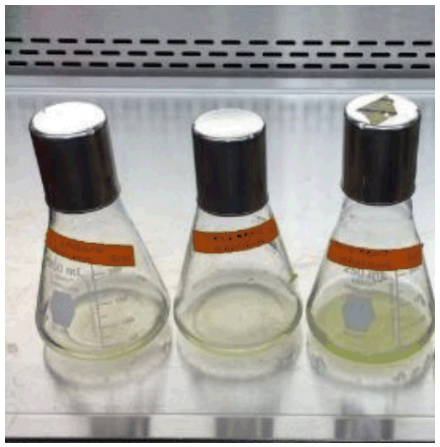

Investigating the chemical and biological landscape of microalgae cultures to mitigate pond crashes

Carolyn Fisher, PhD
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Systems Biology Department

National Nuclear Security Administration

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

BIOENERGY TECHNOLOGIES OFFICE

August 25, 2017

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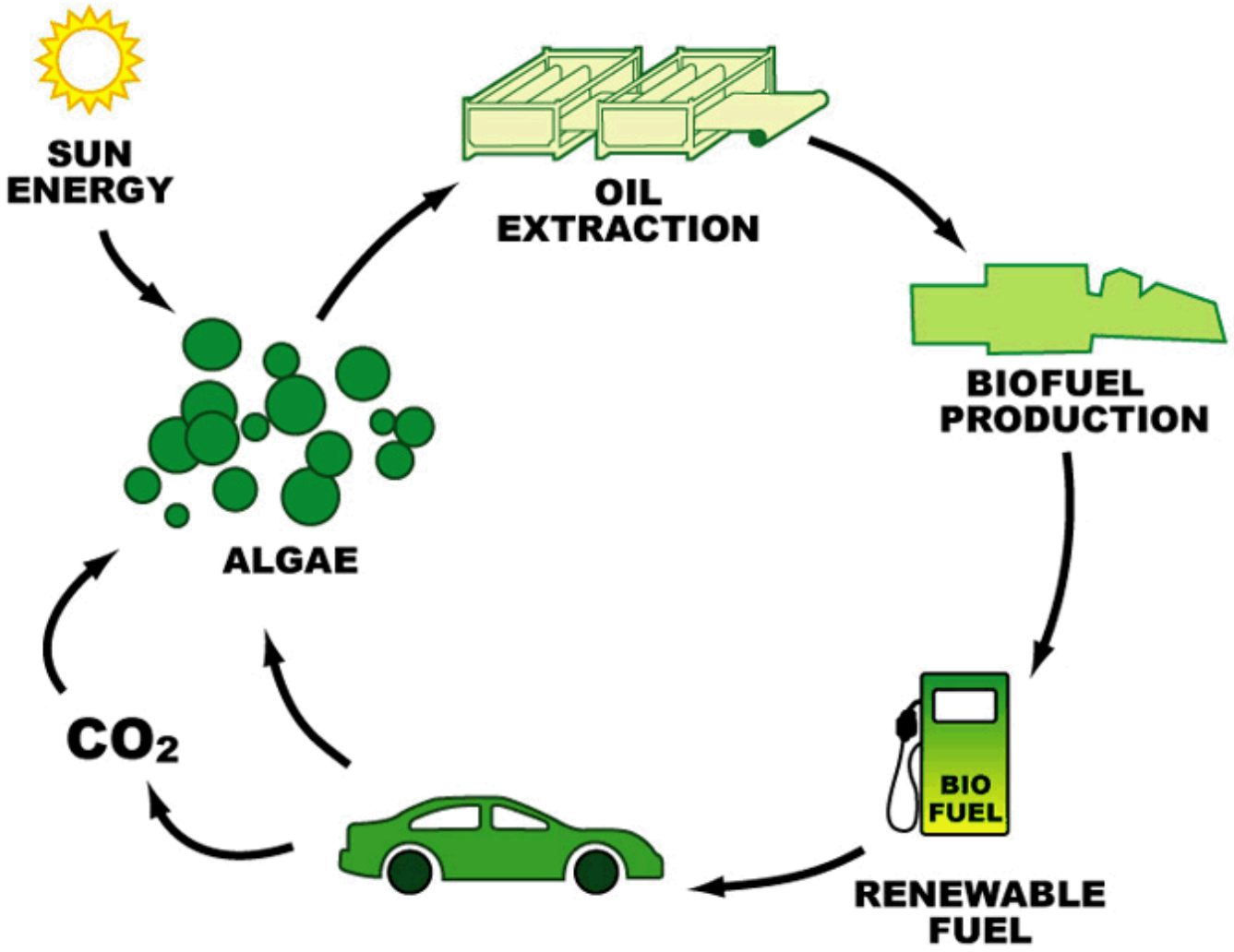
Biofuel is the future, but there are serious economic barriers before it becomes reality.



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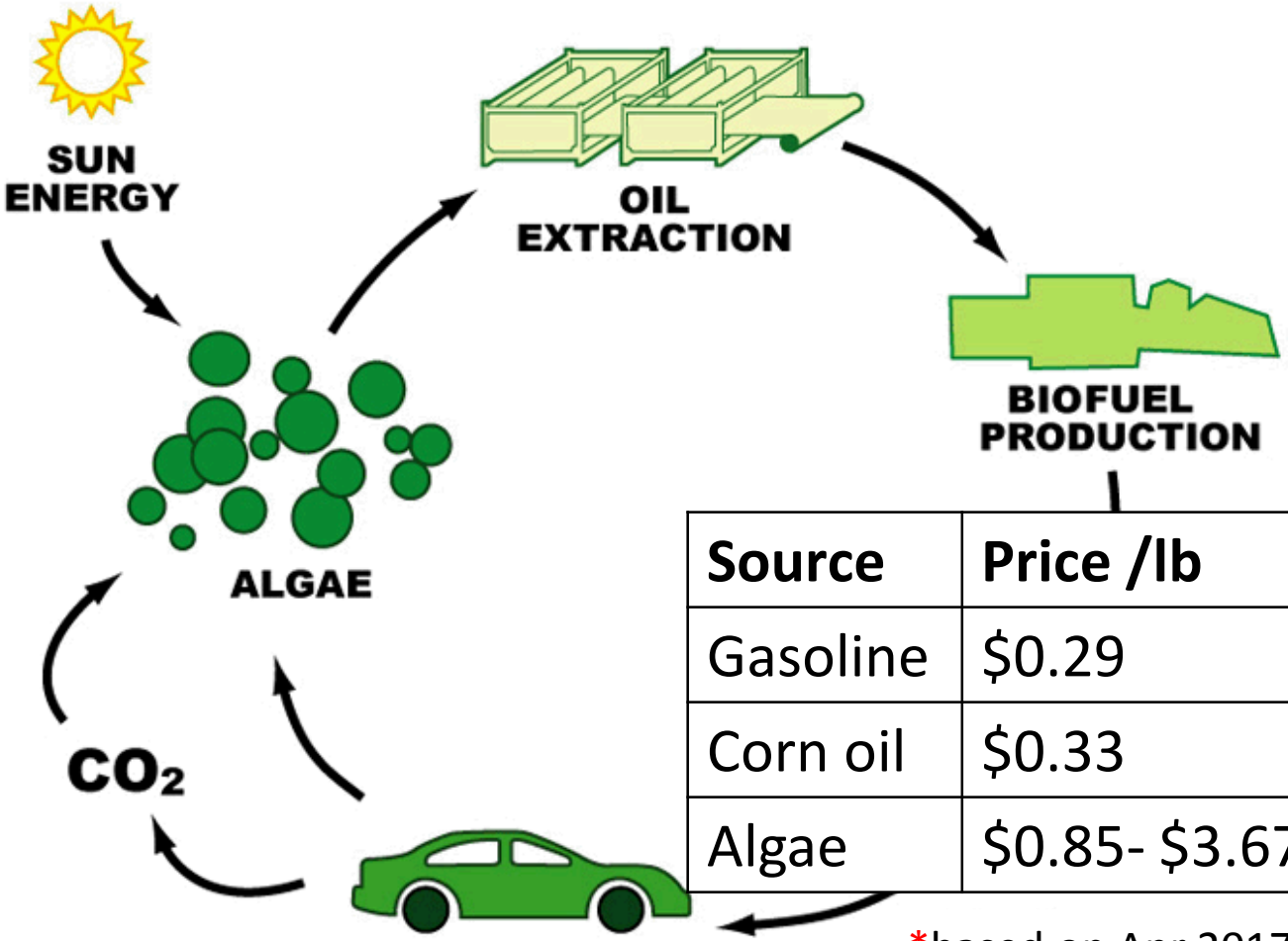
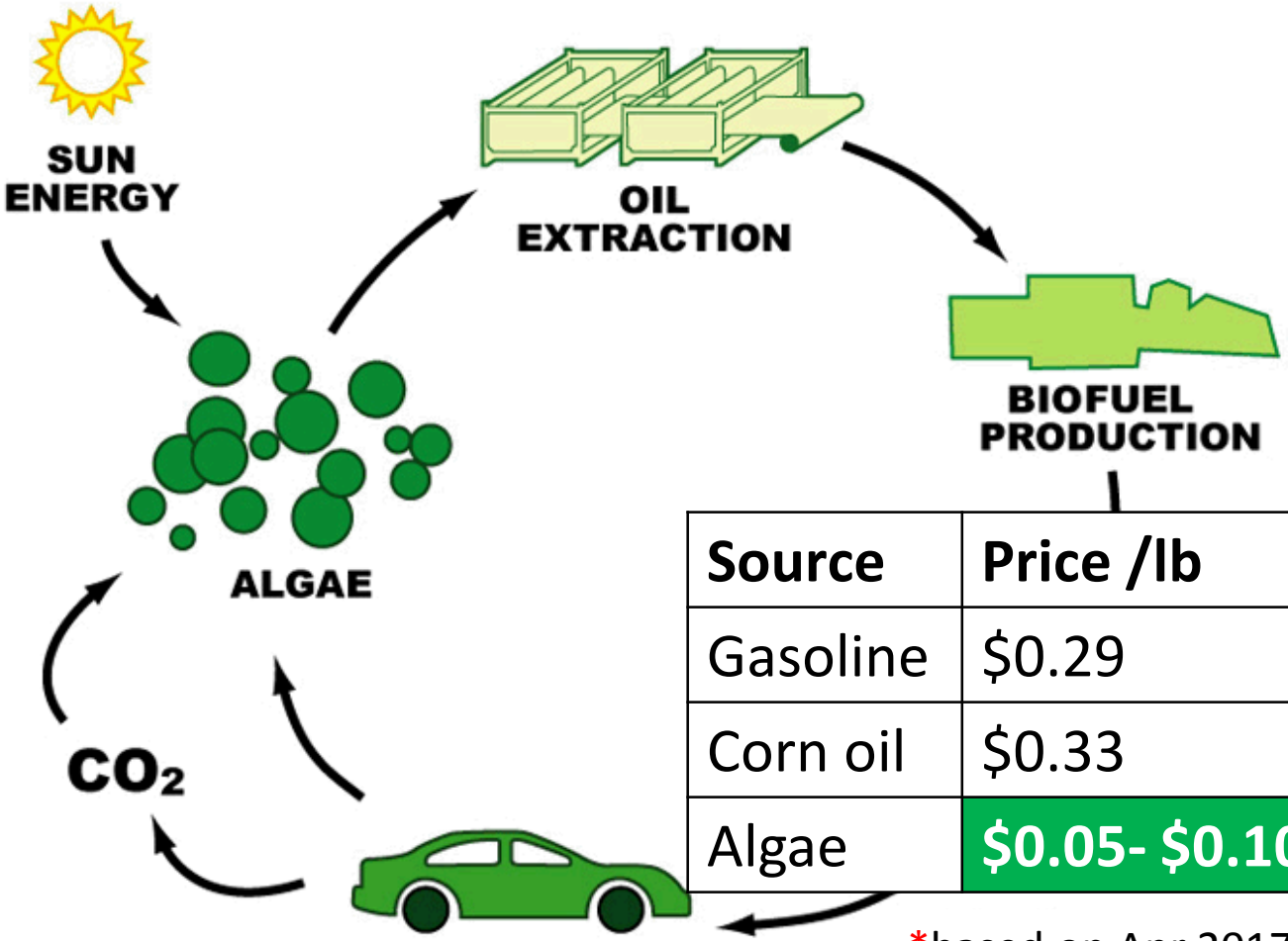


