

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

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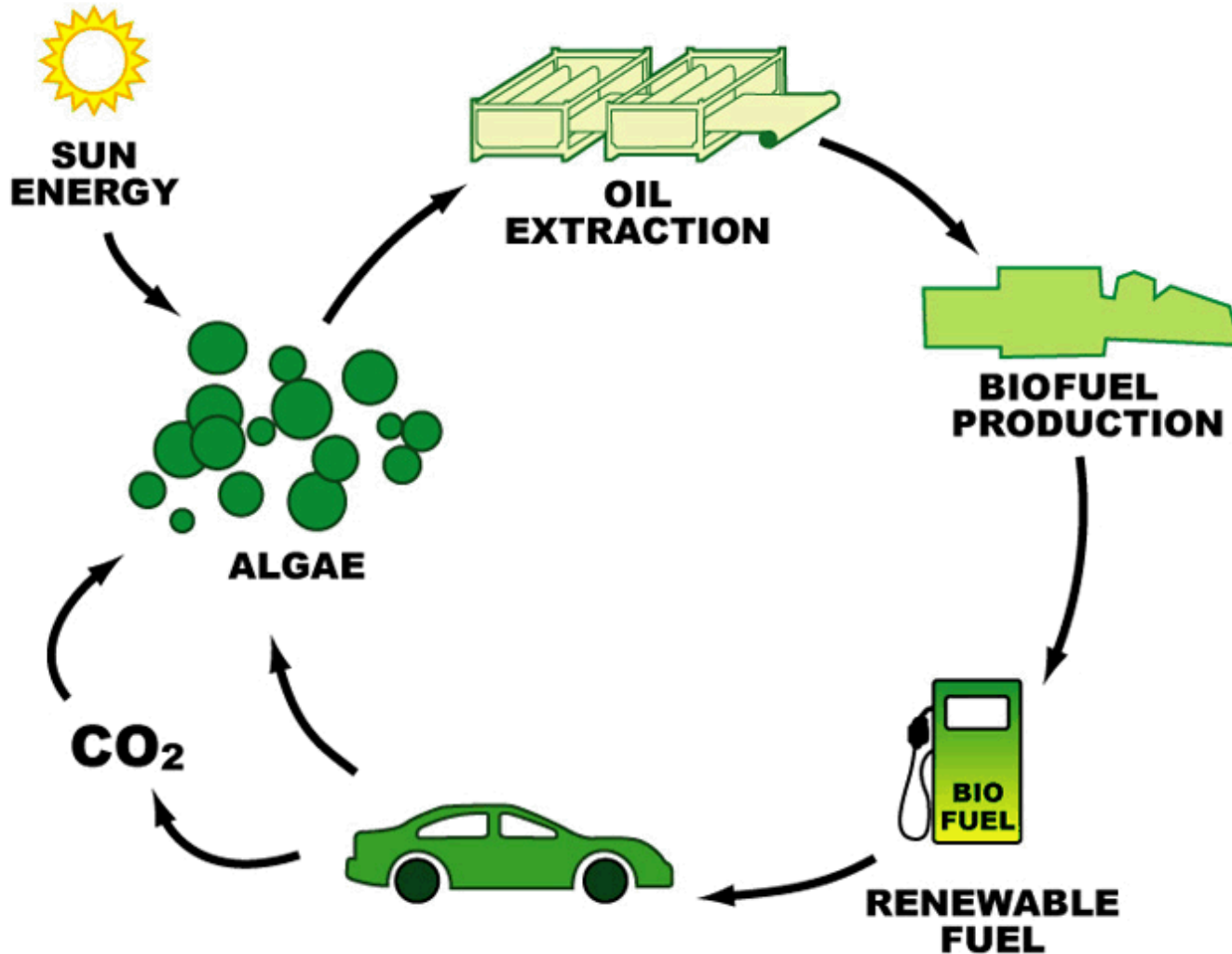
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ENERGY | Energy Efficiency &
Renewable Energy

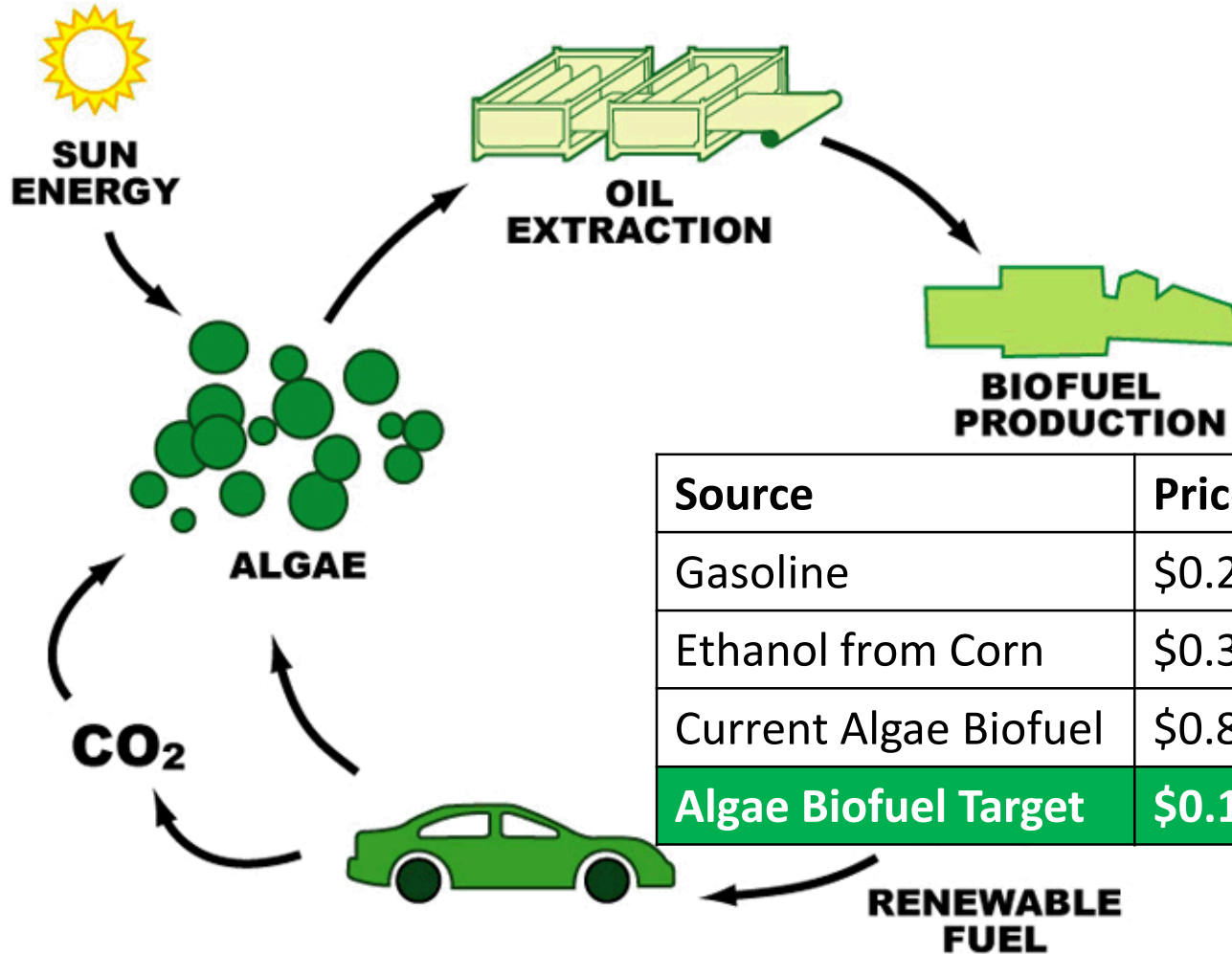
BIOENERGY TECHNOLOGIES OFFICE

February 14, 2018

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	\$2.49*
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

