DOE Bioenergy Technologies Office (BETO) 2021 Project Peer Review

Prevention of Low Productivity Periods in Large-Scale Microalgae Cultivation (PEAK)

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2:05 PM EST
Advanced Algal Systems

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Global Algae Innovations

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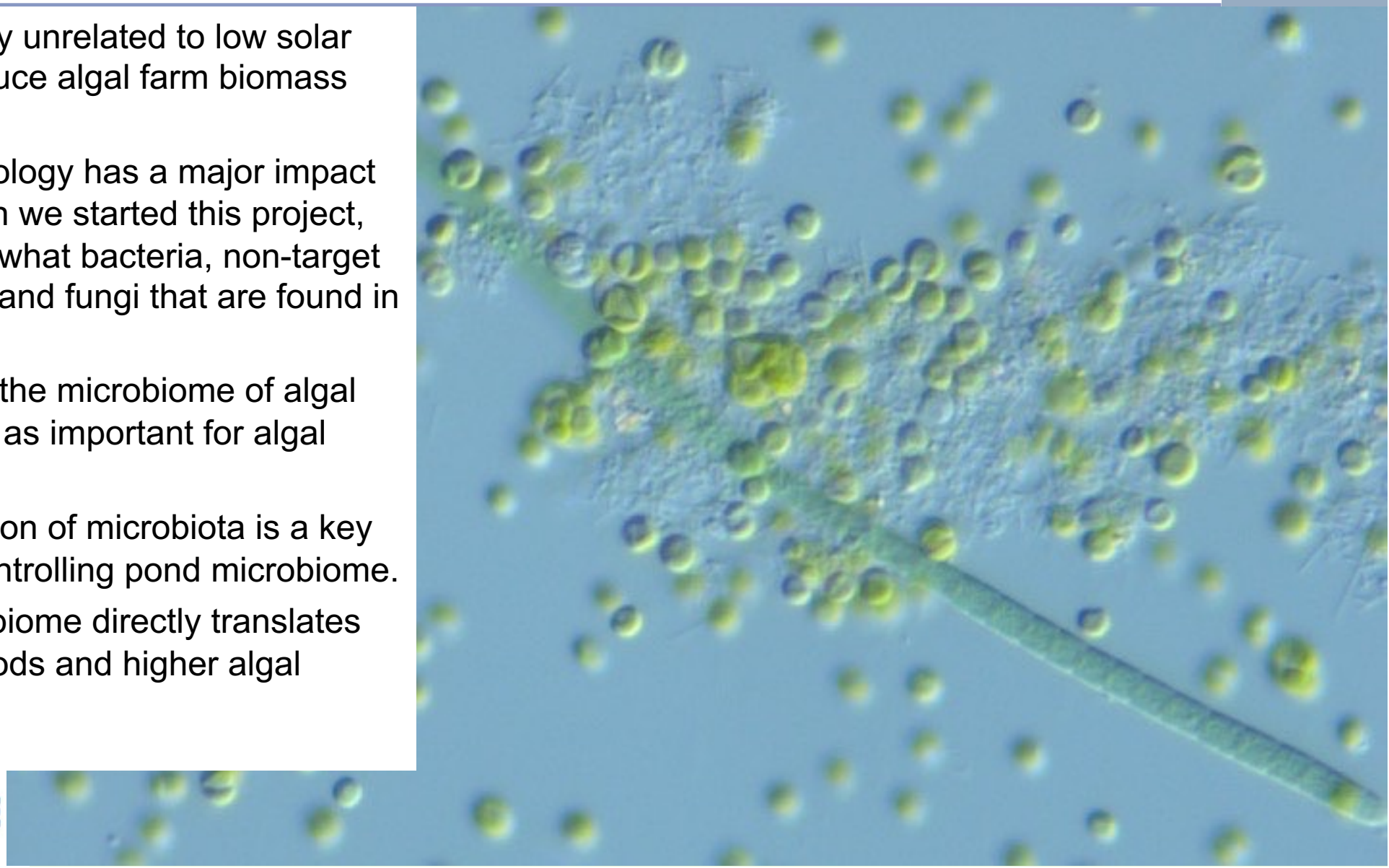
GLOBAL ALGAE

Project Overview

Project Overview

- Periods of low productivity unrelated to low solar radiation significantly reduce algal farm biomass production.
- We suspect that pond ecology has a major impact on algal health. But, when we started this project, we knew very little about what bacteria, non-target algae, viruses, protozoa, and fungi that are found in cultivation ponds.
- Recently, phycosphere – the microbiome of algal cell has been recognized as important for algal growth.
- Detection and quantification of microbiota is a key for understanding and controlling pond microbiome.
- Understanding the microbiome directly translates into new cultivation methods and higher algal productivity.



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Project Goals

The goal is to reduce periods of unexplained low pond productivity by identification and control of microbiota cultivated with target algae

- Measure the microbiota (viral, bacterial, algae, protozoa, fungi)
- Develop a tool for low cost, rapid analysis of pond microbiota
- Utilize the tool and microbiota information to develop cultivation methods to achieve algal productivity of > 25 g/m^2d.

Traditional approach to identification of pond microbes and treatments

- Microscopy is time consuming and relevant to detection of Eukaryotes
- qPCR is effective but you need to know your contaminants to develop your qPCR probes and ideally the contaminant is isolated and in culture
- Treatments are non-specific - how and if they work is not well understood

Project Goals

Relevance to bioenergy industry

- Crop protection and productivity is crucial to economic viability and sustainability of algal biofuel production
- Understanding and controlling microbiota is a necessity
- There is little publicly available information on microbiota control in algae cultivation
- Tools to measure microbiota are needed to accelerate development of cultivation advances and treatment protocols

Project risks

- Project required development of many new methods
- We are attempting to isolate and cultivate many microorganisms that were not isolated before
- Microorganism quantitative detection is needed to develop treatments

1 - Management

Team communication

- Well defined roles and milestones
- Bi-weekly conference call
- Data exchange through box.com
- Bi-monthly review: budget, milestones, issues, opportunities, risks

Milestones

- Method development for genomic sample collection
- Isolation of microbes
- Challenge testing
- Treatment methods for pathogenic microbes and use of probiotics
- SpinDX successfully deployed and application for control

Project team responsibilities

GAI (cultivation, sampling, analyses, data integration & testing)

- Outdoor cultivation, sampling, non-genomic analysis, Spin DX testing
- Data integration, isolation of eukaryotes, testing of microbiota control

JCVI - Dr. Lisa Zeigler (sequencing, viruses)

- Genomic sample preparation, sequencing, and data processing
- Viral data analysis, isolation, challenge testing and control methods

SIO - Dr. Eric Allen (bacteria, DOM control, baseline sequencing)

- Genome sequence of two cultivated algae strains (green GAI-247 and diatom GAI-229)
- Bacterial data analysis, isolation, challenge testing and control methods
- Reduction of dissolved organic material (DOM) during lab-scale cultivation

Sandia - Dr. Todd Lane and Dr. Krissy Mahan (eukaryotes, SpinDX)

- Eukaryotes data analysis, isolation, challenge testing and control methods. Development of **SpinDX**

Dr. Scott Fulbright (data integration & analysis)

- Consultation on genomic data integration and data analysis.

Project Risk Management

Risk	Pr - Sv	Mitigation	Pr - Sv
Sampling problems & coordination issues	5 - 5	All subs assist with first sampling for real-time solutions and coordination	1 - 5
Can't obtain correlations with productivity	3 - 4	Forcing off-normal operation, multiple seasons, many targets (bacteria, fungi, viruses, protozoa)	1 - 2
Can't identify cause and effect	3 - 3	Multiple targets, extensive isolation effort	2-5
Can't complete project within budget	4 - 4	Combine genomic analyses; division of data analysis to leading experts; bimonthly reviews – status, synergy opportunities, & adjustments	1 - 2
Can't make SpinDX work	3 - 5	Early test; accelerate feedback loop: two prototypes for outdoor testing in parallel with Sandia modifications	1 - 5

2 - Approach

Microorganisms associated with algae

- Construct a database of eukaryotes, bacteria and viruses found in algae ponds
- Identify potential key organisms by correlating organism abundance with algal productivity
- Isolate organisms of interest and test for cause/effect on productivity

Spin DX instrument to detect and quantify microbiota

- Develop instrument and protocols for rapid measurements

Microbiota treatments

- Test effects of isolated microbes on algae in the laboratory
- Test remediation treatments on pathogenic microbes in the laboratory
- Test the most promising probiotics and treatments for pathogens at the algae farm

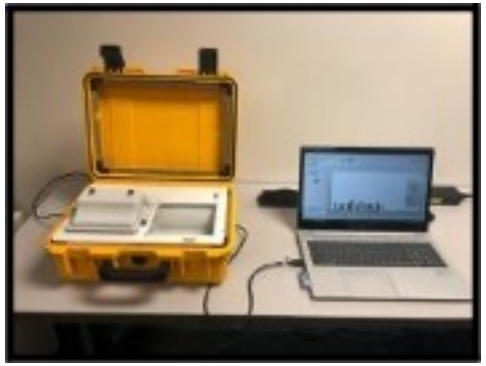
2 – Approach
Microorganisms associated with algae

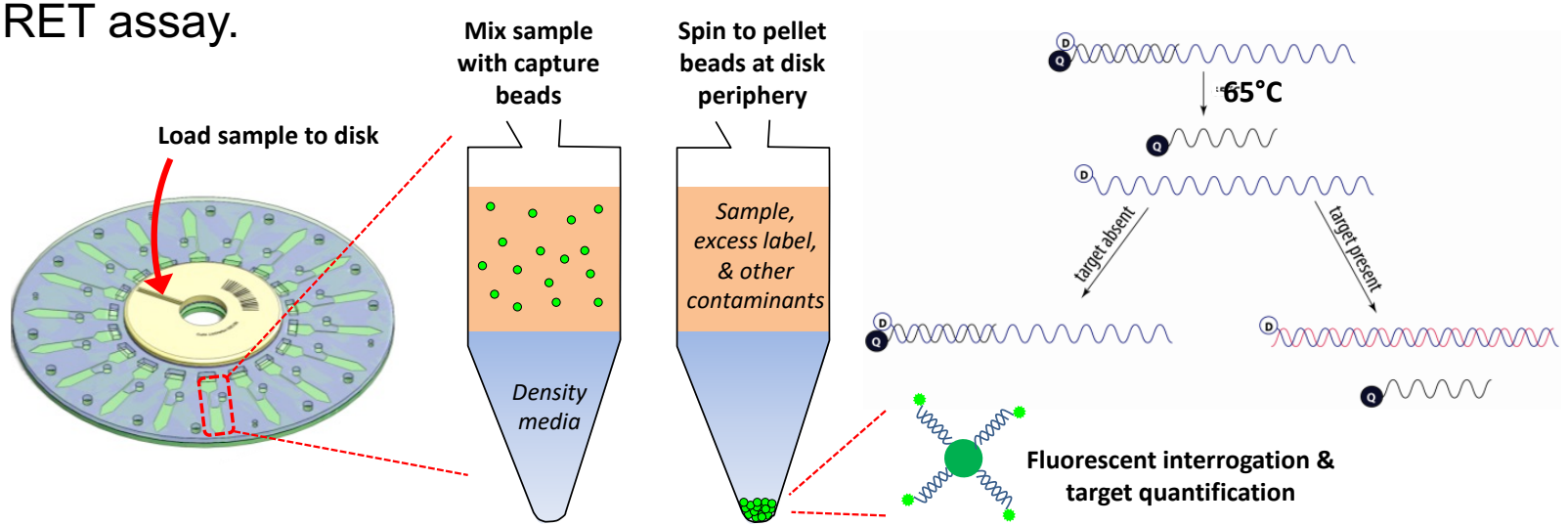
Broad data set

- Samples were collected from cultivation of 2 biofuels strains (green and diatom), during different growing seasons.
- Samples were collected daily during the grow out starting with laboratory inoculum and ending on samples collected in the large outdoors ponds.
- Samples were collected in growth phase and during lipid formation.
- Samples were collected for viral DNA and RNA, bacterial DNA, and eukaryote DNA – fungi and protozoa Metagenomic sequencing included 18S, 16S and transcriptomic data.
- During initial grow out both algae strains were cultivated side by side and nitrogen source varied between treatments.
- Since algae sequences formed the majority of data, genomes of both algal strains were fully sequenced to separate them from sequences of microorganisms

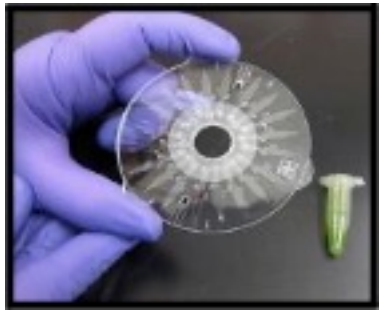
2 – Approach
SpinDX

- Fluorescence Resonance Energy Transfer (FRET) -based bead hybridization assay enabling capture and quantification of pathogen-specific RNA/DNA signatures
- Goal is to use SpinDx to provide early and rapid detection of positive and negative members of the pond microbiome using FRET assay.





