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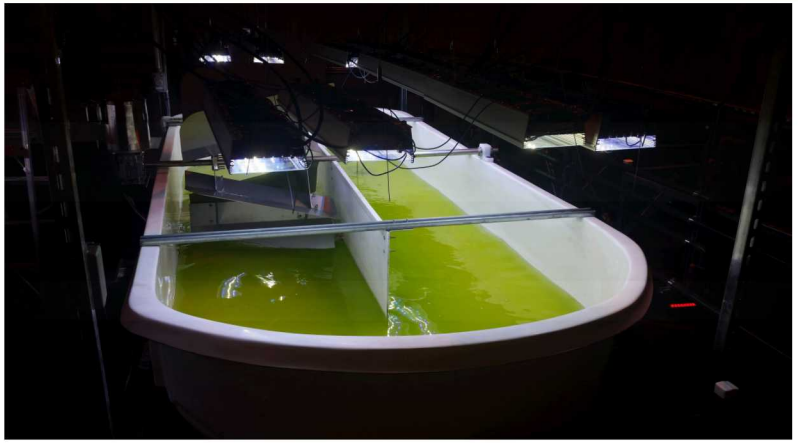

Investigating the chemical and biological landscape of *Nannochloropsis salina* cultures to mitigate pond crashes

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National Nuclear Security Administration

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

BIOENERGY TECHNOLOGIES OFFICE

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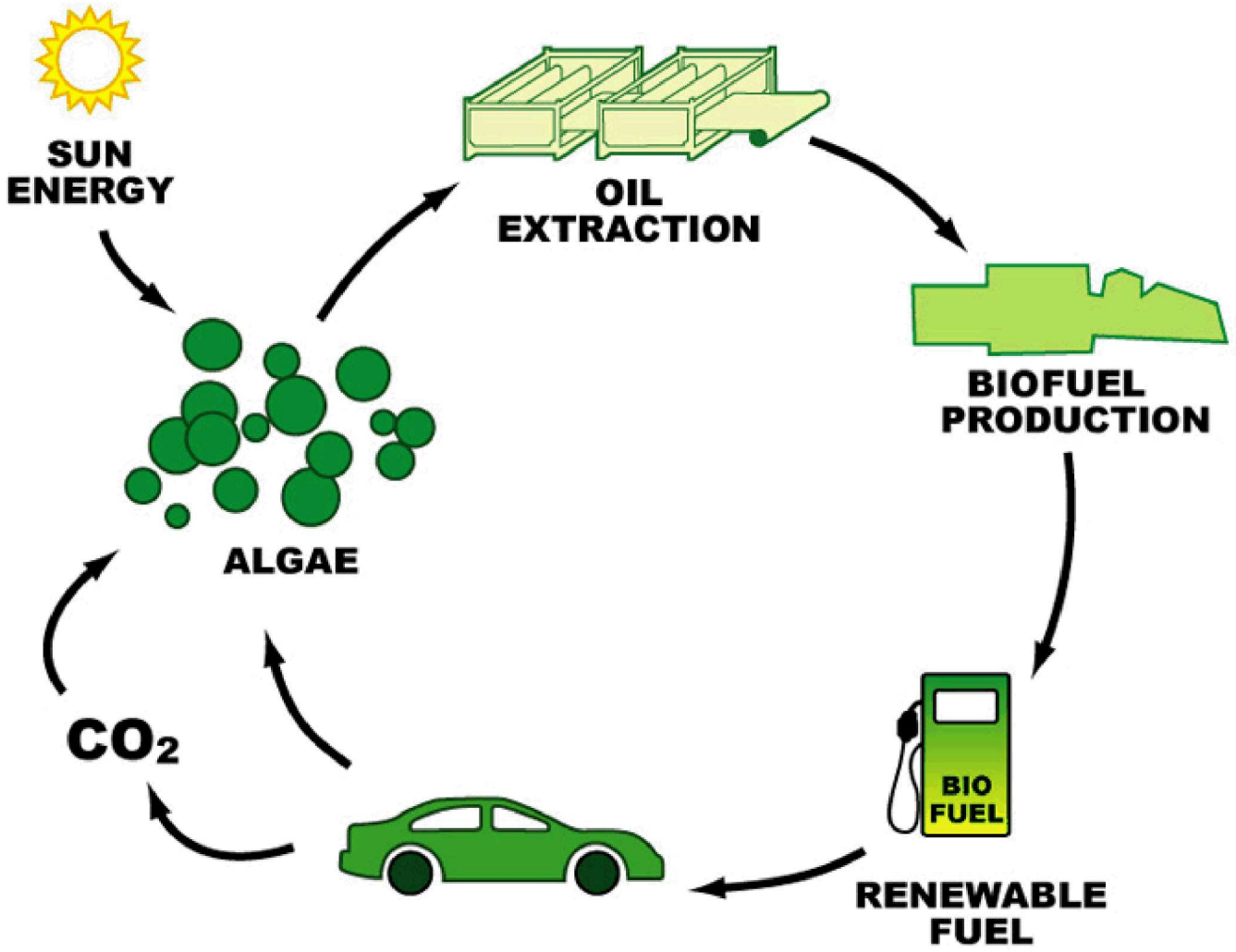
Biofuel is the future, but there are serious economic barriers before it becomes reality.



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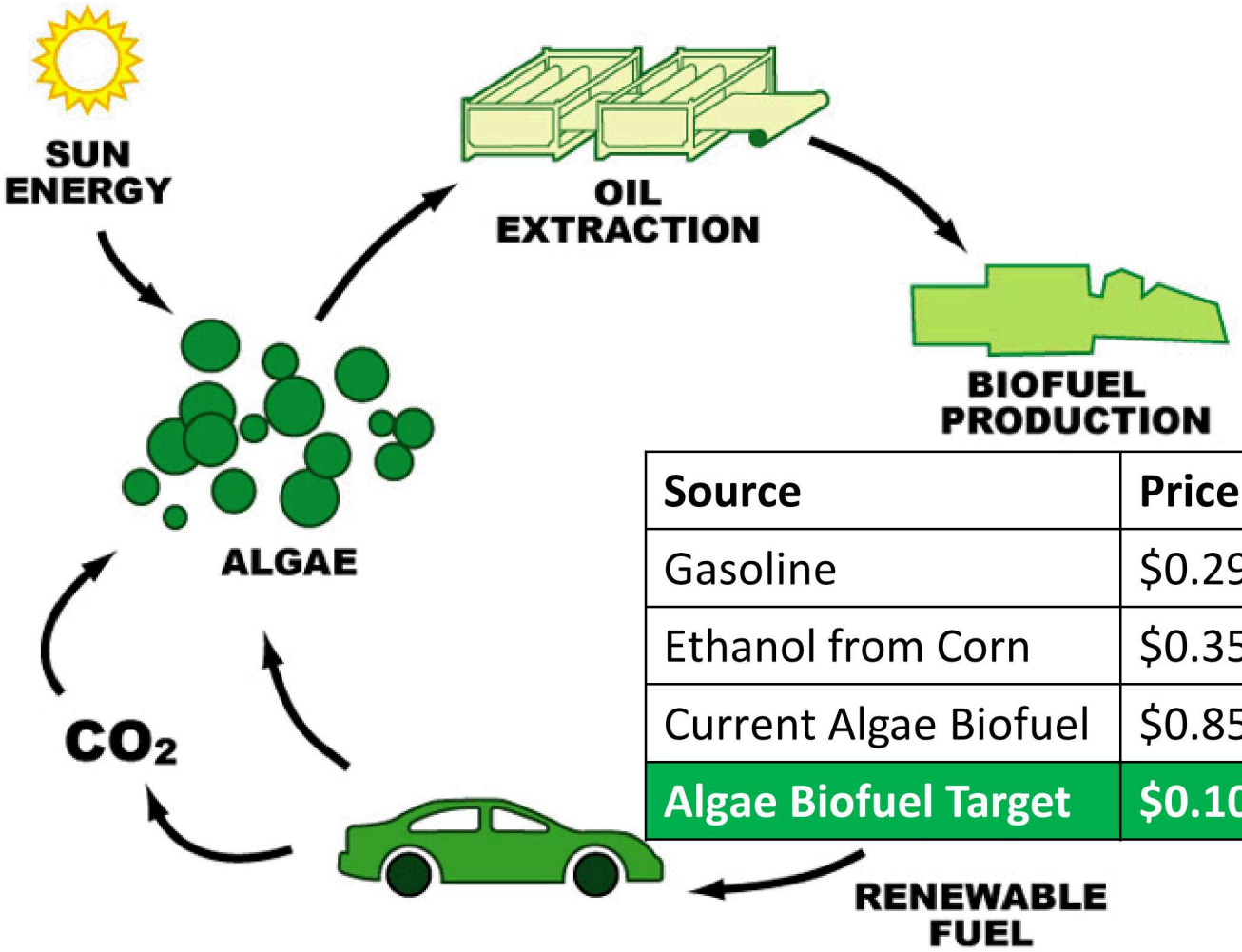


