BIOSCIENCE RESEARCH FOUNDATION

SAND2017-11827C

Algal crop protection strategies and technologies

Todd W. Lane Ph.D., Distinguished Member of Technical Staff
Sandia National Laboratories, Livermore California USA

1 November 2017
Algae Biomass Summit
Salt Lake City Utah

Sandia National Laboratories is a multimission laboratory managed and operated by National Technology and Engineering Solutions of Sandia, LLC, a wholly owned subsidiary of Honeywell International, Inc., for the U.S. Department of Energy's National Nuclear Security Administration under contract DE-NA-0003525.

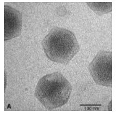
U.S. DEPARTMENT OF ENERGY | NNSA National Nuclear Security Administration | U.S. DEPARTMENT OF ENERGY Office of Science | U.S. DEPARTMENT OF ENERGY Energy Efficiency & Renewable Energy BIOENERGY TECHNOLOGIES OFFICE

Sandia National Laboratories

Algal ponds at risk from “unknown biothreats”

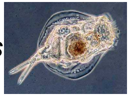
Agent + *Algae* + *Environment* = *Collapse*

Viruse s

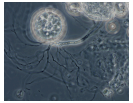


Bacteria

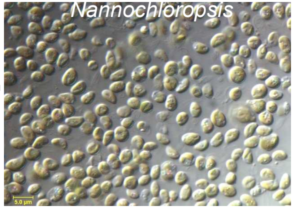
Predators



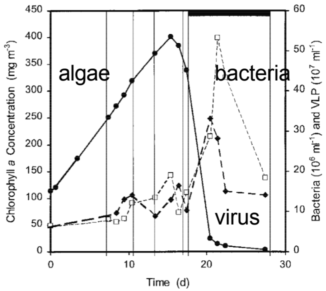
Fungi



Patterson & Laderman, 2001.



Environment (Temp, salinity, pH, CO_2, nutrients)



Herman Gons et al., Antonie van Leeuwenhoek, 81: 319-326, 2002.

“Perhaps the most worrisome component of the large-scale algal cultivation enterprise is the fact that algal predators and pathogens are both pervasive and little understood.”

- DOE Draft Algal Biofuels Technology Roadmap (2009)

A few agents are well known: *Brachionus*. Others are novel: *Chaetonotus*

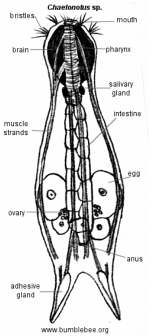
Voracious predator: Ingests >200 cells/min, Can clear a pond in 48 hrs
The presence of 1 organism per 1000L can eventually lead to a crash

Two BETO funded projects:
- Pond Crash Forensics
- ATP3

Sequencing of >1000 samples from healthy and crashed ponds

Identification of deleterious species present in pilot-scale production ponds

Data informing DISCOVR



Protist information server 1995-2016

Rotifers:
- Cultured for aquaculture feed
- Raised commercially on *Nannochloropsis*
- Similar to species used in biofuels research

Chaetonotus
- gastrotrich
- Not previously reported to crash eukaryotic algal production ponds

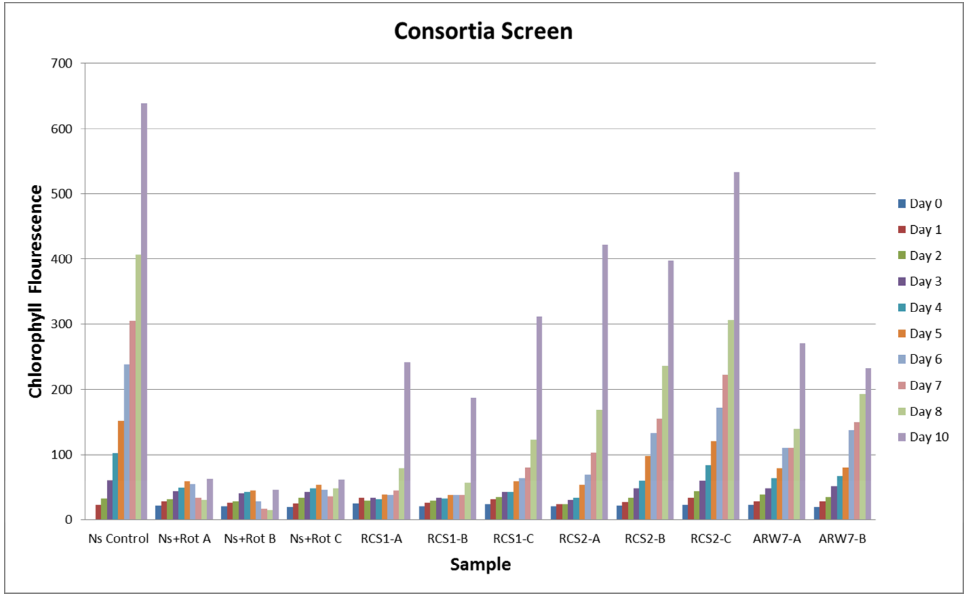
TABB Project: "Engineering" the pond microbiome to defend algal mass cultures