Source	Price /lb	Price /gal
Gasoline	$0.29	$2.38*
Corn oil	$0.33	$2.74
Algae	$0.85- $3.67	$7.06 - $30.46

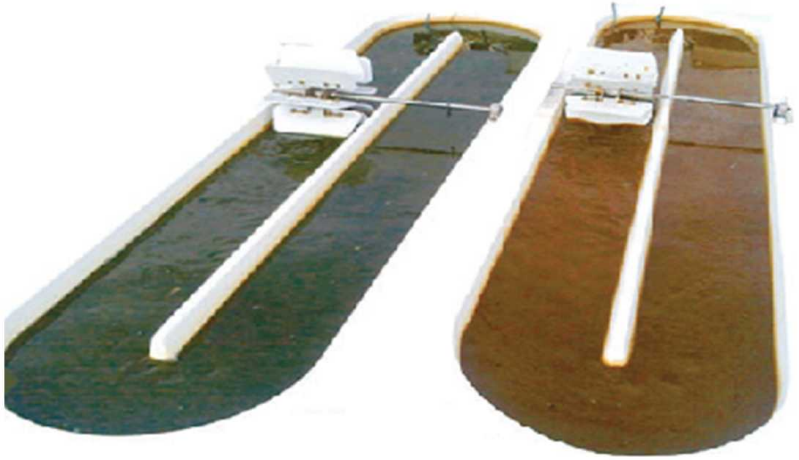
*based on Apr 2017 national average

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



Source	Price /lb	Price /gal
Gasoline	$0.29	$2.38*
Corn oil	$0.33	$2.74
Algae	**$0.05- $0.10**	**$0.42 - $1.50**

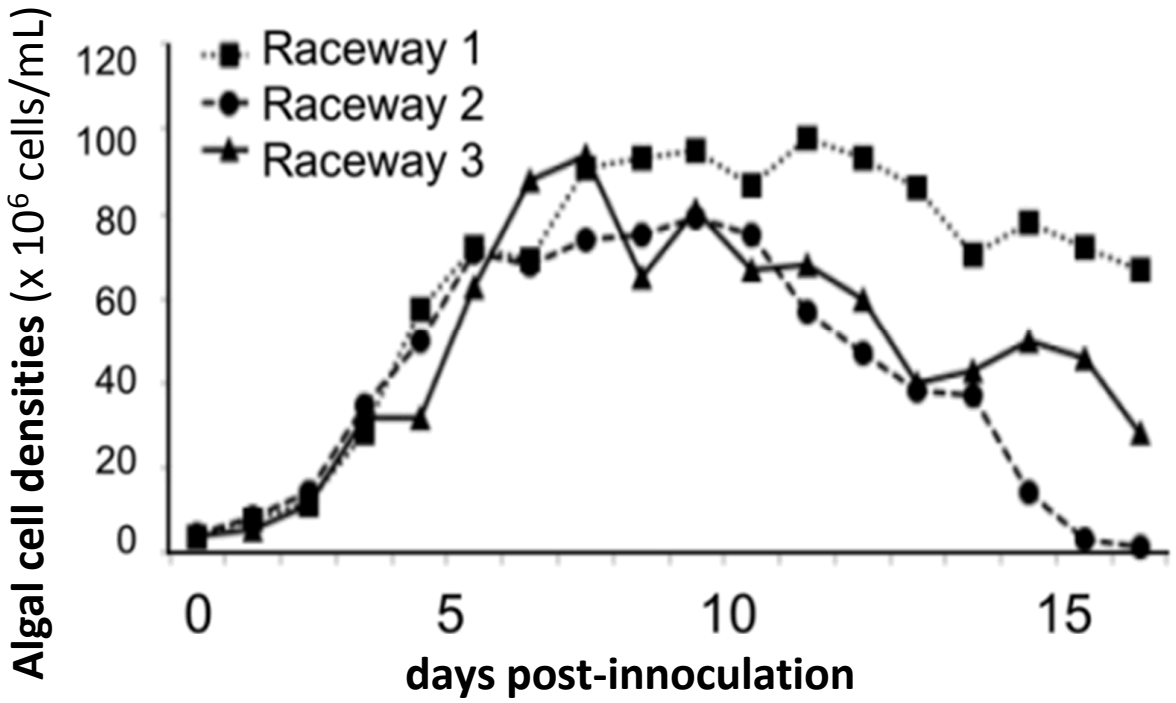
*based on Apr 2017 national average



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.

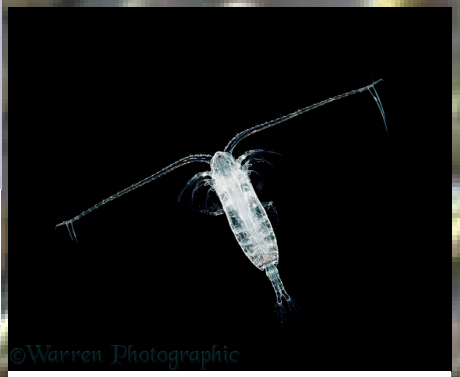


Carney et al. 2016

Nannochloropsis salina

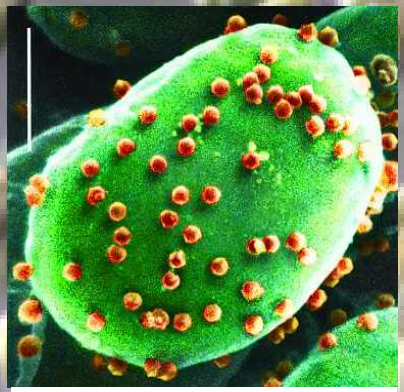
Predators of microalga

Brachionus plicatilis, marine rotifer



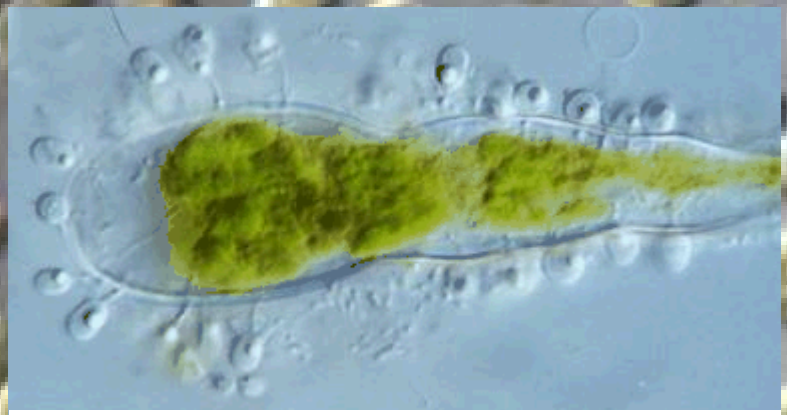
Marine planktonic copepod, *Calanus*

Oxyrrhis marina, dinoflagellate

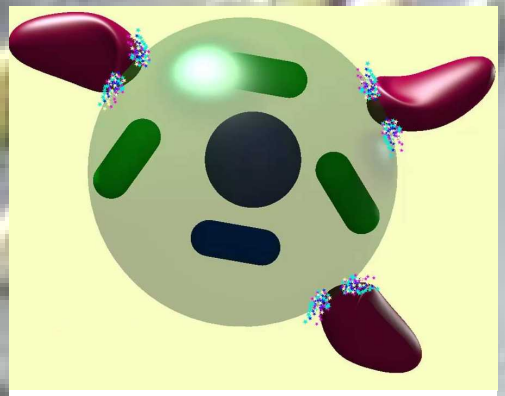


alga infected with chlorovirus

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?

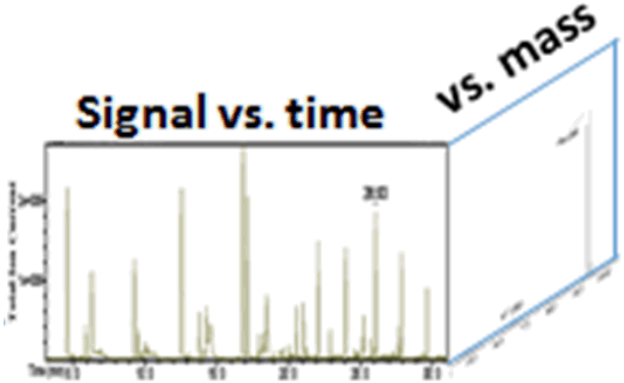
3) Can we isolate and identify chemicals from these probiotic bacteria to understand the mechanism of algae protection?