Source	Price /lb	Price /gal
Gasoline	$0.29	$2.49*
Ethanol from Corn	$0.35	$2.92*
Current Algae Biofuel	$0.85- $3.67*	$7.06 - $30.46
Algae Biofuel Target	**$0.10 – 0.25***	**$1.50 - $2.10**

*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

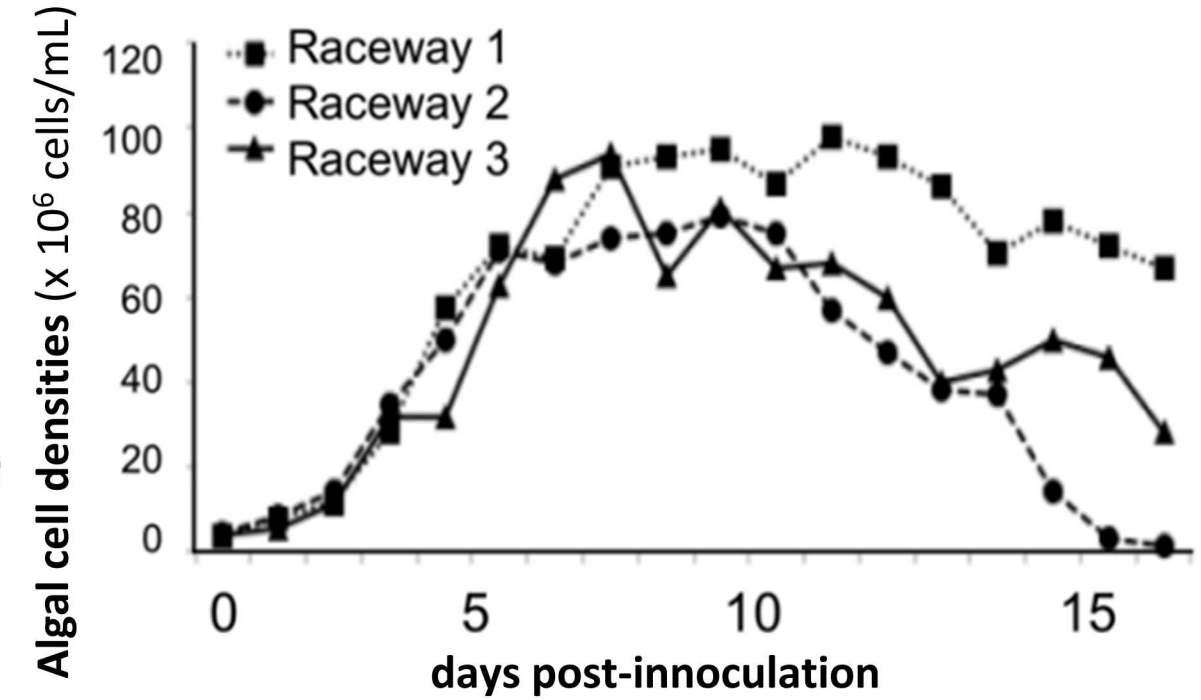
Target selling prices for algae biomass in 2022 is $494/ton AFDW (ash-free dry weigh; 2014$) **to achieve $5/GGE** (gasoline gallon equivalent).

- 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.

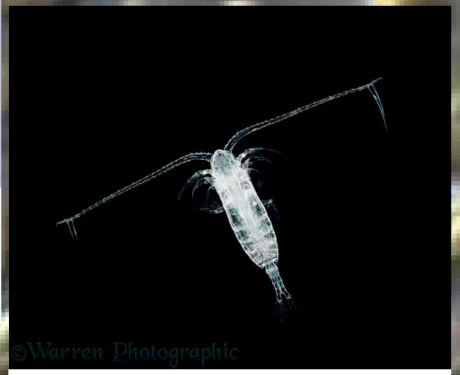


Carney et al. 2016

Nannochloropsis salina

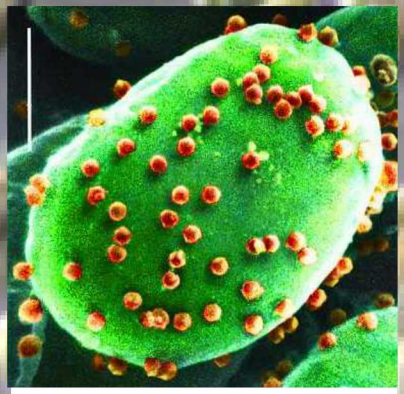
Predators of microalga

Brachionus plicatilis, marine rotifer



Marine planktonic copepod, *Calanus*

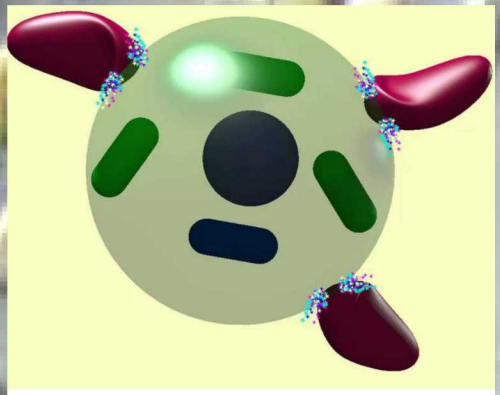
Oxyrrhis marina, dinoflagellate



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?

Today's talk will focus on…

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!

Our Approach

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

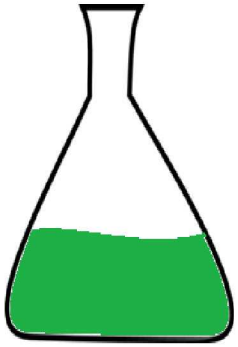
TABB/SFA

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

POSSE LDRD

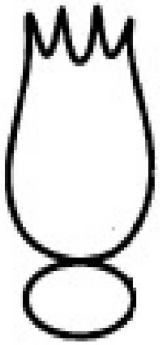
Consortia experiment: screen

Algae survival assay (Rotifer live/dead assay)

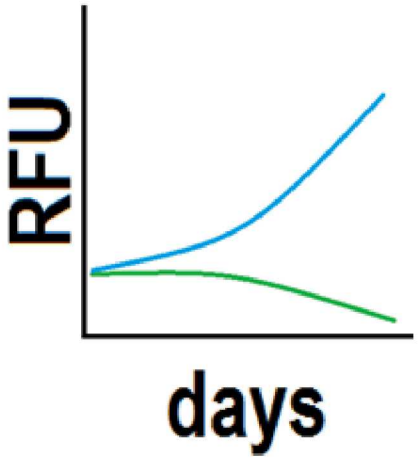


protective algal-bacterial consortia

+/-



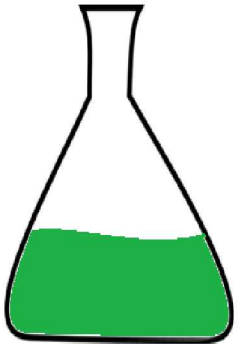
rotifer



consortia-protected algae with rotifers

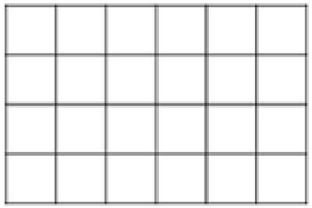
control algae, not protected from rotifer predation