Lead institution: Lawrence Livermore National Lab
Partner institutions: Heliae LLC and UCSC

SNL goal is to isolate individual microbes or microbial consortia that

- Persist in outdoor cultivation with algae production strains
- Protect those production strains from attack by deleterious species
- Have no impact on productivity of production strains in the absence of deleterious species
- Have no effect on downstream use of algal biomass

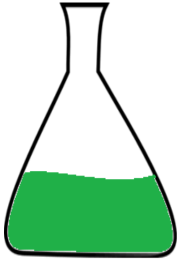
Postdoctoral researcher:
Carolyn Fisher



Microbial consortium: Defense against rotifer predation

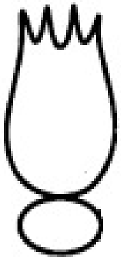
Consortia experiment: screen

Algae survival assay (Rotifer live/dead assay)



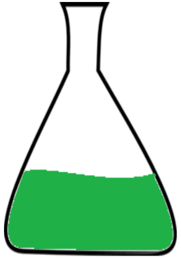
protective algal-bacterial consortia

+/-

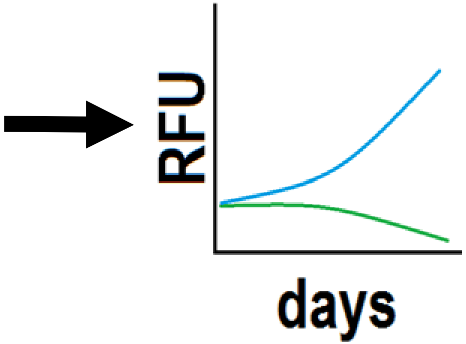


rotifer

+/-



Ns control

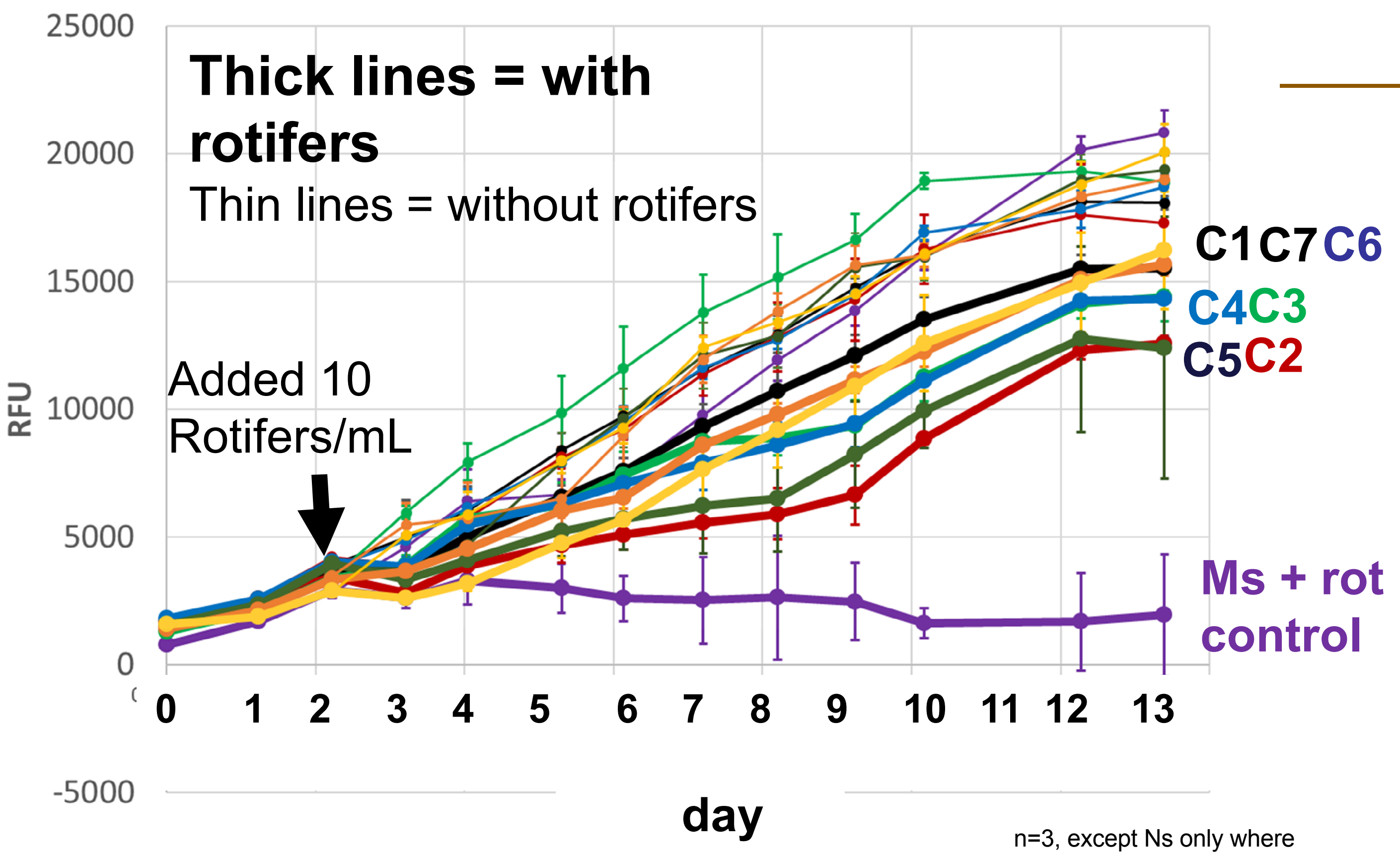


consortia-protected algae with rotifers

control algae, not protected from rotifer predation

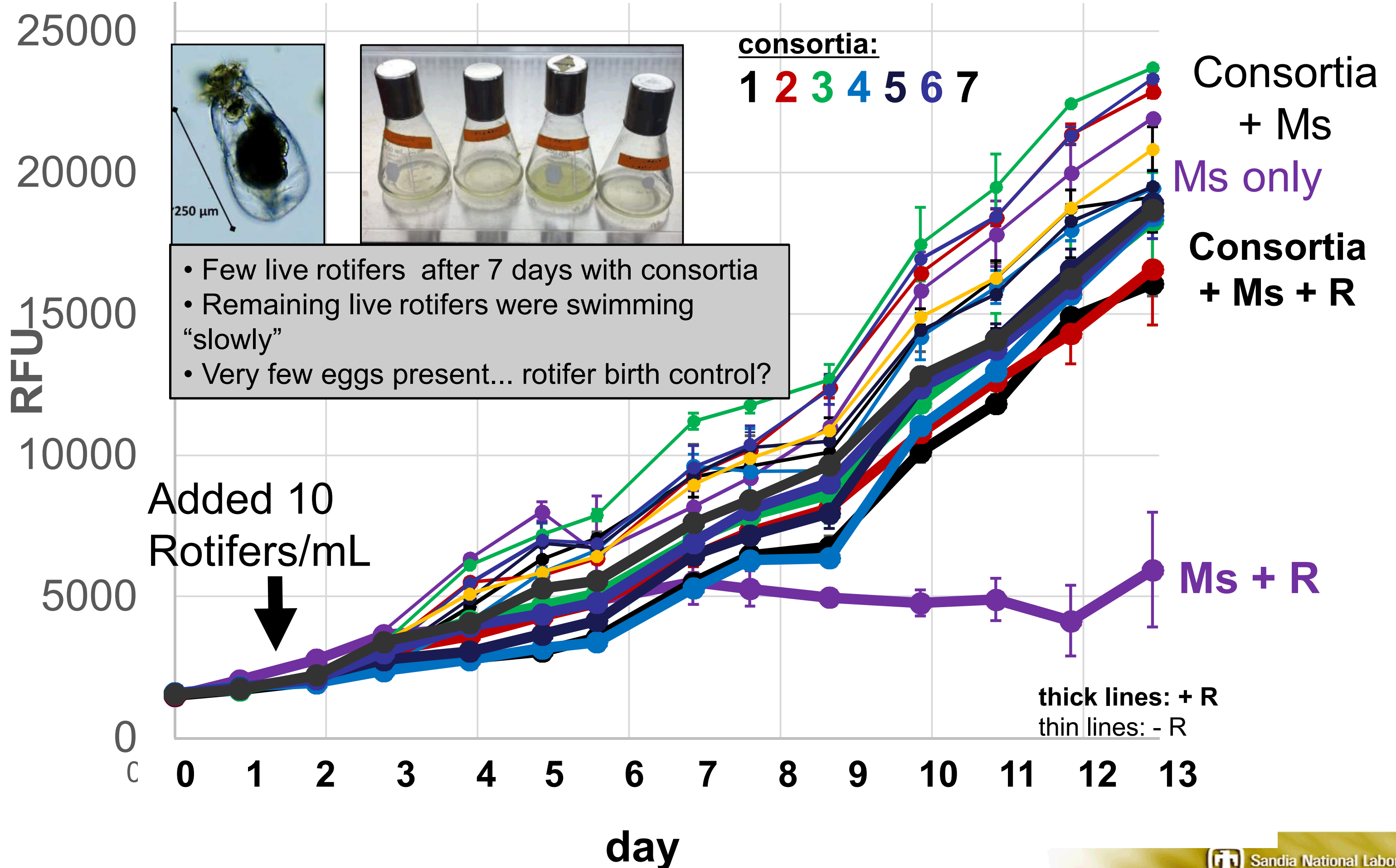
- *Microchloropsis salina:* 1-2 M Ns cells/mL
- *Brachionus plicatilis:* 10 Rotifers/mL
- Daily timepoints, ex/em: 430/685 nm

