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Chemical and Genetic Detection Systems for Early Warning of Pond Crashes

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Distinguished Member of Technical Staff, Sandia National Laboratories

September 16, 2020 Algae Biomass Summit.
The Ether

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Project Goal: Can we monitor chemical signals from algal ponds in order to determine pond health?

grazer attacks

chemical signals released

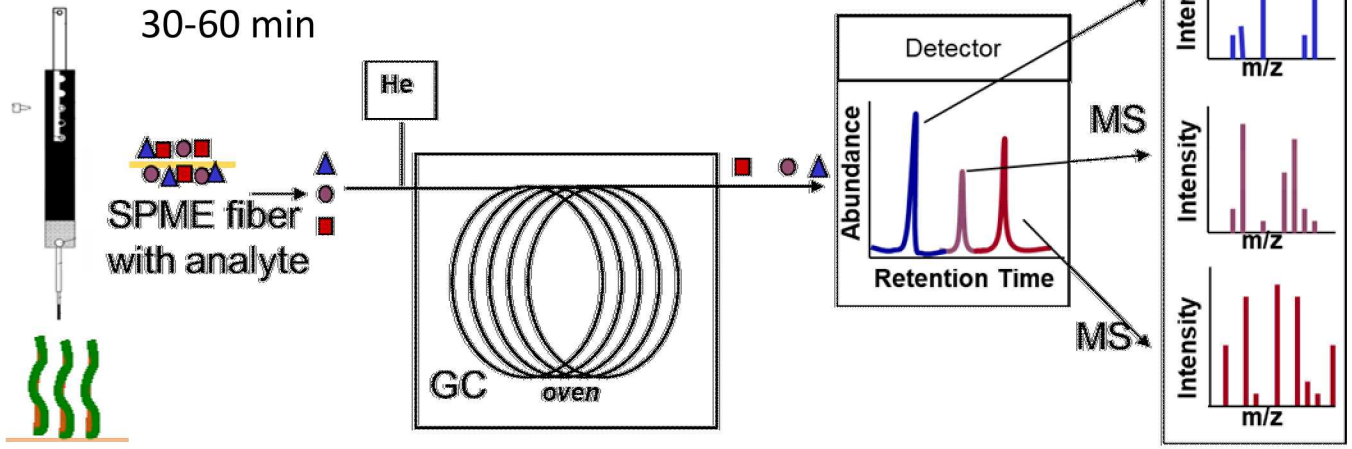
Detection and analysis

Early detection of algal grazers means earlier treatment and higher likelihood to save the pond.