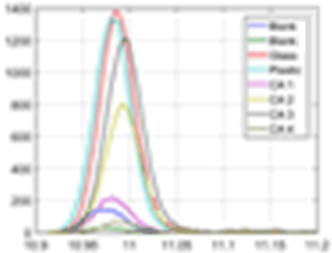
AVOCs experiment

Algal Volatile Organic Compounds

AVOCs sampling

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





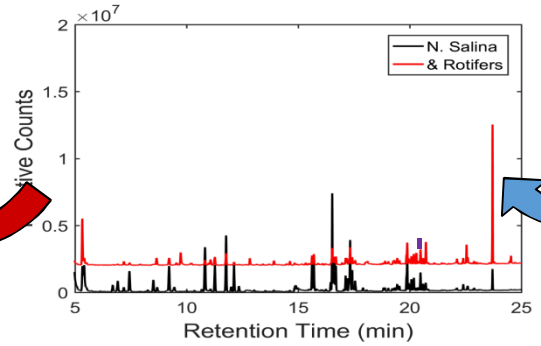
Monitor AVOCs of algal production systems

Algal Pond Monitoring in the Field

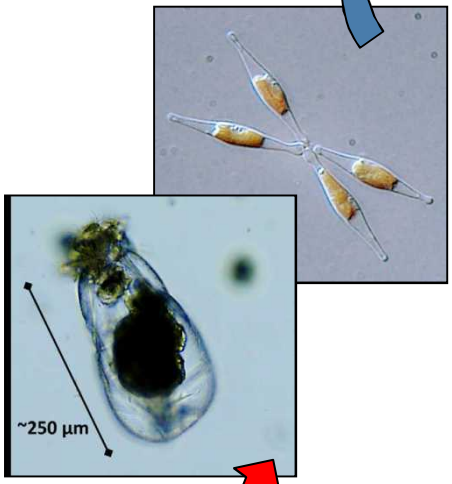
Predator attack

AVOC chemical markers released

Detection and analysis



Remediation

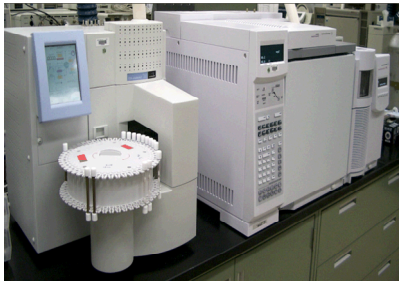


Field Analysis Methods for Algal Pond Monitoring

Field VOC Sampling
(inexpensive)

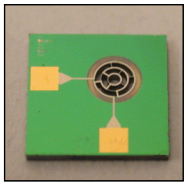
OR

Onsite Laboratory Analysis
(~$100K)



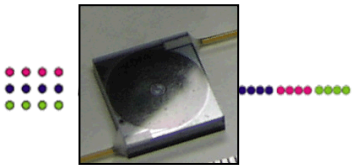
Integrated Sensor System Solution
($3K-$10K in quantity)

MicroPreconcentrators (µPC)



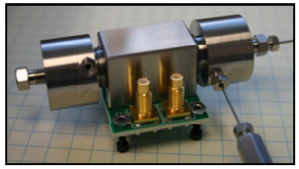
- **Non-contact sample collection**

MicroChromatography (µGC)



- **Separates complex chemical mixtures**

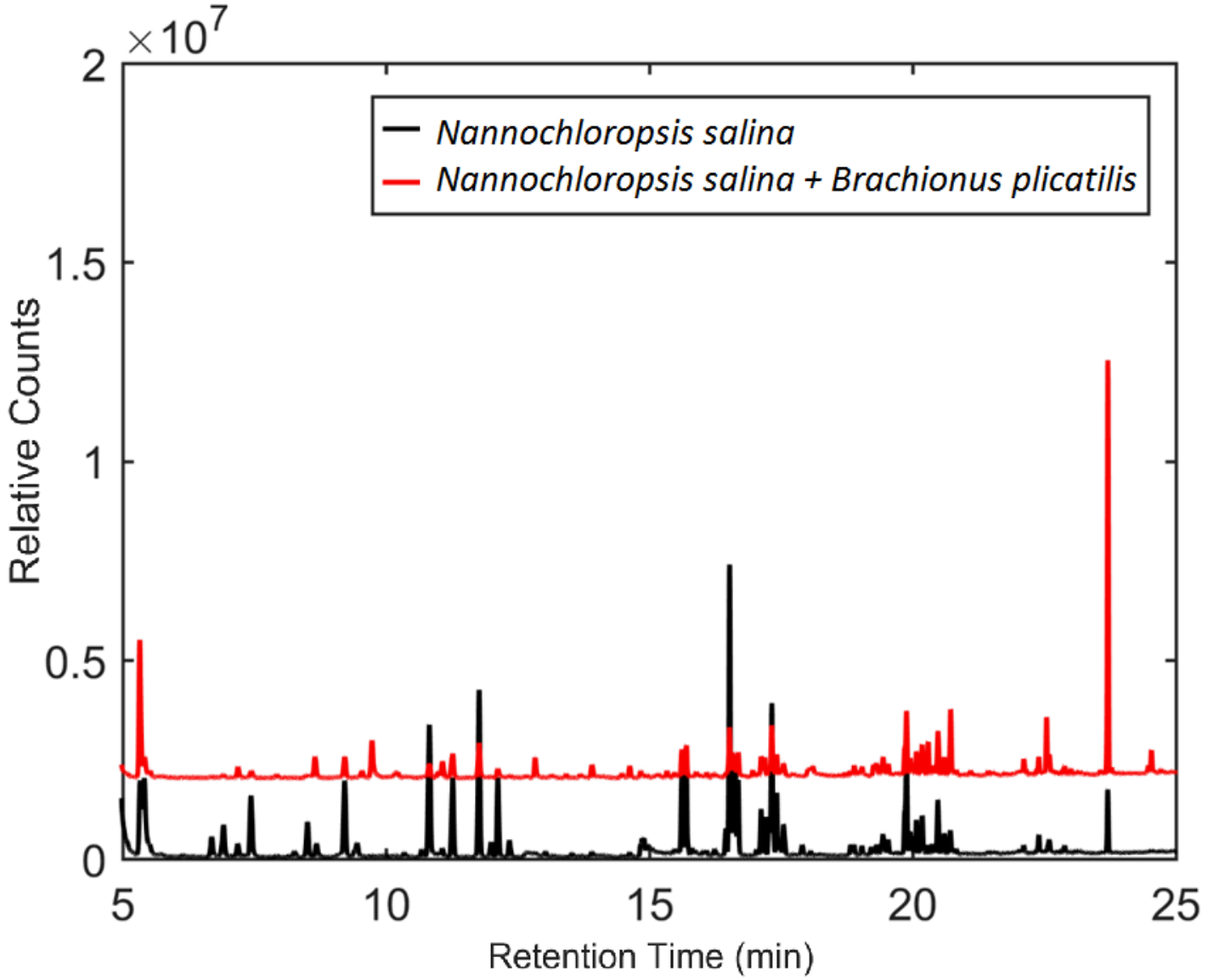
Pulsed Discharge Ionization Detector (PDID)



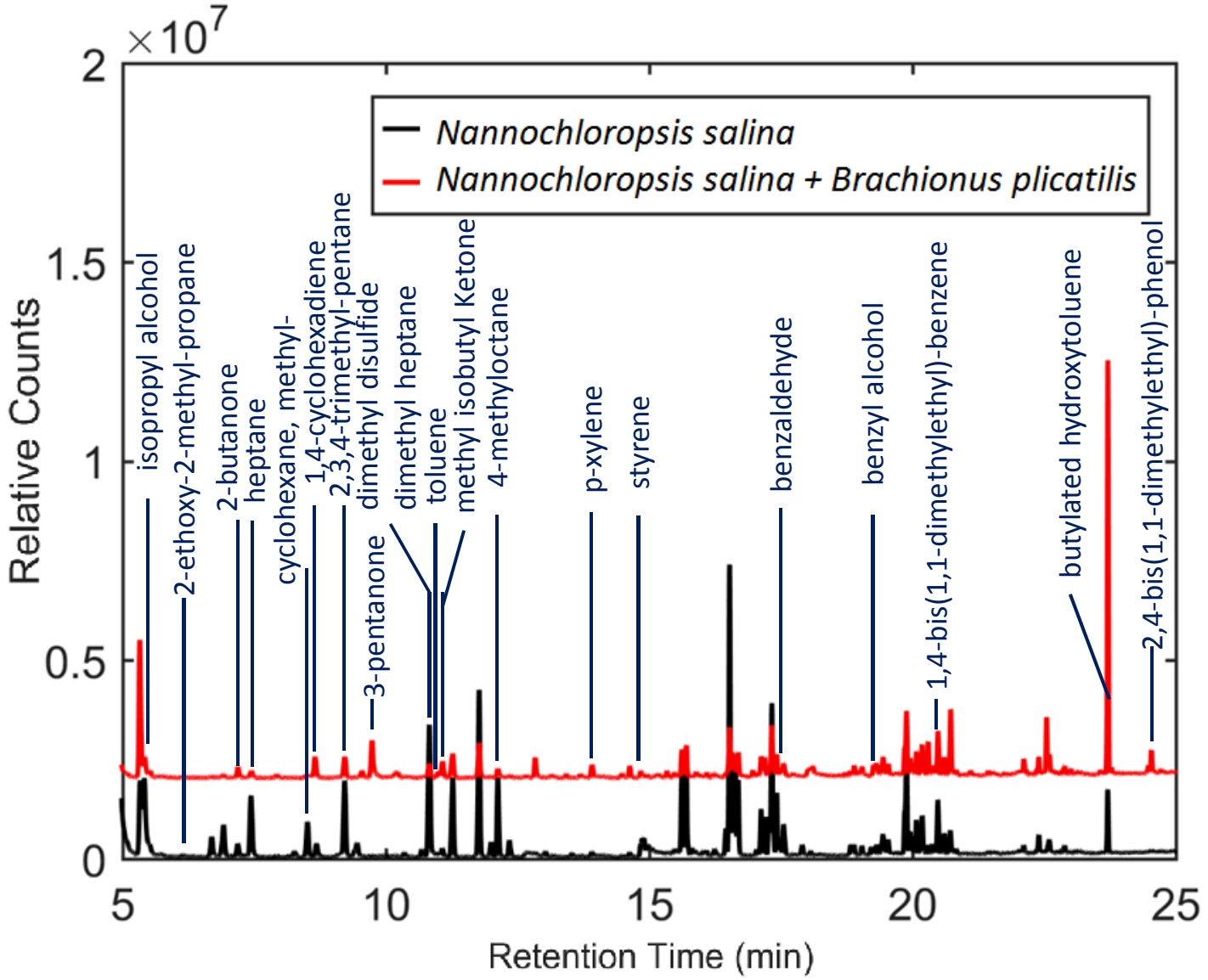
- **High sensitivity (sub-parts per billion)**