+/-

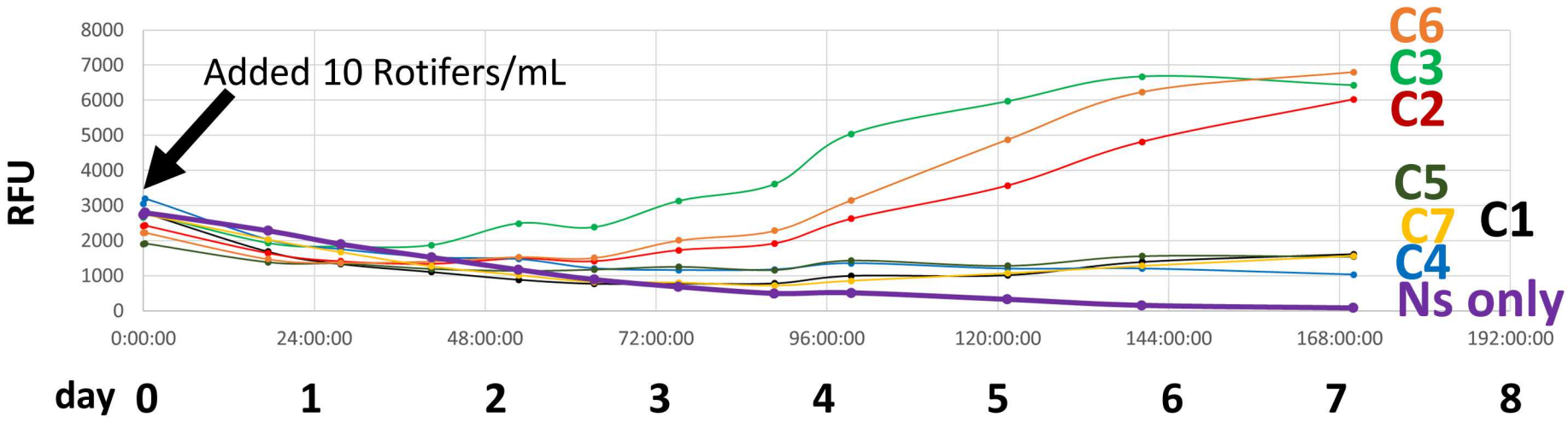


Ns control

- *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**

Plate assay:

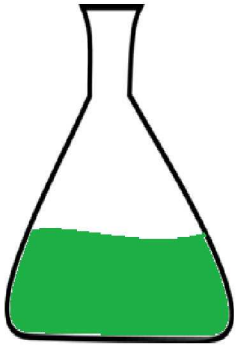
- Not many live rotifers present after 7 days with consortia
- Several rotifers were swimming "slowly"
- Very few eggs present

- Rotifer birth control?!

SNL CONSORTIA

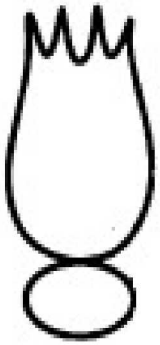
Consortia experiment: screen

Algae survival assay



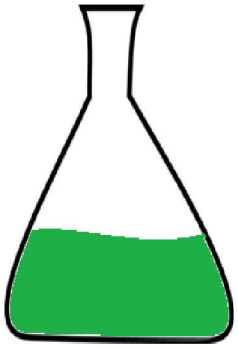
protective algal-bacterial consortia

+/-

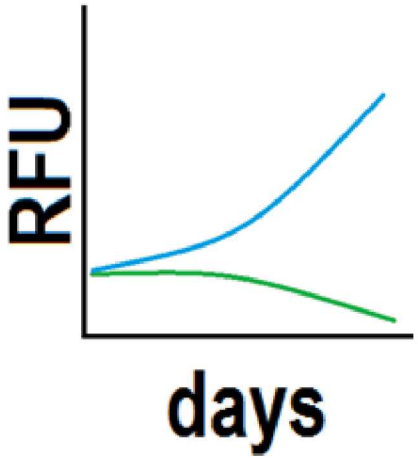


rotifer

+/-



Ns only

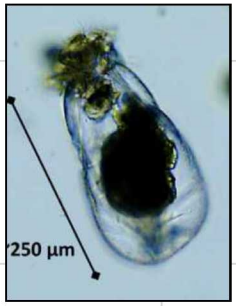


consortia-protected algae with rotifers

control algae, not protected from rotifer predation

- Plate assay
- 2 mL samples, n=2 for each
- *Nannochloropsis salina*
- 1-2 M Ns cells/mL and 10 Rotifers/mL, *Brachionus plicatilis*, all added day 1
- Daily timepoints, ex/em: 430/685 nm

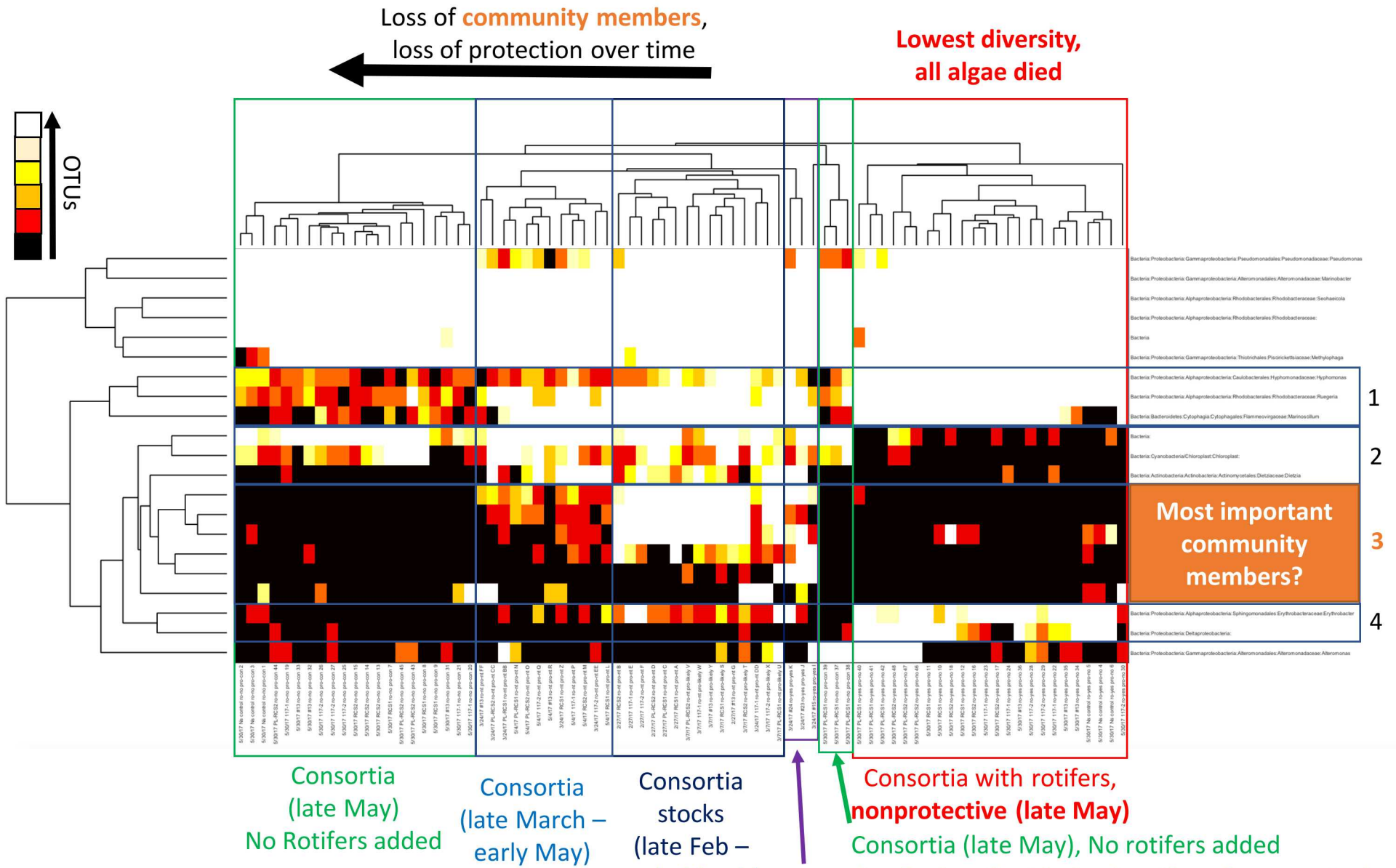
Bacterial consortia protect algae from rotifers