- **Assay time: approximately 30 min**
- **20 channels per disc**
- **Potential for multiplexed assays in each channel**
- **Low reagent costs**
- **Low material costs**
- **Low instrument cost**
- **Fieldable**



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2 – Approach
Microbiota treatments

- Metagenomic sequencing data allow identification of key microorganisms in algae cultivation ponds
- In order to develop probes for SpinDX microorganisms should be isolated but alternatively sequences of interest can be put on plasmids for probe development and testing
- Isolate and test organisms to identify potential cause and effect interactions with algae (positive or negative)
- Learn to control key organisms affecting algal productivity through new cultivation strategies or treatments.
- Test control strategies and treatments in the laboratory
- Test control strategies and treatments outdoors

Challenges

Pinpointing and isolation of key organisms

- Isolation is necessary to develop good probes for SpinDX.
- Correlation does not necessarily mean causation
- Many of the contaminants are difficult to isolate

Large data sets

- Overwhelming amount of data from sequencing and limited time to analyze it.
- Priority was given to look for organisms indicative of good and bad growth.

Translating lab results to large-scale outdoor cultivation

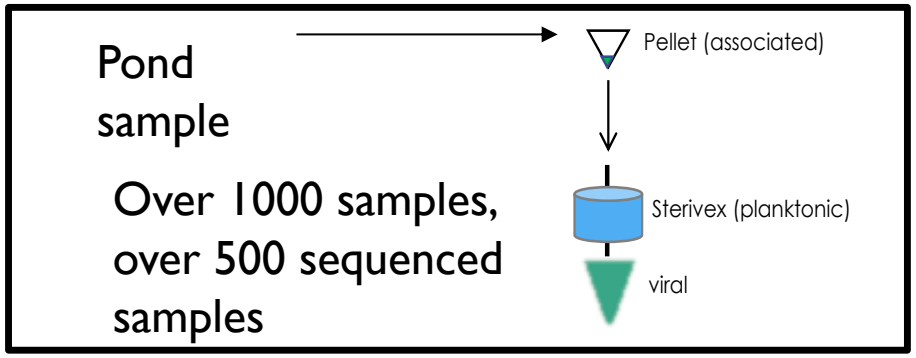
- Application of probiotics or treatment protocols outdoors
- SpinDX

3 - Impact

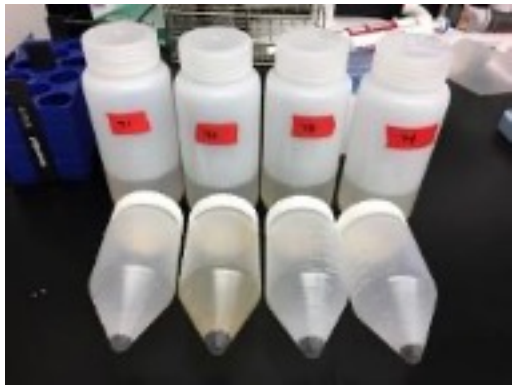
- Understanding microbiome in algae cultivation is the new frontier that is going to have a major effect on improving algal productivity.
- Identification and isolation of key organisms will facilitate work on how the algae pond ecology works. This is the first step to conduct any kind of pond microbiome manipulation to improve productivity.
- Since more scientists are studying the phycosphere and initial results show that many of the microorganisms are found across different environments, the data generated in this project may have a much broader application than originally considered.
- Even without catastrophic deviations such as pond crashes, microbial food webs are affected by local scale community and environmental variation, which can lead to differential ecosystem functioning. This study represents one of the first multi-omic approaches aimed at understanding the interactions between elite algae strains and their microbiomes, including the viral component, in a commercial operational setting.
- Fast and affordable detection and quantification of microorganisms is critical for monitoring algae cultivation ponds and for pathogen treatment.
- The results of this project will allow the start of pest management and biological control of algae cultivation ponds.

4 -Progress and Outcomes
Microbiota associated with algae ponds

We developed a sampling protocol for collecting genomic sample from algae associated microbiota (pellet), planktonic fraction (Sterivex 0.22µm filter) and viruses. Samples were process within 30 min from collection and flash frozen in LN2 and shipped on dry ice. This sampling protocol resulted in high quality genomic data. We have plenty of extracted DNA and RNA saved for future work.

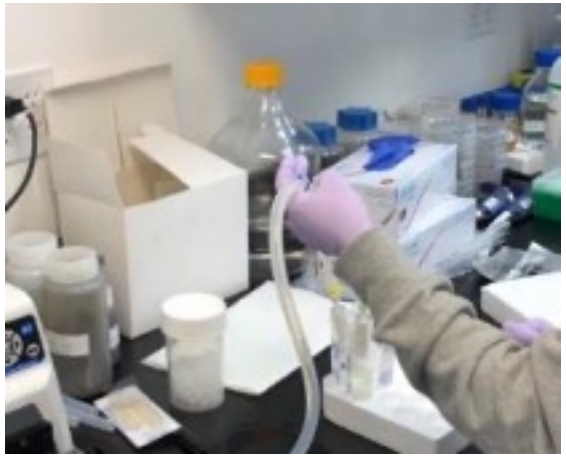


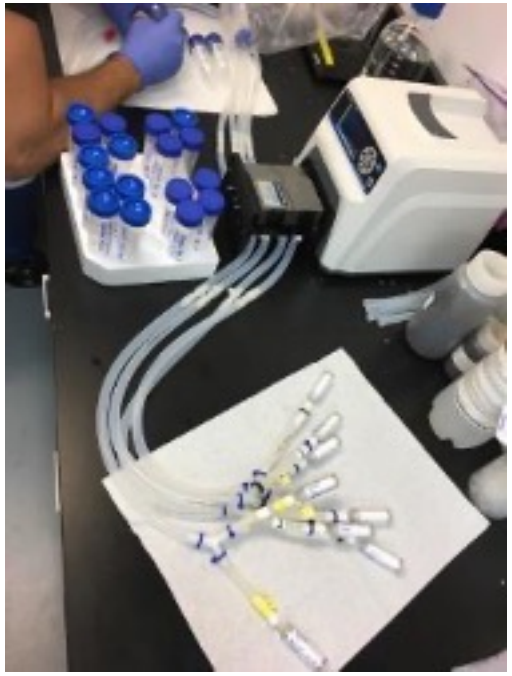
Pond Sample and pellet



Final Pellet

Collect <0.22 µm filtrate (Virus)





Filter through 0.22 µm Sterivex filter

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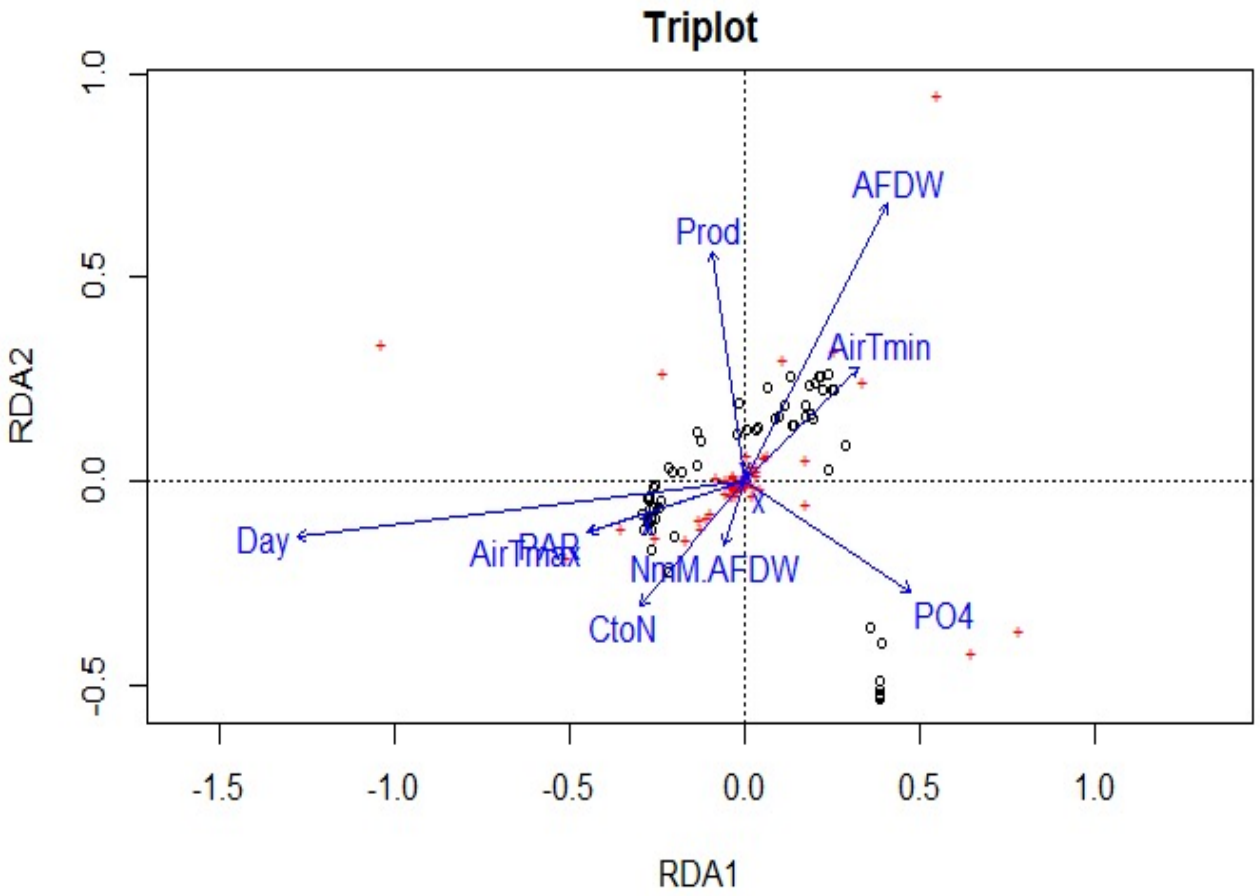
4 -Progress and Outcomes
Grow-outs and sampling

March 2018:	Both species, nitrogen sources, lipid formation
June/July 2018:	Green (GAI-247), high productivity
October 2019:	Green, crash
Jan/Feb 2019:	Diatom (GAI-229), high bacteria-low growth, low temperatures
May 2019:	Diatom, high productivity

Grow out	Days of genomic sampling	Max scale	Species	Samples	Sequencing schedule	Non-genomic analysis
March 2018	5	1,214L	229, 247	84	Completed	Completed
June/July 2018	32	101,705L	247	67	Completed	Completed
October 2018	19	2,500L	247	34	Completed	Completed
January/February 2019	40	7,200L	229	118	Not sequenced	Completed
April/May 2019	40	200L	229	64	Not sequenced	Completed

4 -Progress and Outcomes
Analysis of GAI-247 grow-outs



62 sampling events with algae culture conditions and 16S sequencing data with 118 OTUs were used.

Environmental conditions and algae condition explained well variability within bacterial community.

The first two RDA axes explained 55.9% of variability within OTUs data (RDA 1 43% and RDA 2 12.9%)

Algal productivity alone explained only 4.4% of variability within bacterial OTUs.

Distinct OTUs were associated with low and high algal productivity.

Decrease or increase in bacteria associated with good growth occurred before the drop in algal productivity was observed.

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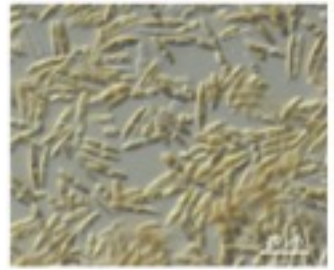
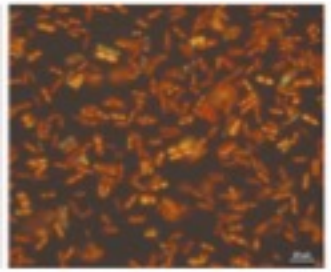
4 -Progress and Outcomes
Analysis of GAI-247 grow-outs

- Bacterial variability was better explained by environmental factors then algal productivity.
- Changes in bacterial community were preceding changes in algal productivity. Detection of bacterial community changes could be an early warning system of potential decrease in algal productivity.
- Bacterial community was strongly affected by how long culture was outdoors.
- A lot of the OTUs were undefined and therefore their genus and species level taxonomy is unknown.
- There were also large seasonal differences even though the algae, media, and cultivation process was the same.

4 -Progress and Outcomes
Genomic insights from *Nitzschia hildebrandi* str GAI293

- **High quality, telomer-to-telomer assembly**
  - 99.7 Mbp (diploid) in 14 syntenic chromosome pairs
  - Paired alleles with low heterozygosity (2.7%)
- **Expanded families of bicarbonate transporters and carbonic anhydrases**
  - Enhanced carbon concentration ability under varying environmental conditions
- **High numbers of surface adhesion proteins**
  - Promote biofilm formation
- **Duplication of glycolysis and fatty acid synthesis enzymes**
  - May enable elevation of peak metabolic activity, providing a competitive advantage over other organisms in mixed cultures
- **Anticipated manuscript submission in February 2021**





Oliver A, Podell S, Pinowska A, Traller JC, Smith SR, *et al.* Diploid genomic architecture of *Nitzschia hildebrandi* str. GAI293, an elite biomass production diatom.