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

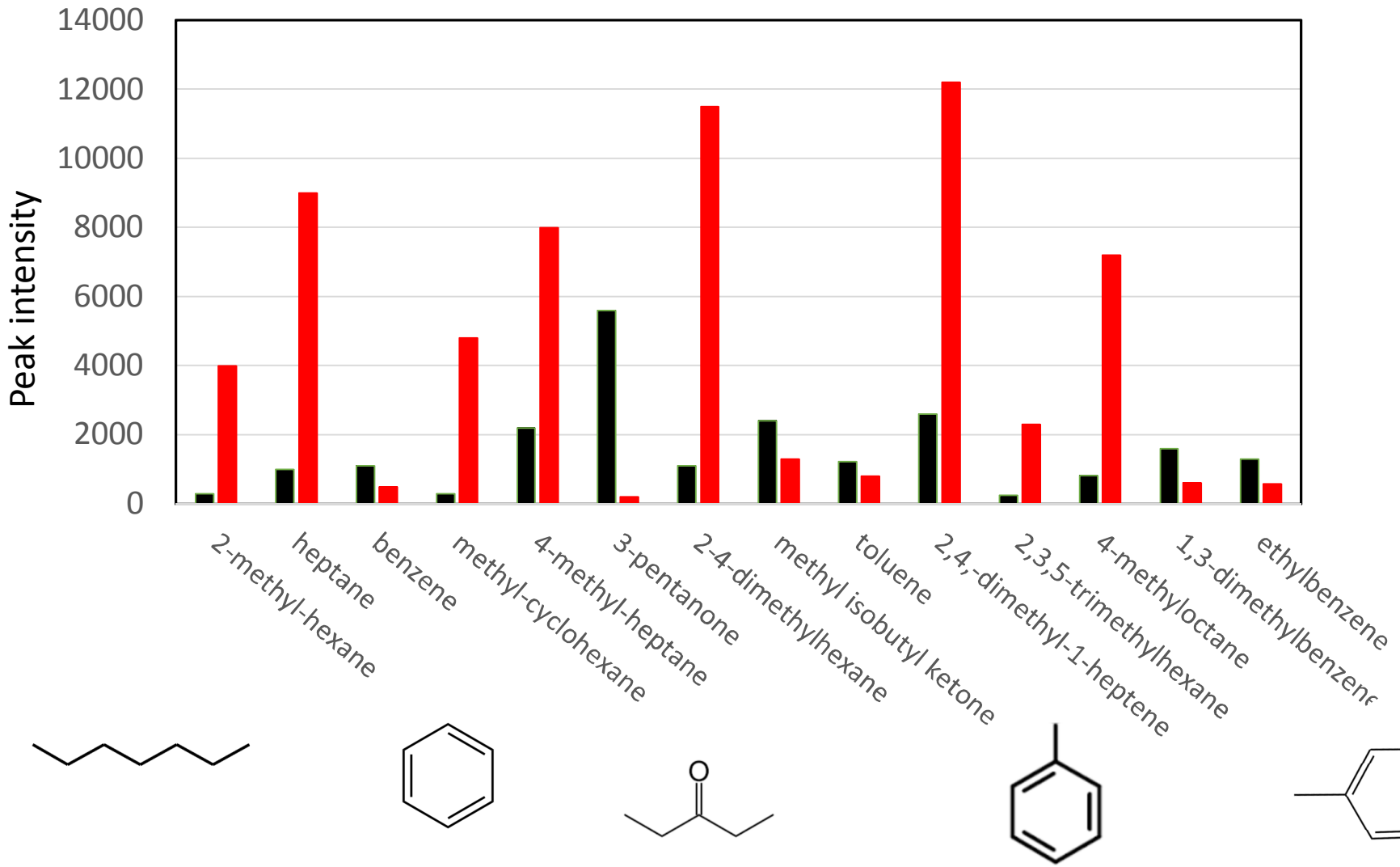
Very different AVOCs for Ns vs. Ns+R



Very different AVOCs for Ns vs. Ns+R

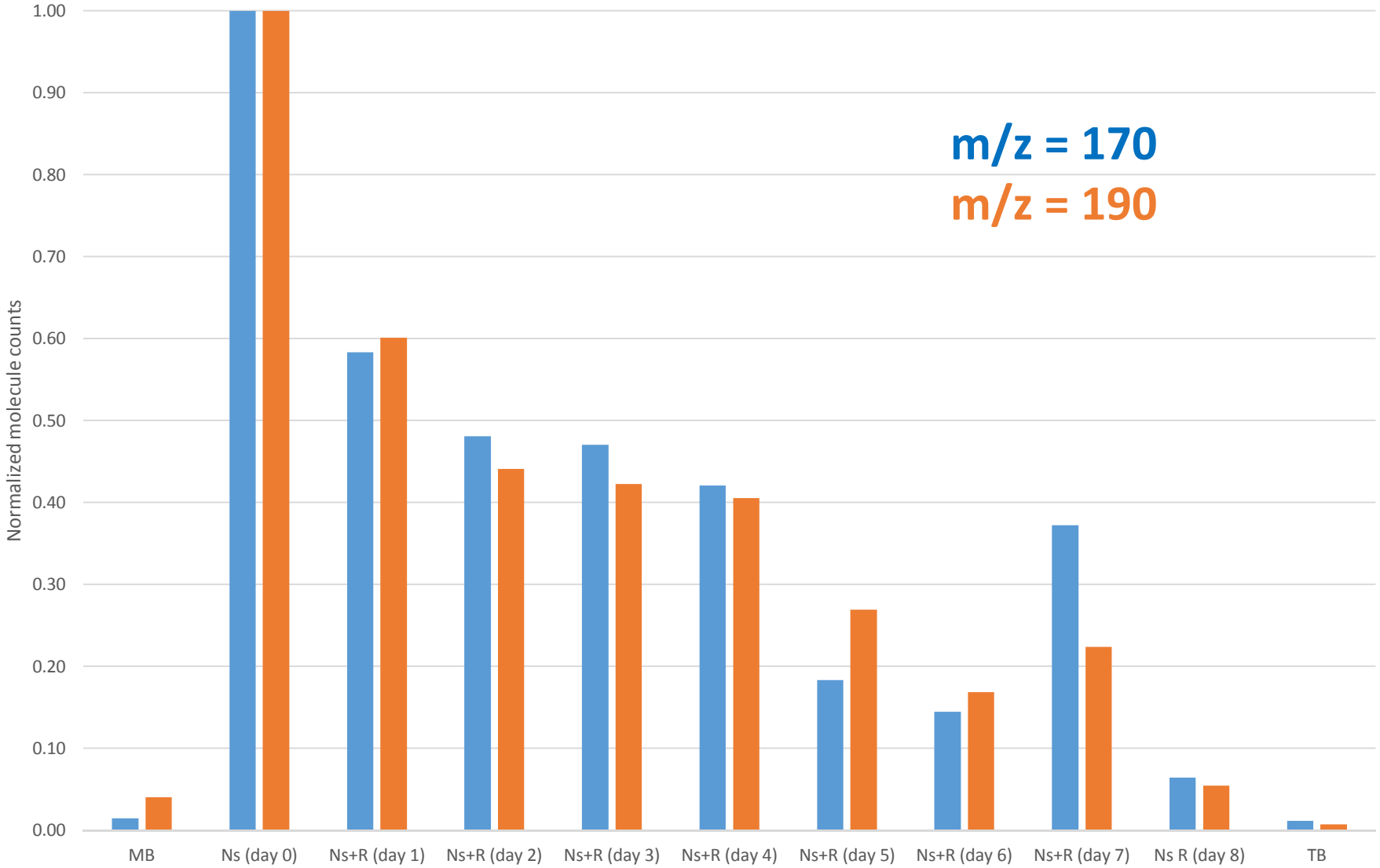


Very different AVOCs for Ns vs. Ns+R



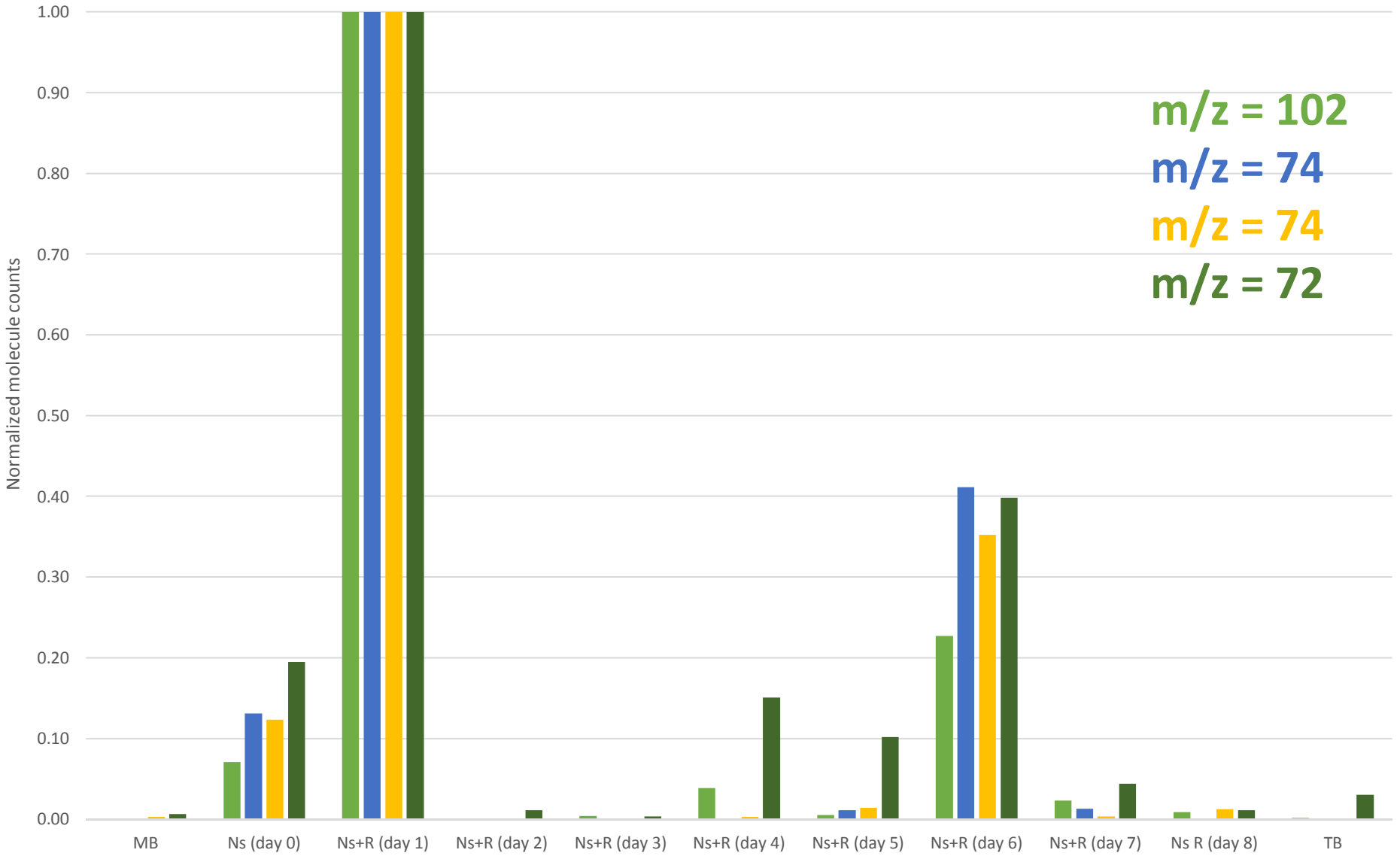
Preliminary Results:

Some AVOCs seem to decrease as incubation period with rotifers increases



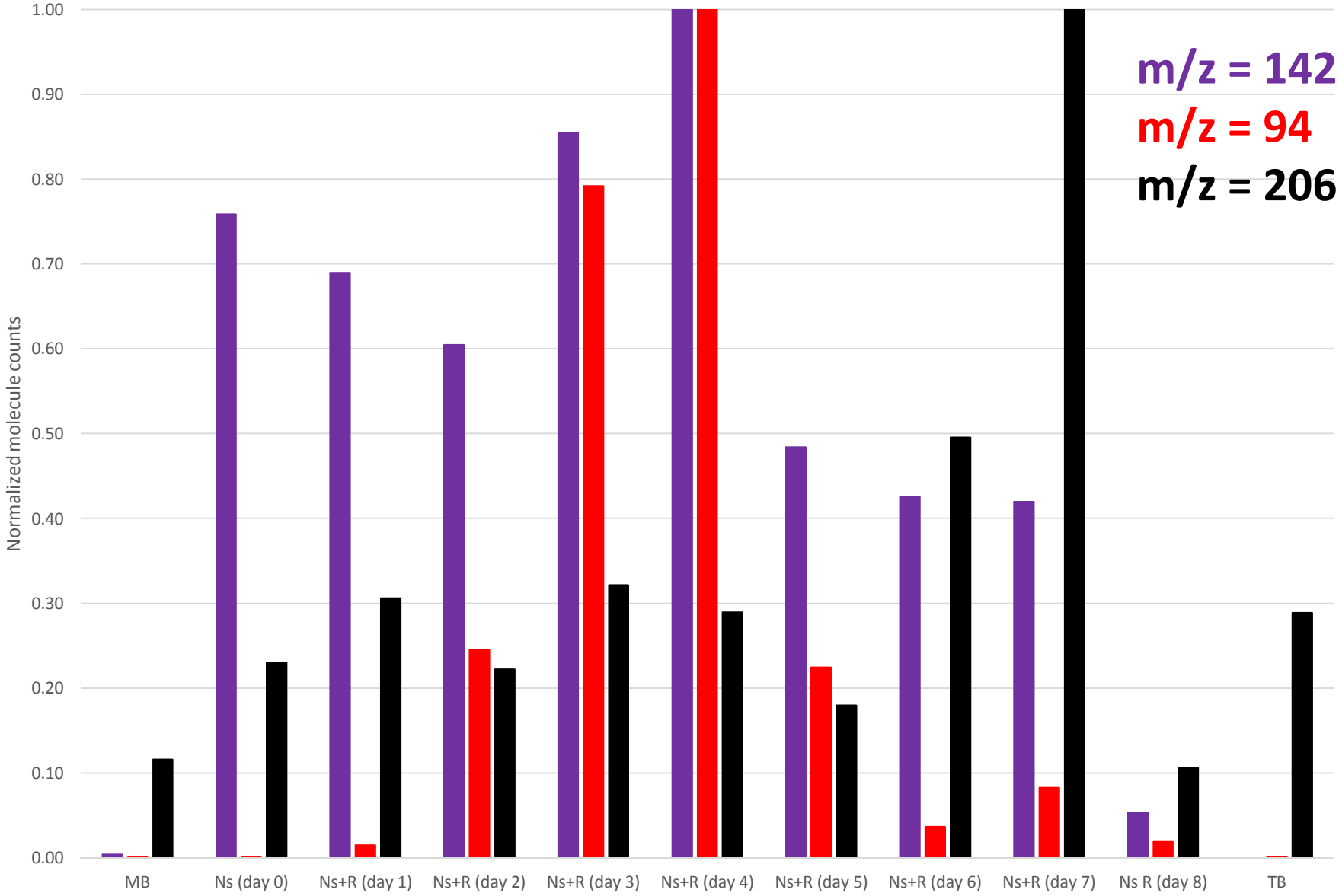
Preliminary Results:

Some AVOCs seem to initially increase then rapidly decline



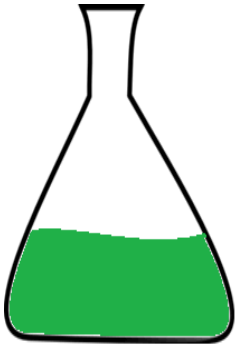
Preliminary Results:

Some AVOCs experience other changes