*2017 DOE Alternative Fuel Price Report

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

*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

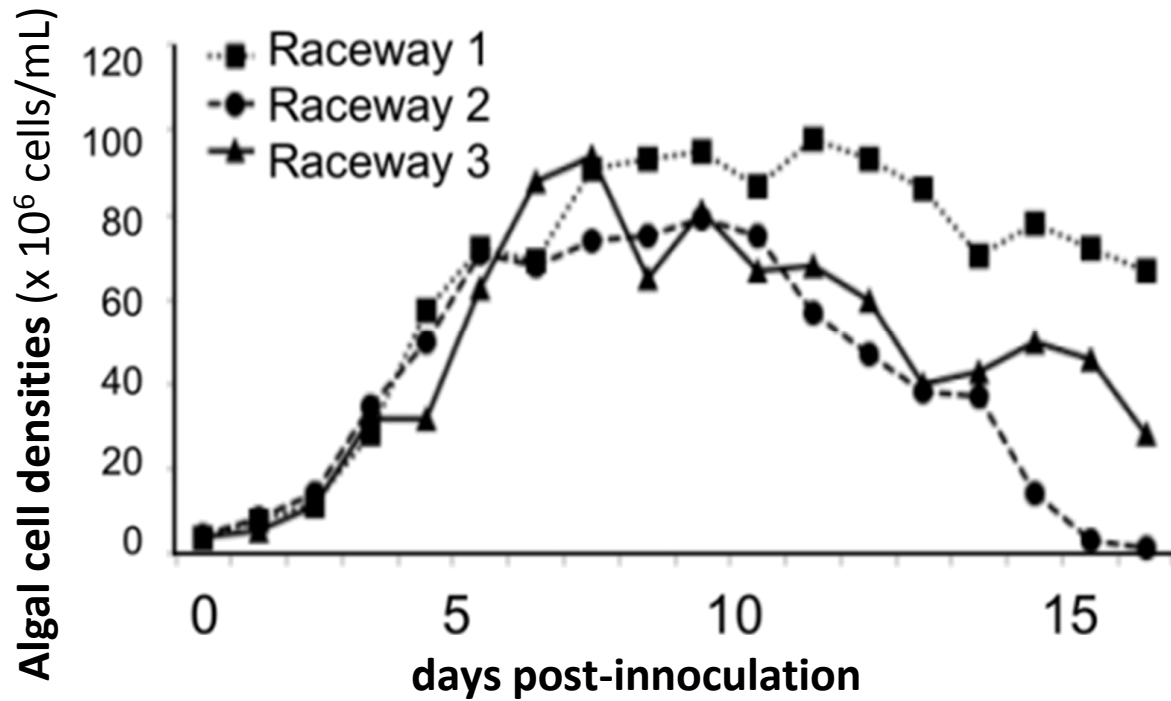


Healthy pond

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



5.0 μ m

Predators of microalga



Brachionus plicatilis,
marine rotifer

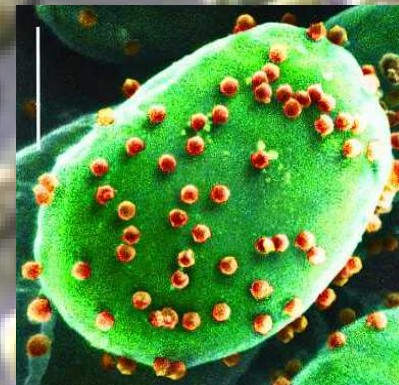


©Warren Photographic

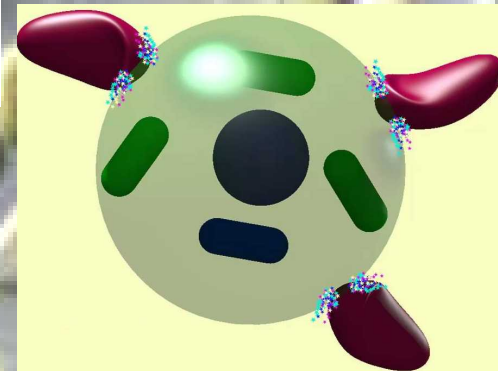
Marine planktonic
copepod, *Calanus*



Oxyrrhis marina,
dinoflagellate



alga infected with
chlorovirus



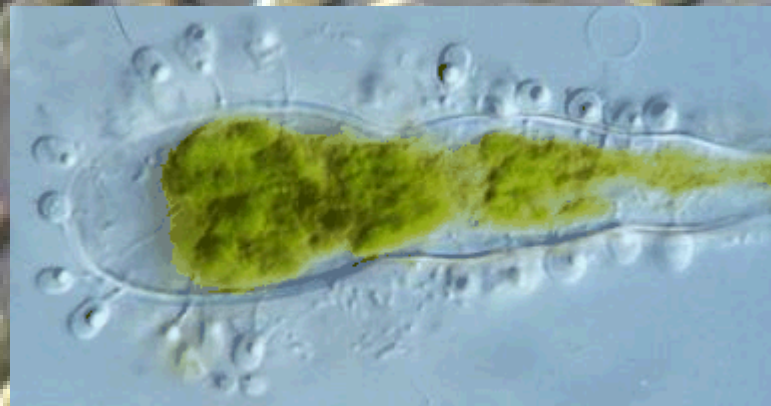
*Vampirovibrio
chlorellavoras*
bacterial predation on
green alga, *Chlorella*.



CCMP3181

5 μ m

Poterioochromonas,
a golden alga or
chrysophyte



Numerous parasitic chytrids attack
the filament of a green alga

Our Approach

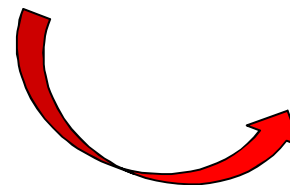
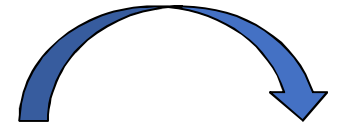
- 1) Can we identify and monitor volatile chemicals that indicate when algae is infected with predators?
- 2) Can we stabilize algae culture and prevent algal predation with probiotic bacteria?
- 3) Can we isolate and identify chemicals from these probiotic bacteria to understand the mechanism of algae 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?