Consortia yield protection from predation by rotifers



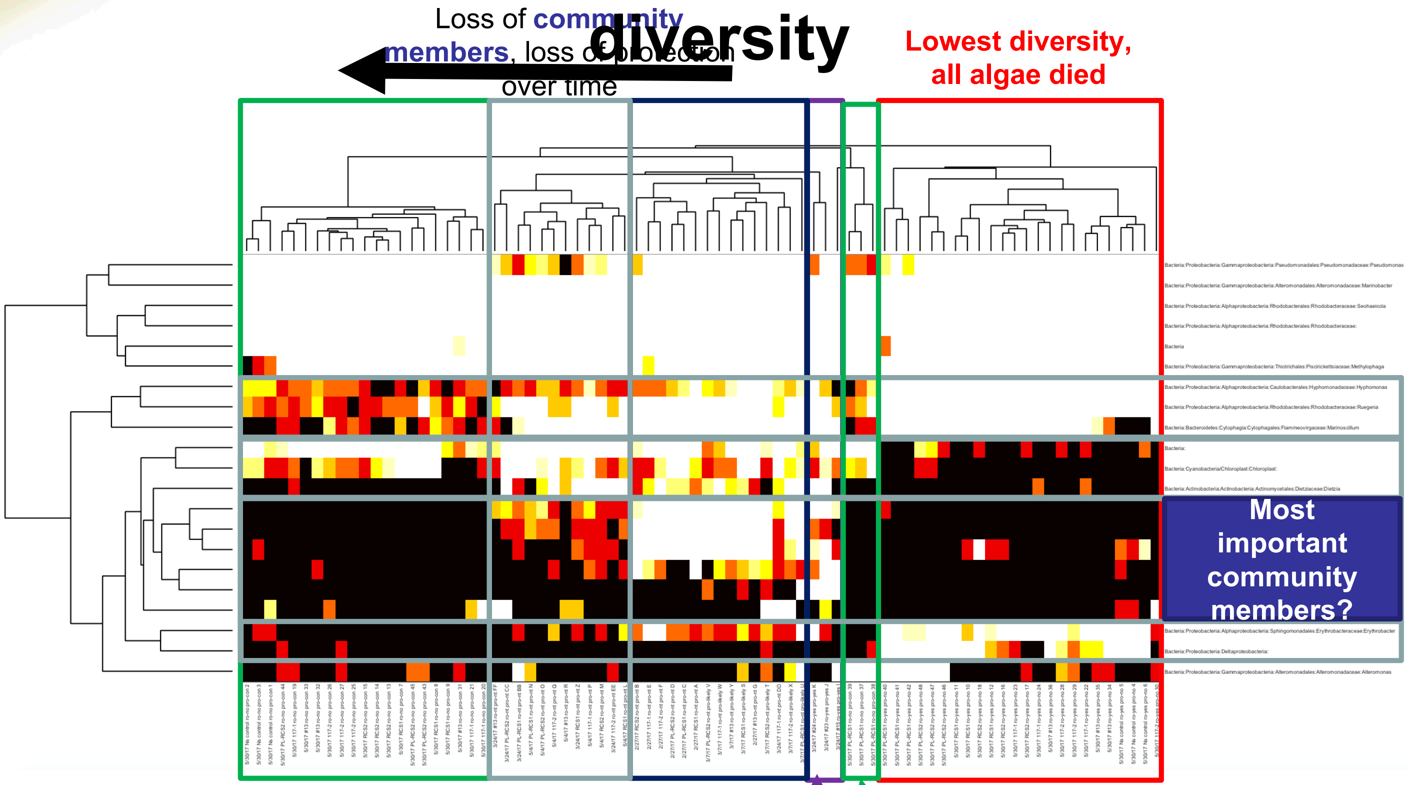
n=3, except Ns only where n=2

Bacterial consortia protect algae from rotifers



- Few live rotifers after 7 days with consortia
- Remaining live rotifers were swimming "slowly"