1) Sample algal headspace with SPMEs or TD tubes for 30-60 min

2) Run SPMEs and TD tubes on GCMS

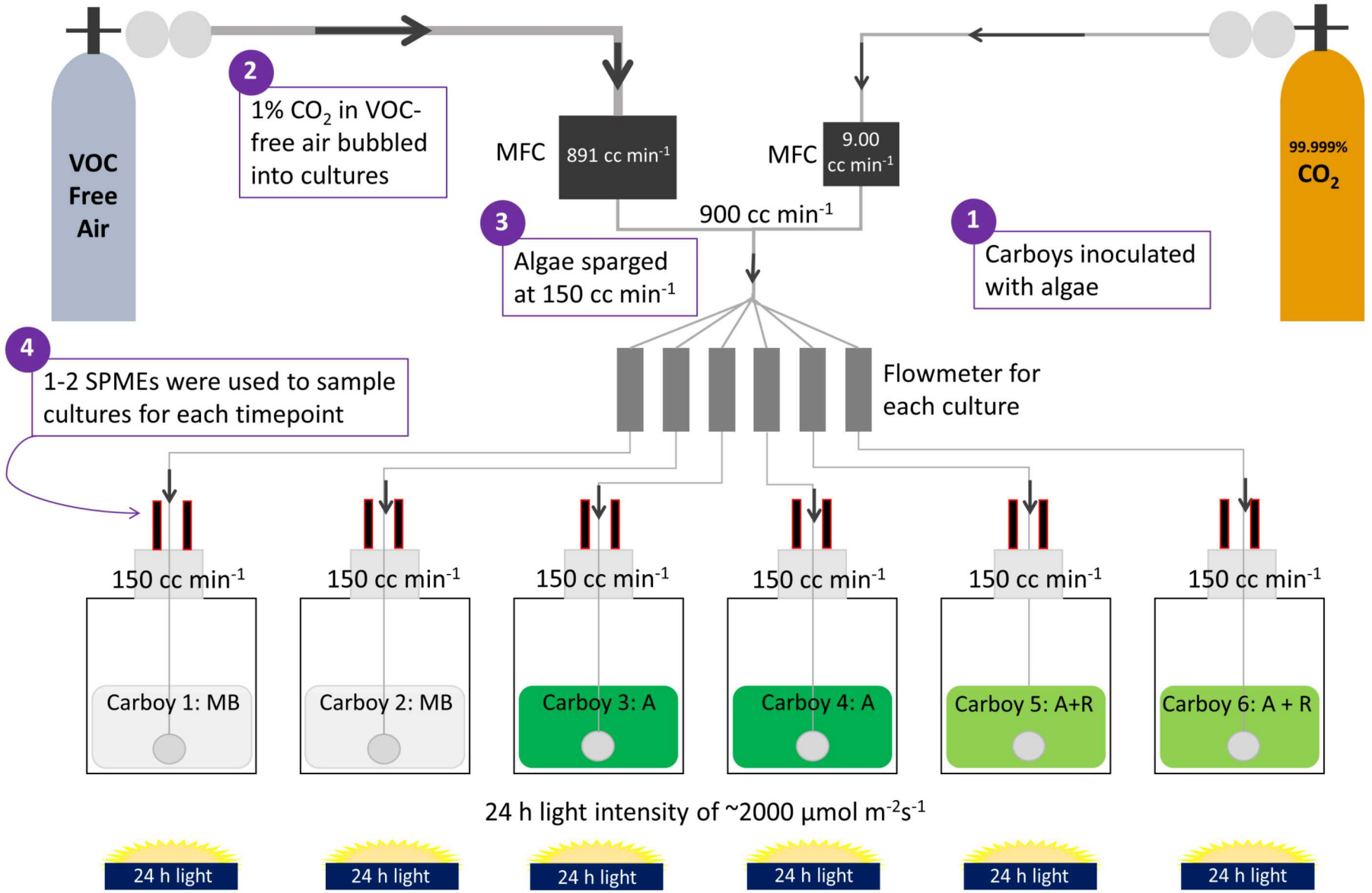
3) Analyze chemical differences



AVOCs sampling using SPMEs and Carbopack thermal desorption tubes → GCMS analysis

Effort led by: Carolyn Fisher and Kristen Reese

We designed a system to effectively emulate an algal mass production system.



AVOCs = algal volatile organic compounds

AVOCs sampling using:

- SPMEs (solid phase microextraction fibers)
- Carbopack thermal desorption (TD) tubes
- GCMS metabolomics analysis using Agilent MassHunter and Mass Profile Professional.

Reese KL, Fisher CL, Lane PD, Jaryenneh JD, Moorman MW, Jones AD, Frank M, Lane TW, Chemical Profiling of Volatile Organic Compounds in the Headspace of Algal Cultures as Early Biomarkers of Algal Pond Crashes, *Scientific Reports*, **9**:13866 (2019). DOI: 10.1038/s41598-019-50125-z