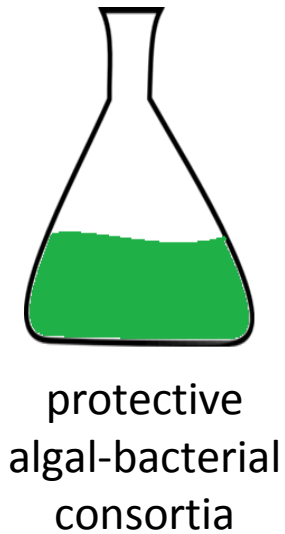
TABB/SFA

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

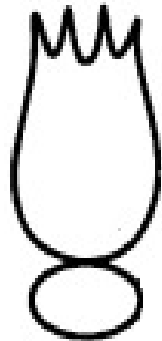
POSSE LDRD

Consortia experiment: screen

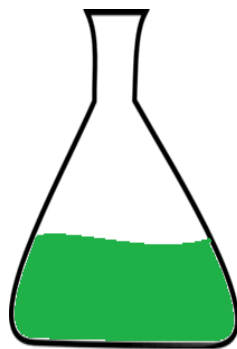
Algae survival assay (Rotifer live/dead assay)



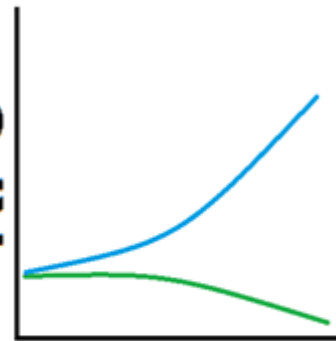
+/-



+/-

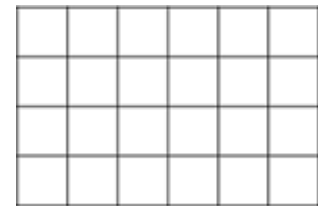


RFU

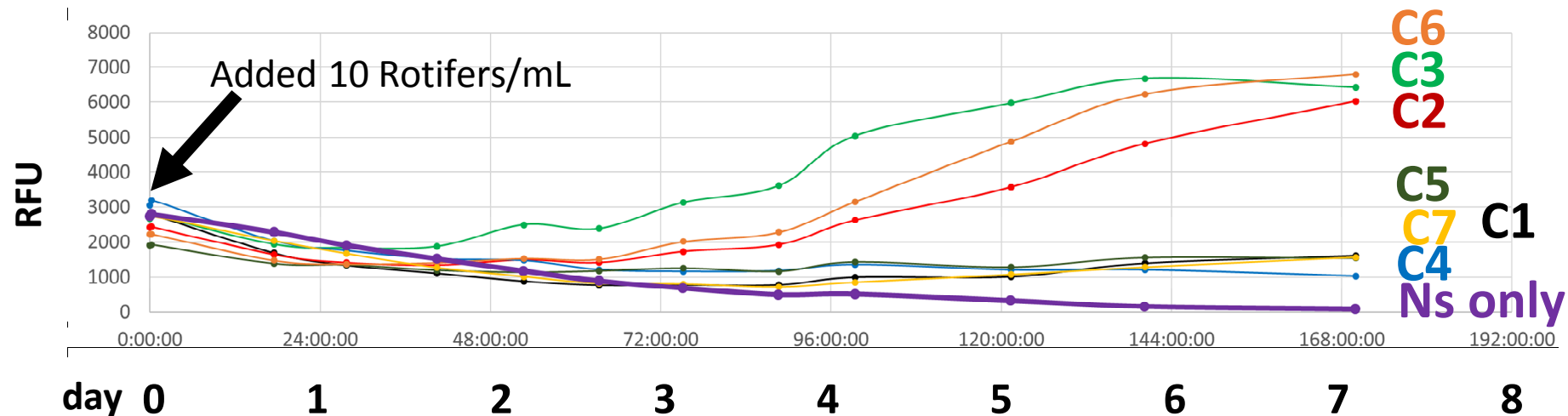


days

- *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:

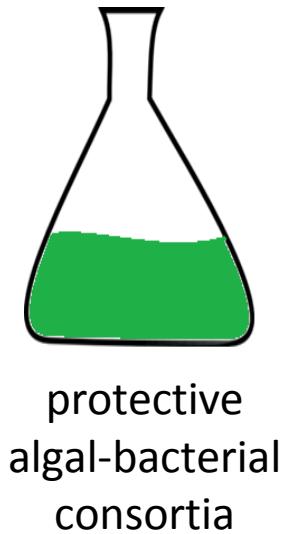
- Not many live rotifers present after 7 days with consortia
- Several rotifers were swimming "slowly"
- Very few eggs present
- Rotifer birth control?!



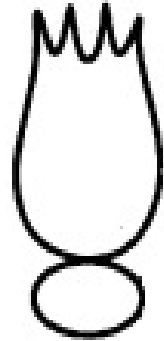
SNL CONSORTIA

Consortia experiment: screen

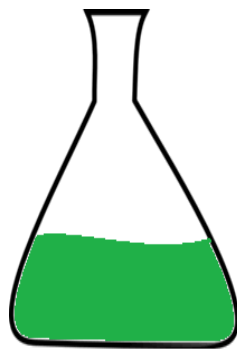
Algae survival assay



+/-



+/-



RFU

days

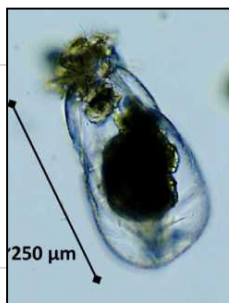
consortia-
protected
algae with
rotifers

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

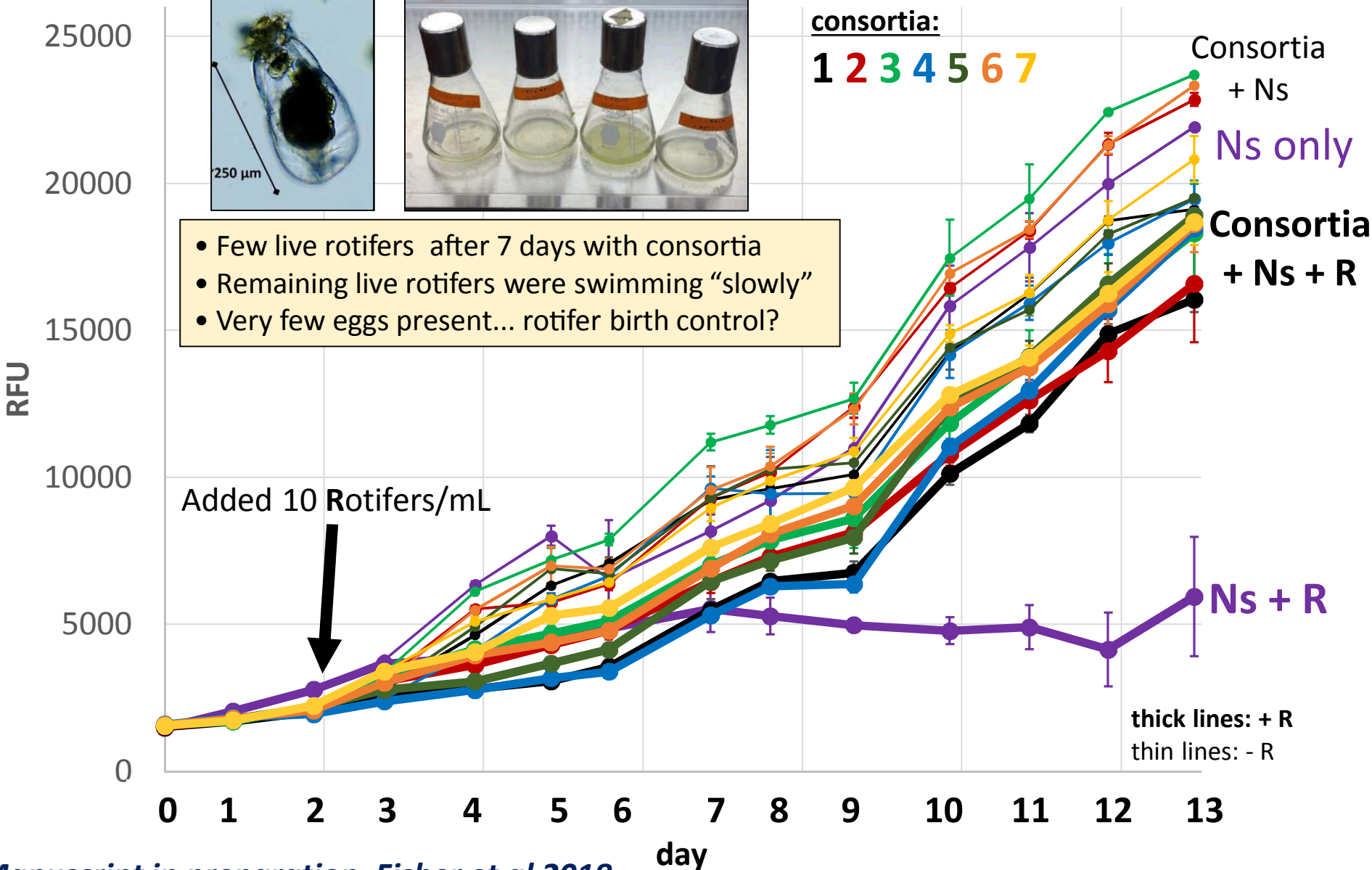


Bacterial consortia protect algae from rotifers



consortia:

1 2 3 4 5 6 7



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

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:

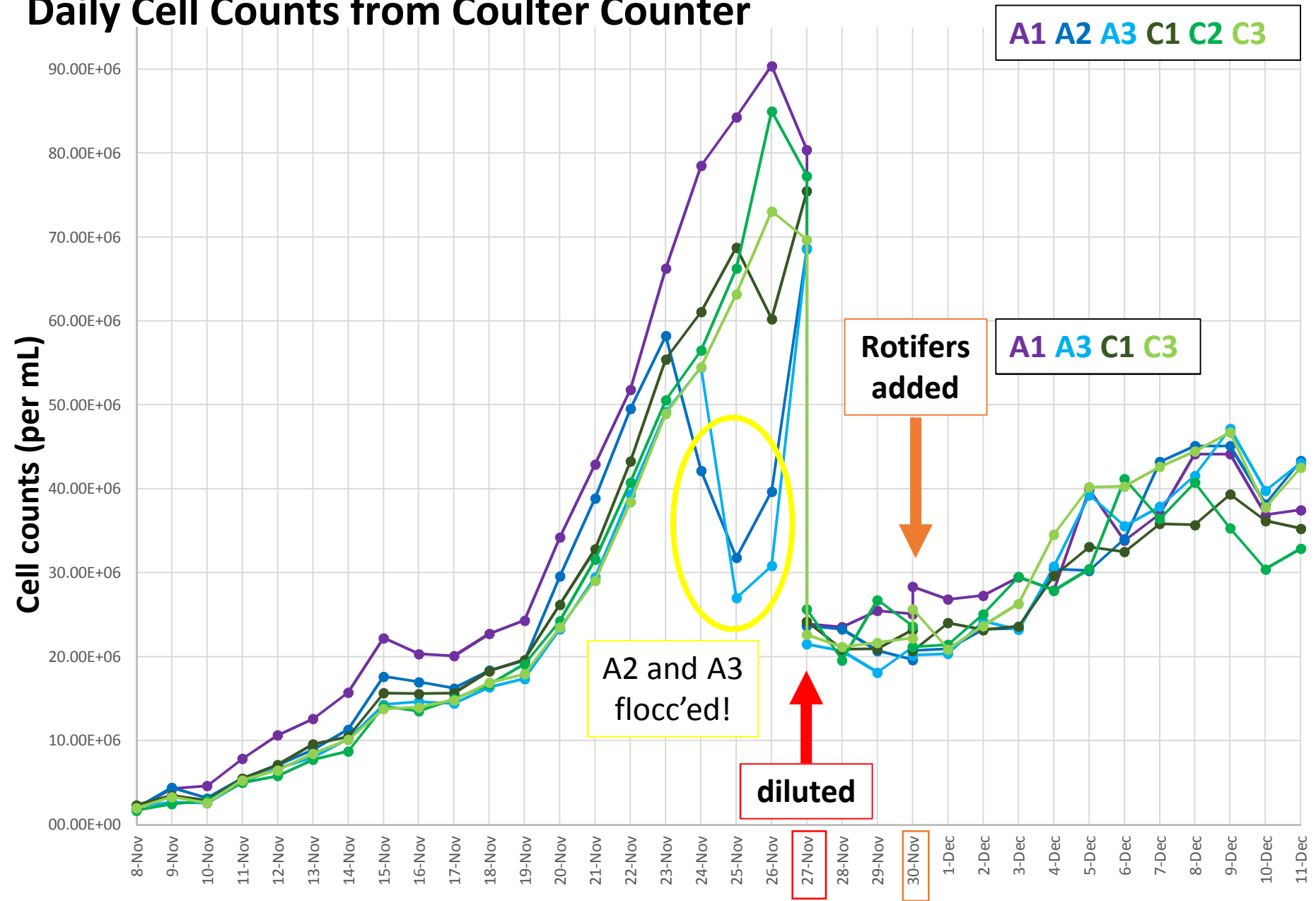
- Fluorescence
- Cell counts
- OD 750

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.