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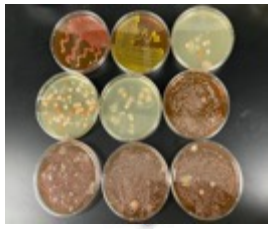
4 -Progress and Outcomes
Algae-Bacteria Interactions - SIO

Single Isolate Interactions


Farm samples


Enrichments


Plating & screening

Culture collection of representative bacteria isolates from mixed community pond samples

Objective: Discover positive and negative interactions between elite algae strains and their associated bacteria

Experimental Design:

- Monitor growth of algae in co-culture with bacteria.
- Analyze transcriptome data in co-cultures to identify synergistic or antagonistic mechanisms impacting growth.

Collection includes hundreds of strains representing ~20 genera (and counting)

Genus	Significance
Exiguobacterium	Plant growth promoting bacteria
Halomonas	Enhanced growth of Nannochloropsis
Microbacterium	Enhanced growth of green algae
Cyclobacteria	Highly abundant strain in farm ponds
Bacillus	Growth inhibition of green algae
Halomonas	Inhibition of red tide dinoflagellates
Alishewanella	
Alkalimonas	
Dietzia	
Jonsia	
Listeria	
Luteimonas	
Planococcus	
Pseudomonas	
Roseomonas	

GLOBAL ALGAE

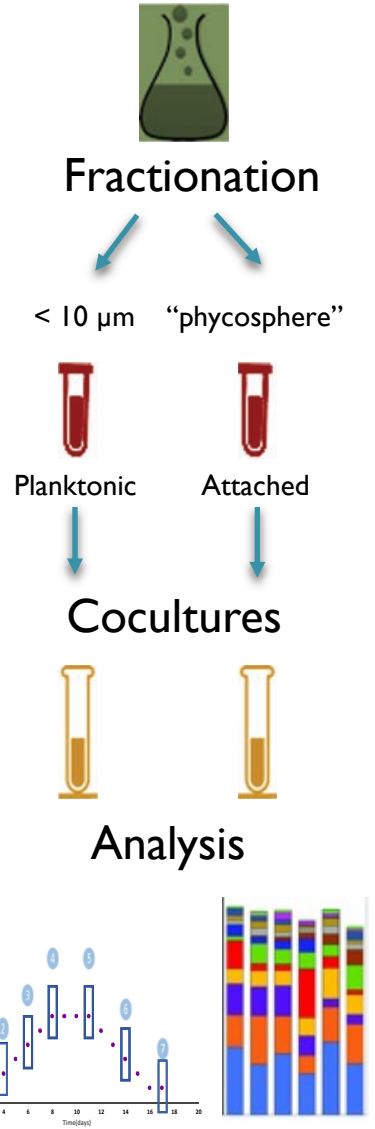
4 -Progress and Outcomes
Algae-Bacteria Interactions - SIO

Mixed Community Interactions

Objective: Characterize the growth and microbiome dynamics of *Nitzschia*

Experimental Design:

- Separate algae-associated bacterial communities from farm samples into “planktonic" and "attached" fractions.
- Grow axenic diatoms in coculture with the planktonic and attached fractions. Monitor changes in growth by measuring changes in cell numbers and fluorescence over time.
- Analyze metagenomic and metatranscriptomic data to profile microbiome community and functional changes in mixed cocultures.



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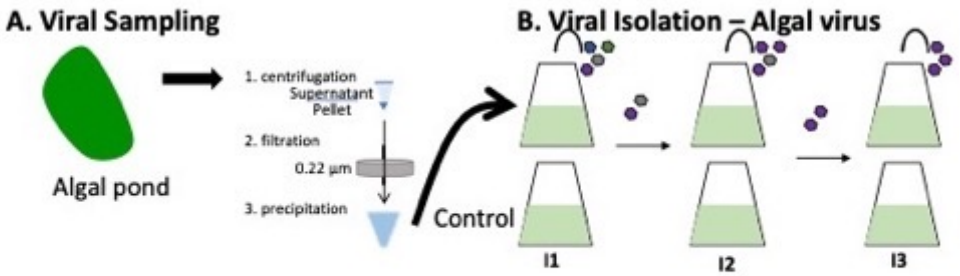
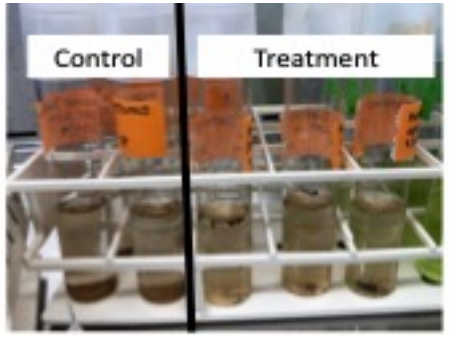
4 -Progress and Outcomes
Viruses - JCVI

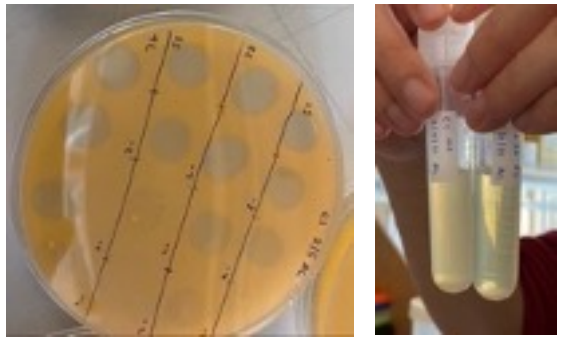
Viral bio-prospecting: virus isolation and sequence-based identification



Algae



Bacteria



Algae

- **Diverse virus isolates for each algal strain** Phycodnaviridae (dsDNA; *Nannochloris* sp. GAI247) and Bacillarnaviridae (+ssRNA; *Nitzschia* sp. GAI229)

Bacteria

- **Interrogated viral concentrates from 2018 grow-outs (n= 34) against 7 bacterial isolates from ponds** (SIO; E. Allen Lab)
- **Combined cultivation approach** for isolation and phage titer

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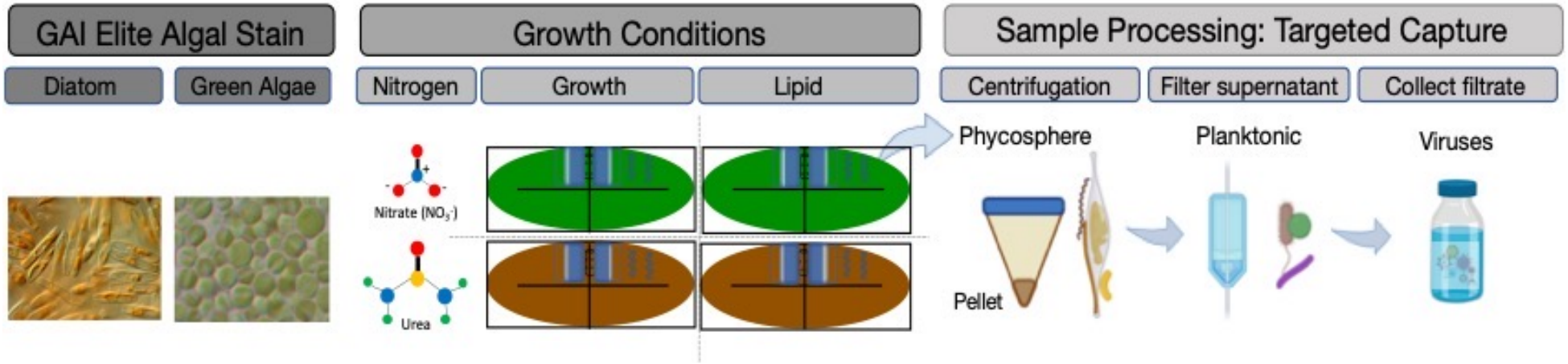
4 -Progress and Outcomes
Microbiome (Bacteria and Viruses) - JCVI

Growth and sampling scheme for multi-omic monitoring of commercial algae cultivations



Microbiome sequencing and analysis

- **Multi-omic dataset**: viromes (n=32), 16S and 18S rDNA amplicons (n=256), and total RNA (n=268)
- **Flow-cytometry methods further developed for high throughput enumeration of microbiome constituents** and genomic-based sequencing were applied to assess community composition
- **Wholistic microbiome approach**: Phycosphere and planktonic communities; ambient (free) viromes and potential infectious (active) viruses were extracted from total RNA sequencing (quantitative RNAseq) of the cellular (pellet and sterivex) fractions

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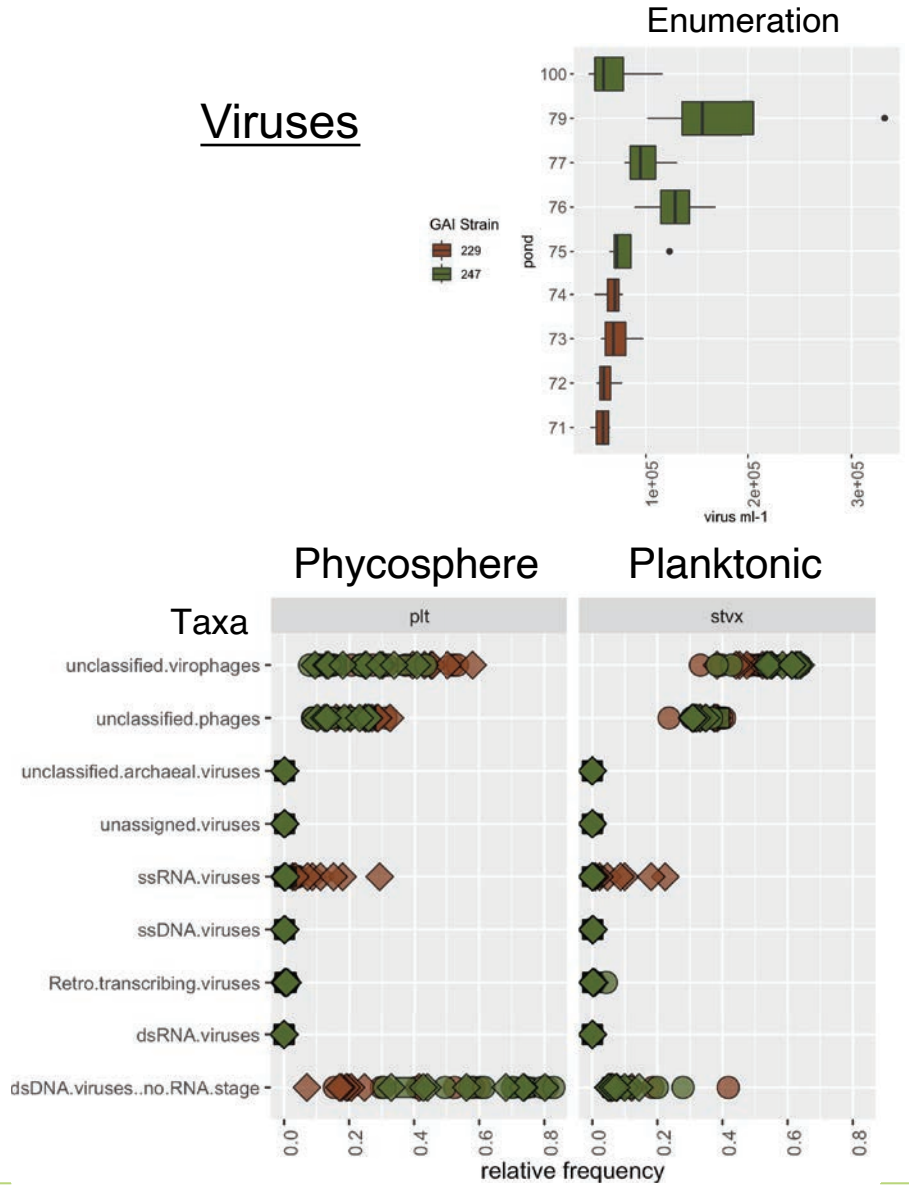
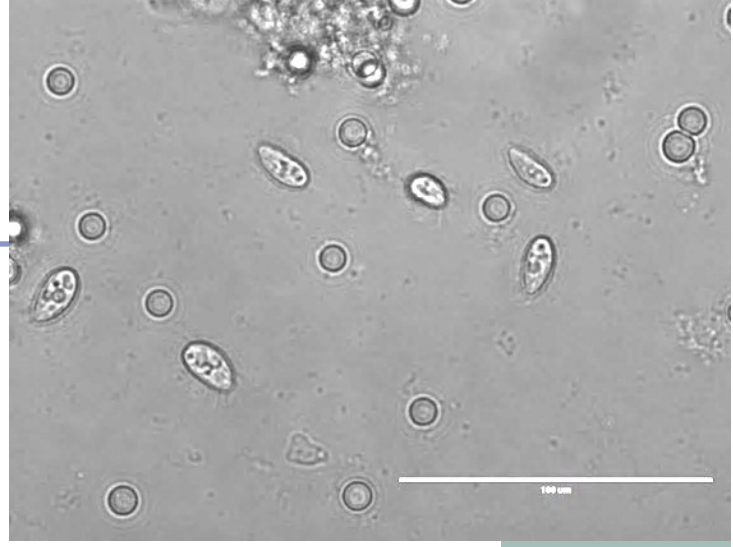
4 -Progress and Outcomes
Microbiome (Bacteria and Viruses)