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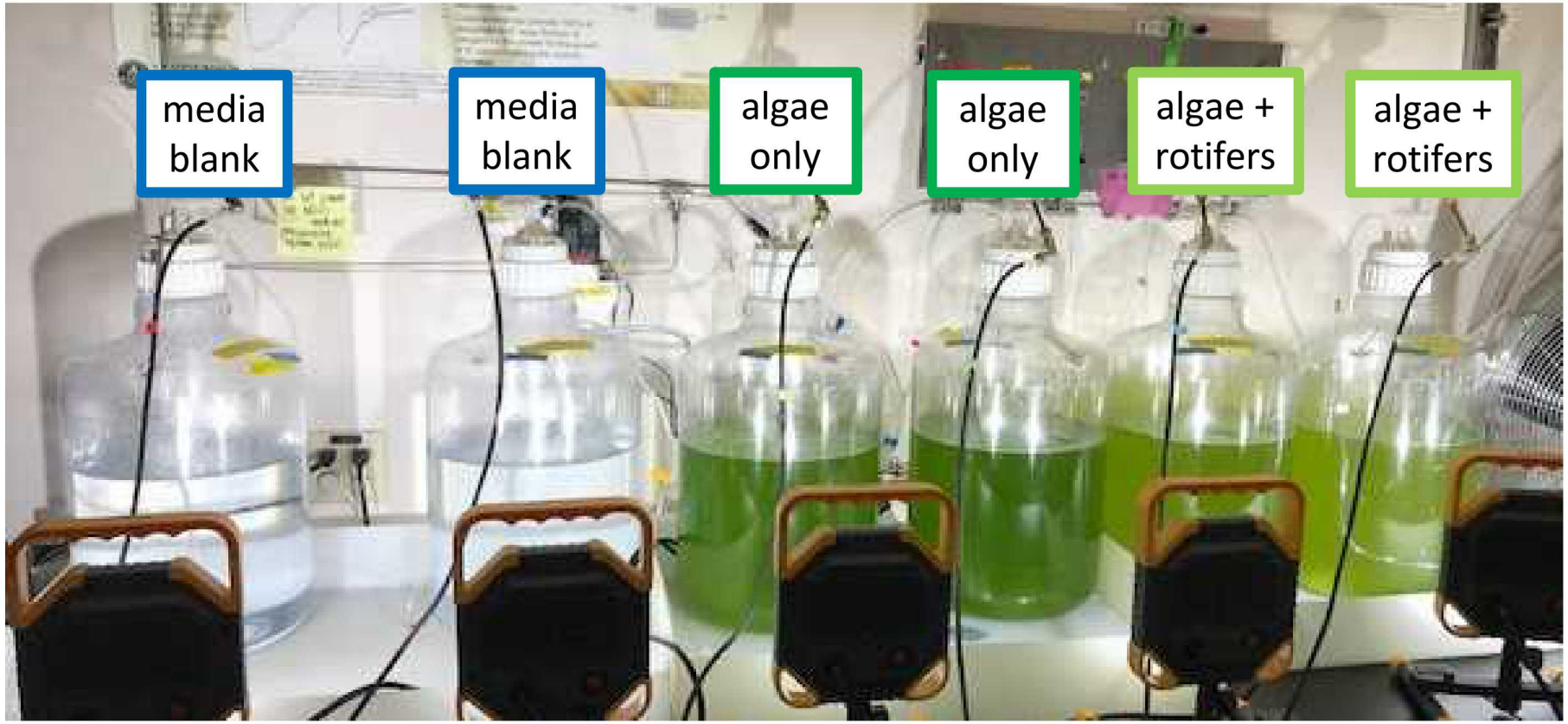

In situ sample collection under physiologically-relevant conditions via SPMEs and TD tubes.