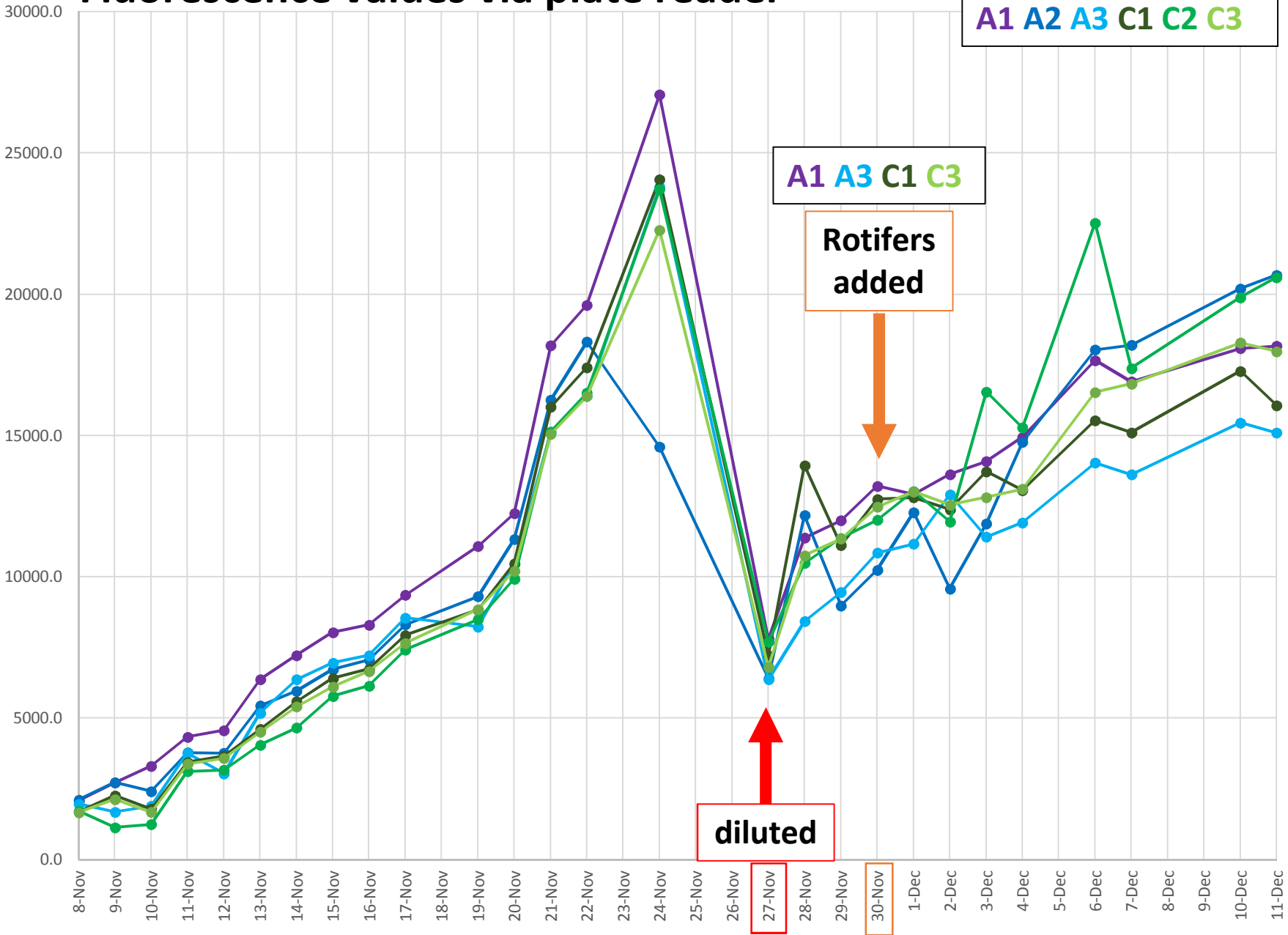
Daily Cell Counts from Coulter Counter



Fluorescence values via plate reader

A1 A2 A3 C1 C2 C3

RFU at 680nm

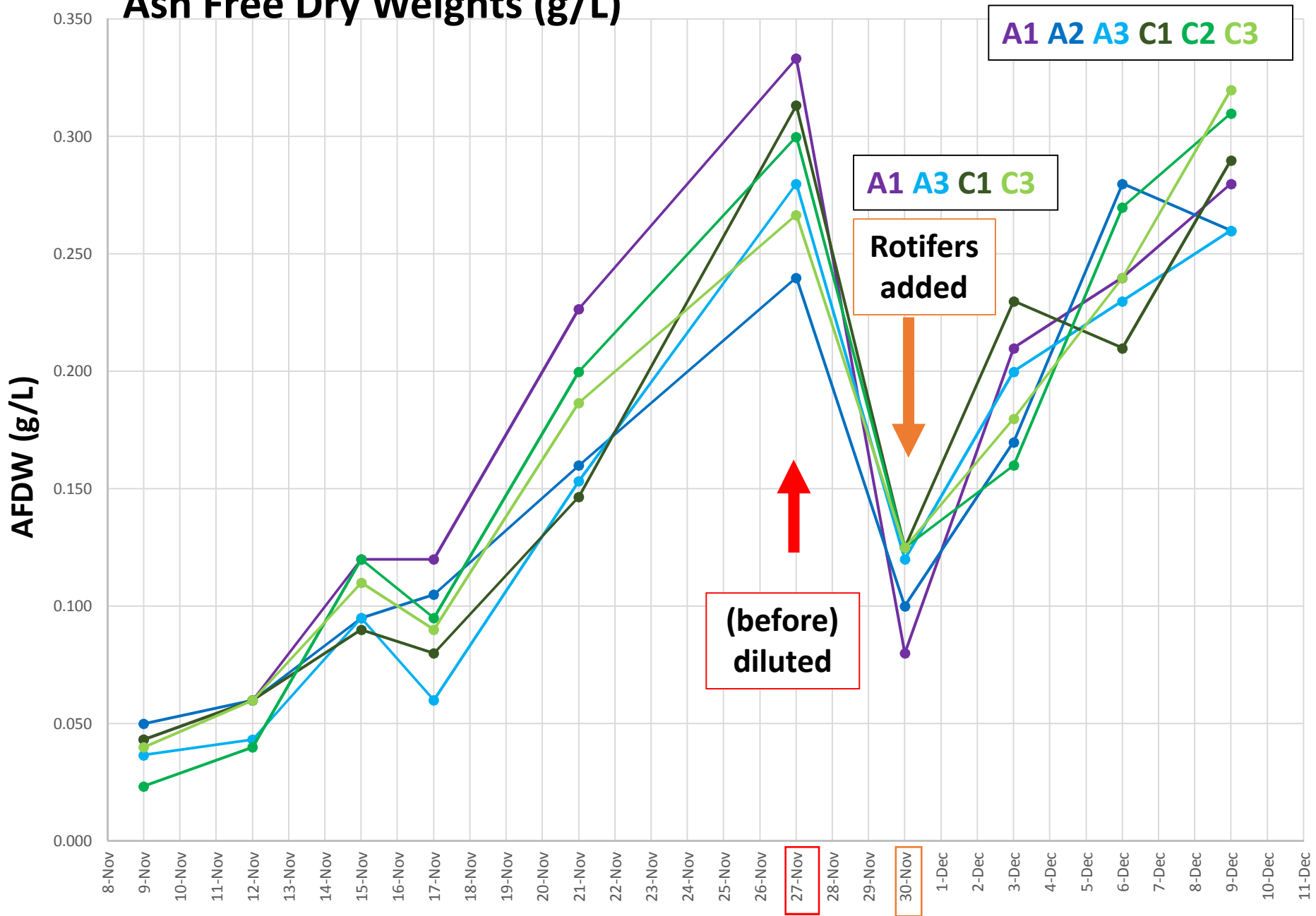


diluted

Rotifers added

A1 A3 C1 C3

Ash Free Dry Weights (g/L)