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

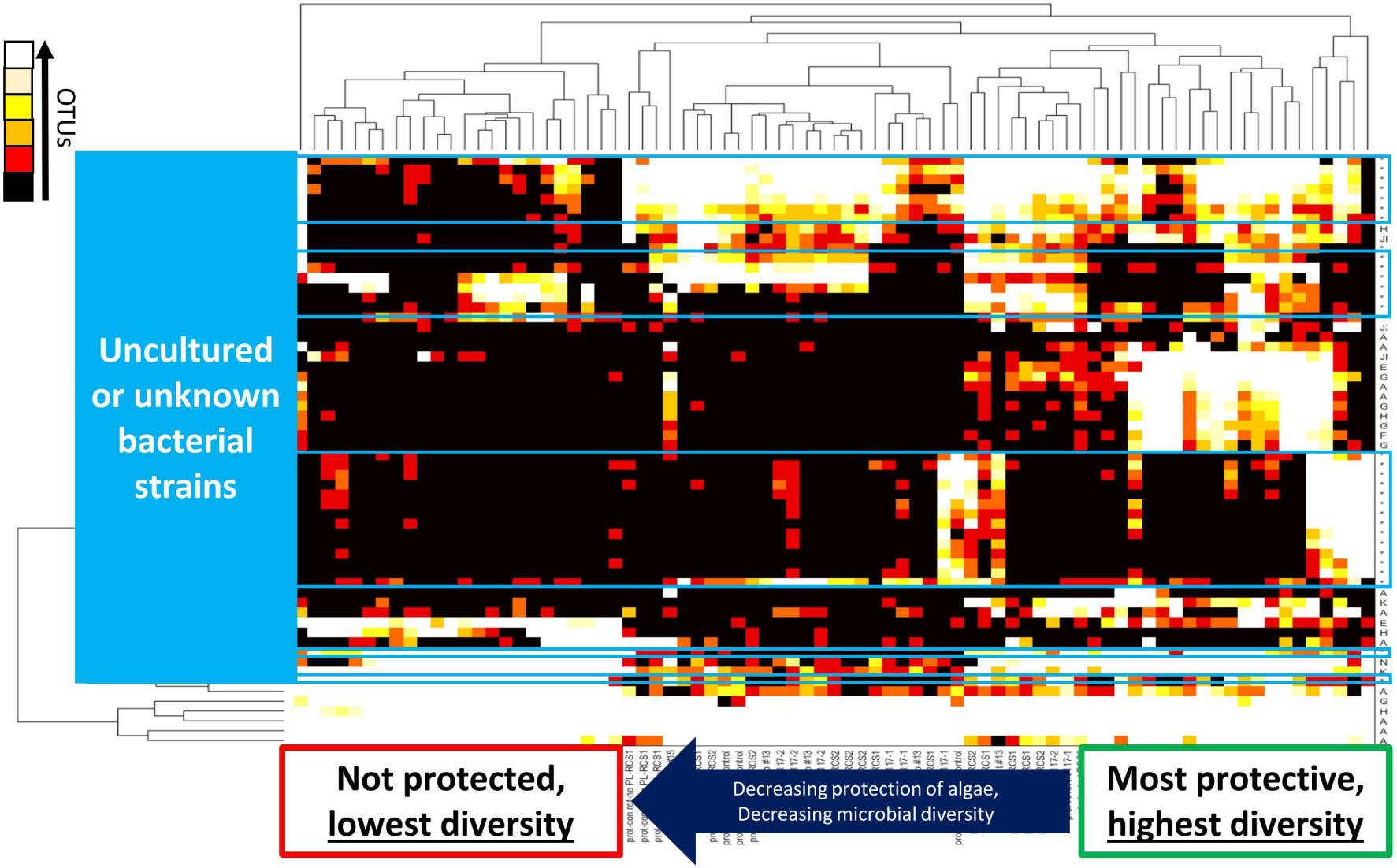
Manuscript in preparation, Fisher et al 2018

Protective consortia have more bacterial diversity



Manuscript in preparation, Fisher et al 2018

Protective consortia have more bacterial diversity



Manuscript in preparation, Fisher et al 2018

Sandia Outdoor Mesocosm Setup

Objective:
Do consortia stabilize algal culture in outdoor mesocosm culture?

Do consortia retain protective qualities outside the laboratory?

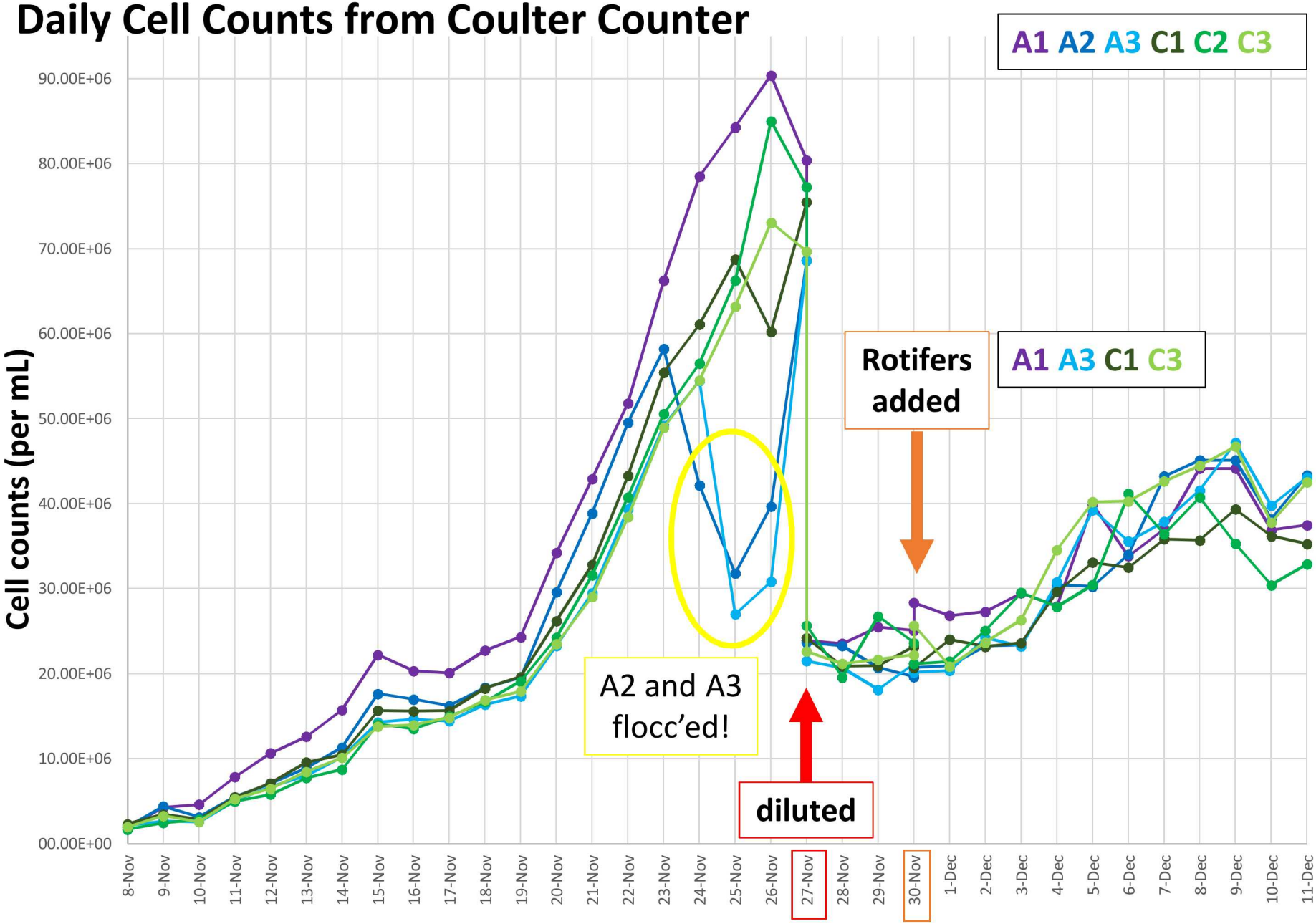
3 axenic Ns samples - **A1 A2 A3**
3 Ns + consortia samples - **C1 C2 C3**
17L volume for each