- Very few eggs present... rotifer birth control?

Protective consortia have more bacterial diversity



Consortia (late May) No Rotifers added

Consortia (late March – early May)

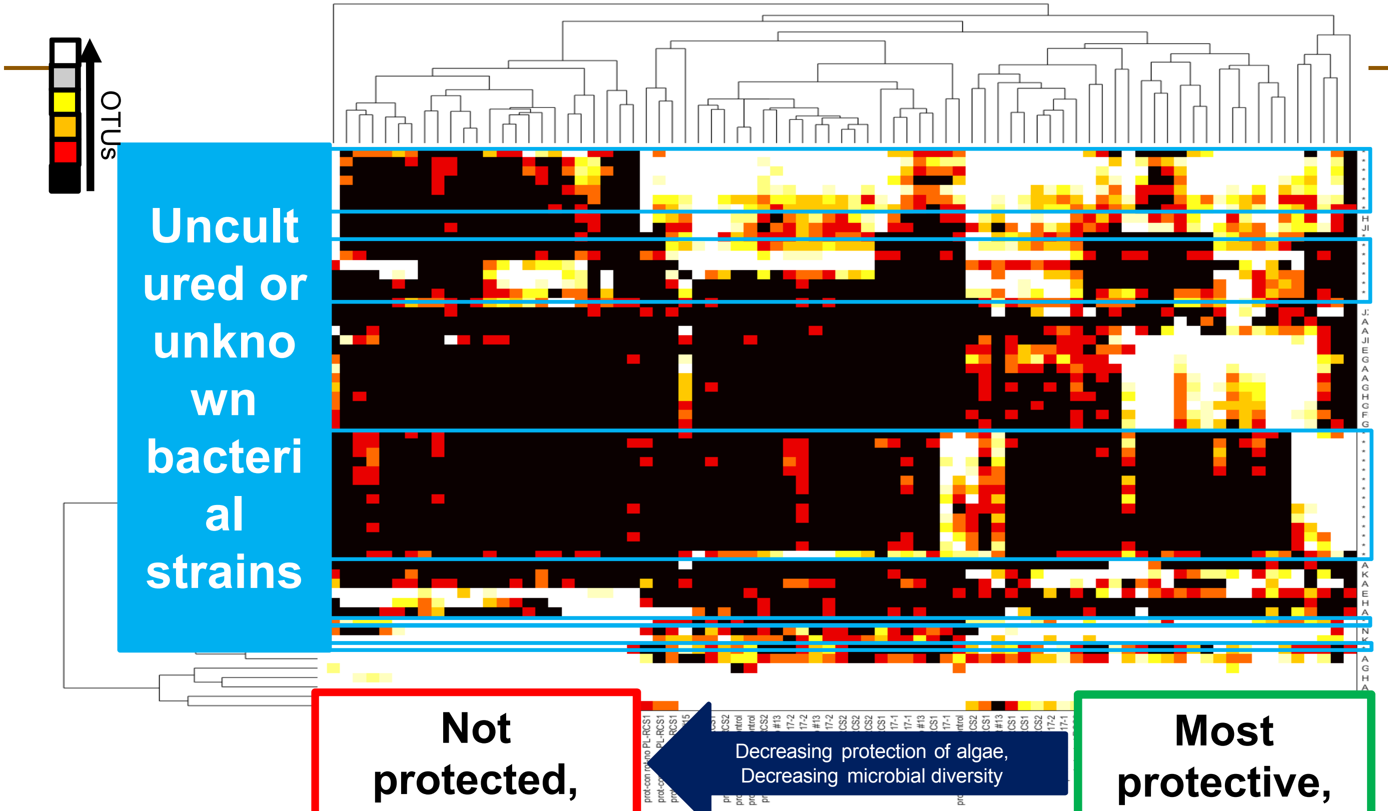
Consortia stocks (late Feb – early March)