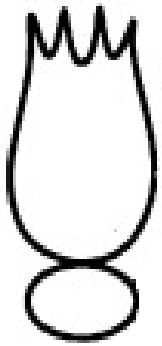
Consortia experiment: screen

Algae survival assay (Rotifer live/dead assay)

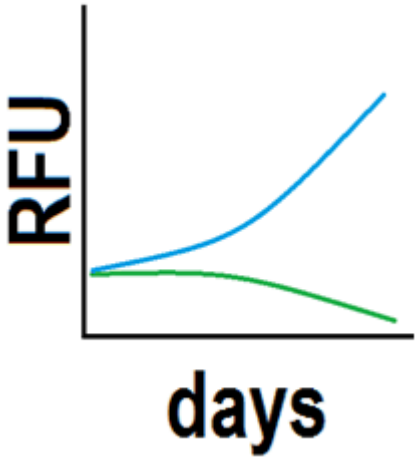


protective algal-bacterial consortia

+/-



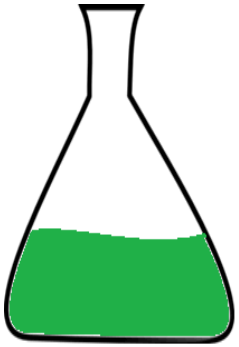
rotifer



consortia-protected algae with rotifers

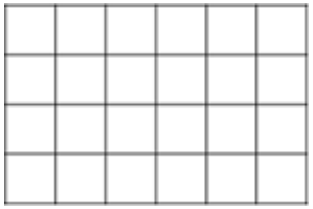
control algae, not protected from rotifer predation

+/-

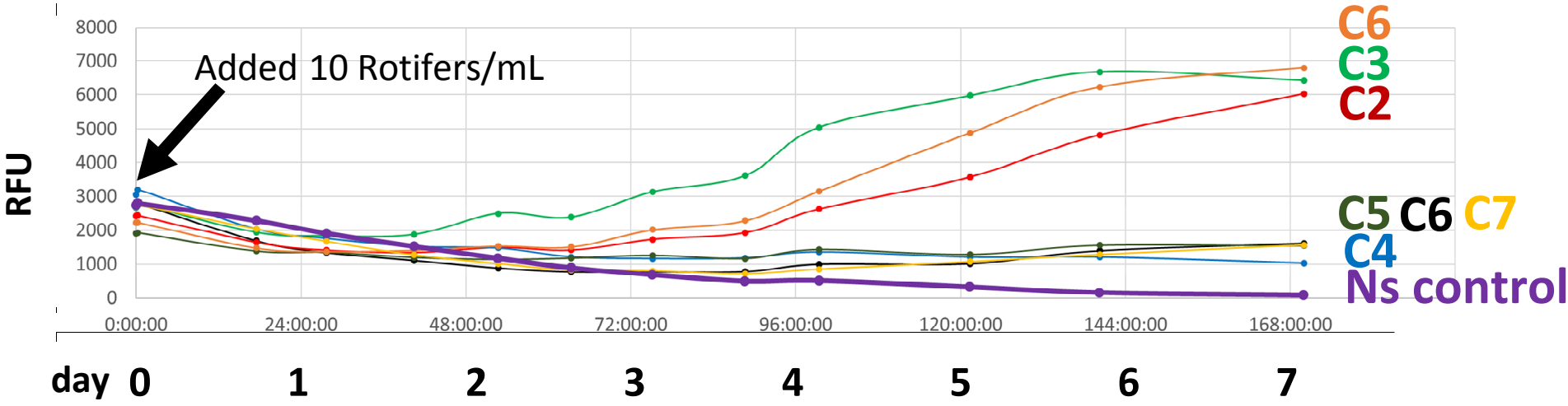


Ns control

- *Nannochloropsis salina:* 1-2 M Ns cells/mL
- *Brachionus plicatilis:* 10 Rotifers/mL
- Daily timepoints, ex/em: 430/685 nm
- Microtiter plate, 2 mL per well