Differing viral particle abundance between algal species

- **Virus populations reflect algal host and varied community structure between strain**
  - *Nitzschia :* Majority of +ssRNA sequences were found similar to *Labyrnavirus* and *Bacillarnavirus*
  - *Nannochloris*: high frequencies of dsDNA viruses within the Phycodnaviridae (phytoplankton) and Caudovirales (bacteria)

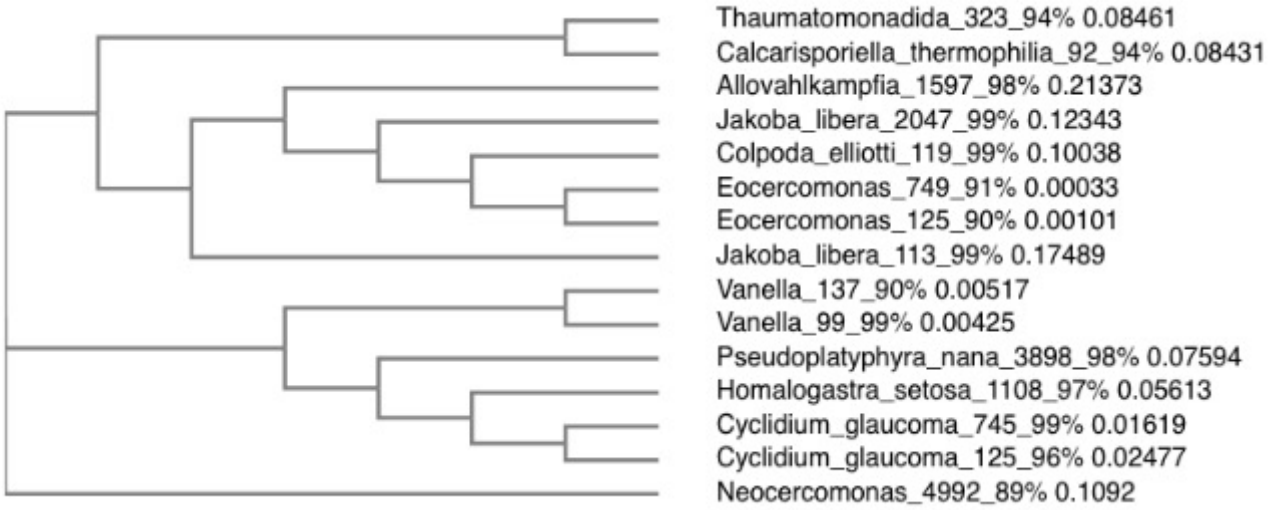


4 -Progress and Outcomes
Eukaryote Enrichments



PacBio long amplicon sequencing of 18S regions of enrichments cultured from GAI ponds

- **Ciliates (5 hits)**
  - *Pseudoplatyphyra nana* (98% sequence identity)
  - *Cyclidium glaucoma* (99% & 96%)
  - *Colpoda elliotti* (99%)
  - *Homalogastra setosa* (97%)
- **Amoeba (5 hits)**
  - Allovahlkampfia (98%)
  - Eocercomonas (91% & 90%)
  - Neocercomonas (89%)
  - Vanella (90%)
- **Flagellates (2 hits)**
  - Jakoba libera (99%)
  - Thaumatomonadida (94%)
- **Fungi (1 hit)**
  - Calcarisporiella thermophilia (94%)



Sandia has enriched cultures containing numerous ciliates. We have probes constructed to three different ciliates. Two ciliate probes work with Sandia's enriched cultures.

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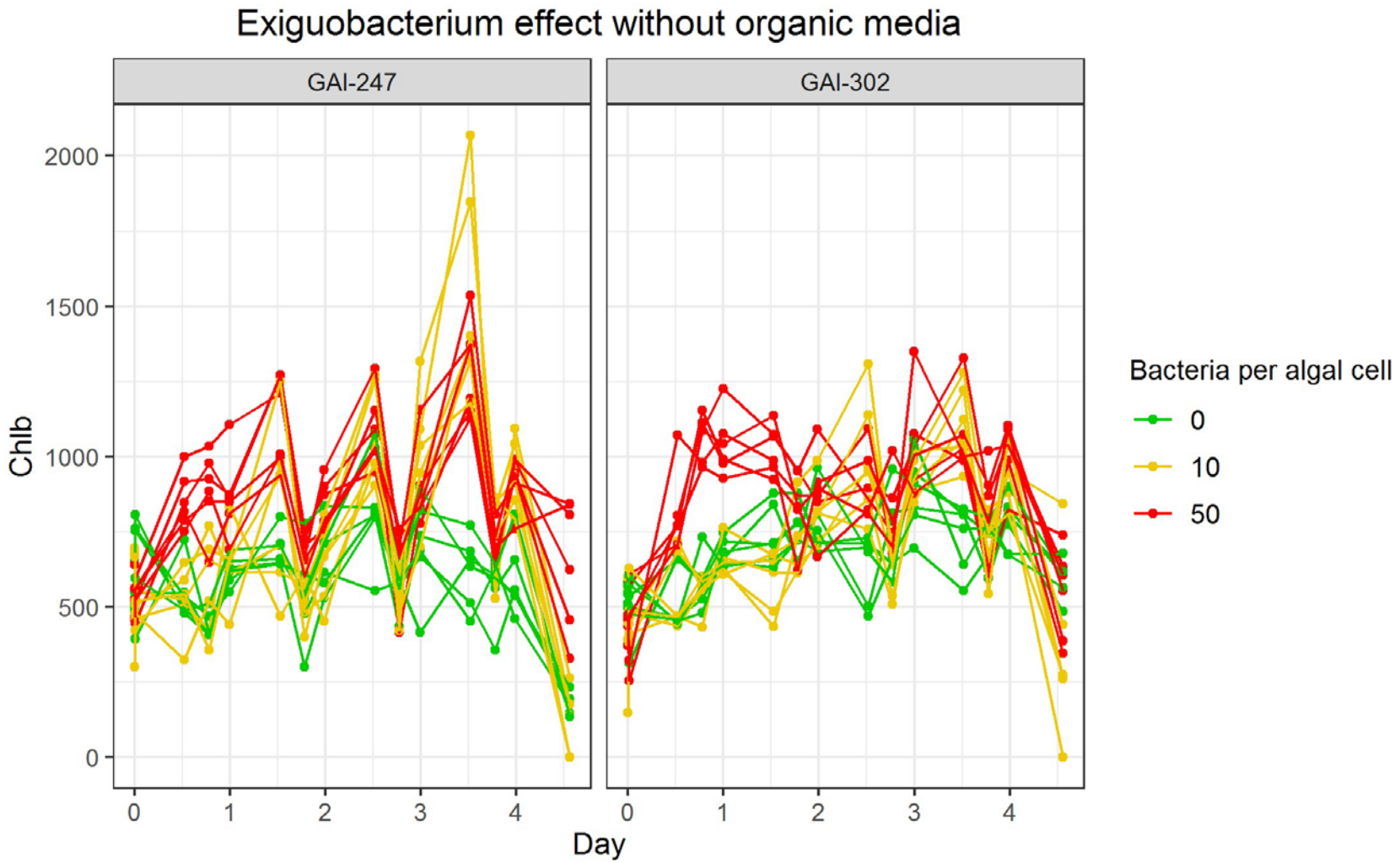
4 -Progress and Outcomes
Effect of probiotic bacterium on growth of GAI-247



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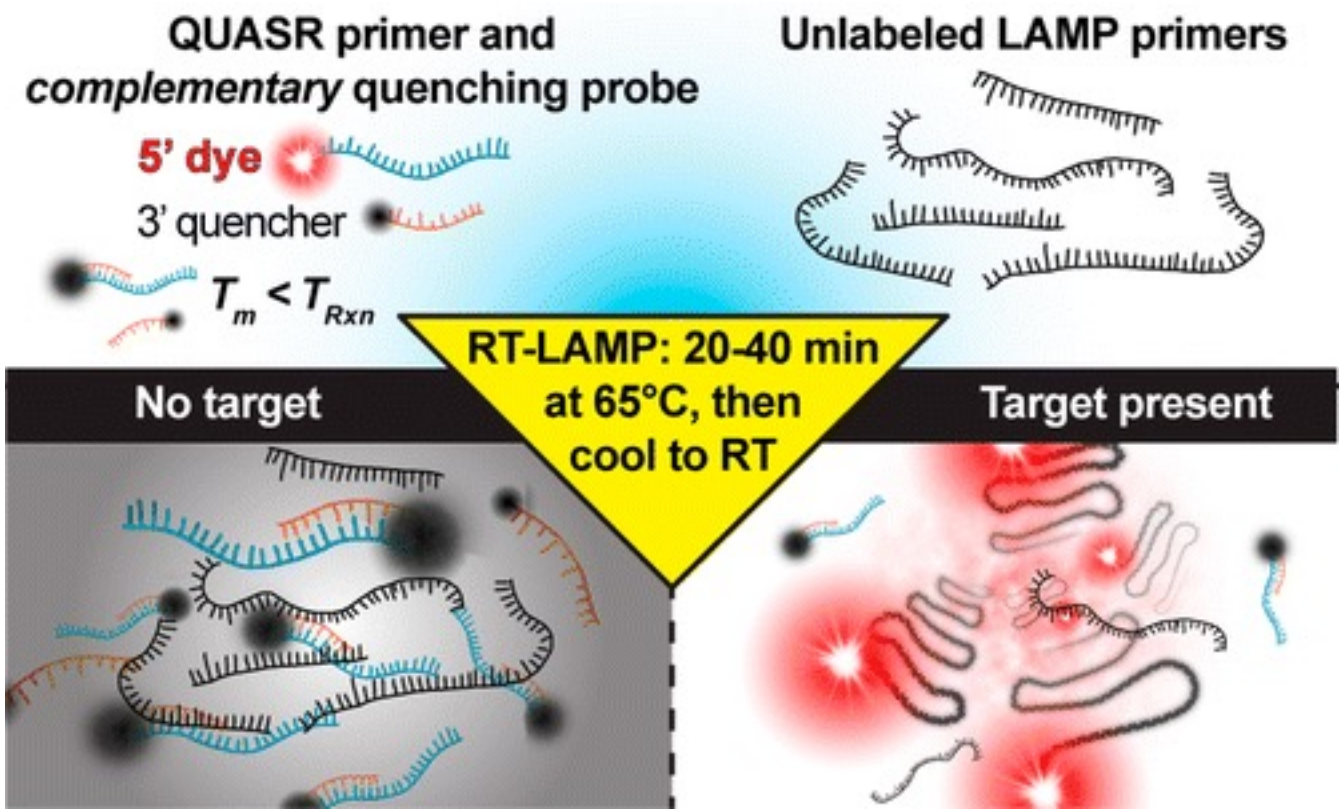
4 -Progress and Outcomes
SpinDX - Sandia

