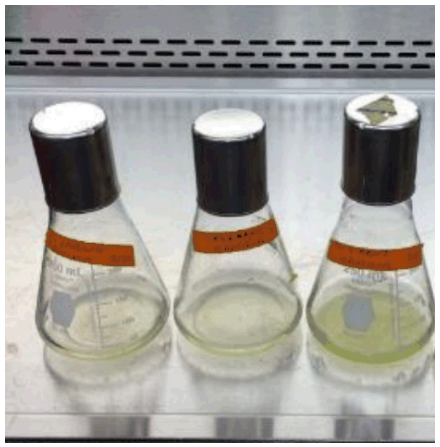


Investigating the chemical and biological landscape of microalgae cultures to mitigate pond crashes

ALGAE BIOMASS SUMMIT 2017

SALT LAKE CITY • OCTOBER 29 – NOVEMBER 1

Carolyn Fisher, PhD

Postdoctoral Researcher

Systems Biology Department

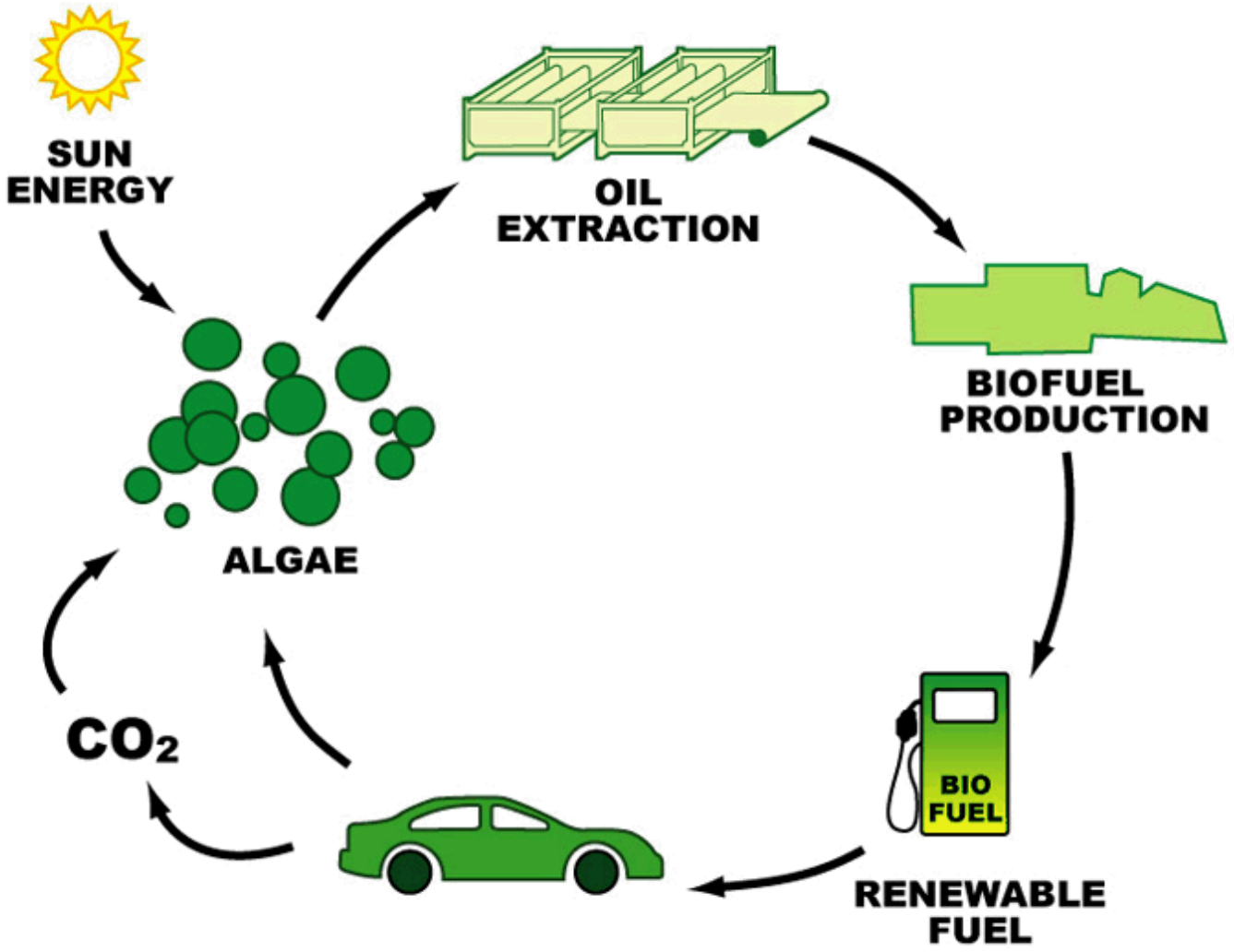
U.S. DEPARTMENT OF ENERGY | Energy Efficiency & Renewable Energy

BIOENERGY TECHNOLOGIES OFFICE

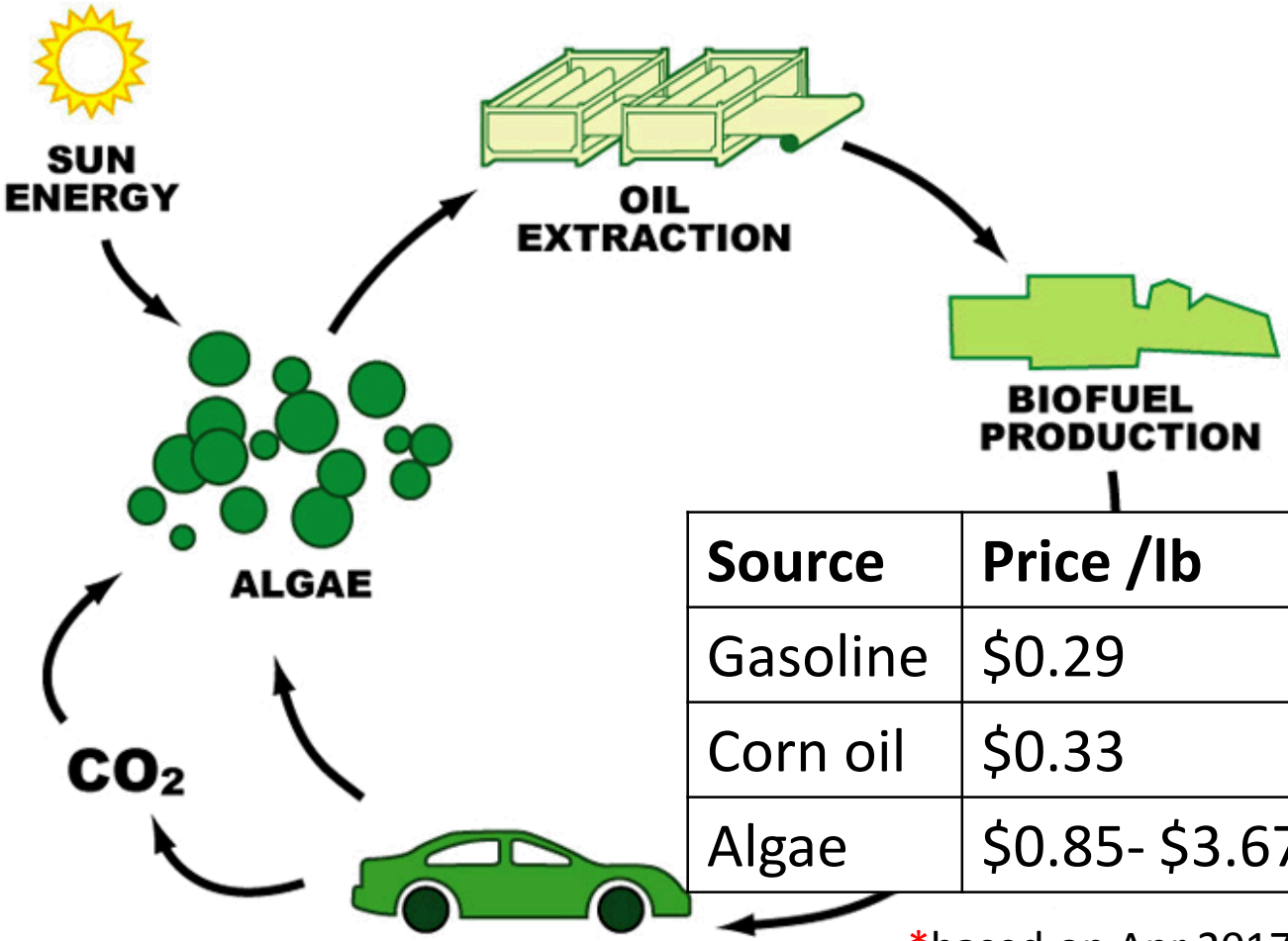
National Nuclear Security Administration

August 25, 2017

Biofuel is the future, but there are serious economic barriers before it becomes reality.