Rotifer and egg counts per mL

A1 A3 C1 C3

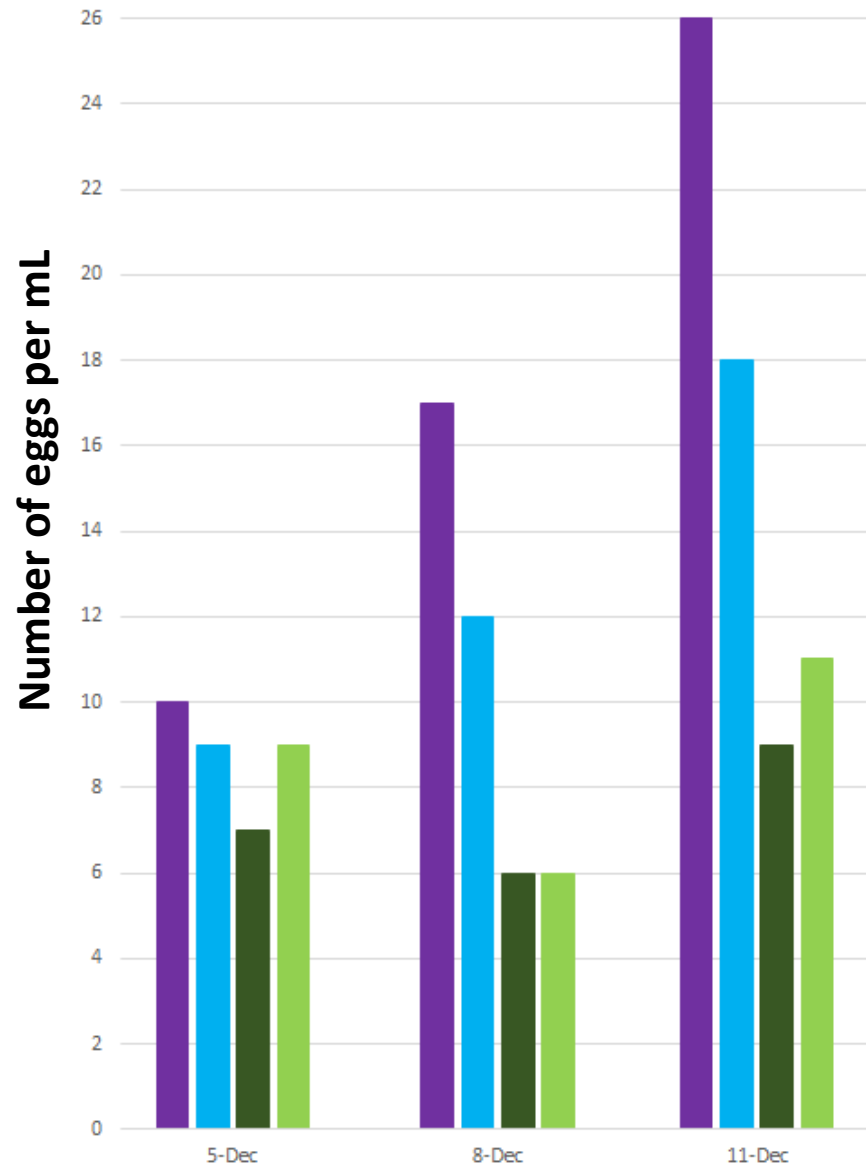
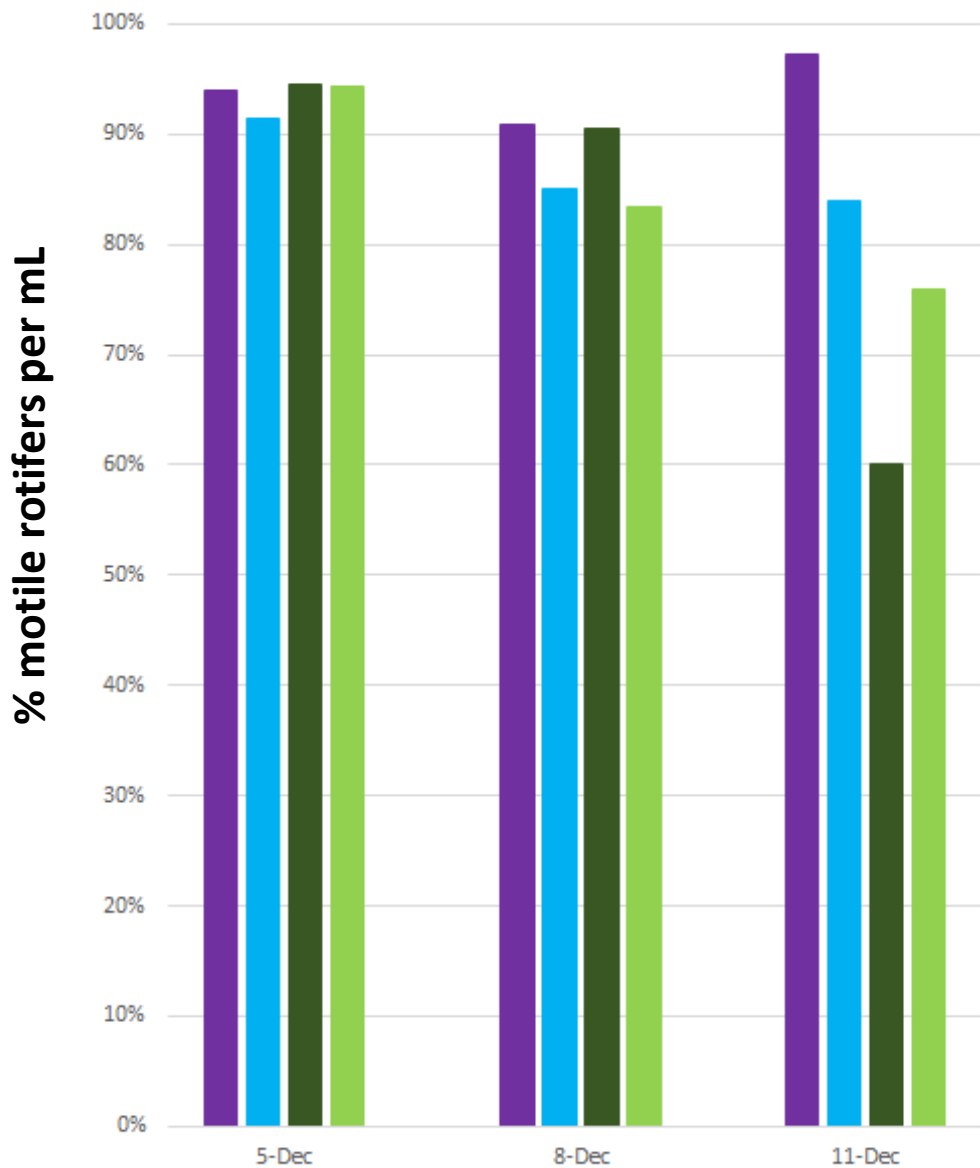
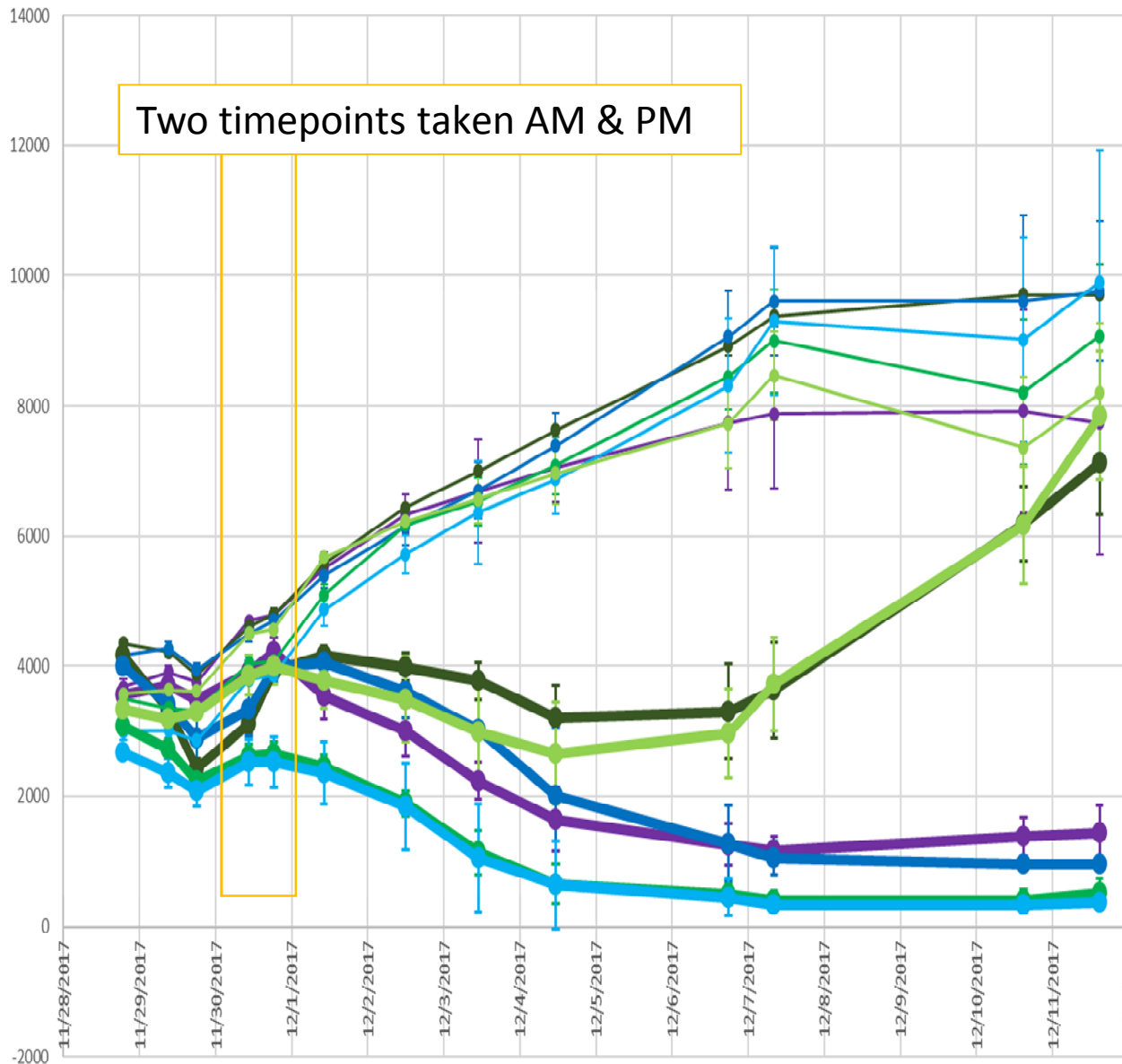