Pond-side Capability



Sandia Technology: Analytical Chemistry 2016, 88, 3562-3568

Fluorescence detector

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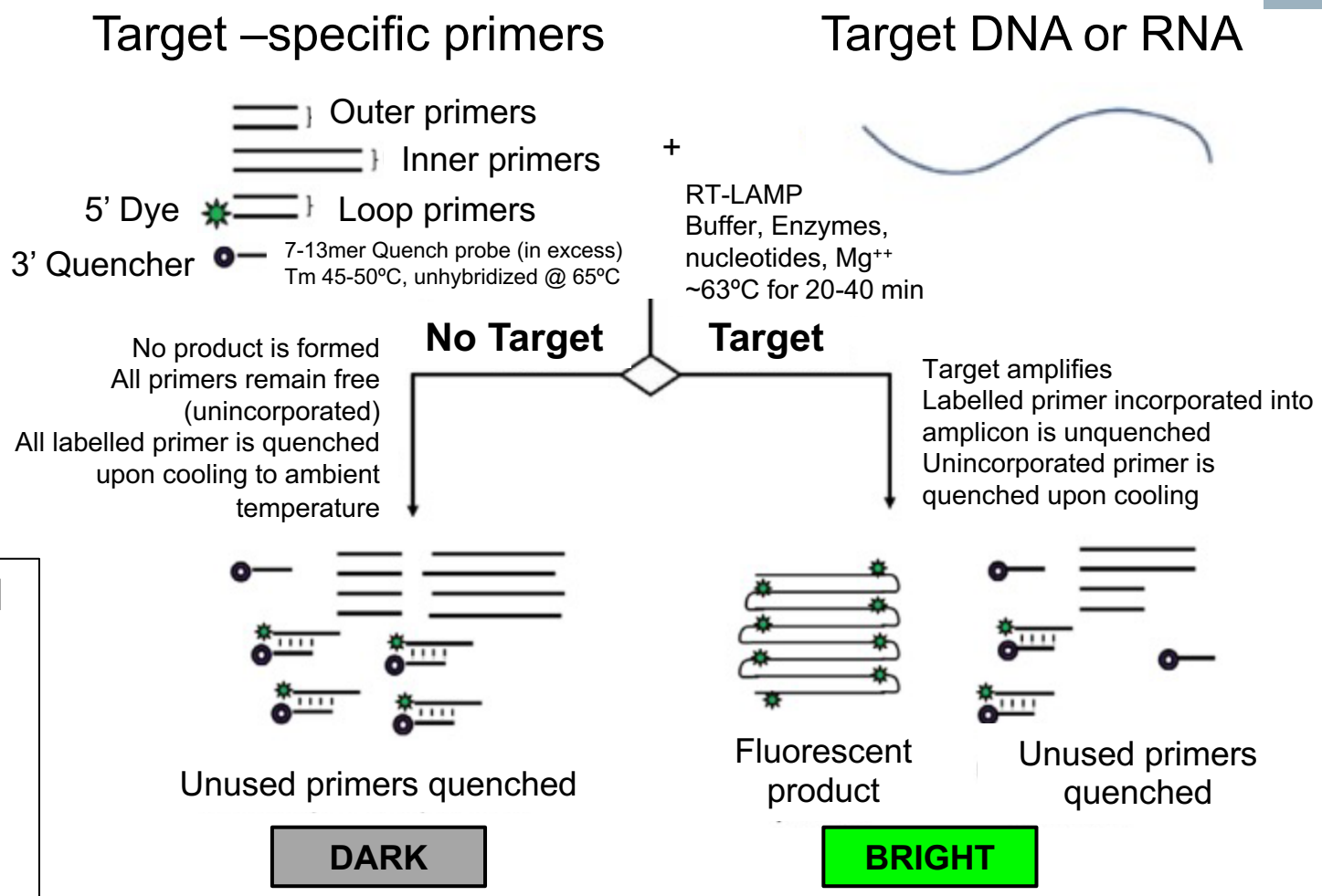
4 -Progress and Outcomes SpinDx QUASR Assays
Quenching of Unincorporated Amplification Signal Reporters

Sandia Technology: Analytical Chemistry 2016, 88, 3562-3568

- **Six primers allow for enhanced target specific detection**
- **Assays are fast < 40 min**
- **Amplification of target allows for very sensitive detection**
- **Can be multiplexed if using a plate reader**
- **Can be monitored using the pond-side diagnostic equipment: SpinDx**



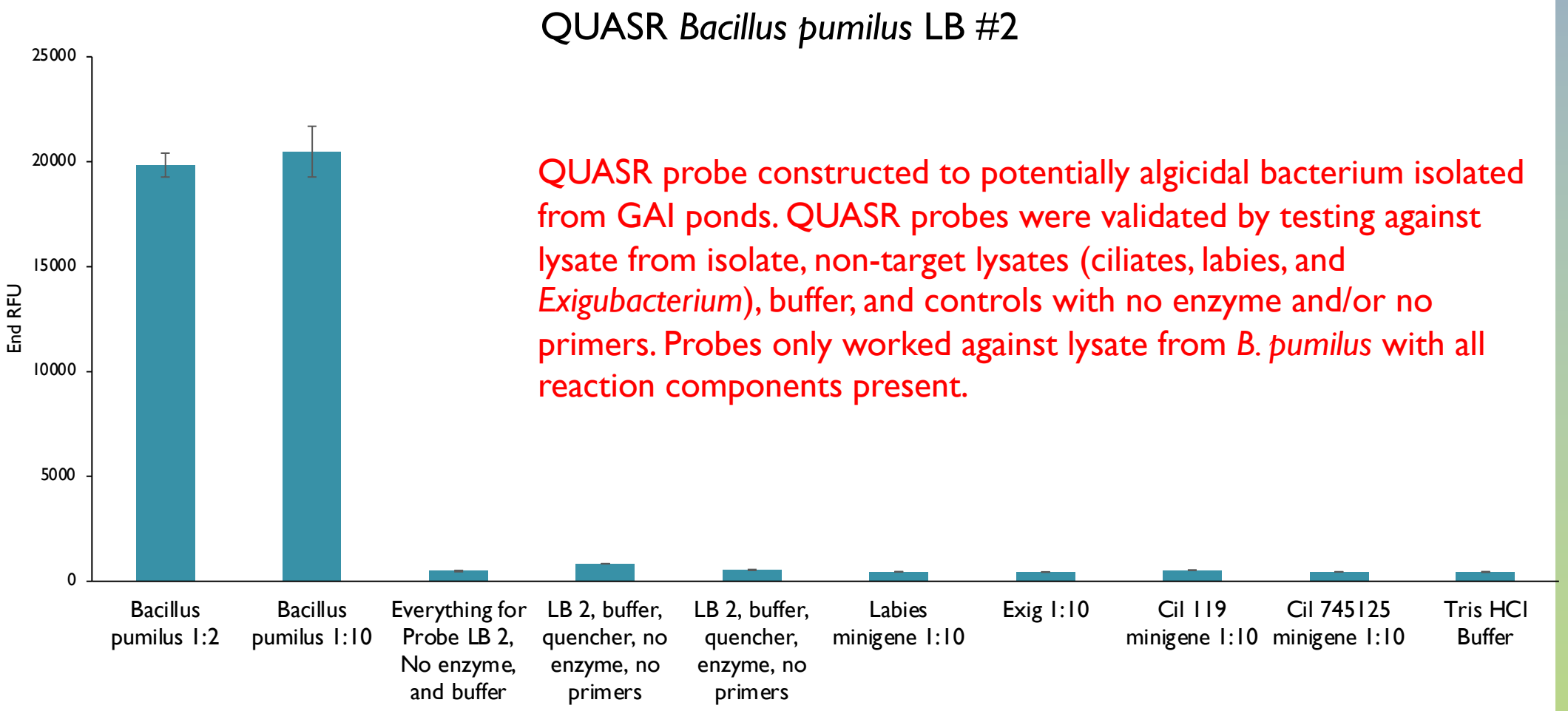
Assay reagents are pre-mixed and provided to GAI staff so that protocol is:

1. 9 µl assay mix + 1 µl pond lysate
2. Heat in plate reader or on SpinDx @ 63ºC, 20-40 min
3. Cool to room temperature
4. Detect fluorescence using plate reader or on SpinDx box

4 -Progress and Outcomes

Probes tested with suspected GAI algicidal pond isolate

QUASR probe sets were tested with all controls and either their respective lysate or cloned minigene



QUASR probe constructed to potentially algicidal bacterium isolated from GAI ponds. QUASR probes were validated by testing against lysate from isolate, non-target lysates (ciliates, labies, and *Exigubacterium*), buffer, and controls with no enzyme and/or no primers. Probes only worked against lysate from *B. pumilus* with all reaction components present.

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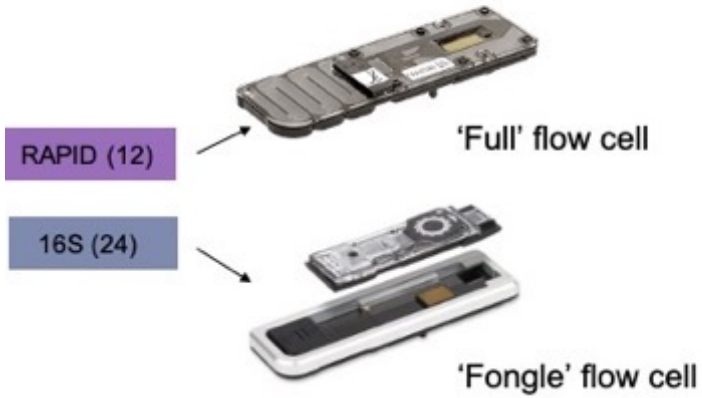
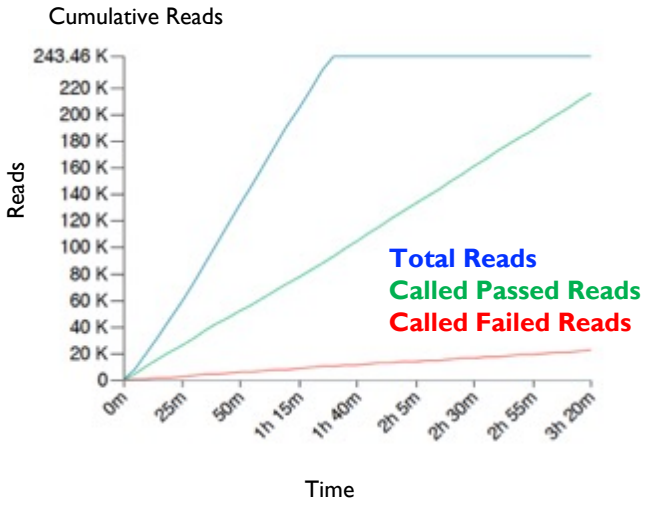
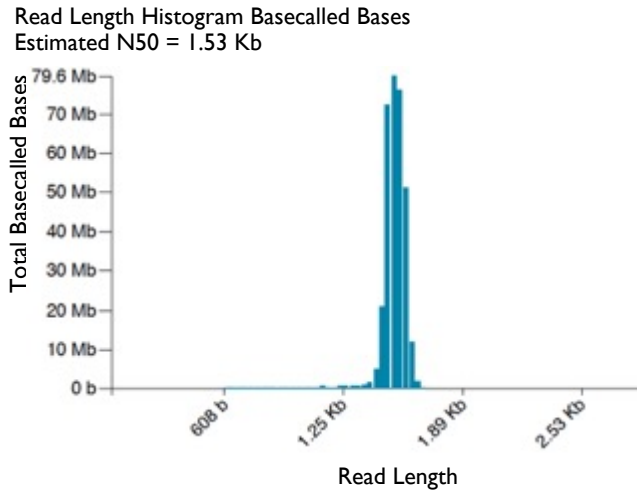
4 -Progress and Outcomes
Real-time monitoring of pond microbiomes - JCVI

Oxford Nanopore Sequencing

- **MinION:** Portable, real-time, potential for actionable results
- **Long read, 16S barcoded and full-length genome-based sequencing**
- **Investigate the utility of this technology to assess pond communities, in (or near) real-time** as well as provide an unbiased breadth of taxa not restricted to a priori knowledge
- **Test run**: DNA sequences were amplified, barcoded and sequenced in less than 2 hr. and generated >200k sequence reads with an average read length of ~1.4kb, near full gene length for 16S rDNA.







GLOBAL ALGAE

Summary

- A database of microorganisms associated with algal cultivation ponds in Kauai was generated (microbial populations are not very diverse as compared to natural environments).
- Genomes of the two algal elite strains were sequenced and assembled – this allowed to separate sequences of cultivated strains from contaminant sequences.
- Multiple strains of organisms from all taxonomic groups were isolated and are in culture.
- We identified microorganisms associated with low and high algal productivity.
- Initial testing shows potential probiotic and pathogenic effect of isolated bacteria and viruses.
- Flowcytometry was used to quantify bacteria and viruses in samples.
- We were able to detect presence and absence of microorganisms in cultivation ponds using SpinDX but the detection was not quantitative. Possibly due to interaction with pond media. Developing probes without having microbe in culture was difficult.
- Oxford Nanopore MinION NGS technology is the next advance in pond microbial detection. It was successfully tested for real time detection of 16S sequences from pond samples. This approach is the future of pond diagnostics. It detects all taxa in the pond and is not restricted to a priori knowledge. It is also affordable.