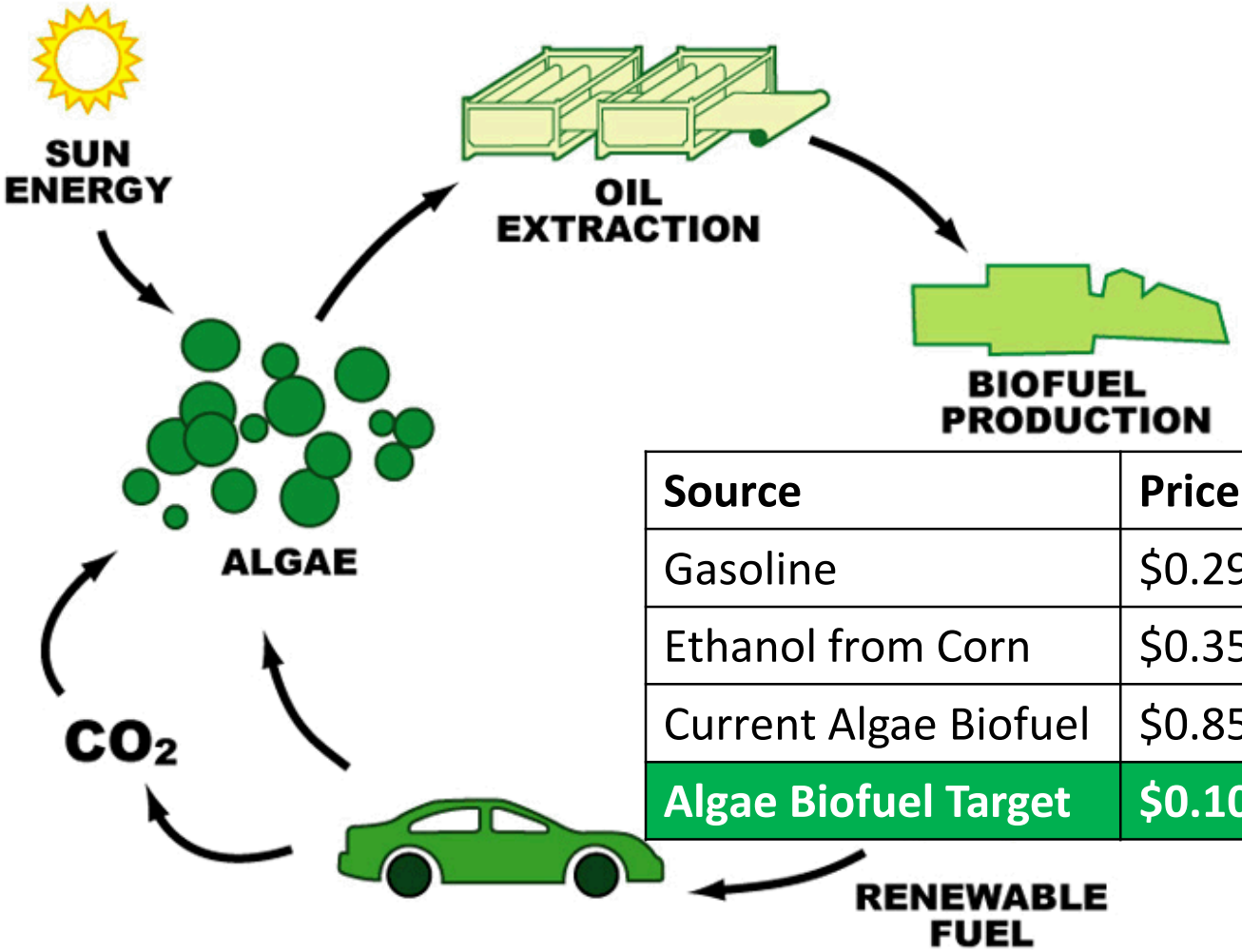
Biofuel is the future, but there are serious economic barriers before it becomes reality.



Source	Price /lb	Price /gal
Gasoline	$0.29	$2.38*
Corn oil	$0.33	$2.74
Algae	$0.85- $3.67	$7.06 - $30.46

*based on Apr 2017 national average

Biofuel is the future, but there are serious economic barriers before it becomes reality.



Source	Price /lb	Price /gal
Gasoline	$0.29	$2.49*
Ethanol from Corn	$0.35	$2.92*
Current Algae Biofuel	$0.85- $3.67*	$7.06 - $30.46
Algae Biofuel Target	**$0.10 – 0.25***	**$1.50 - $2.10**

*Approximate national average for 2017

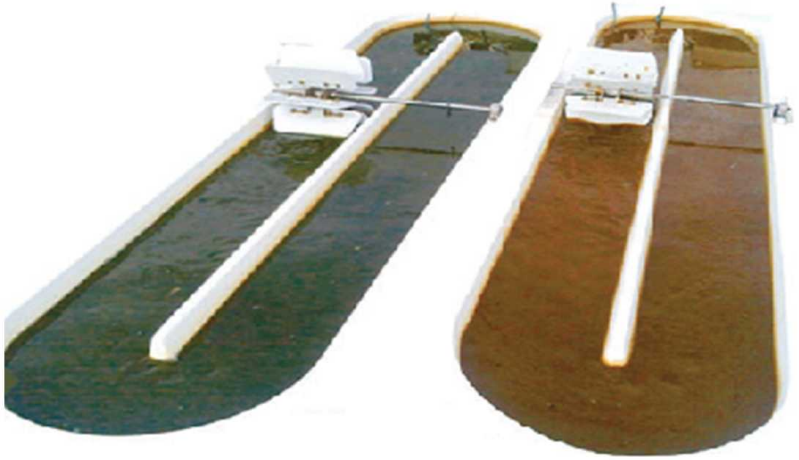
*Sun et al, *Energy,* 36 (2011) 5169-51795.

*2017 DOE Alternative Fuel Price Report

*BETO Multi-Year Program Plan, March 2016

Target selling prices for algae biomass in 2022 is $494/ton AFDW (ash-free dry weigh; 2014$) **to achieve $5/GGE** (gasoline gallon equivalent).