Plate assay of mesocosms

A1 A2 A3 C1 C2 C3

RFU at 685nm



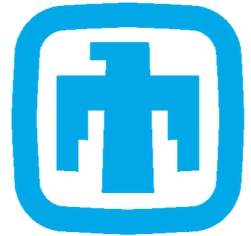
20M/mL Ns
50 Rots / well
20C
16/8 light/dark

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

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

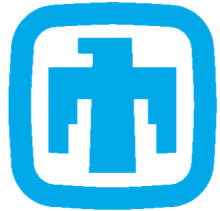
... consortia probiotic for algae?
(outgrows control)

SNL CONSORTIA: CONCLUSIONS



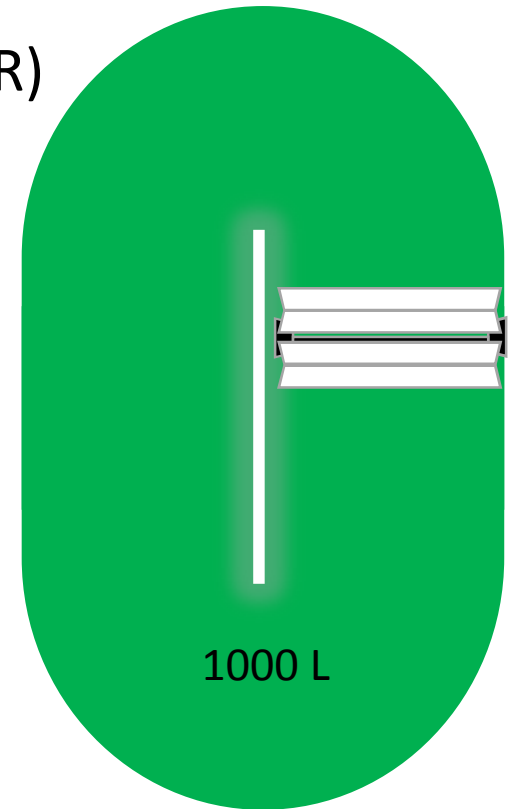
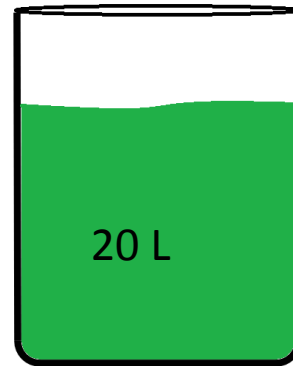
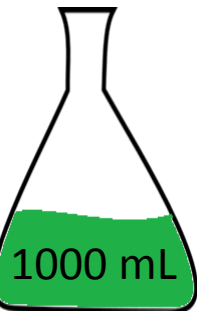
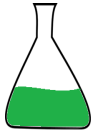
- ❖ **Low sun, low temperatures Nov – Dec: not ideal**
 - Affected algal growth ... consortia growth?
 - Affected rotifer feeding and mesocosm crash
 - Algae doubled every 3-4 days, only 1 dilution needed
- ❖ **Rotifers purchased, not axenic...**
 - High quantity of rotifers → Reed aquaculture
 - Did 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 help us learn this information).
- ❖ **16S Sequencing data will be really interesting!**
- ❖ **Nutrient analysis will also be interesting**

SNL CONSORTIA: FUTURE WORK



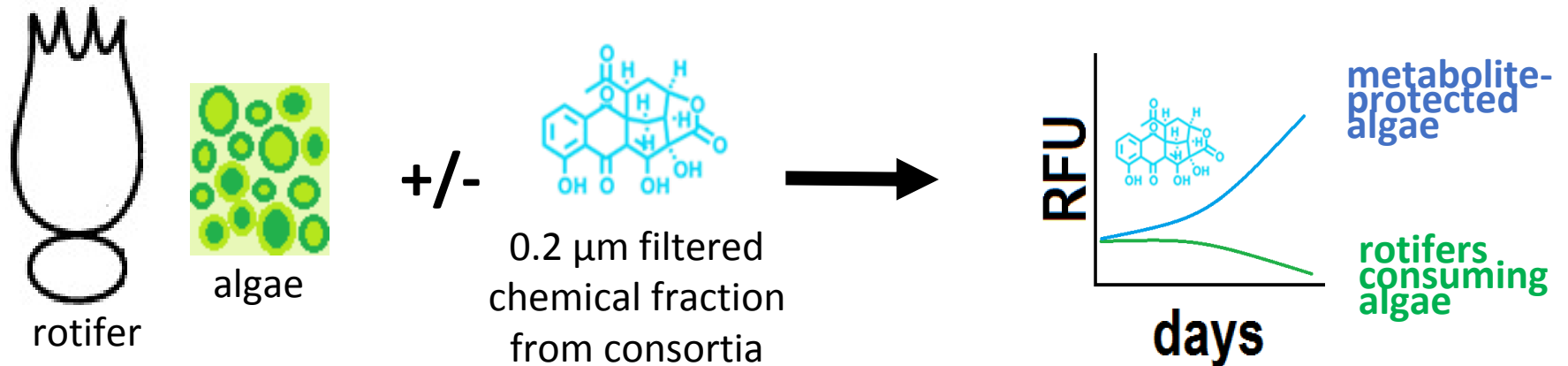
- **State of Technology for DISCOVER**
 - Test consortia in outdoor ponds at ASU and/or TAMU in 2018
 - 16S amplicon sequencing (Illumina MiSeq) analysis of consortia
 - Consortia simplification experiments
 - Determine mechanism (UPLC-MS and NMR)
- **Test consortia with more algae and more predators...**