Consortia yield protection from predation by rotifers

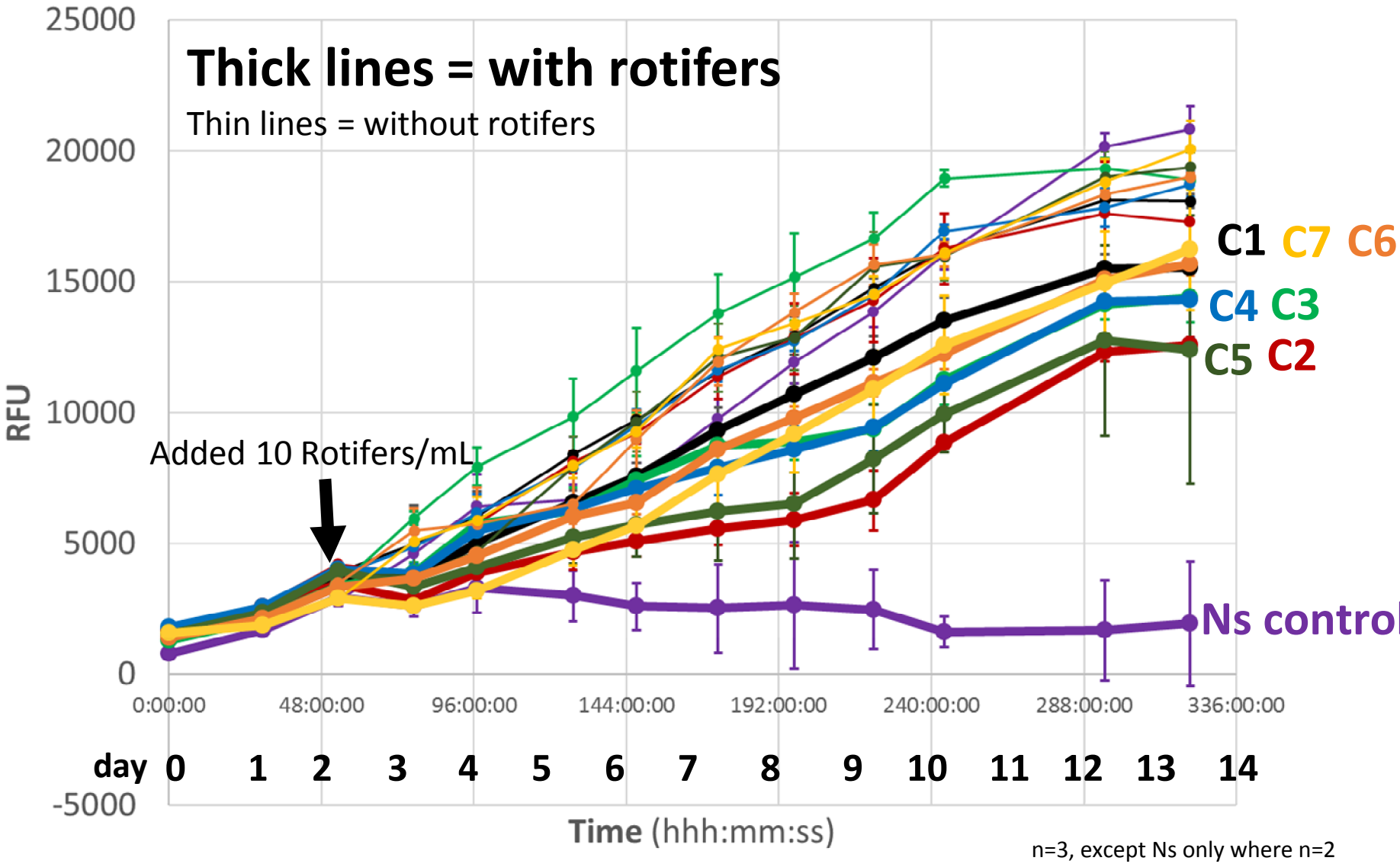


# rotifers in each well		sample name
~10	4	**Consortia 1**
0	2	**Consortia 2**
0	9	**Consortia 3**
>20, fast	1	**Consortia 4**
3	5	**Consortia 5**
0	3	**Consortia 6**
>20, fast	4	**Consortia 7**
>50, fast	>50, fast	**Ns control**

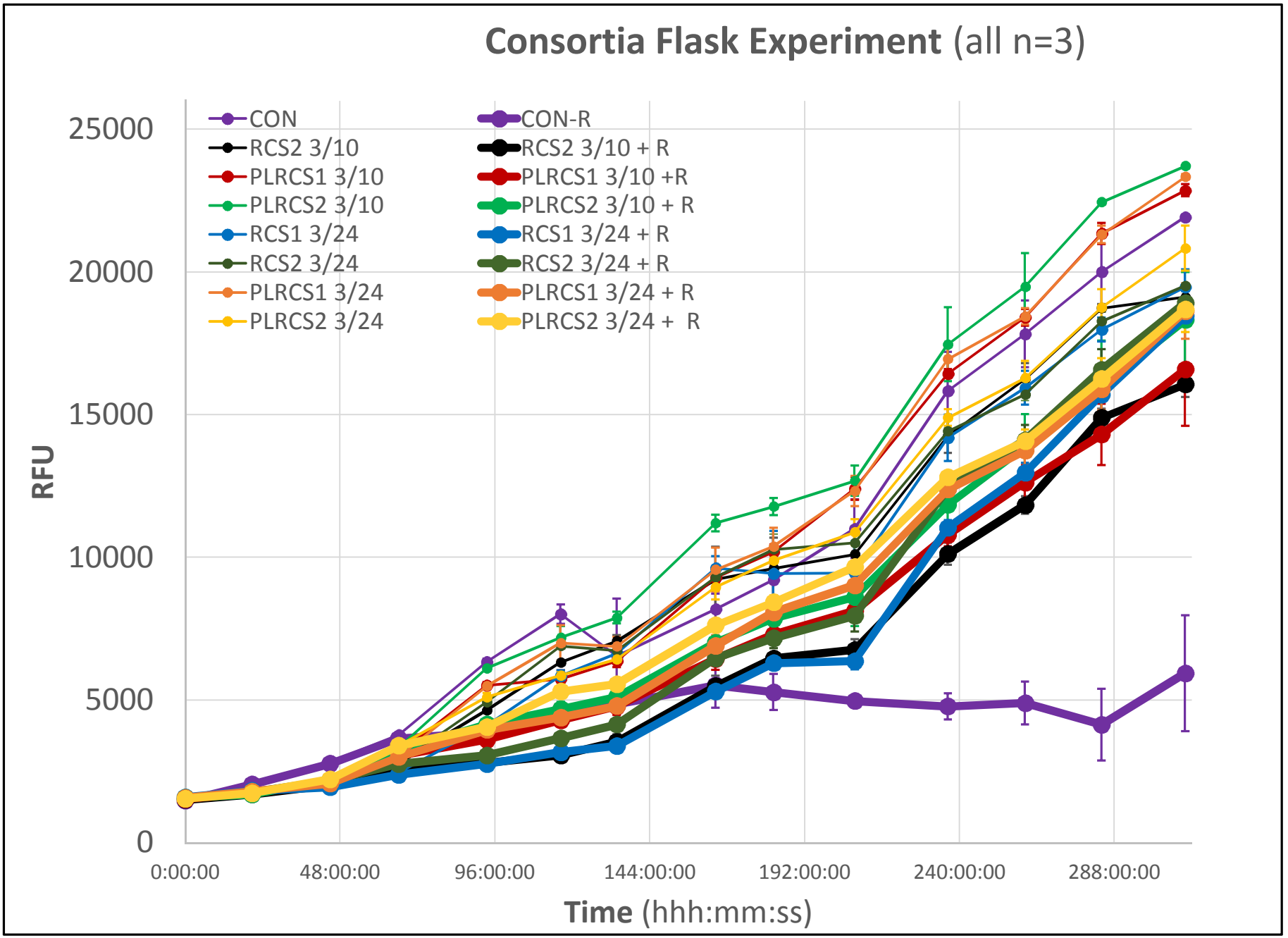
- Not many live rotifers present after 7 days with consortia
- Several rotifers were swimming "slowly"
- Very few eggs present