Consortia with rotifers, **nonprotective (late May)**

Consortia (late May), No rotifers added

3 outlier flasks, all with dead rotifers → protective!

Protective consortia have more bacterial diversity



Not protected, lowest diversity

Most protective, highest diversity

PEAK Project: PondDx

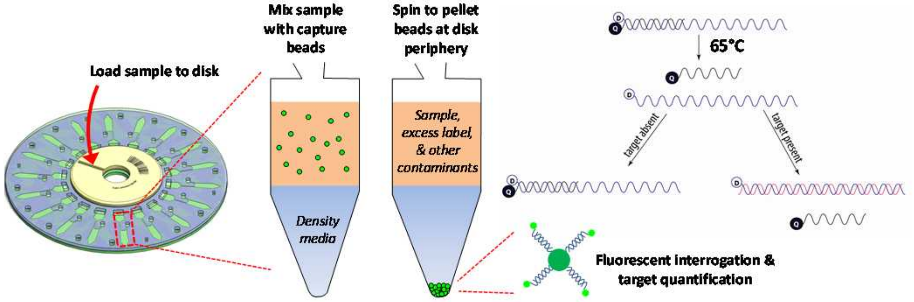
Project lead: GAI

Goal is to use PonDx provide early and rapid detection of positive and negative members of the pond microbiome

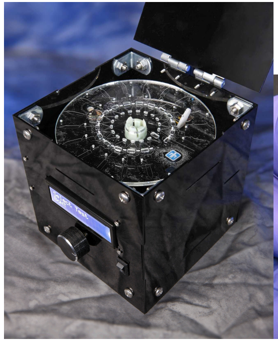
Including grazers and probiotic species

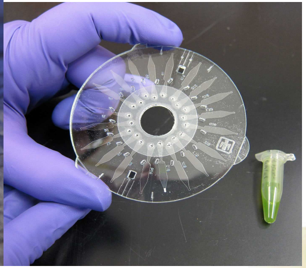
Postdoctoral researchers: Krissy Mahan

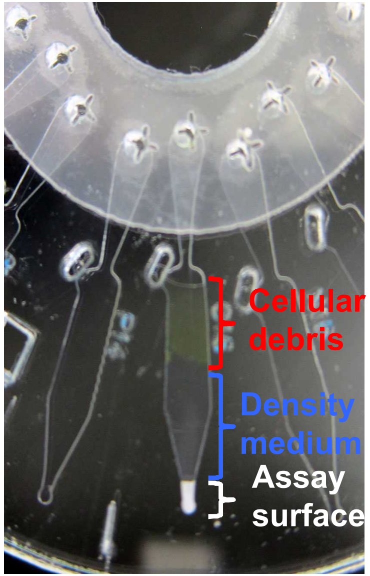
- FRET-based bead hybridization assay enabling capture and quantification of pathogen-specific RNA/DNA signatures