Daily sampling:
- Fluorescence
- Cell counts
- OD 750

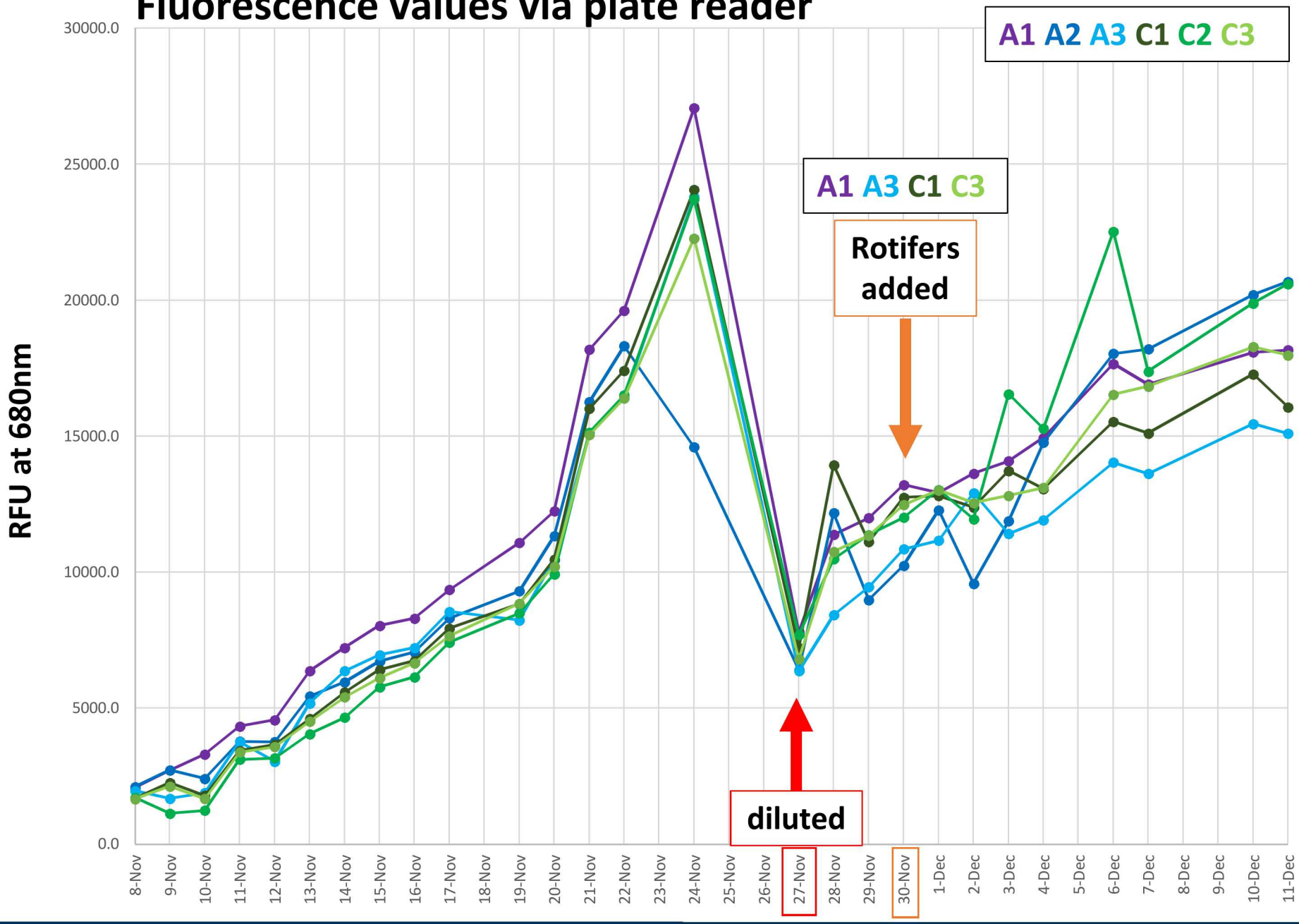
Sample every 3 days:
- AFDW
- Nutrient analysis
- Sequencing

Added non-axenic rotifers at 1 per 500,000 algal cells (retained same ratio from lab experiments) to test protective ability of consortia in outdoor mesocosm.