Quad Chart Overview

Timeline

- Project start 10/2017
- Project end 9/2021

	FY20	Total Award
DOE Funding	1,231,820	2,625,000
Project Cost Share	421,816	750,017

Project Partners

- Scripps Institution of Oceanography, UCSD 32%
- J Craig Venter Institute 13%
- Sandia National Lab. 11%
- Scott Fulbright 2%

Barriers addressed

Aft-B. Sustainable Algae Production
Aft-C. Biomass Genetics and Development
Aft-A. Biomass Availability and Cost

Project Goal

Achieve systematic high algal productivity through analysis and control of pond microbiota

End of Project Milestone

- Data on pond microbiota and correlations with productivity
- Low cost, rapid analytical tool for measuring microbiota
- New cultivation methodology resulting in algal productivity of >25 g/m2/day on AFDW basis

Funding Mechanism

DE-FOA-0001628

Year 2017

Topic Area 2: Cultivation Biology Improvement

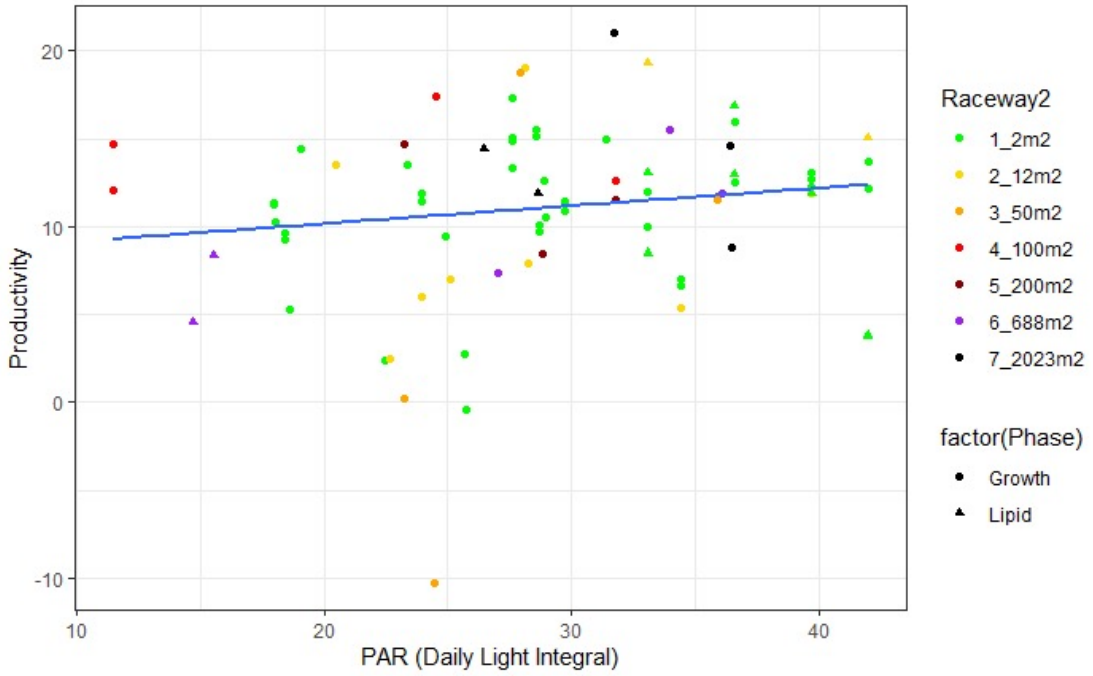
GLOBAL ALGAE

Additional slides

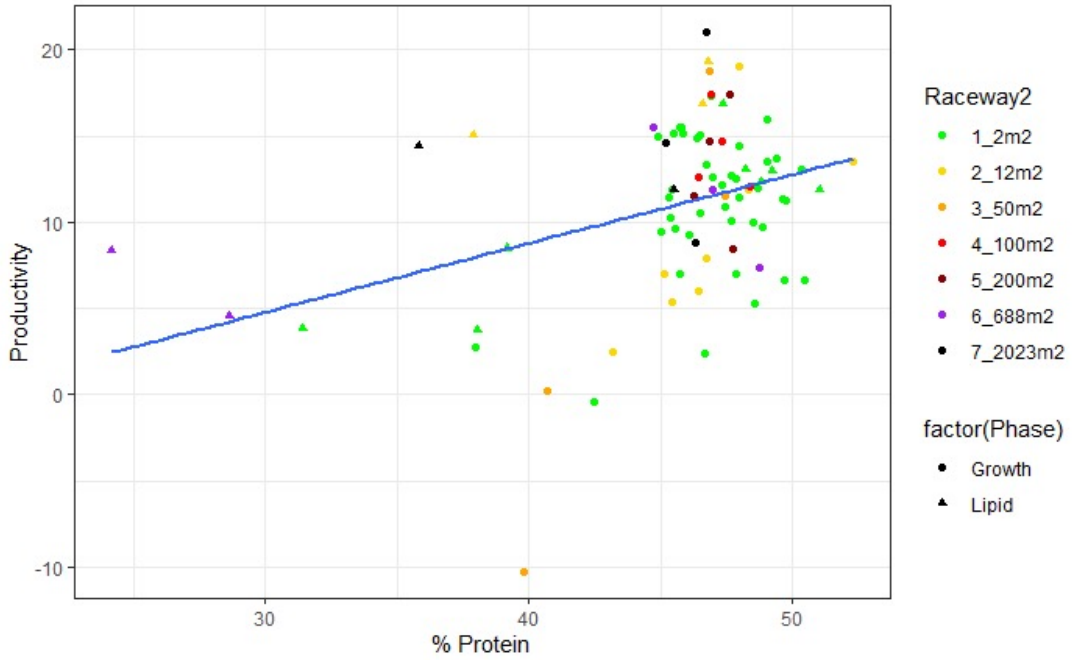
4 -Progress and Outcomes
Analysis of GAI-247 grow-outs

- 62 sampling events with algae culture conditions and 16S sequencing data with 118 OTUs were used for the analysis.
- Algal productivity was measure as g AFDW/m²/day

Productivity vs environmental data



Productivity vs algae condition



GLOBAL ALGAE

4 -Progress and Outcomes
Analysis of GAI-247 grow-outs

Measurement	r	P
Oil index	-0.48	0.000
Phycocyanin	-0.36	0.001
P per AFDW	-0.30	0.005
C/N	-0.27	0.013
N per AFDW	-0.19	0.088
PO4 in the media	-0.16	0.142
Rainfall_mm	-0.12	0.261
AirTmin_C	-0.04	0.749
PC_H	0.05	0.661
TN in alga&media combined	0.11	0.329
AirTmax_C	0.14	0.191
PAR (Daily Light Integral)	0.15	0.173
pH	0.19	0.083
AFDW g/L	0.22	0.042
PC_Protein	0.36	0.001

- Environmental conditions such as solar radiation, temperature and rainfall do not explain well observed variability in algal productivity.
- Measurements of algae conditions such as oil index and %Protein correlated with algal productivity.

Algae-Bacteria Interactions: *Single Isolates*

Isolation of Bacteria From Bulk Microbial Community

Xenic Diatom Farm Water

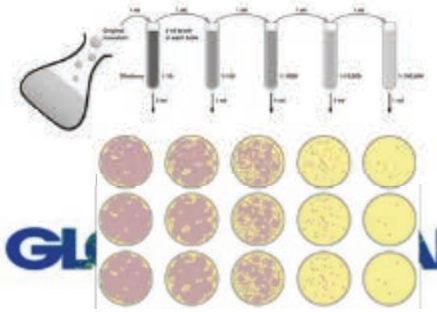
Transfer to sterile media

Batch farm water

Filter algae out and quick rinse

Bulk microbial community

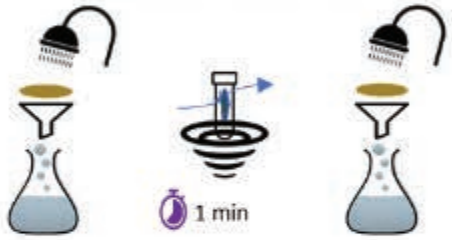
Dilute and plate filtrate



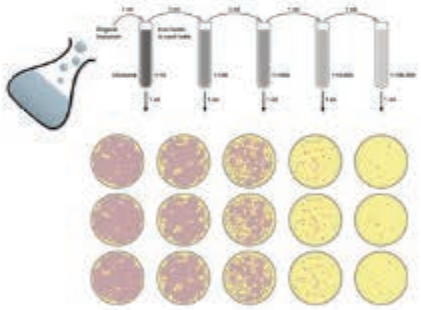
Isolation of Bacteria From Attached Microbial Community

Filtered Algae from Xenic Farm Water

Long rinse, vortex, rinse

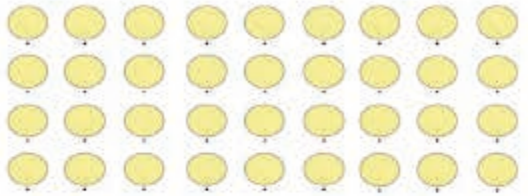


Dilute and plate filtrate

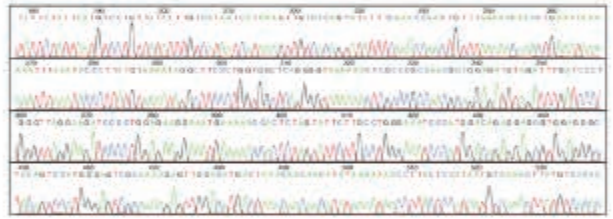


Purification and Identification of Bacterial Isolates

Purify colonies from bulk and attached mixed communities



Identify isolates using DNA extraction and 16s rRNA amplicon sequencing

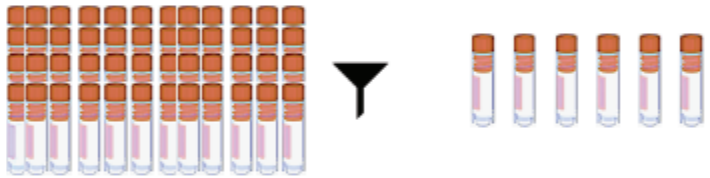


Preserve isolates

Algae-Bacteria Interactions: *Single Isolates*

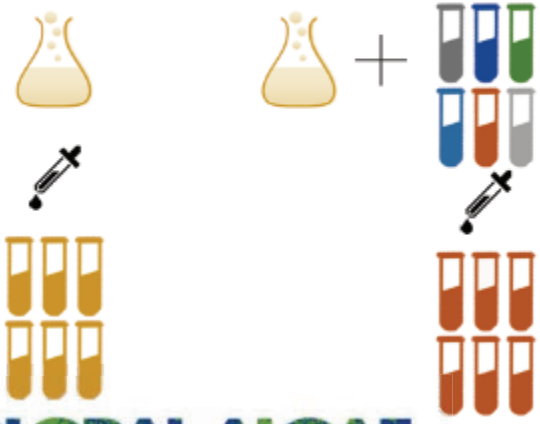
Selection of Candidate Isolates

From preserved samples select isolates of interest using the literature and preliminary data from farm water transcriptome and mixed community experiments.



Transfer isolates to liquid media

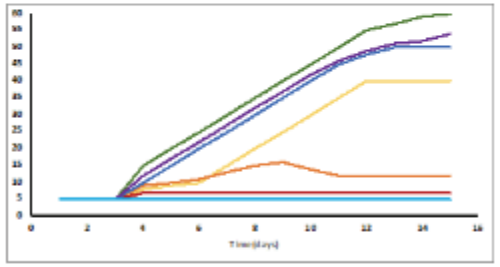
Strat control and cocultures of isolated bacteria and diatom



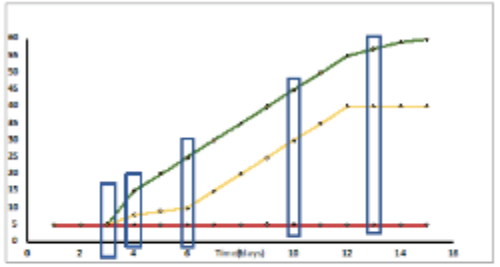
Control diatom + bacterial isolates] Coculture [diatom + bacterial isolates]

Diatom Growth Monitoring

Preform cell counts on control and coculture samples



Determine isolates with significant effect on diatom growth and timepoints of interest.



Utilize commercial kits for extraction and purification of RNA.

Generate diatom single-cell transcriptome data.

Data Analysis

Utilize bioinformatics for data analysis and visualization.

Interpret data, share, publish.