125 mL



Chemical fraction experiments

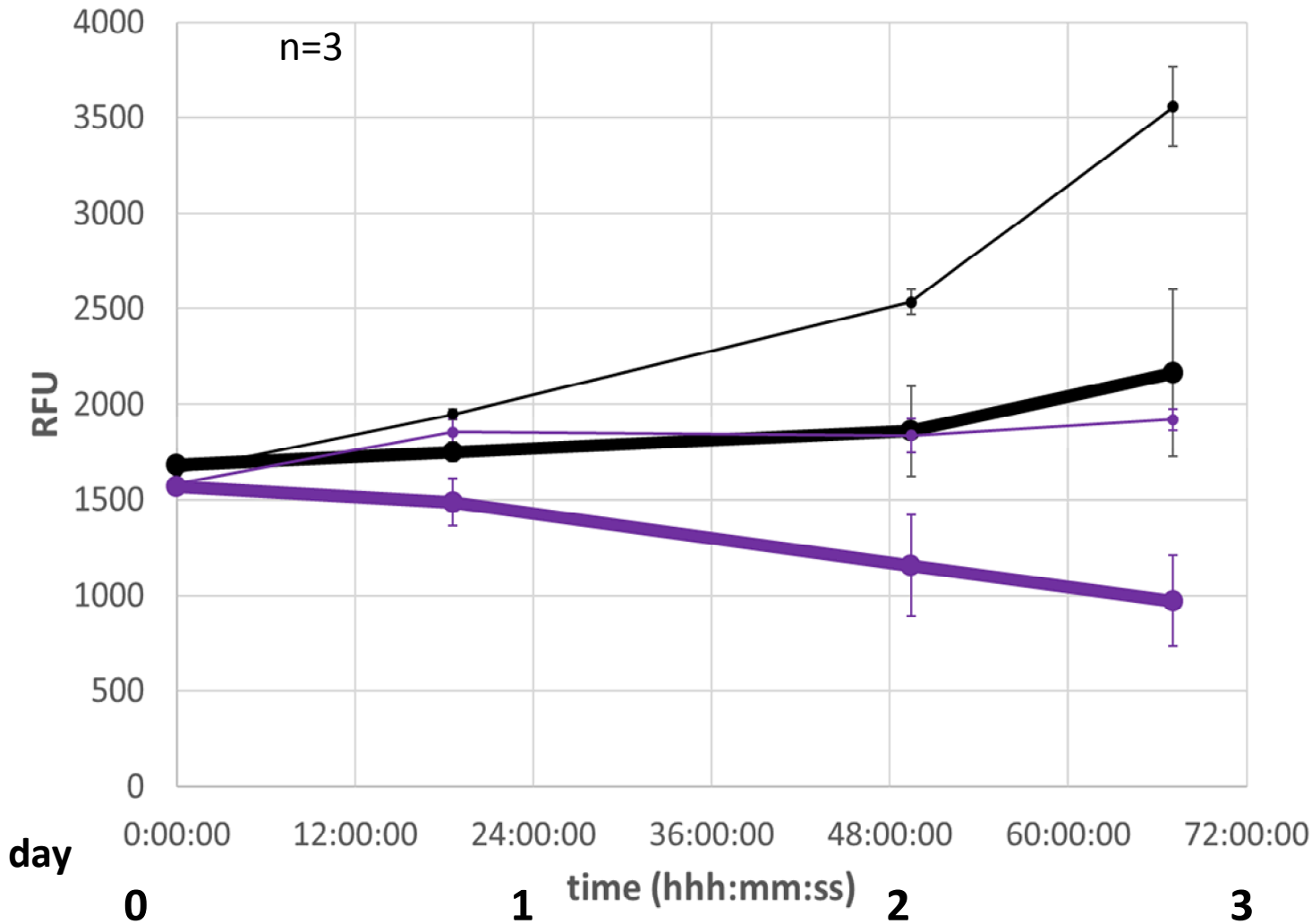
Algae survival assay (Rotifer live/dead assay), sans bacteria



- **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

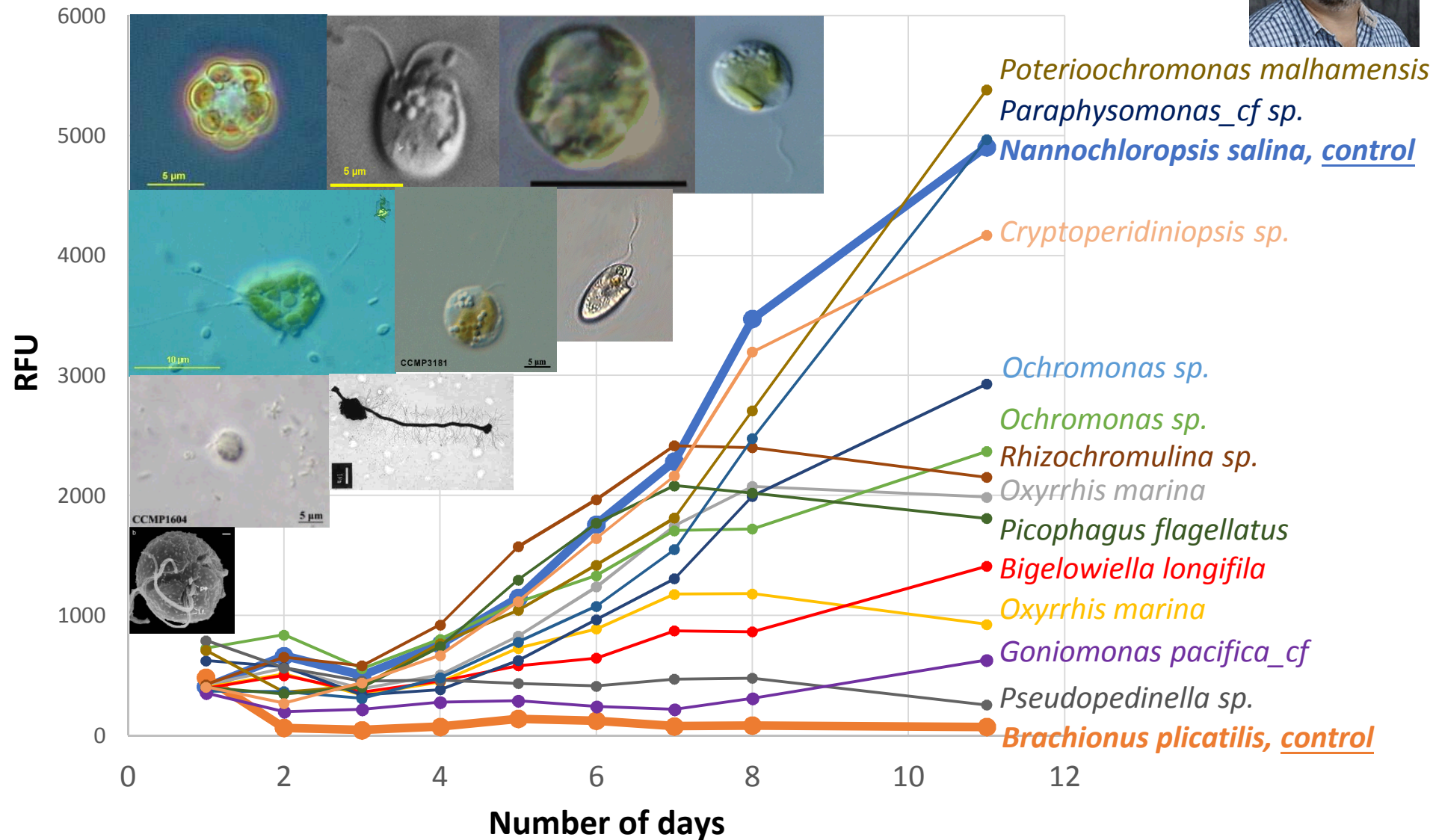
Ns only

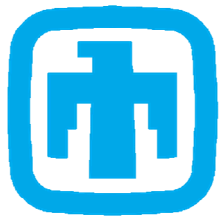
Ns control
+ Rotifers

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



Effect of various predators on *Nannochloropsis salina* concentration





**Sandia
National
Laboratories**

CA

Todd W. Lane
Pamela D. Lane
Mary Tran-Gyamfi
James Jaryenneh
Franny Carcellar
Kunal Poorey
Peter McIlroy
Krissy Mahan
Nataly Beck

NM

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

Thank you!



Algae Testbed
Public-Private Partnership



**Lawrence Livermore
National Laboratory**

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



heliae
Laura T. Carney

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