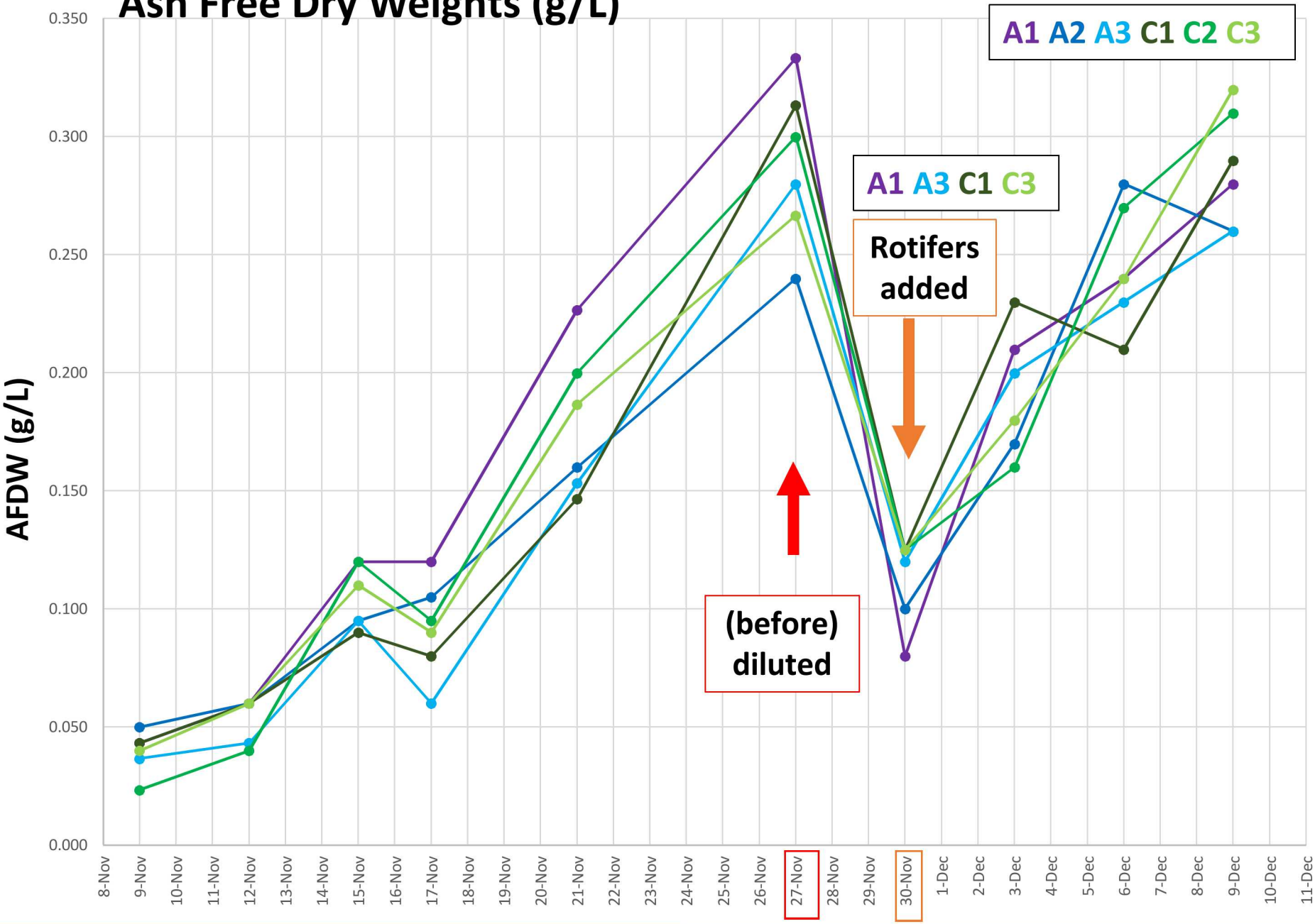
Daily Cell Counts from Coulter Counter



Fluorescence values via plate reader

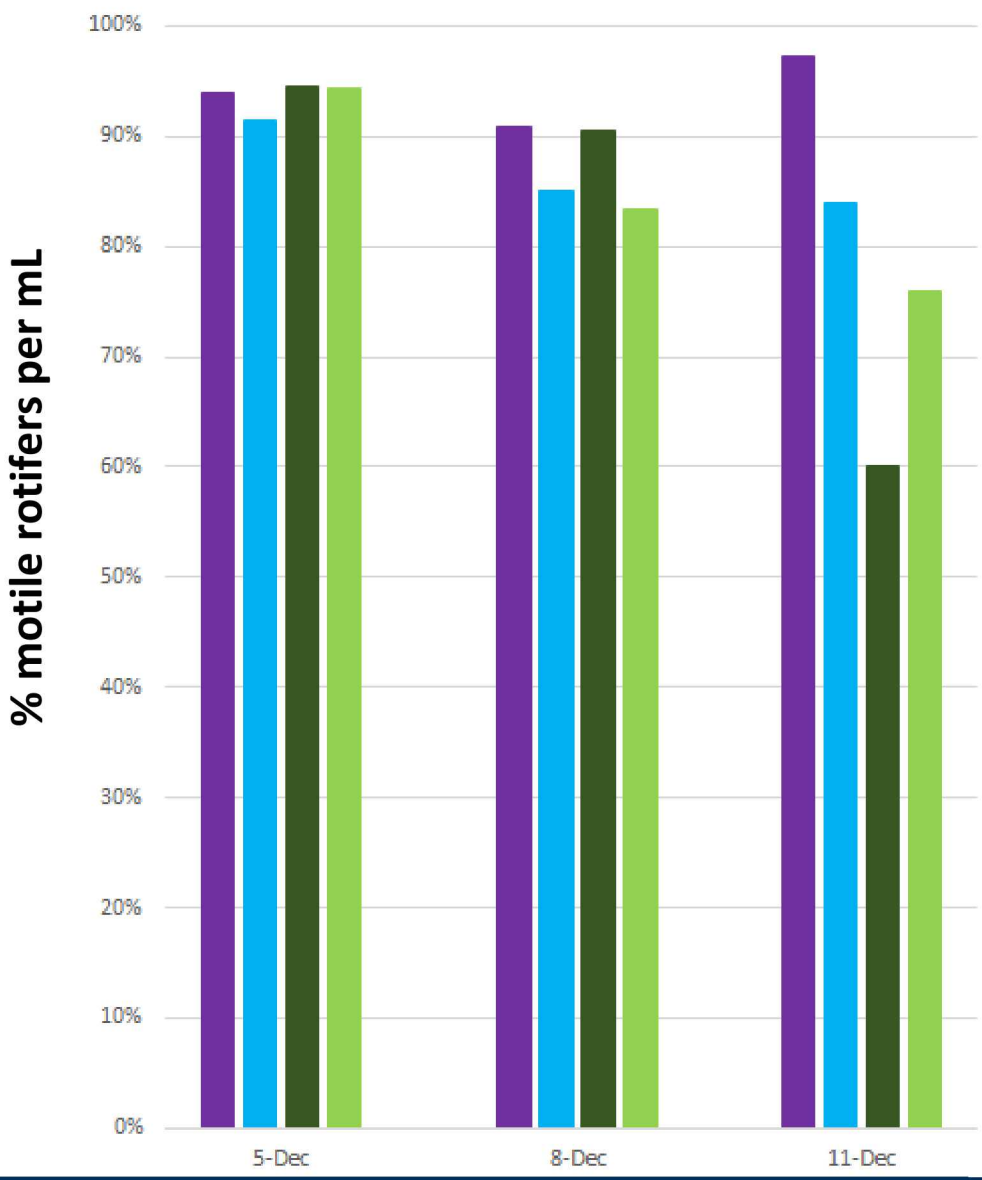


Ash Free Dry Weights (g/L)



Rotifer and egg counts per mL

A1 A3 C1 C3



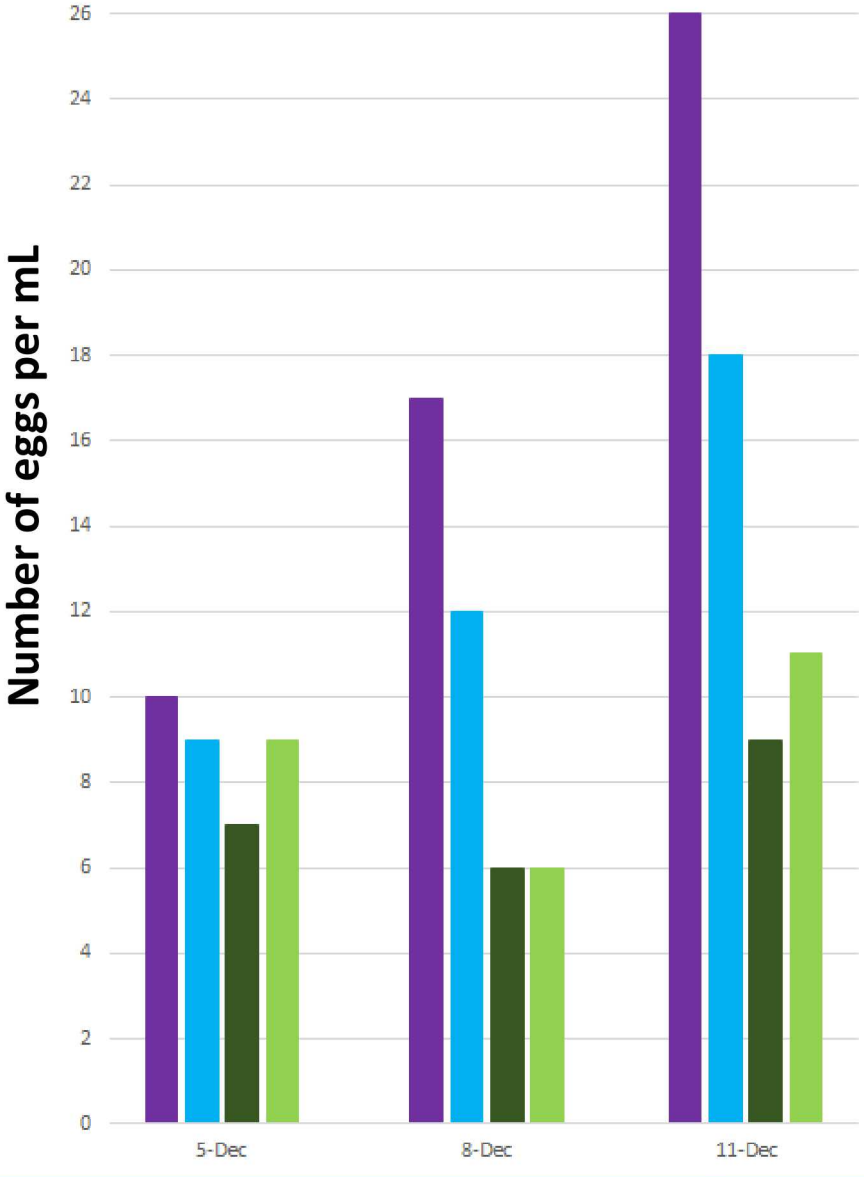
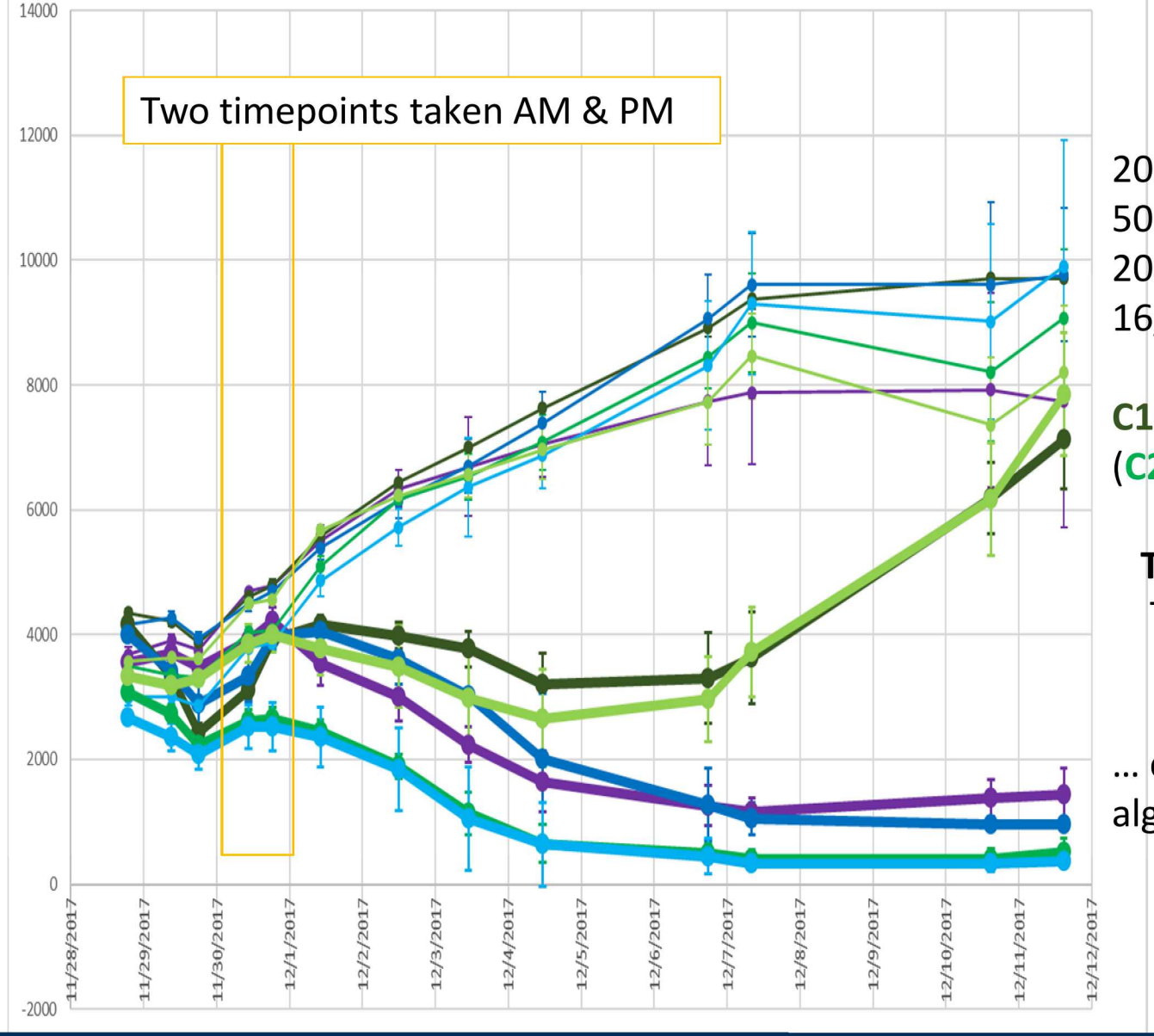