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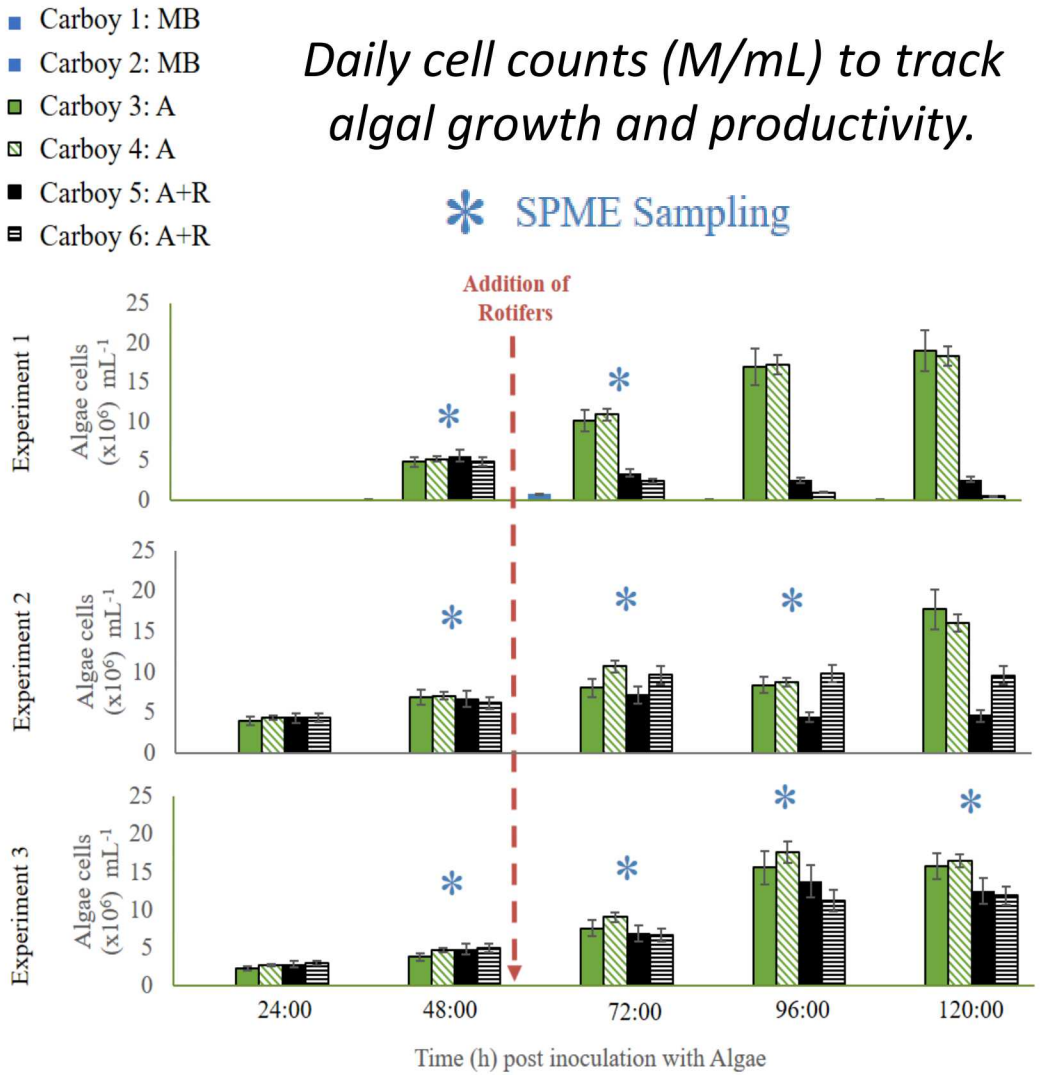
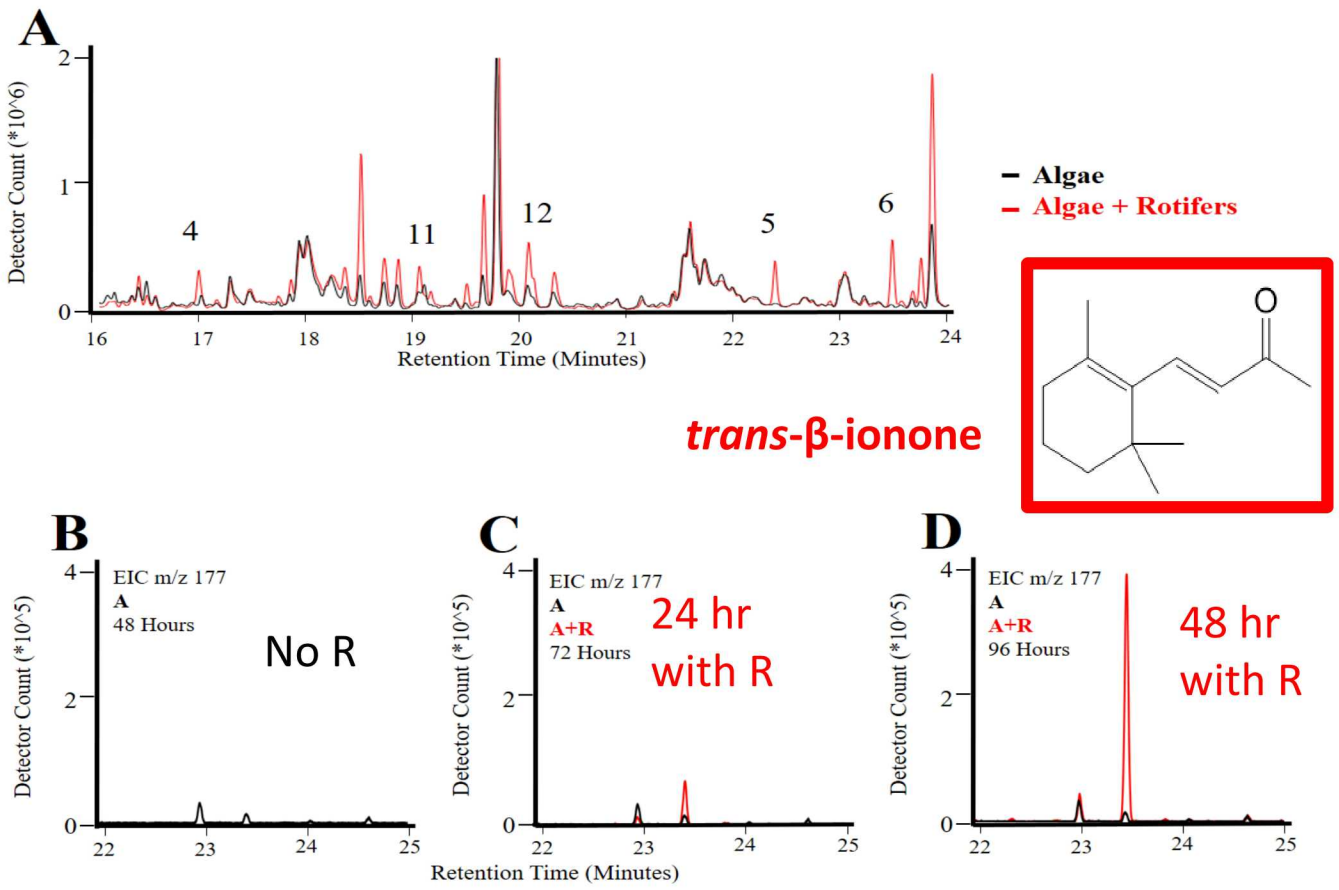
RESULTS: Different chemical signals are detected for algae only vs. algae + rotifers cultures