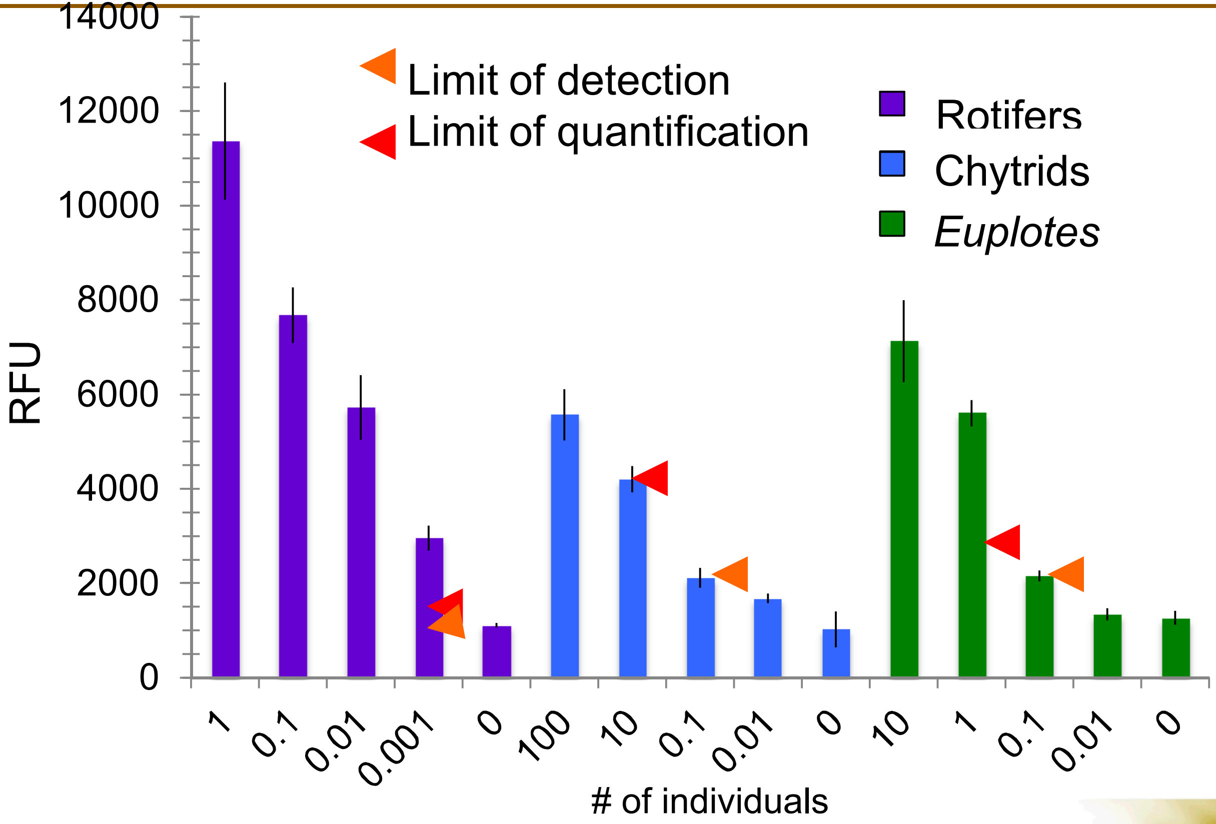
- Assay time: approx 30 min
- 36 channels per disc
- Potential for multiplexed assays in each channel
- Low reagent costs
- Low material costs
- Low instrument cost ($1000)
- Fieldable







PonDX detection/quantification of pest species



Carney et al 2017

Cheap detection is important for biofuels

BETO Harmonized Model System- $5/gge Scenario

Annual Productivity (calculated)	13g-AFDW/m2/day
Daily production per pond (40,000m2 per pond)	0.52 MT/day
Annual production per pond (330 days)	171.6 MT/pond/year
Algae Lipid Content	25%
Overall Algal Oil Yield	1000 gal_Algal_oil/acre/yr.
Biomass loss through predation	10-30%
Annualized Biomass lost per pond	17.2-51.5 MT/pond/year
Annualized Biomass lost per farm (101 ponds)	1737-5201 MT/farm/year
Algae Lipid Content	25%
Annualized lipid loss per farm per year	434-1300 MT/farm/year
Total value of lost algal lipid per farm per year	$608K -$1.82M/farm/year

Must double

$3000/acre
@ current values

@ $5 gge

Harmonized model from ANL, NREL, and PNNL. (2012);
Richardson et al. (2014) Algal Research 4, 96-104;
McBride et al. (2014). Industrial Biotechnology 10, 221-227

PonDx versus PCR

Number of analytes	#	Number of deleterious species to be tracked	12
Assay frequency			Daily
Days of operation per year	# days per year		330 days
Number of ponds assayed per farm	# ponds per farm		101 per farm
Total annual number of assays per farm	**# assays per farm per year**		**400000 per farm per year**
Cost of thermocycler	$	Assuming one PCR machine per farm	$30,000 - 50,000
Reagent cost per assay	$		$1.70
Total annual reagent cost of surveillance by qPCR	**$**	**Per farm per year**	**$680K**
Cost of PonDx boxes	$	Assuming one PonDx machine per farm	$1.5k-3k
Reagent cost per assay	$	National Lab Prices	$0.1-0.2
Total annual reagent cost of surveillance by PonDx per farm	**$**	**Per farm per year**	**$40K-80K**

