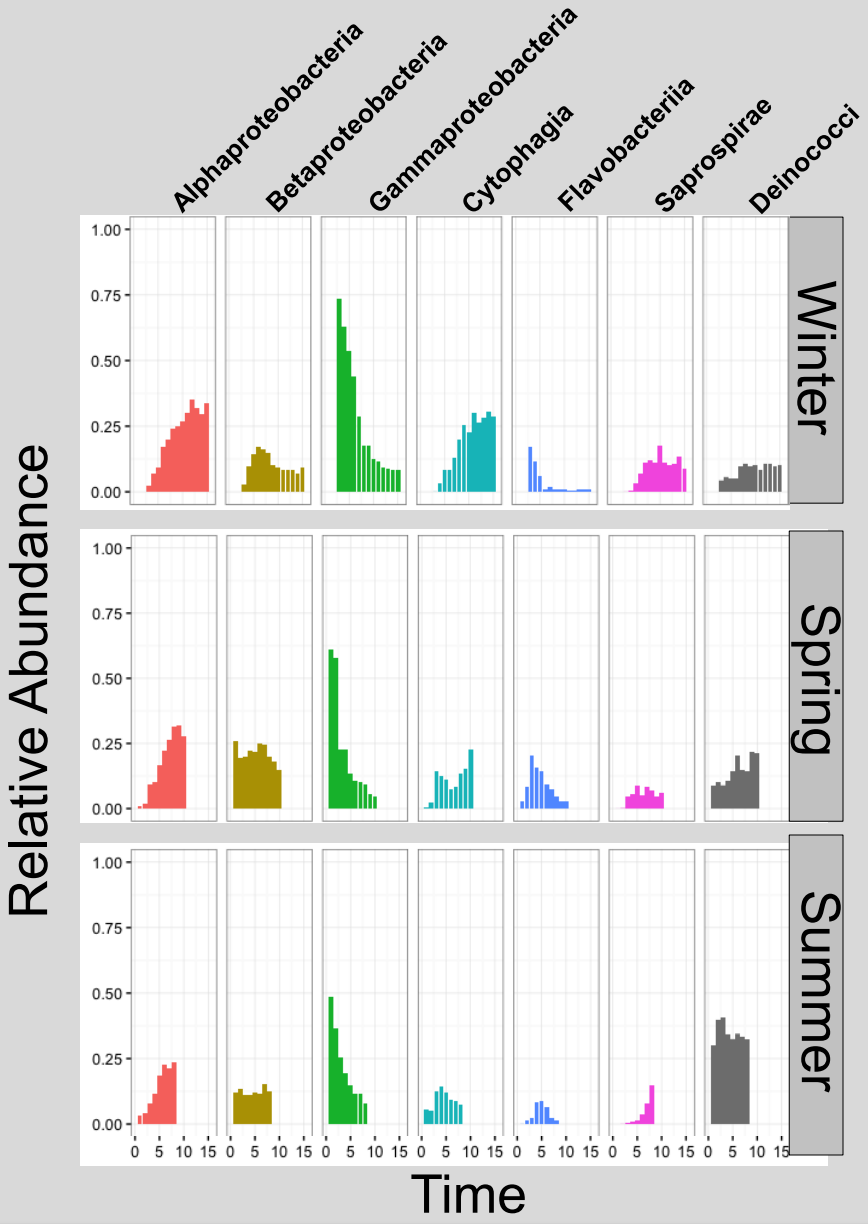


We have profiled industrial ponds over an entire year to identify core taxa

➢ Core taxa belong to taxonomic groups frequently associated with high-density algal growth/algal blooms

POND MICROBIOMES FOLLOW SUCCESSIONAL TRAJECTORIES





Time and algal physiology influence microbiome, with predictable trends, regardless of season

FUTURE WORK—18 MONTHS

- Scaling up testing to outdoor mesocosms and optimizing at 1000 L testbed.
- Complete TEA based on collected data
  - Kinetic model of algal biomass accumulation
  - Kinetic model with rotifer
  - Kinetic model with rotifer and probiotics
- Key Milestones:
  - Demonstrate probiotic persistence in outdoor mesocosms with 5 or less inoculations
  - Demonstrate increased algal protection in response to added deleterious species in open mesocosm
- Go/No-Go in Oct.2017: Demonstrate probiotic persistence at validated protective levels throughout pilot stage pond development

Relevance: Generating Biological Controls to Prevent Algal Crop Loss

- **GOAL**: Improve the resilience of algal crops to predators and pathogens by using probiotic bacteria that will increase annual algae biomass yields by 5-30%.
- **Competitive award granted under Targeted Algal Biofuels and Bioproducts (TABB) FOA, directly addresses barriers from FOA:**
  - One key barrier stated is "*low yields of target biofuel and bioproduct feedstocks*" and "***biological contamination*** *presents one of the greatest challenges in cultivating robust, reliable algal cultures*" which requires "***novel***, ***safe***, *and* ***effective*** *strategies need to be developed to control culture contamination events*". Our probiotic approach directly addresses these challenges.
  - Another barrier is in "*translating laboratory success to demonstrated, scalable, outdoor cultivation environments that capture all of the variables not present in laboratory systems*", which our pipeline strategy addresses.
- FOA sought "*bench and process development scale applied R&D that focuses on: ...increased biomass productivity leading to higher yields by developing* ***crop protection****...strategies*" and contributing to decreasing cost of algal biofuels from $8 per gallon gasoline gallon equivalent (gge) to $5 gge.
- Objectives are also aligned the Bioenergy Technologies Office, MYPP goals, and are relevant to and algal biomass industry
  - **Advanced Algal Systems WBS (Production):** "Integrate fundamental learning from community and systems ecology into cultivation design and practice to maximize productivity and resilience".
- The success of this project will advance the state of technology and positively impact the commercial viability of algal biofuels
  - Increasing algal resistance to predators and pathogens
  - Pipeline to screen for novel probiotics

SUMMARY

- We aim to improve the resilience of algal crops to predators and pathogens by using probiotic bacteria to increase annual algae biomass yields through pond crash prevention.
- Our approach includes a step-wise scale-up from lab to industrial scale tests
- Our technical accomplisments include:
  - Successful assay development for measuring algal resistance to both rotifer grazing and chytrid infection (2 key culprits of pond crashes)
  - A selection-based pipeline to identify protective probiotics
  - Idenfication and characterization of a robust protective isolate and a protective consortia at laboratory scale
  - A unique dataset of industrial pond microbial communities over growth through 4 seasons over a year
- Relevance summary: Crop protection through increasing pond stability, without the need for chemical management
- In the coming 18 months we will:
  - field test our promising probiotics (in a step wise scale up)
  - Complete our TEA analyses, incoporating data gathered in years 1 and 2
  - Refine our pipeline for novel probiotic discovery

Acknowledgements

U.S. DEPARTMENT OF ENERGY | Energy Efficiency & Renewable Energy
BIOENERGY TECHNOLOGIES OFFICE

DOE EERE BioEnergy Technology Office

LLNL

- Chris Ward*
- Xavier Mayali
- Amy Gryshuk

SNL

- Todd Lane
- Pamela Lane
- Carolyn Fisher*

Heliae, Inc

- Laura Carney
- Braden Bennett
- Rachel Montoya

UC Davis

- Jean VanderGheynst
- Lauren Jabusch*

BETO

- Amanda Barry, Dan Fishman and Evan Mueller

*postdocs and graduate student