Daily cell counts (M/mL) to track algal growth and productivity.





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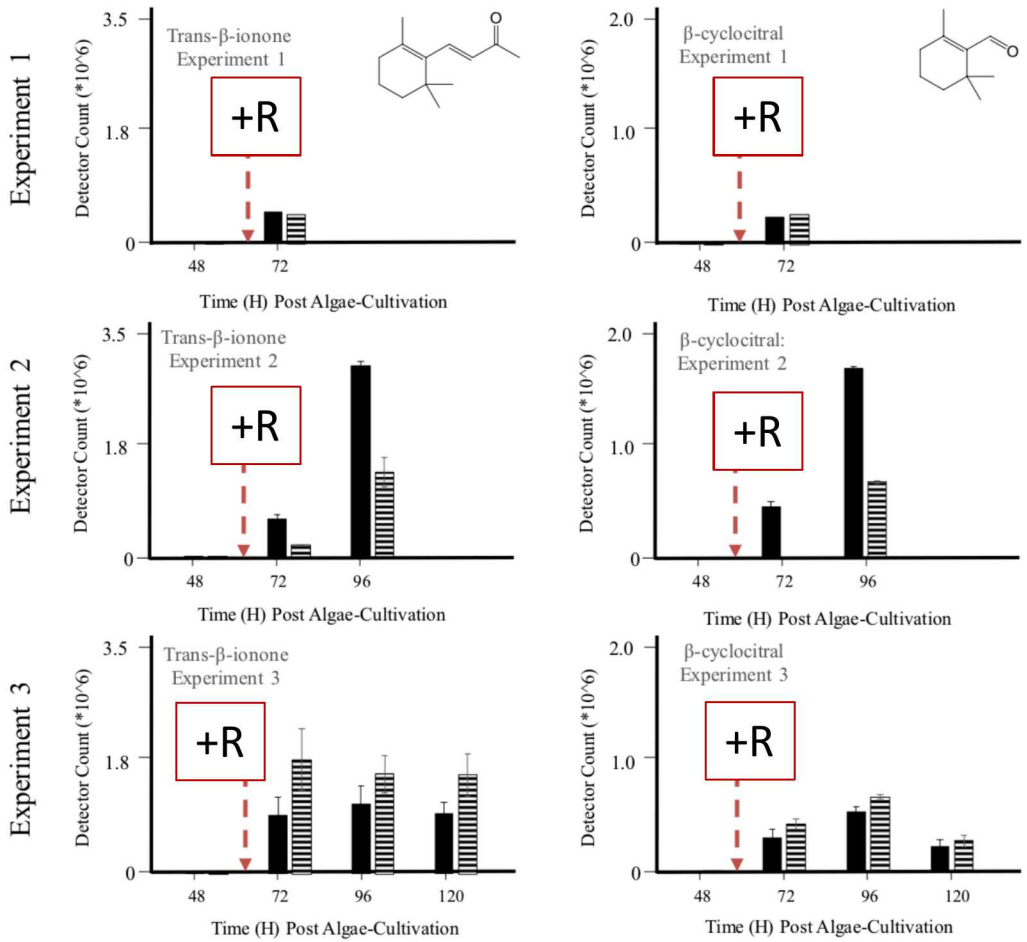
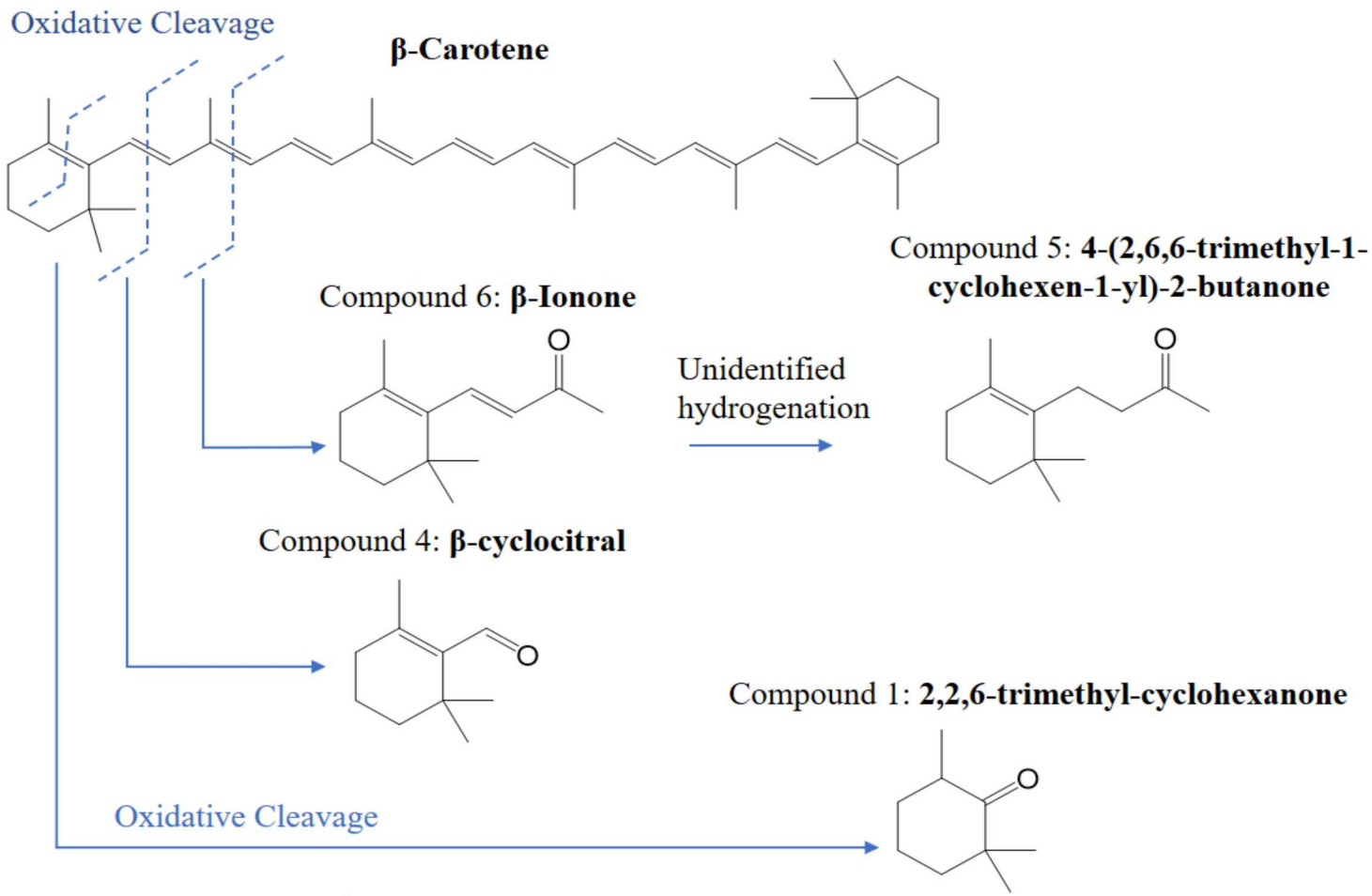
Patent Pending: Reese KL, **Fisher CL**, Lane TW, and Frank M are inventors on a 2019 pending application: Sandia Ref. No. SD14957.0/S162755, by Sandia National Laboratories, that covers the identification of several volatile organic chemical biomarkers for detecting predators in microalgae cultures, grown for biofuel production.