Acknowledgments

Sandia National Laboratories

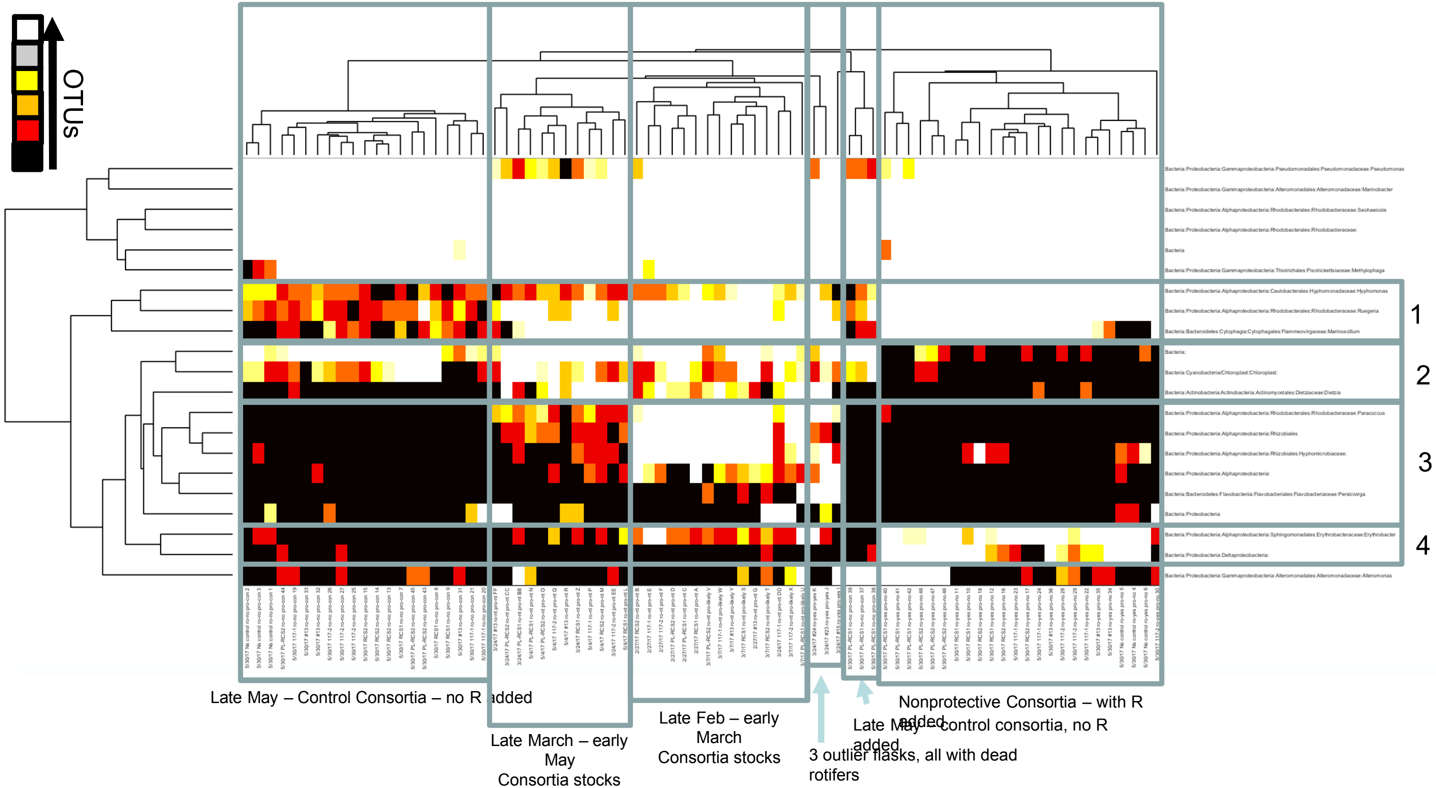
Carolyn Fisher
Laura T. Carney
Kunal Poorey
Pamela D. Lane
Deanna J. Curtis
Krissy Mahan

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

Lawrence Livermore National Laboratory

heliae

Spare slides


OTUs
1
2
3
4
Late May – Control Consortia – no R added
Late March – early May Consortia stocks
Late Feb – early March Consortia stocks
3 outlier flasks, all with dead rotifers
Late May – control consortia, no R added
Nonprotective Consortia – with R added