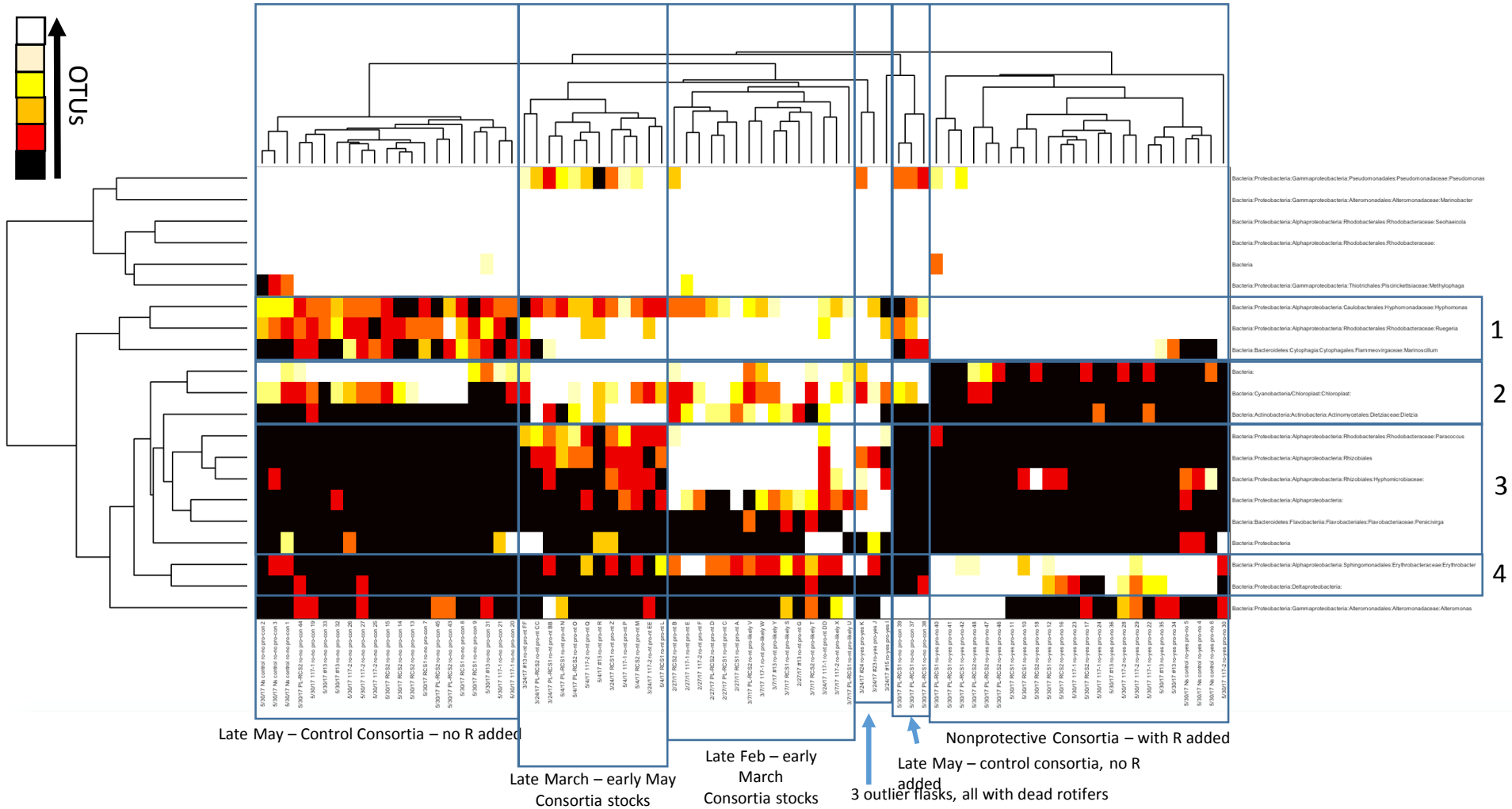
- Rotifer birth control?

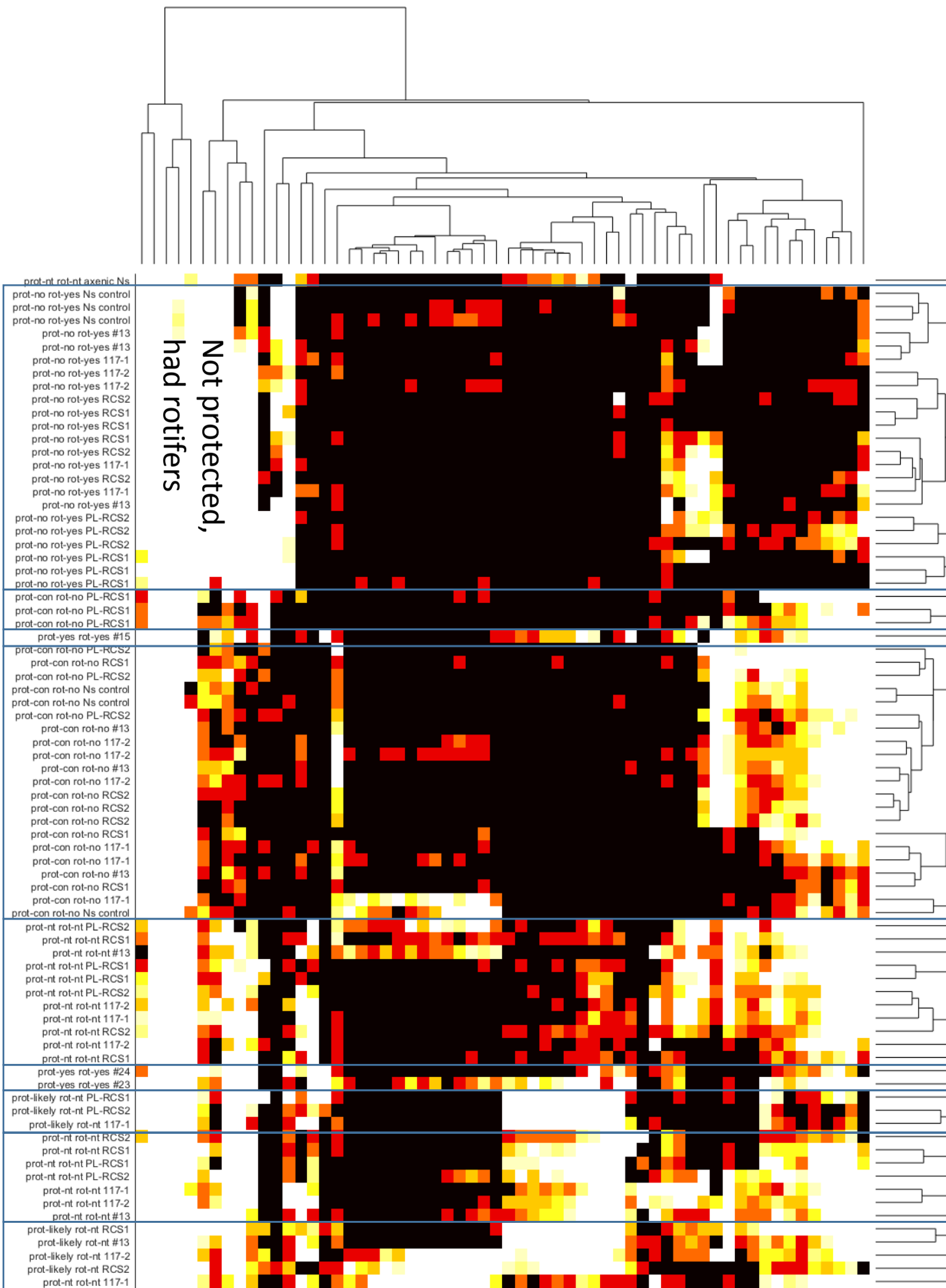
Consortia yield protection from predation by rotifers



n=3, except Ns only where n=2

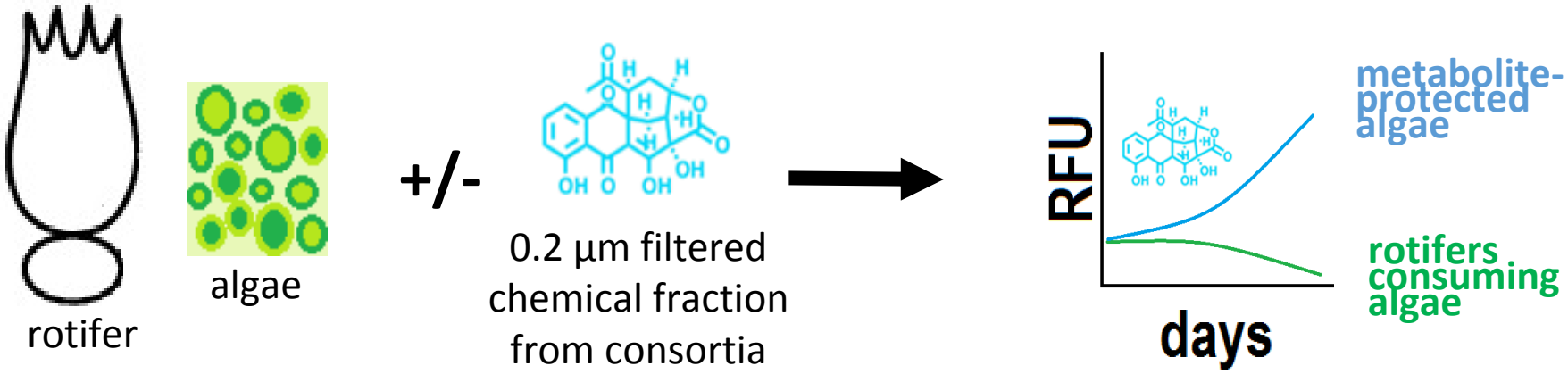

Consortia Flask Experiment (all n=3)
CON
CON-R
RCS2 3/10
RCS2 3/10 + R
PLRCS1 3/10
PLRCS1 3/10 +R
PLRCS2 3/10
PLRCS2 3/10 + R
RCS1 3/24
RCS1 3/24 + R
RCS2 3/24
RCS2 3/24 + R
PLRCS1 3/24
PLRCS1 3/24 + R
PLRCS2 3/24
PLRCS2 3/24 + R
25000
20000
15000
10000
5000
0
RFU
0:00:00
48:00:00
96:00:00
144:00:00
192:00:00
240:00:00
288:00:00
Time (hhh:mm:ss)


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


prot-nt rot-nt axenic Ns
prot-no rot-yes Ns control
prot-no rot-yes Ns control
prot-no rot-yes Ns control
prot-no rot-yes #13
prot-no rot-yes #13
prot-no rot-yes 117-1
prot-no rot-yes 117-2
prot-no rot-yes 117-2
prot-no rot-yes RCS2
prot-no rot-yes RCS1
prot-no rot-yes RCS1
prot-no rot-yes RCS1
prot-no rot-yes RCS2
prot-no rot-yes 117-1
prot-no rot-yes RCS2
prot-no rot-yes 117-1
prot-no rot-yes #13
prot-no rot-yes PL-RCS2
prot-no rot-yes PL-RCS2
prot-no rot-yes PL-RCS2
prot-no rot-yes PL-RCS1
prot-no rot-yes PL-RCS1
prot-no rot-yes PL-RCS1
prot-con rot-no PL-RCS1
prot-con rot-no PL-RCS1
prot-con rot-no PL-RCS1
prot-yes rot-yes #15
prot-con rot-no PL-RCS2
prot-con rot-no RCS1
prot-con rot-no PL-RCS2
prot-con rot-no Ns control
prot-con rot-no Ns control
prot-con rot-no PL-RCS2
prot-con rot-no #13
prot-con rot-no 117-2
prot-con rot-no 117-2
prot-con rot-no #13
prot-con rot-no 117-2
prot-con rot-no RCS2
prot-con rot-no RCS2
prot-con rot-no RCS2
prot-con rot-no RCS1
prot-con rot-no 117-1
prot-con rot-no 117-1
prot-con rot-no #13
prot-con rot-no RCS1
prot-con rot-no 117-1
prot-con rot-no Ns control
prot-nt rot-nt PL-RCS2
prot-nt rot-nt RCS1
prot-nt rot-nt #13
prot-nt rot-nt PL-RCS1
prot-nt rot-nt PL-RCS1
prot-nt rot-nt PL-RCS2
prot-nt rot-nt 117-2
prot-nt rot-nt 117-1
prot-nt rot-nt RCS2
prot-nt rot-nt 117-2
prot-nt rot-nt RCS1
prot-yes rot-yes #24
prot-yes rot-yes #23
prot-likely rot-nt PL-RCS1
prot-likely rot-nt PL-RCS2
prot-likely rot-nt 117-1
prot-nt rot-nt RCS2
prot-nt rot-nt RCS1
prot-nt rot-nt PL-RCS1
prot-nt rot-nt PL-RCS2
prot-nt rot-nt 117-1
prot-nt rot-nt 117-2
prot-nt rot-nt #13
prot-likely rot-nt RCS1
prot-likely rot-nt #13
prot-likely rot-nt 117-2
prot-likely rot-nt RCS2
prot-nt rot-nt 117-1
Not protected, had rotifers