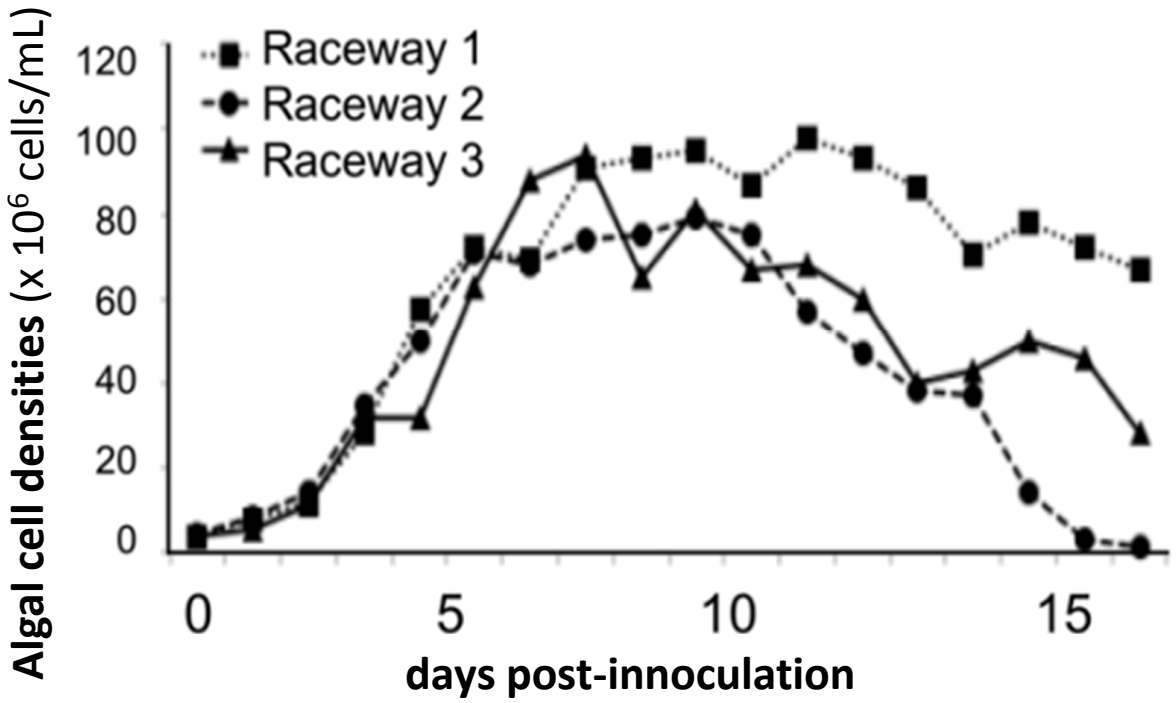
- BETO MYPP, 2014



Healthy pond Crashed pond

Algal population crashes cause **losses of up to 30% of annual crop production** from the typical open raceway system.

Pond crashes: *N. salina* growth in biological replicate raceways at Texas Agrilife. Raceways show moderate to severe **biomass loss** as a result of algal predation.

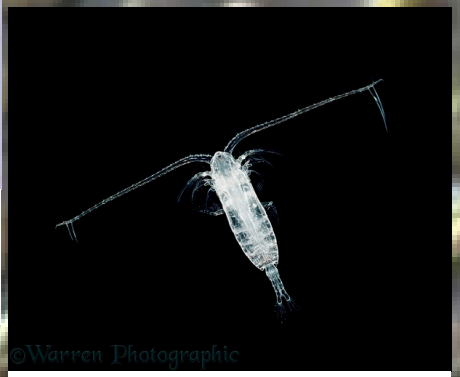


Carney et al. 2016

Nannochloropsis salina

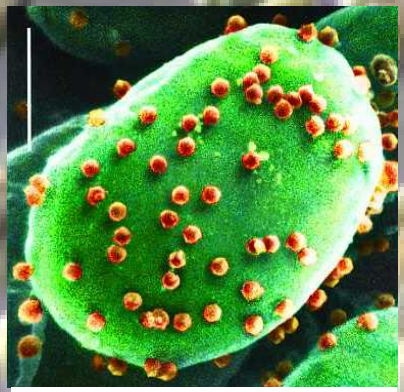
Predators of microalga

Brachionus plicatilis, marine rotifer



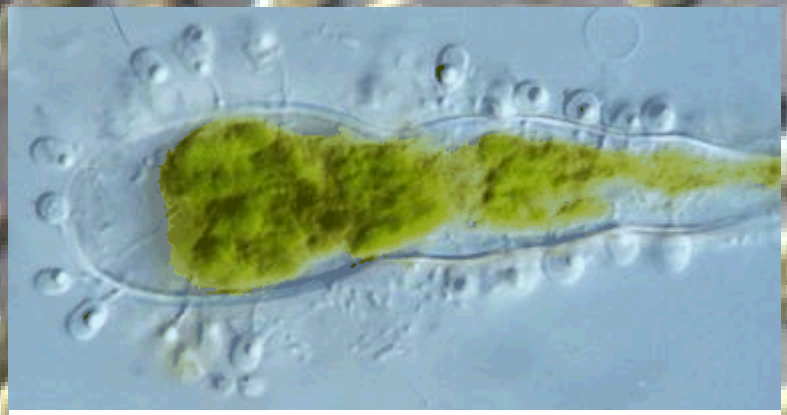
Marine planktonic copepod, *Calanus*

Oxyrrhis marina, dinoflagellate

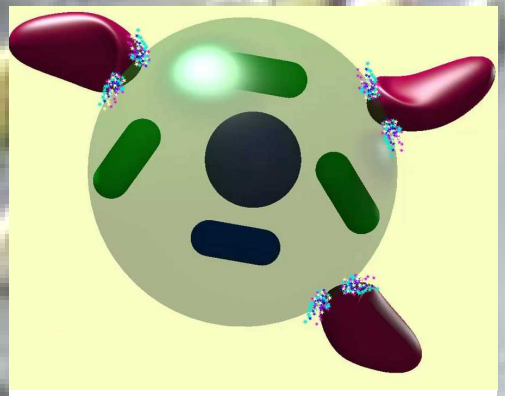


alga infected with chlorovirus

Poterioochromonas, a golden algae or chrysophyte



Numerous parasitic chytrids attack the filament of a green alga



Vampirovibrio chlorellavoras bacterial predation on green alga, *Chlorella*.

Our Approach

1) Can we identify and monitor volatile chemicals that indicate when algae is infected with predators?

2) Can we stabilize algae culture and prevent algal predation with probiotic bacteria?

3) Can we isolate and identify chemicals from these probiotic bacteria to understand the mechanism of algae protection?

Our Approach

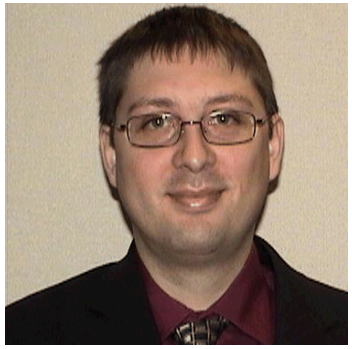
1) Can we identify and monitor volatile chemicals that indicate when algae is infected with predators?

2) Can we stabilize algae culture and prevent algal predation with probiotic bacteria?

3) Can we isolate and identify chemicals from these probiotic bacteria to understand the mechanism of algae protection?

Come talk to me in person!

Matthew W. Moorman
R&D S&E, Mechanical Engineering



Stephen M. Anthony
R&D S&E, Biological Sciences & Engineering

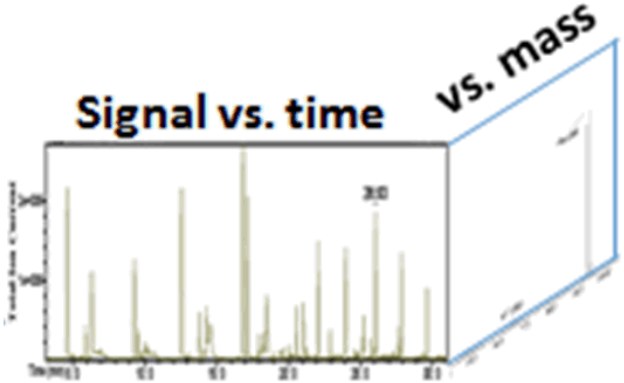
Curtis D. Mowry
R&D S&E, Materials Science

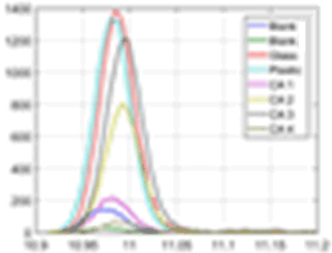
AVOCs experiment

Algal Volatile Organic Compounds

AVOCs sampling

Thermal Desorption
Gas Chromatography
Mass Spectrometry
(TD/GC/MS)