Plate assay of mesocosms



A1 A2 A3 C1 C2 C3

20M/mL Ns
50 Rots / well
20C
16/8 light/dark

C1+R & **C3+R** are protective (**C2+R** is not protective) ?

Thick lines = +R (n=4)
Thin lines = -R (n=2)

… consortia probiotic for algae? (outgrows control)

SNL CONSORTIA: CONCLUSIONS

- **Low sun, low temperatures Nov – Dec: not ideal**
  - Affected algal growth … consortia growth?
  - Affected rotifer feeding and mesocosm crash
  - Algae doubled every 3-4 days, only 1 dilution needed

- **Rotifers purchased, not axenic…**
  - High quantity of rotifers → Reed aquaculture
  - Did non-axenic rotifers "come with" bacteria that helped rotifers survive better than they would have normally…?
  - What bacteria did rotifers arrive with? How were consortia dynamics affected? (only 16S sequencing will hep us learn this information).

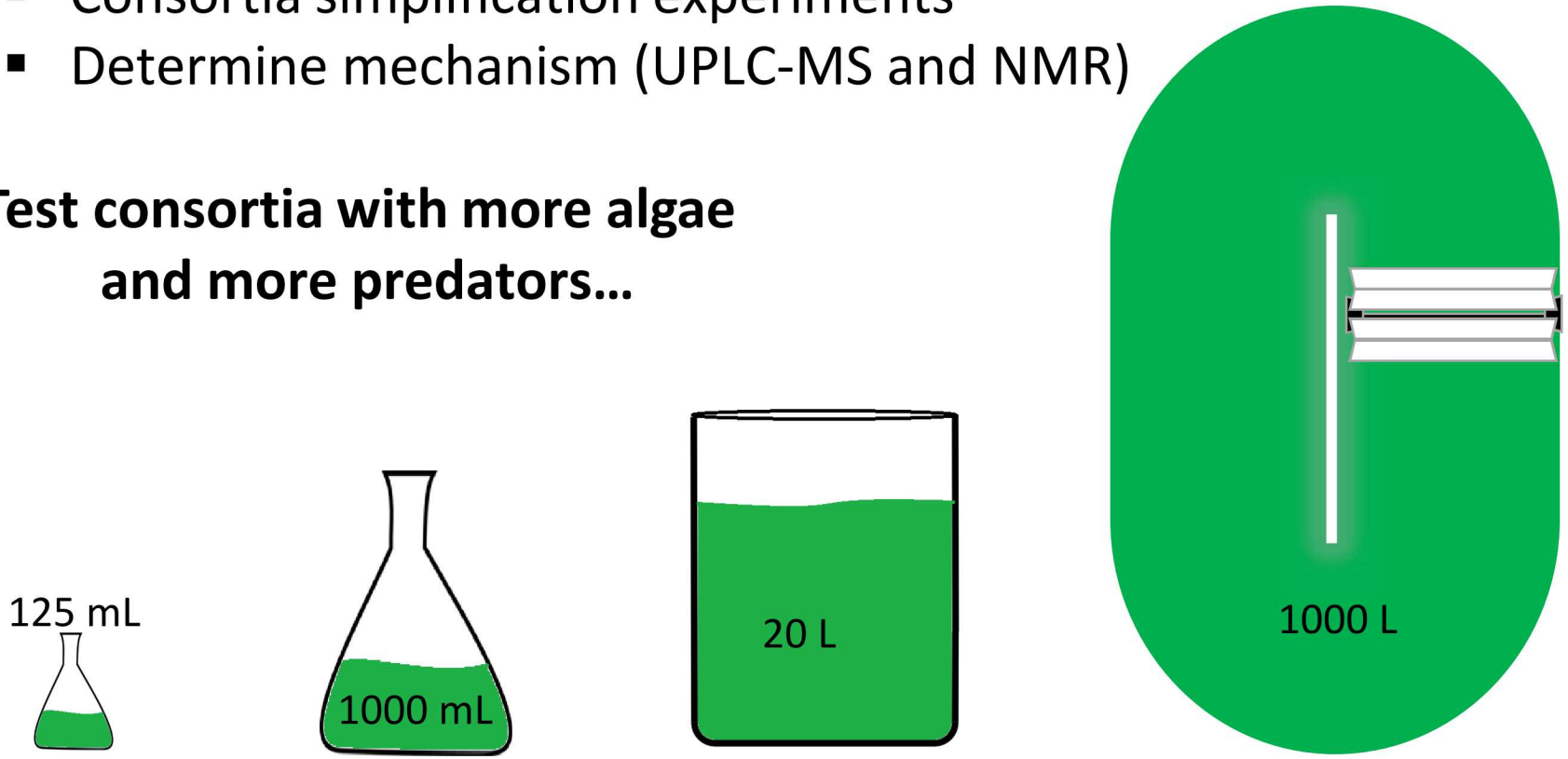
- **16S Sequencing data will be really interesting!**