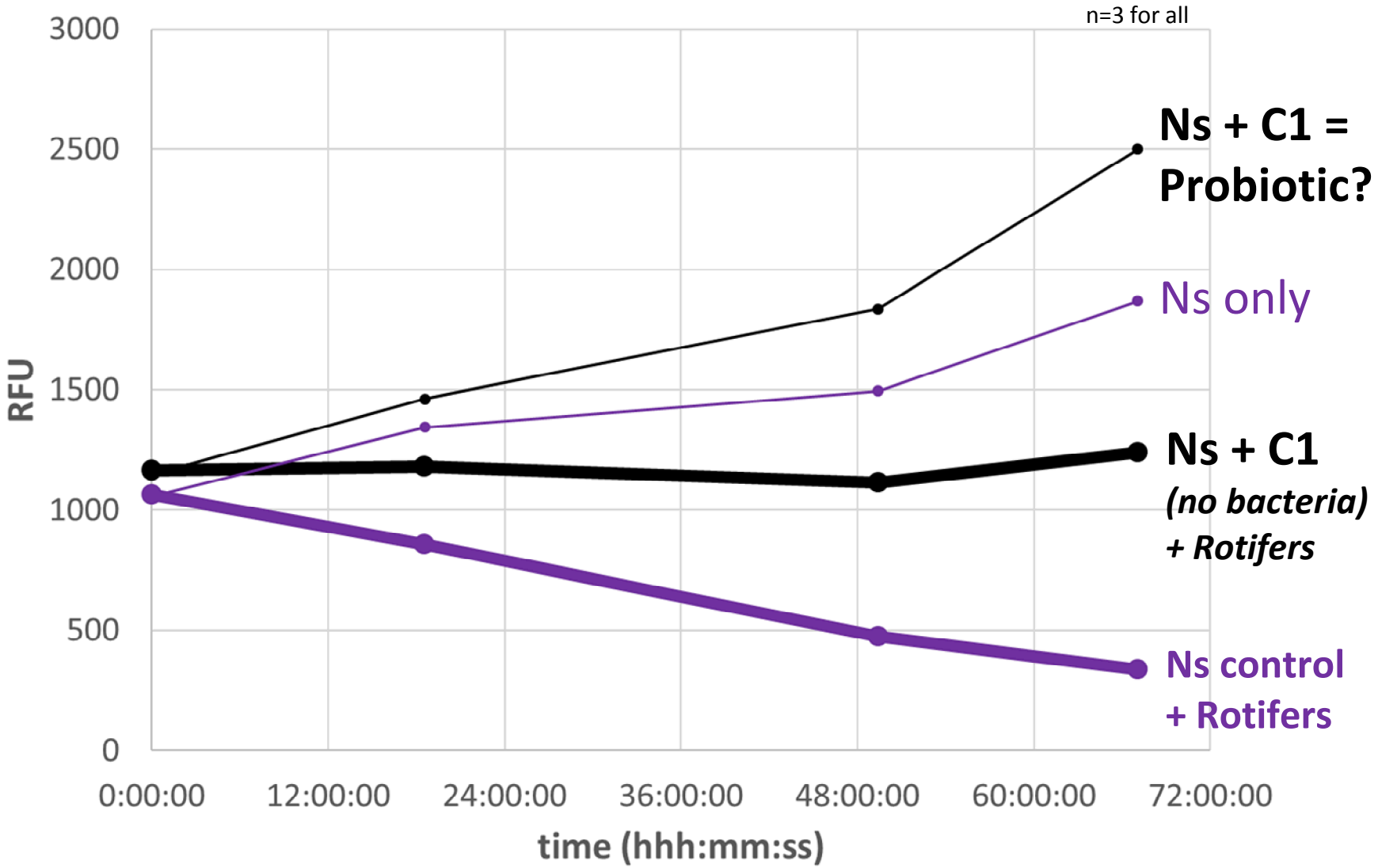
Chemical fraction experiments

Algae survival assay (Rotifer live/dead assay), sans bacteria



- **0.2 µm filter protective consortia + Ns only control**
- Used filtrate; added in Ns and rotifers
- *Nannochloropsis salina:* 3 M Ns cells/mL
- *Brachionus plicatilis:* 8 Rotifers/mL
- Daily timepoints, ex/em: 430/685 nm

Chemical fraction from C1 consortia yield protection for 3 days



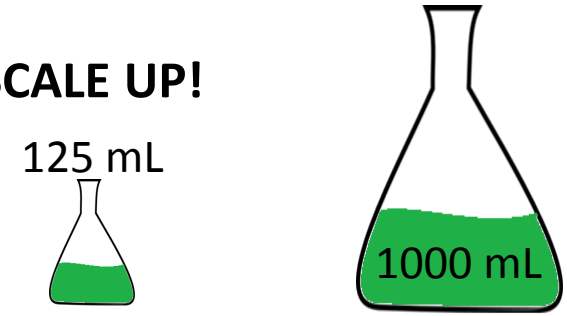
Summary & Future Work

- **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

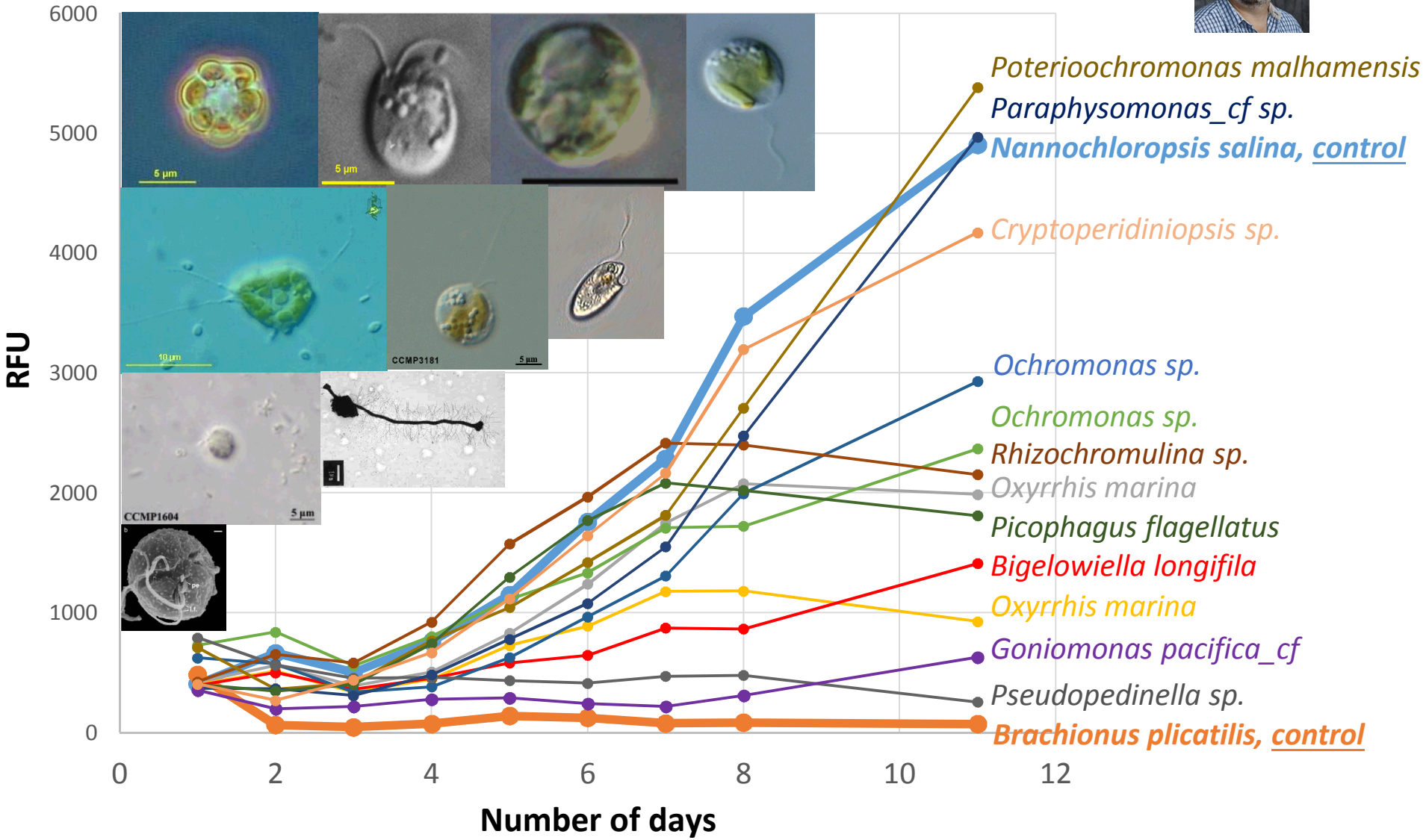
- **Chemical fraction was protective**
  - Identify the active chemical(s) → LC/MS & NMR
  - Identify the bacteria that create the chemical(s)
  - Dosage experiments

- **SCALE UP!**



- **Test more predators…**

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



Thank you!

Sandia National Laboratories

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Laura T. Carney

ATP3 Algae Testbed Public-Private Partnership

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