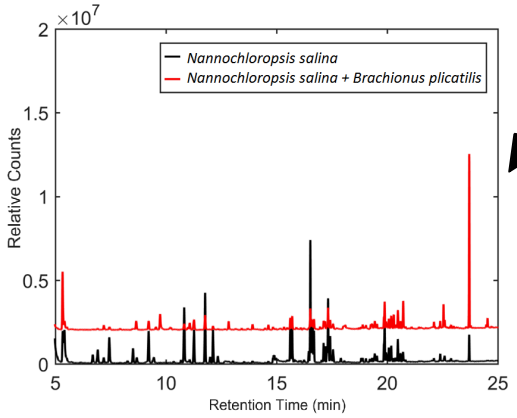
Monitor AVOCs of algal production systems

Algal Pond Monitoring in the Field

Predator attack

AVOC chemical markers released

Detection and analysis

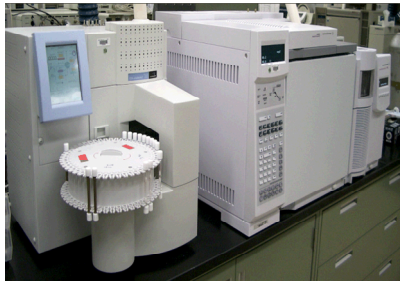


Remediation to save the pond

Sandia is developing a dedicated field analysis system for algal VOCs with an emphasis on usability and low cost.

Field VOC Sampling
(inexpensive)

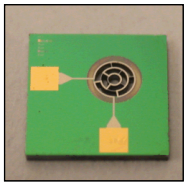
Onsite Laboratory Analysis
(~$100K)



OR

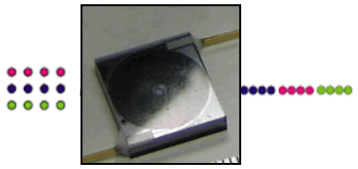
Integrated Sensor System Solution
($3K-$10K in quantity)

MicroPreconcentrators (µPC)



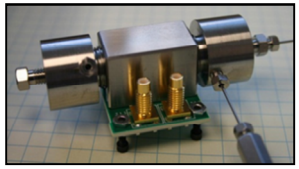
- **Non-contact sample collection**

MicroChromatography (µGC)



- **Separates complex chemical mixtures**

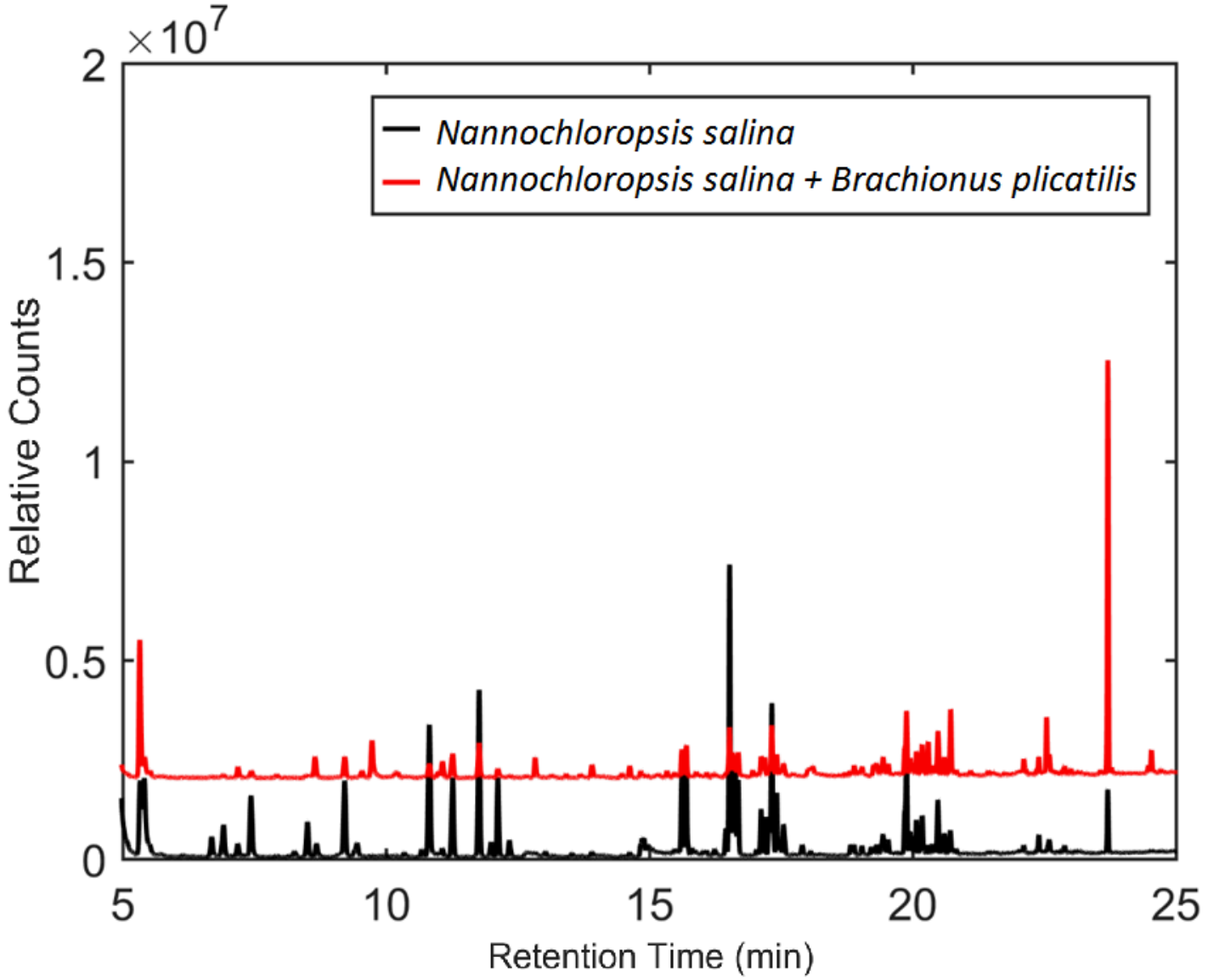
Pulsed Discharge Ionization Detector (PDID)