- **Nutrient analysis will also be interesting**

SNL CONSORTIA: FUTURE WORK

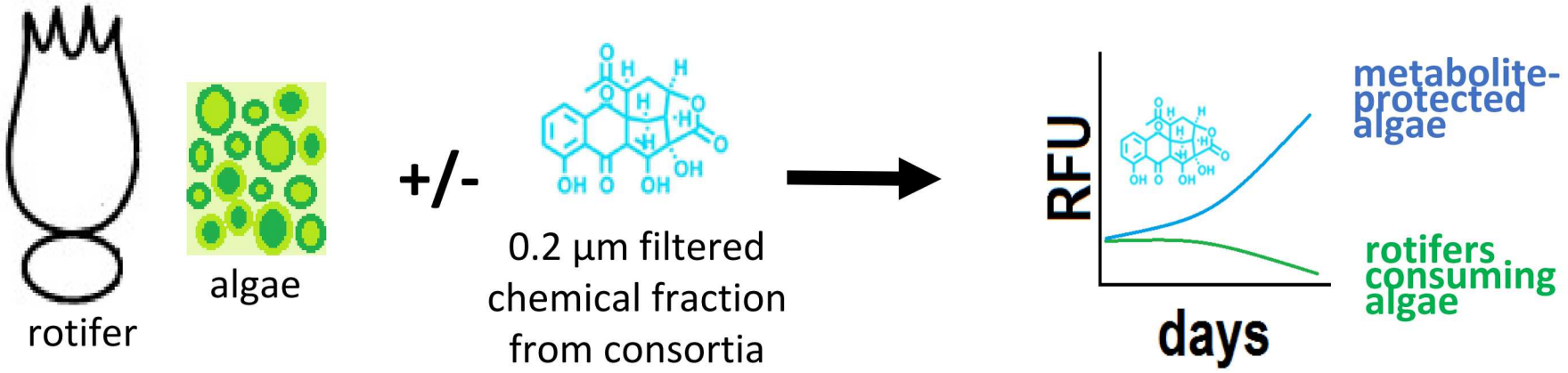
- **State of Technology for DISCOVR**
  - Test consortia in outdoor ponds at ASU and/or TAMU in 2018
  - 16S amplicon sequencing (Illumina MiSeq) analysis of consortia
  - Consortia simplification experiments
  - Determine mechanism (UPLC-MS and NMR)

- **Test consortia with more algae and more predators…**



Chemical fraction experiments

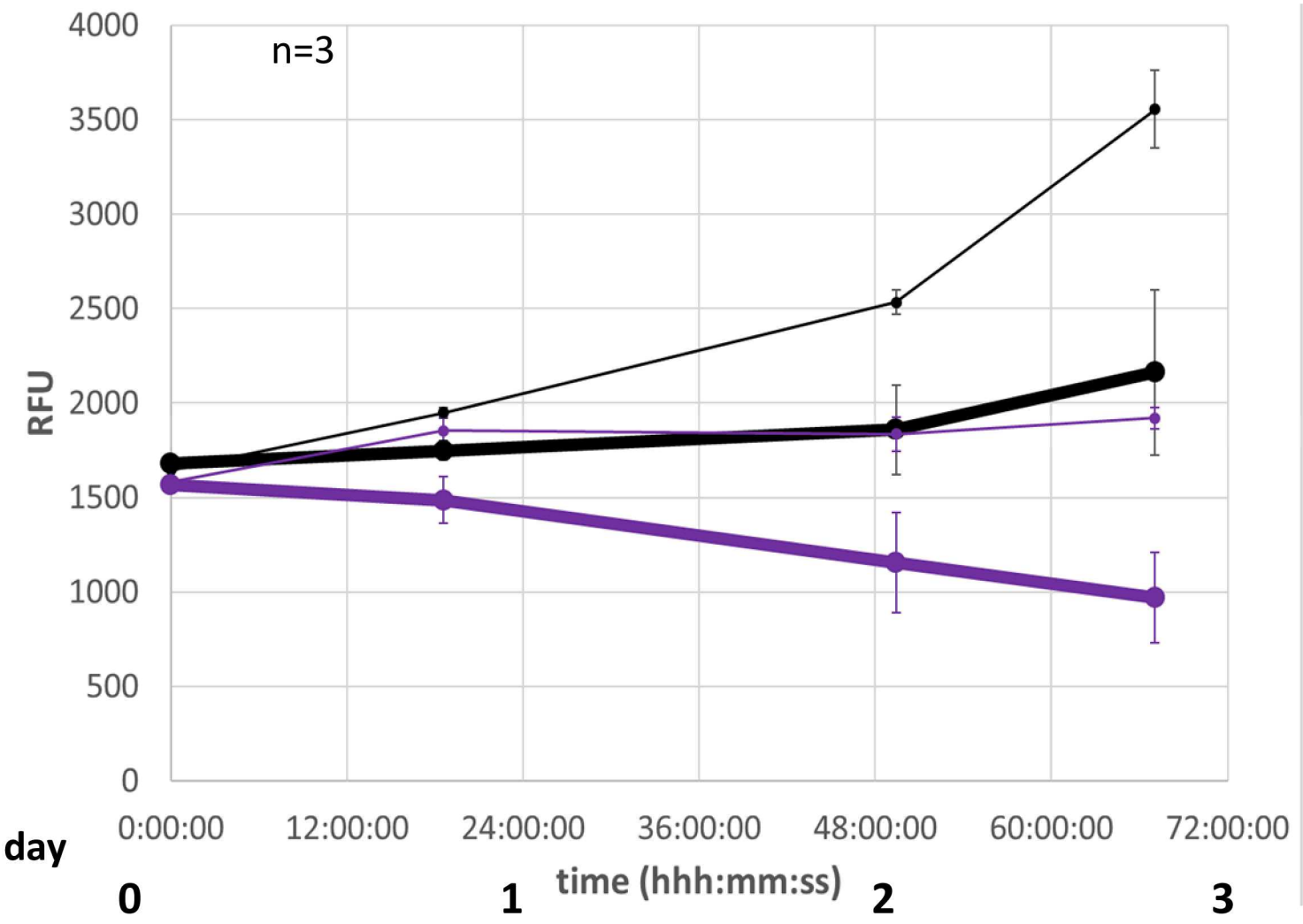
Algae survival assay (Rotifer live/dead assay), sans bacteria



- **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

SNL CONSORTIA: **MECHANISM …**

Chemical fraction from C1 consortia yield protection for 3 days



Ns + C1 = Probiotic?

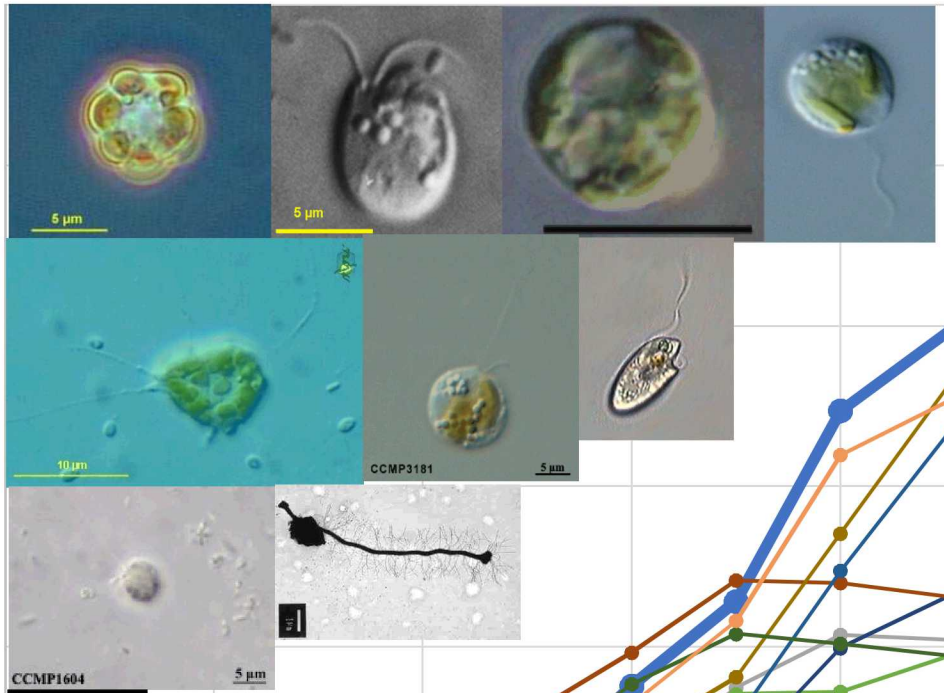
Ns + C1
(no bacteria)
+ Rotifers

Ns only

Ns control
+ Rotifers

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

Effect of various predators on *Nannochloropsis salina* concentration



RFU

6000
5000
4000
3000
2000
1000
0

0
2
4
6
8
10
12

Number of days

Poterioochromonas malhamensis
Paraphysomonas_cf sp.
Nannochloropsis salina, control
Cryptoperidiniopsis sp.
Ochromonas sp.
Ochromonas sp.
Rhizochromulina sp.
Oxyrrhis marina
Picophagus flagellatus
Bigelowiella longifila
Oxyrrhis marina
Goniomonas pacifica_cf
Pseudopedinella sp.
Brachionus plicatilis, control

Thank you!

Sandia National Laboratories

ATP3 Algae Testbed Public-Private Partnership

Lawrence Livermore National Laboratory

CA

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Kunal Poorey
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