Algae-Bacteria Interactions: *Mixed Communities*

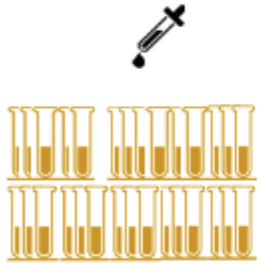
Diatom Control Cultures

Axenic Diatom Stock

Transfer to sterile media

Batch axenic algae

Transfer to sterile media



Experimental diatom control

Bulk Microbial Community

Xenic Diatom Farm Water

Transfer to sterile media

Batch farm water

Filter algae out and quick rinse

Bulk microbial community

Centrifuge filtrate

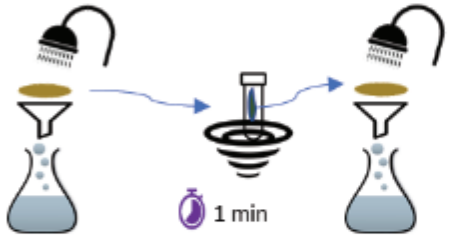
Wash and suspend pellet in sterile media

Bulk microbial community

Attached Microbial Community

Filtered Algae from Xenic Farm Water

Long rinse, vortex, rinse



Centrifuge filtrate

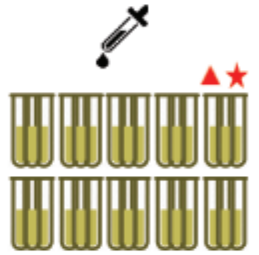
Wash and suspend pellet in sterile media

Attached microbial community

Cocultures

Axenic diatom/Bulk microbial community

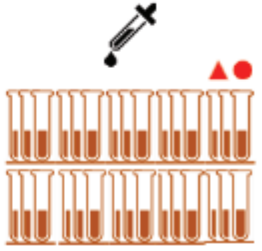
Transfer to sterile media



Experimental coculture (1)

Axenic diatom/attached microbial community

Transfer to sterile media



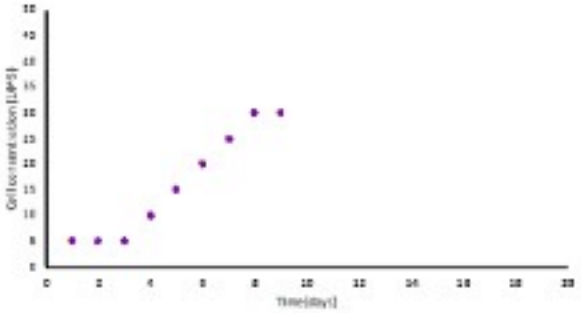
Experimental coculture (2)

GLOBAL ALGAE

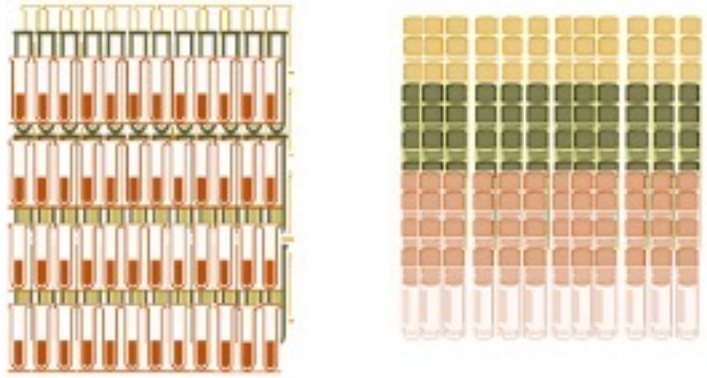
Algae-Bacteria Interactions: *Mixed Communities*

Diatom Growth Monitoring

Preform cell counts on all replicates

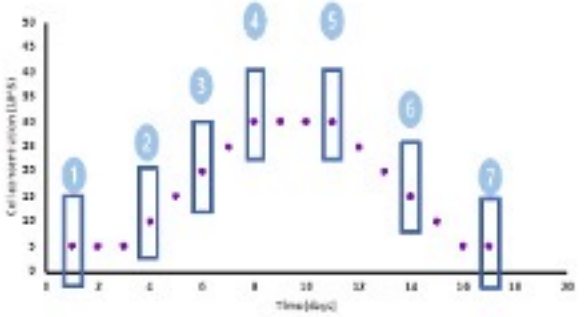


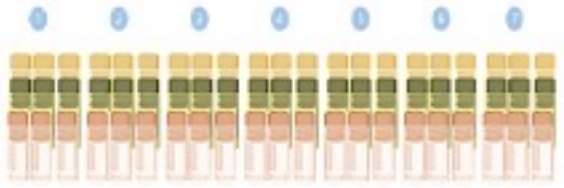
Filter diatom (2 um) and bacteria (0.2 um) and preserve triplicates of all samples at each timepoint measured



DNA and RNA Extraction and Sequencing

Retrieve samples corresponding to predicted shifts in microbial communities.





Utilize commercial kits for extraction and purification of DNA and RNA

Illumina Next Generation Sequencing:

- Miseq sequencing–Library quality control
- Novaseq sequencing–High-throughput sequencing of samples.

Data Analysis

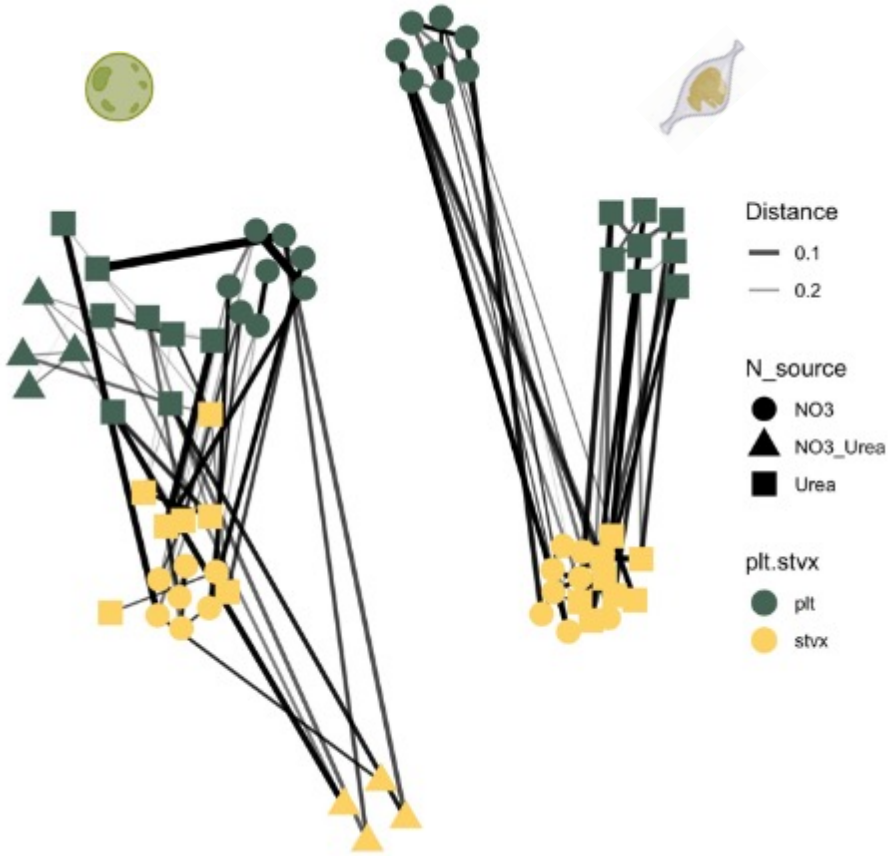
Utilize bioinformatics for data analysis and visualization.

Interpret data, share, publish.

4 -Progress and Outcomes
Microbiome (Bacteria and Viruses)

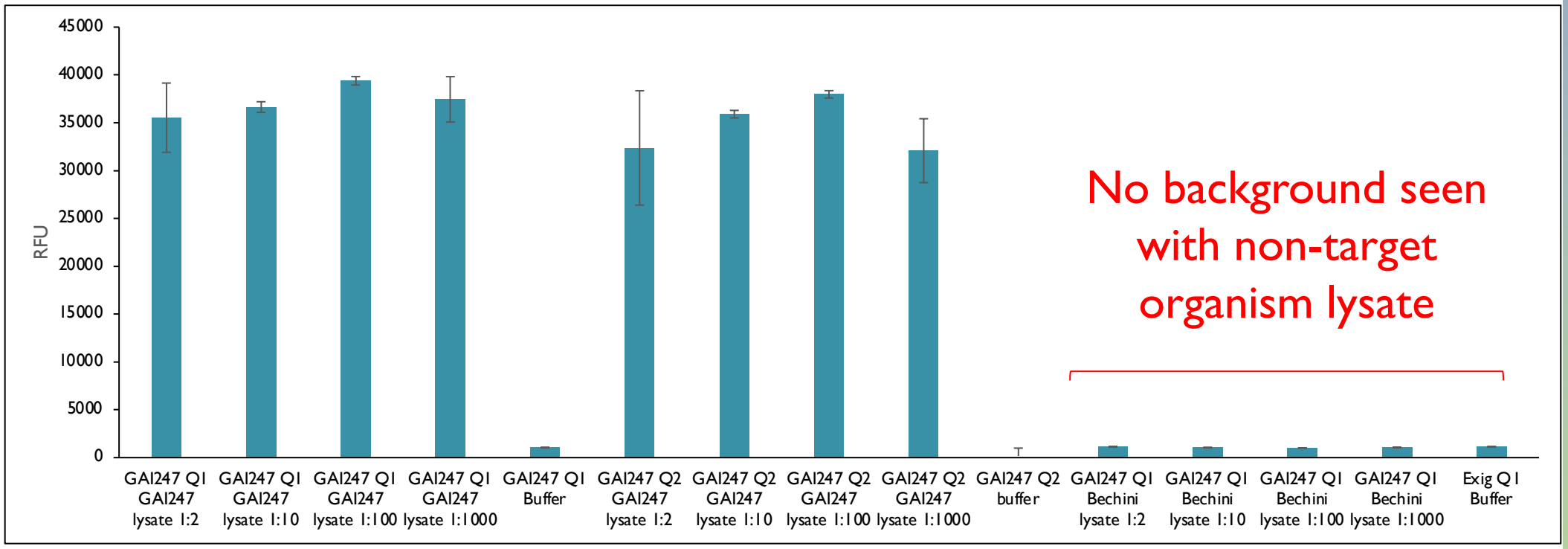
- **Diversity variation** between algal species (diatom vs green) and nitrogen type in growth media
- **Growth conditions and sample processing drive significant differences between communities**
  - However, strain-specific factors may play a larger role in compositional divergence

Bacteria



Growth Conditions Sample Processing

Assays for the detection of algal pond microbiota



QUASR Probes Q1 & Q2 designed to detect the green alga GAI 247
Detected on Tecan Plate Reader: Ex 532 nm Em 571 nm

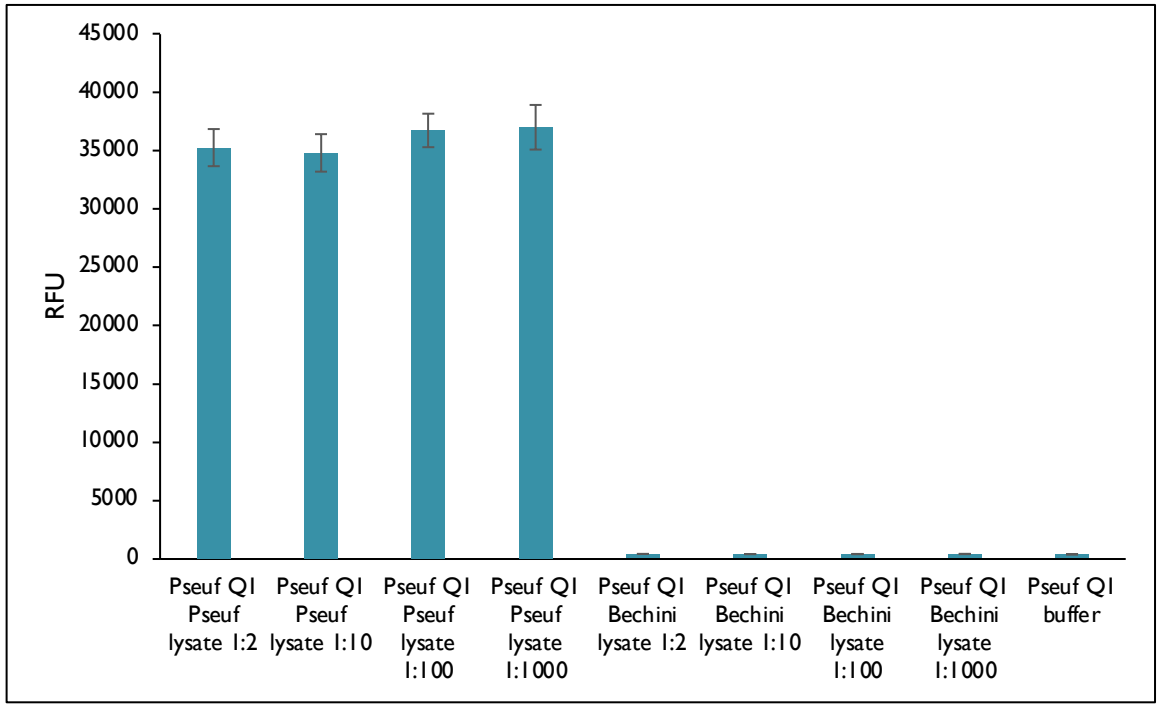
Two different probes designed to detect GAI247 were tested against GAI247 lysate (cells harvested and diluted out 1000x), buffer, and lysate to an off-target bacteria. Both probes only worked against GAI247 lysate at all dilutions tested.