- **High sensitivity (sub-parts per billion)**

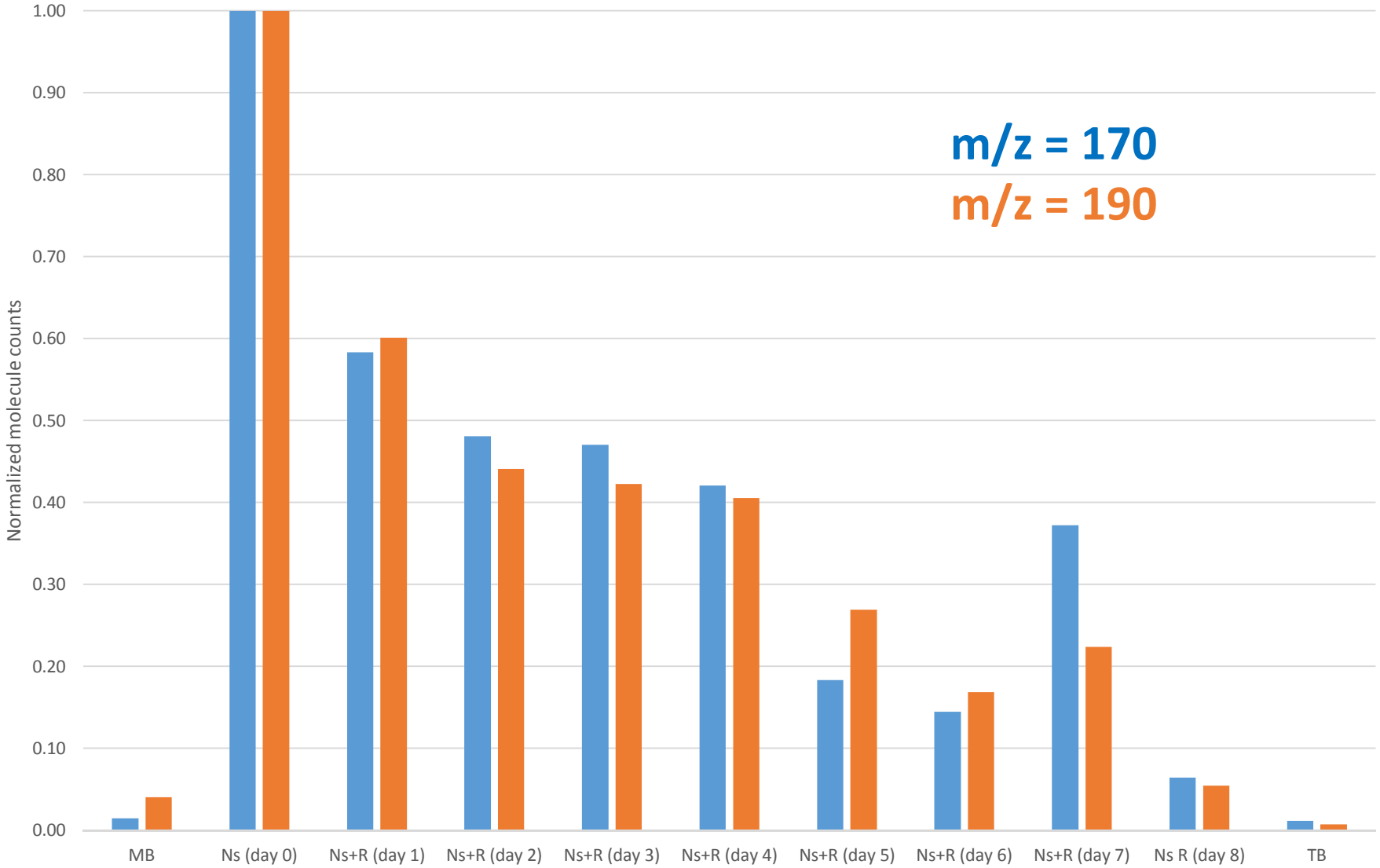

Thermo
SCIENTIFIC
Thermo
SCIENTIFIC
Thermo
SCIENTIFIC
Thermo
SCIENTIFIC
Thermo
SCIENTIFIC
Thermo
SCIENTIFIC

Very different AVOCs for Ns vs. Ns+R



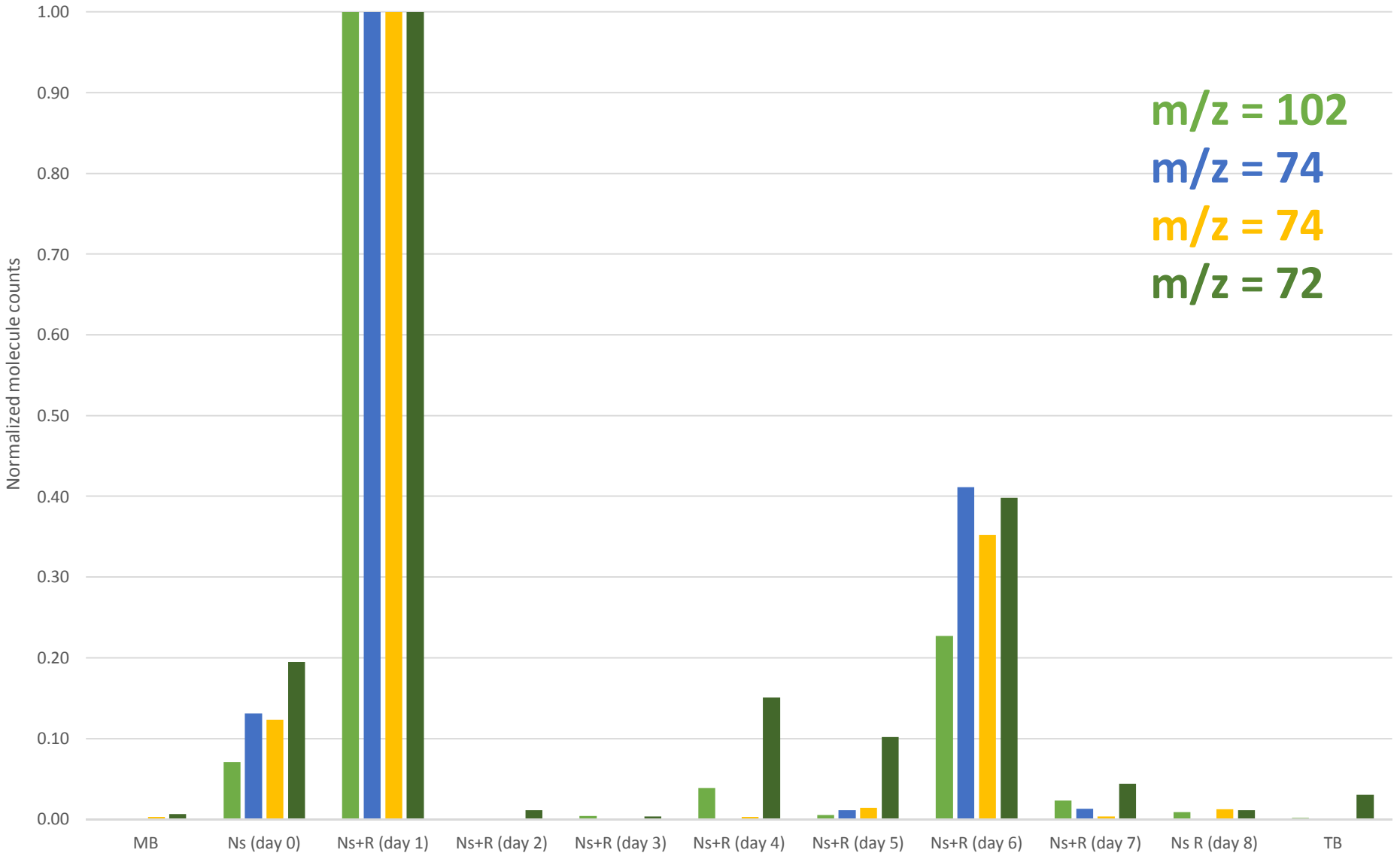
Preliminary Results:

Some AVOCs seem to decrease as incubation period with rotifers increases



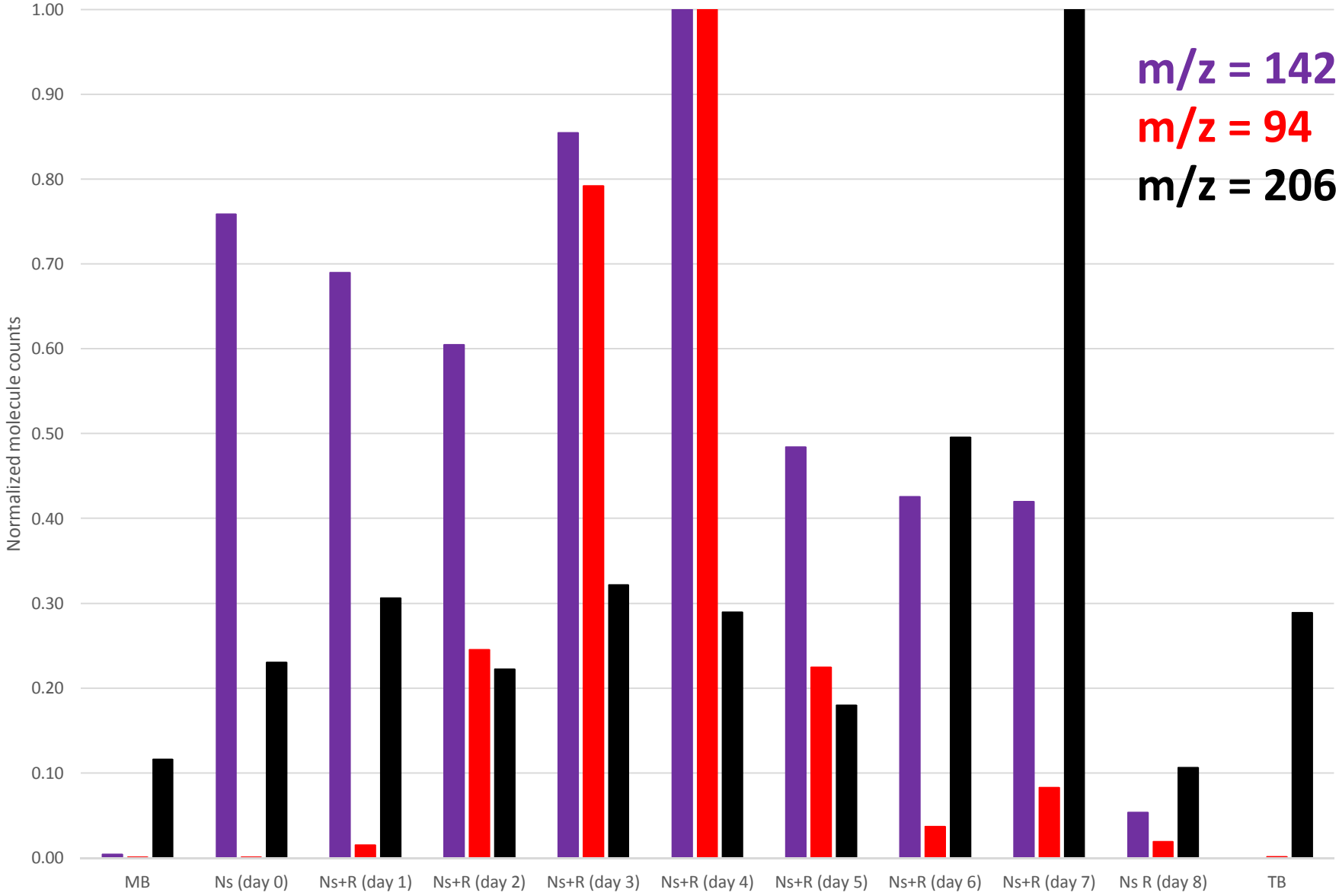
Preliminary Results:

Some AVOCs seem to initially increase then rapidly decline

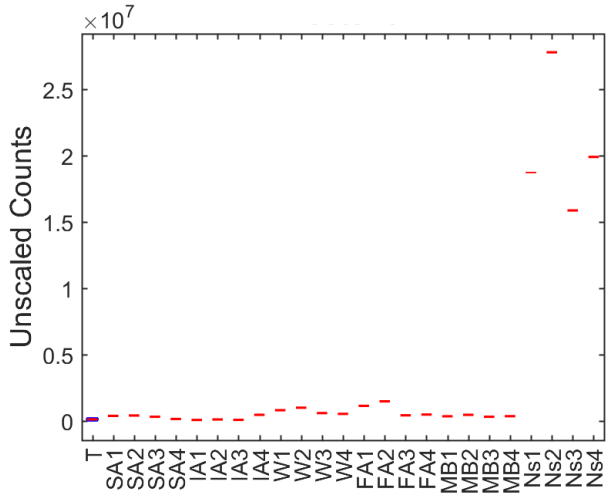


Preliminary Results:

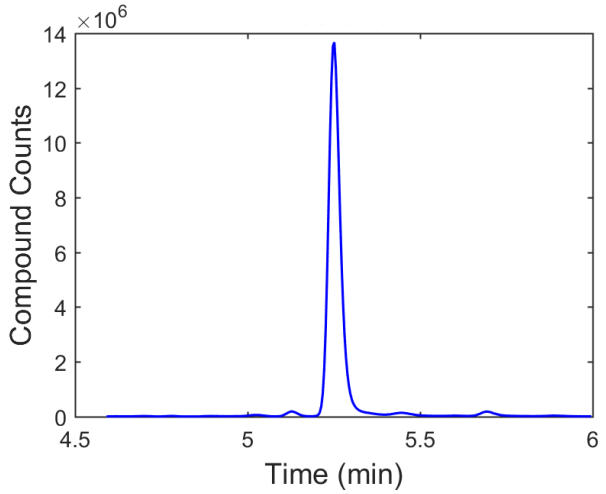
Some AVOCs experience other changes



CA12



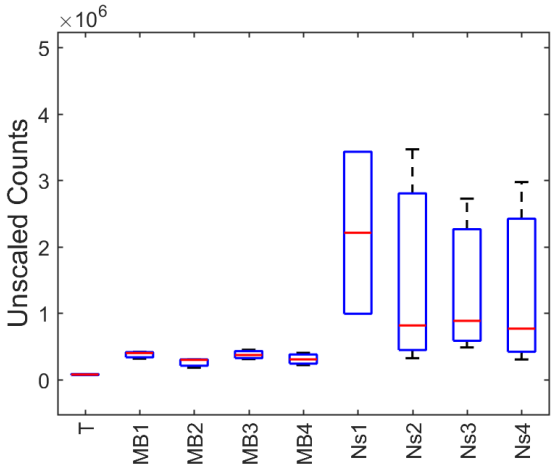
Summed elution



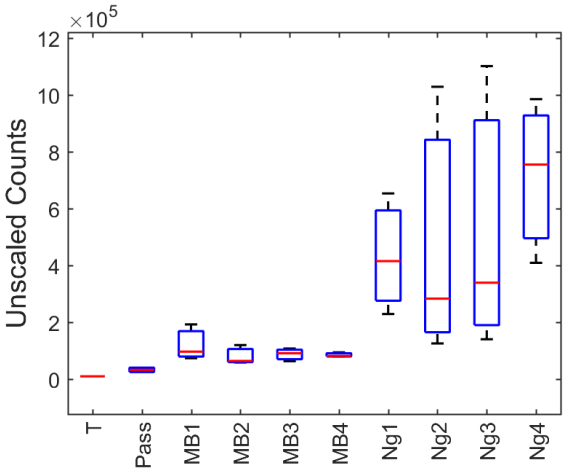
Matt approved

Todd approved

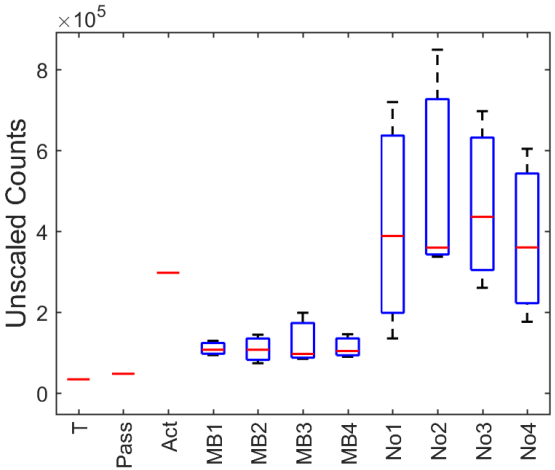
CA13

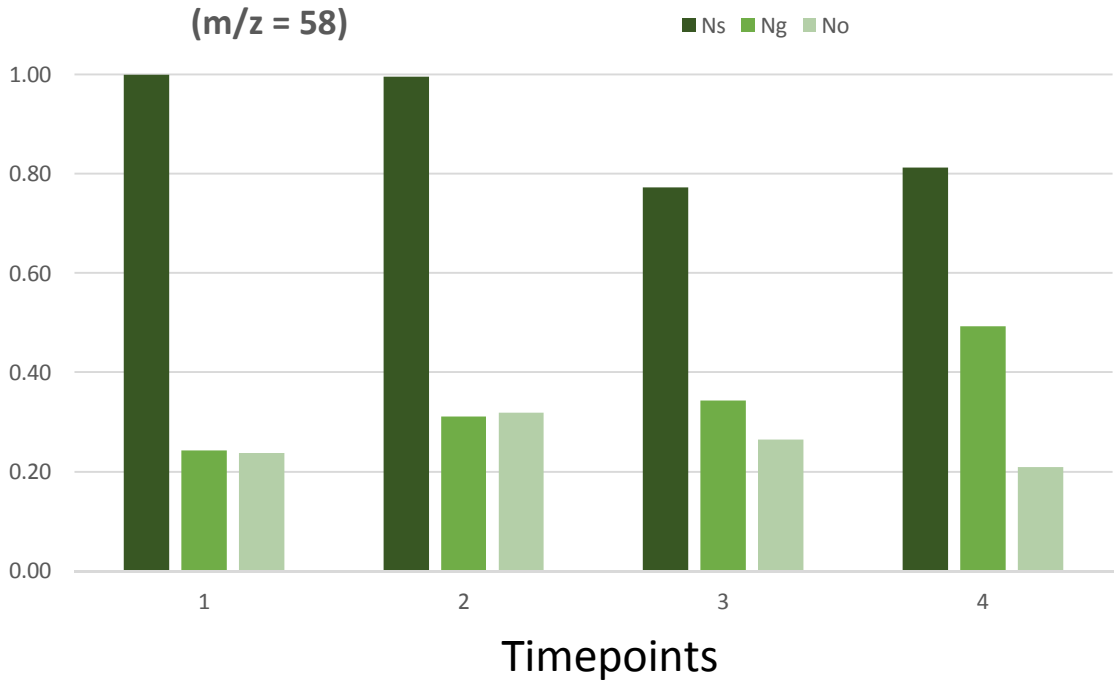


CA14

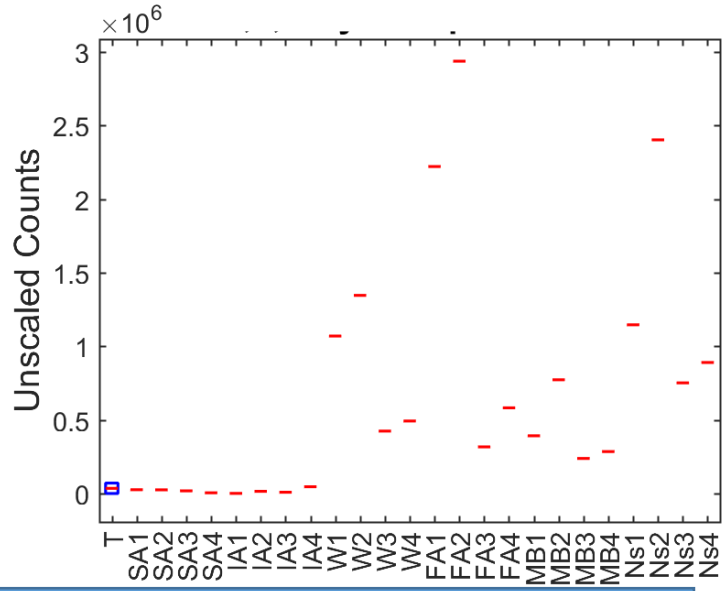


CA15

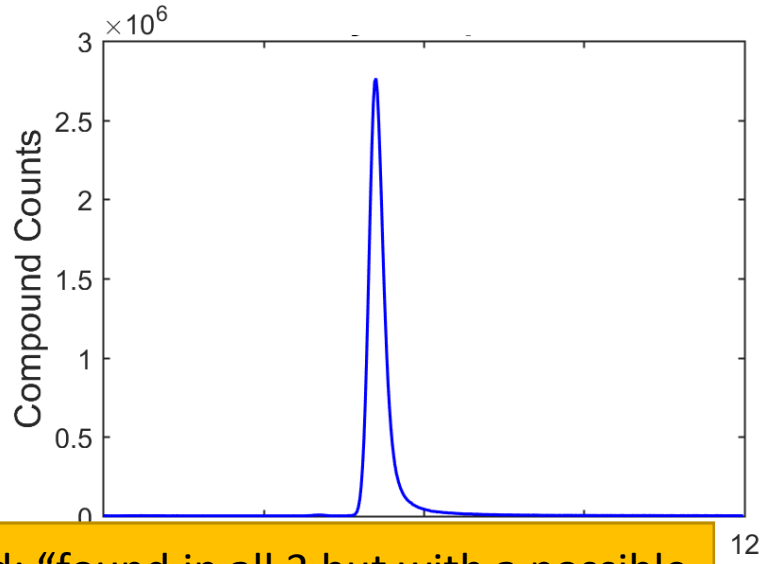



(m/z = 58)
Ns
Ng
No
1.00
0.80
0.60
0.40
0.20
0.00
1
2
3
4
Timepoints

CA12



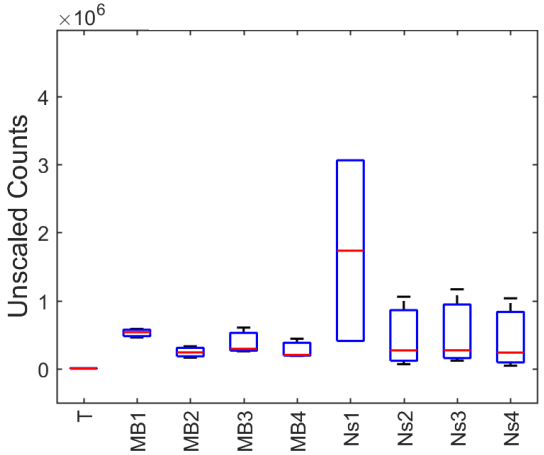
Summed elution



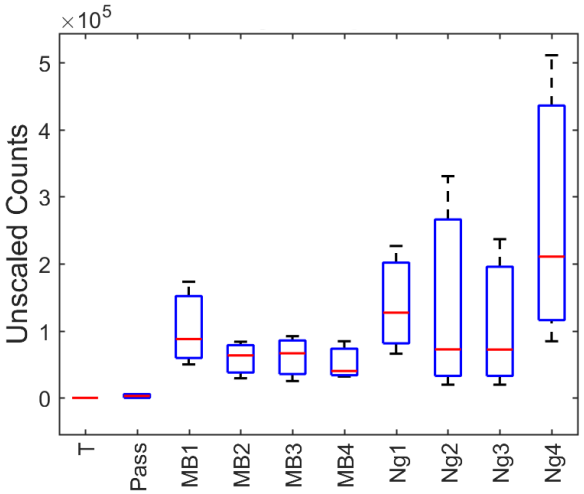
Matt: "I might show CA12 - CA14"

Todd: "found in all 3 but with a possible species specific pattern

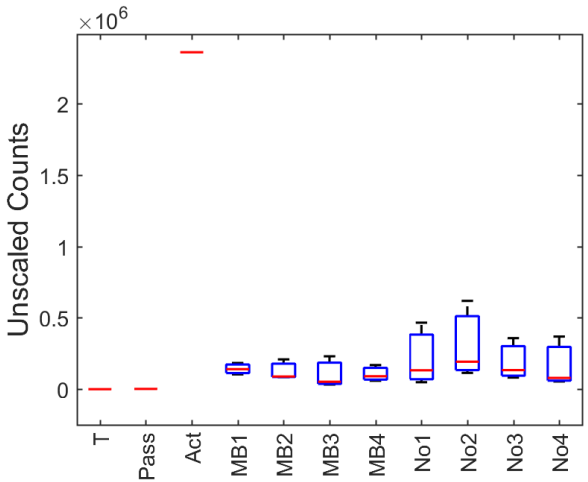
CA13

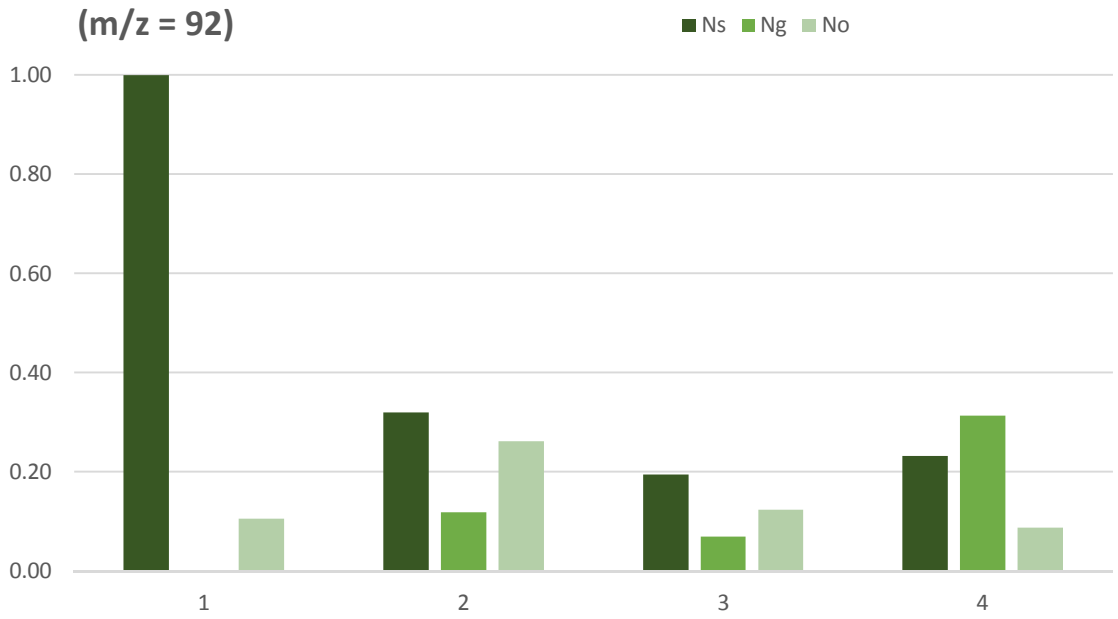


CA14



CA15




(m/z = 92)
Ns
Ng
No
1.00
0.80
0.60
0.40
0.20
0.00
1
2
3
4

Summary & Future Work

- **AVOCs indicate chemical differences between algae +/- rotifers**
  - Identify chemicals and quantify differences
  - Determine which would be the best to monitor

- **Protective consortia**
  - Will use MiSeq for bacteria identification
  - Determine bacterial differences between consortia

Summary & Future Work

- **AVOCs indicate chemical differences between algae +/- rotifers**
  - Identify chemicals and quantify differences
  - Determine which would be the best to monitor

- **Protective consortia**
  - **Will use MiSeq for bacteria identification**
  - **Determine bacterial differences between consortia**

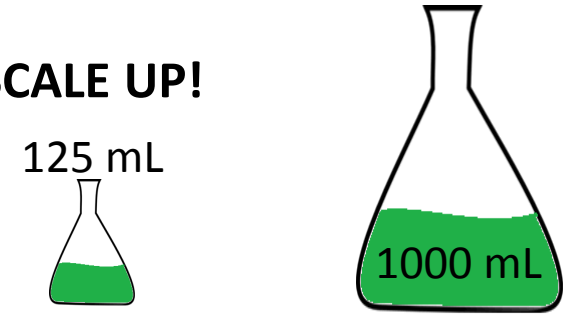
Summary & Future Work

- **AVOCs indicate chemical differences between algae +/- rotifers**
  - Identify chemicals and quantify differences
  - Determine which would be the best to monitor

- **Protective consortia**
  - Will use MiSeq for bacteria identification
  - Determine bacterial differences between consortia
  - Consortia simplification experiments

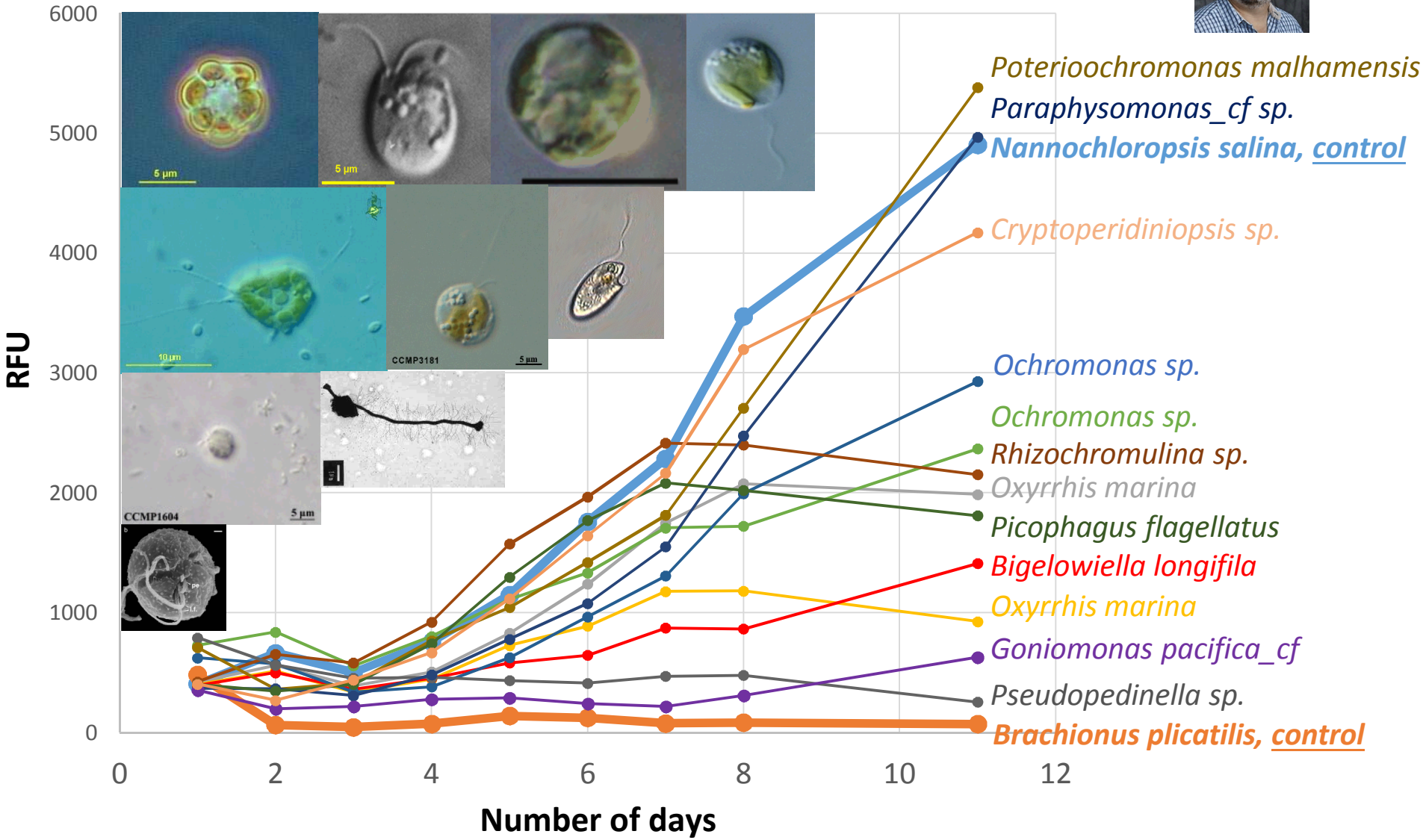
- **Chemical fraction was protective**
  - Identify the active chemical(s) → UPLC-MS & NMR
  - Identify the bacteria that create the chemical(s)
  - Dosage experiments

- **SCALE UP!**



- **Test more predators…**

We are assembling “a diverse panel of nasty things” – Todd W. Lane



Thank you!

Sandia National Laboratories

CA

Todd W. Lane
Kunal Poorey
Pamela D. Lane
Deanna J. Curtis
Nataly Beck
Peter McIlroy
Krissy Mahan

NM

Matt Moorman
Curtis Mowry
Adam Pimentel
Stephen Anthony
Jerilyn Timlin
Jaclyn Murton

Lawrence Livermore National Laboratory

Michael Thelen
Xavier Mayali
Rhona Stuart
Chris Ward
Ty Samo
Jennifer Pett-Ridge

heliae

Laura T. Carney

ATP3 Algae Testbed Public-Private Partnership

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

U.S. DEPARTMENT OF ENERGY | Office of Science