GLOBAL ALGAE

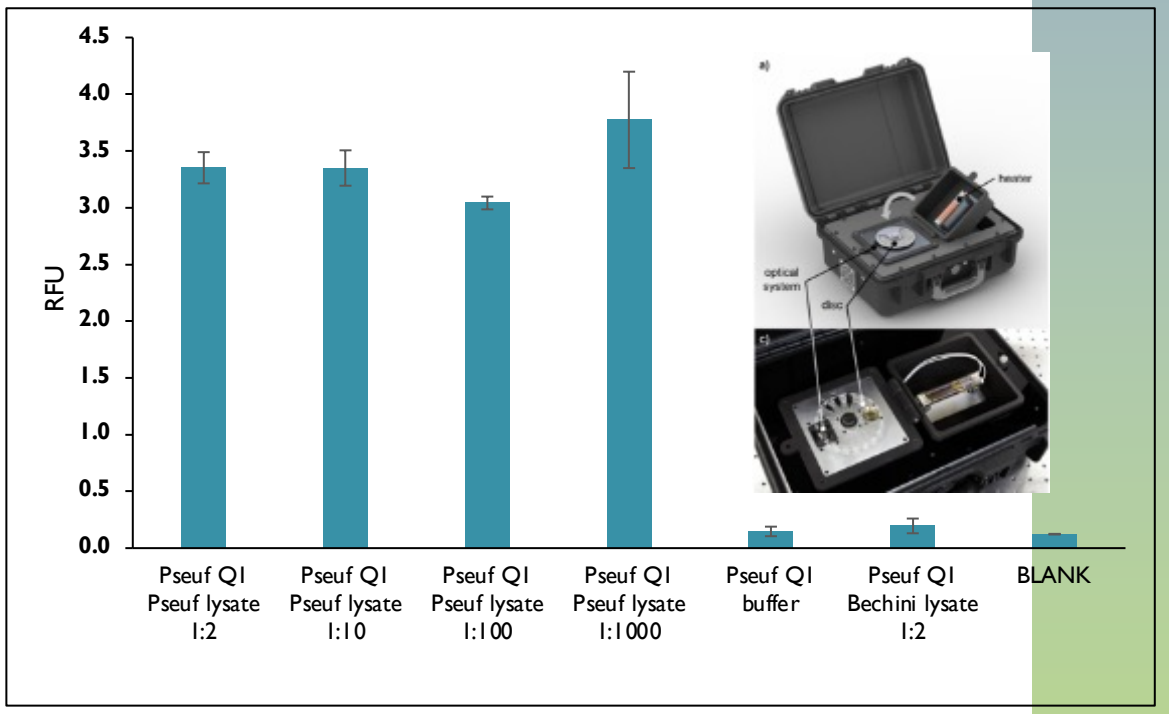
Assays for the detection of algal pond microbiota

Assays can be performed on a plate reader with temperature controls or on the SpinDx box

Tecan



SpinDx



QUASR Probe designed to detect organism of interest: *Pseufofulvimonas gallinarii*
Detected on Tecan plate reader Ex 532 nm Em 571 nm and SpinDx box

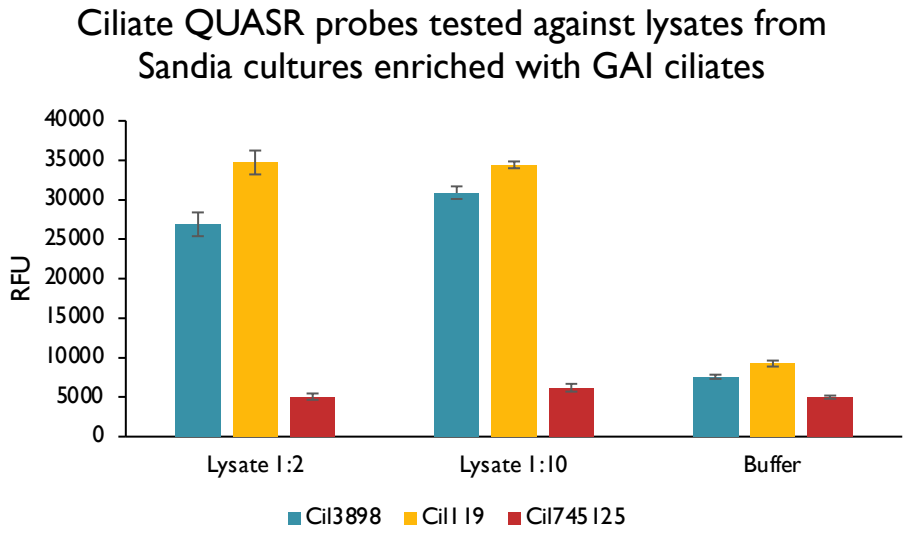
GLOBAL ALGAE

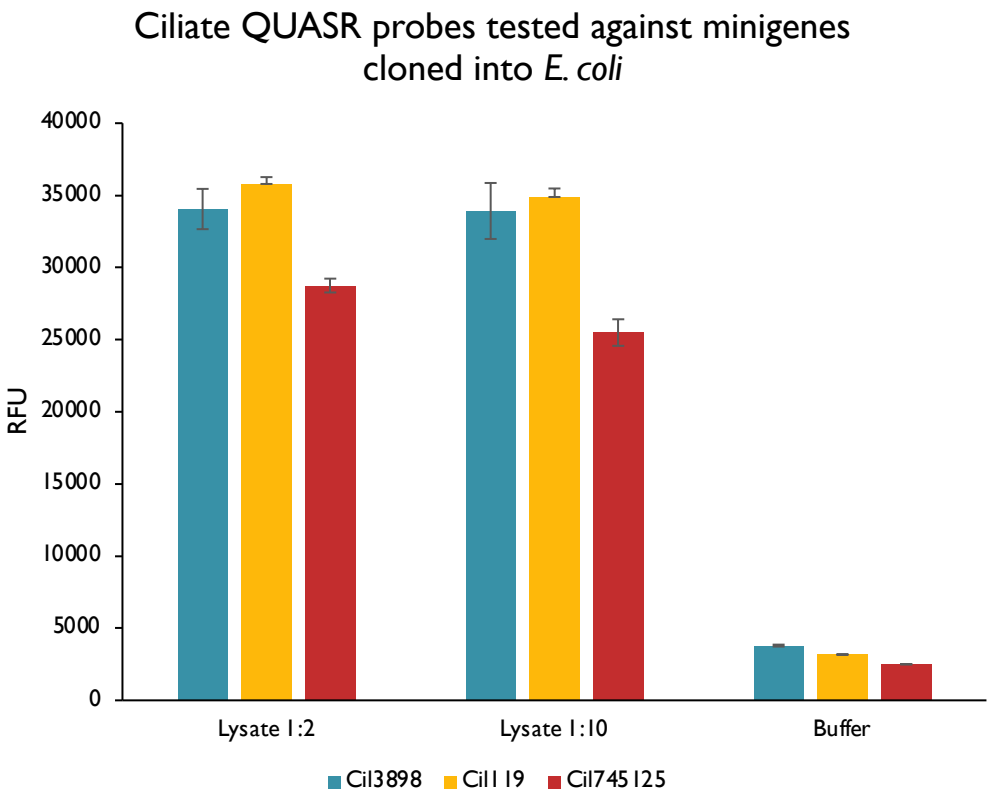
PROBES

Category	Organism	Found in GAI ponds?	In culture?	Rationale
	GAI229	yes	yes	Positive control
Algal	GAI247	yes	yes	Positive control
	Michrochloropsis salina	no	yes	Test probe
Bacterial	*Exiguobacterium*	yes	yes	Pond isolate
	Pseufofulvimonas gallinarii	yes	no	Potential interest
	Bizionia echini	no	yes	Algicidal
Eukaryotes	*Brachionus plicatilis*	no	yes	Test probe
	Pseudoplatyphyra nana	yes	yes	Ciliate probes
	Cyclidium glaucoma	yes	yes	
	Colpoda elliotti	yes	yes	
	Homalogastra setosa	yes	no	
	Allovahlkampfia	yes	yes	Amoeba probes
	Eocercomonas	yes	yes	
	Neocercomonas	yes	no	
	Vanella	yes	no	
	Jakoba libera	yes	no	Flagellate probes
	Thaumatomonadida	yes	no	
	Calcarisporiella thermophilia	yes	no	Fungal probe

* https://pubs.acs.org/doi/pdf/10.1021/acs.analchem.5b04054

QUASR probes made to different ciliates identified in GAI pond samples





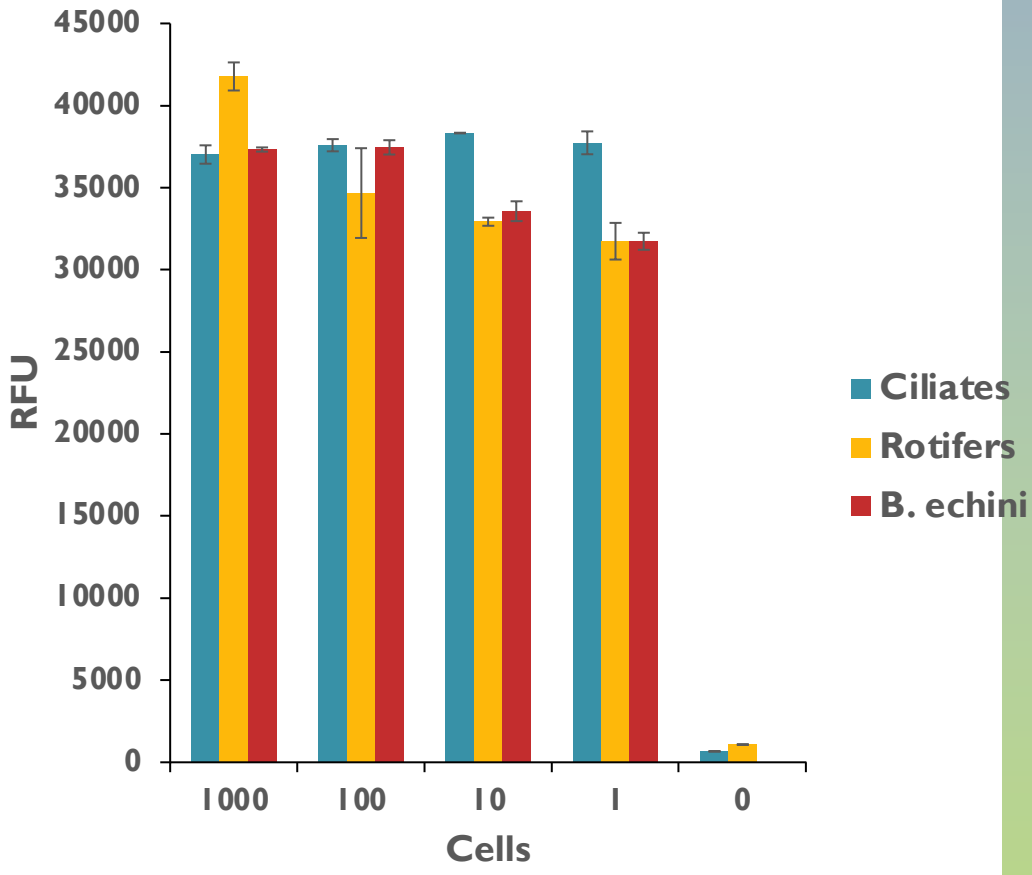
- ***Pseudoplatyphyra nana* (3898)**
- ***Cyclidium glaucoma* (745125)***
- ***Colpoda elliotti* (119)**

Cyclidium glaucoma (745125) constructed but does not detect ciliates in enrichment cultures. This suggests that *Cyclidium glaucoma* is not present in Sandia's enrichments but present in GAI ponds. Validated probe does work with the positive control minigene.

GLOBAL ALGAE

Sensitivity of LAMP for Detection of Pond Microbiota

- Organisms present in dense pond samples were detected using LAMP assays on SpinDx box.
- Organisms were counted using microscopy or by measuring optical density and then concentrated and / or diluted in dense pond backgrounds and lysed using bead beater
- Pond sample volume is 1 µl diluted in total assay volume of 10 µl
- Plot demonstrates sensitivity of assays



GLOBAL ALGAE

Responses to Previous Reviewers' Comments

- Understanding the microbial community data is likely to be complicated and time consuming. The project would benefit from more clear objectives in this area, timeline and mitigation strategies.
- **We generated the database of microbes found in the Kauai ponds. We identified OTUs associated with low and high productivity. We demonstrated on the laboratory scale a potential probiotic and pathogenic effect of bacteria and viruses. Our goal is to demonstrate that we can detect a pathogen and lower its abundance. This requires quantitative detection which we found to be challenging but a new approach using MinION is the solution.**

Responses to Previous Reviewers' Comments

- Specific dates of milestones and go/no-go decisions absent. In the future, how can the team reduce the time and resources required to understand pond crashes of different strains?
- **We have a list of goals and milestones that we are carefully tracking.**
- **The tasks associated with isolation of Labirynthulids and DOM remediations were delayed due to difficulties in isolating a pure culture. Labirynthulids were isolated but are maintained in media prepared with algae.**
- **Microbe treatment development tasks and final PEAK challenge were delayed due to covid 19.**
- **Treatment of pathogens outdoors was delayed due to inaccurate quantitative detection of microbes with SpinDX.**
- **Full list of goals and milestones is on the next slides with delayed tasks marked in red.**

Publications

- We have 3 Manuscripts in Prep

- Oliver A, Podell S, Pinowska A, Traller JC, Smith SR, *et al.* Diploid genomic architecture of *Nitzschia hildebrandi* str. GAI293, an elite biomass production diatom.
- Rabines A, Zeigler Allen L, Pinowska A, Allen E, *et al.* Microbial and Viral Communities Associated with Outdoor Algal Ponds for Bioproducts Production.
- Maham K.M. et al. QUASAR assays