In algae with rotifer samples, specific volatiles increase over time



CONCLUSION: Chemical signals are likely derived from oxidation of algal carotenoids



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RESULTS: Low MW VOCs detected by Carbopack B/X™ thermal desorption tubes differ for algae only versus algae + rotifer samples

Compound 3:

m/z = 82

r.t. = 10.409

NIST14 ID: 2-methylfuran

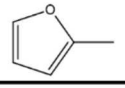
NIST14 % : 79.74

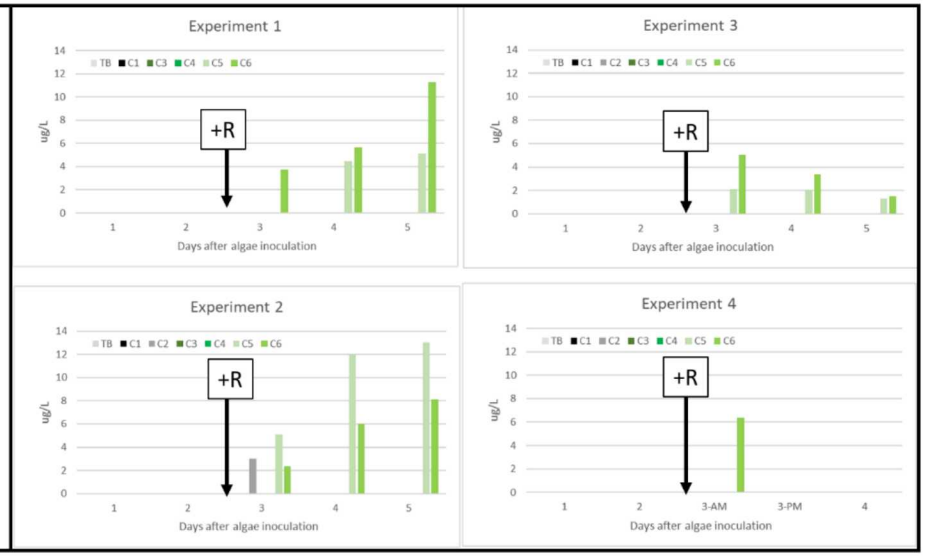
CAS: 534-22-5

Molecular formula: C5 H6 O

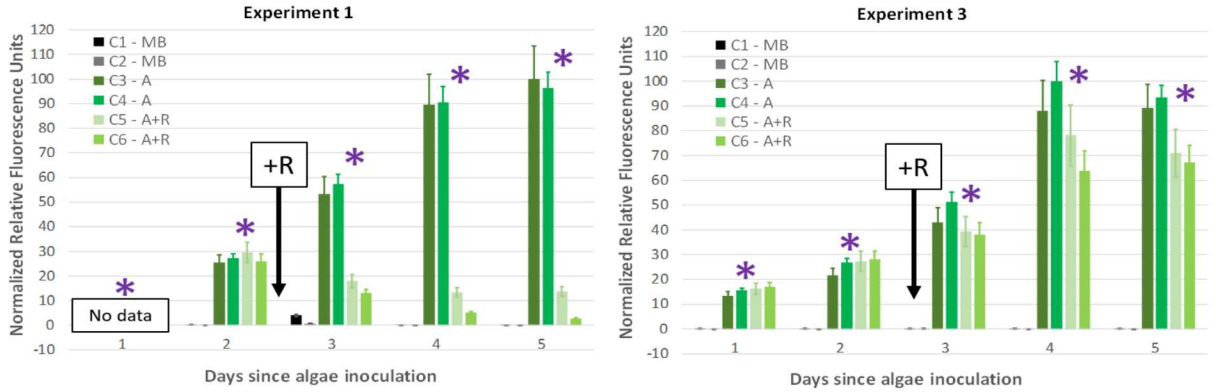
MW: 82.10 g/mol

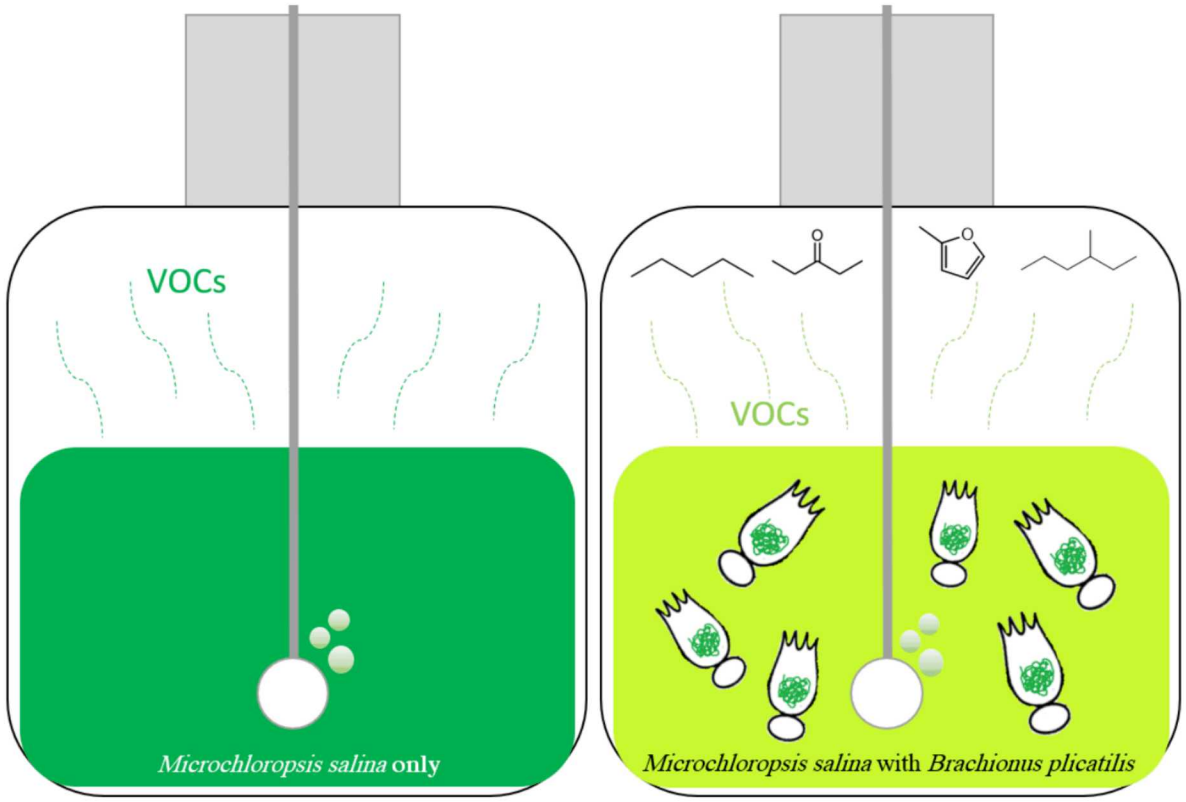
Structure:





Algal growth via chlorophyll fluorescence *indicates Carbopack sampling



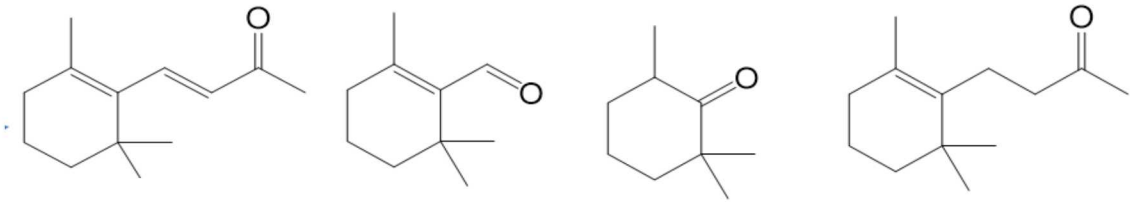


pentane, 2-pentanone,
2-methylfuran, 3-methylhexane

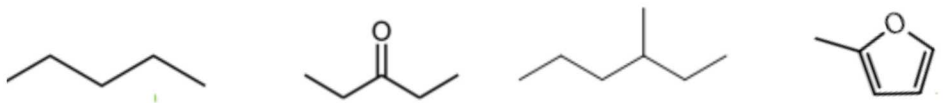
Fisher C.L., Russell M., Maddalena R., Lane, T.W. 2020. Low molecular weight volatile organic compounds (VOCs) indicate grazing by the marine rotifer *Brachionus plicatilis* on the microalgae *Microchloropsis salina*. *Metabolites* 10, 361; doi:10.3390/metabo10090361

Summary of the current AVOCs work:

- Several putative carotenoid oxidation products detected only from cultures of *M. salina* in the presence of active, grazing rotifers, *B. plicatilis,* by SPMEs (Reese KL, Fisher CL, *et al*, 2019)



- Low MW VOCs also identified from *M. salina* in the presence of actively grazing *B. plicatilis* using Carbopack B/X™ thermal desorption tubes (Fisher CL, *et al*, in revision 2020)

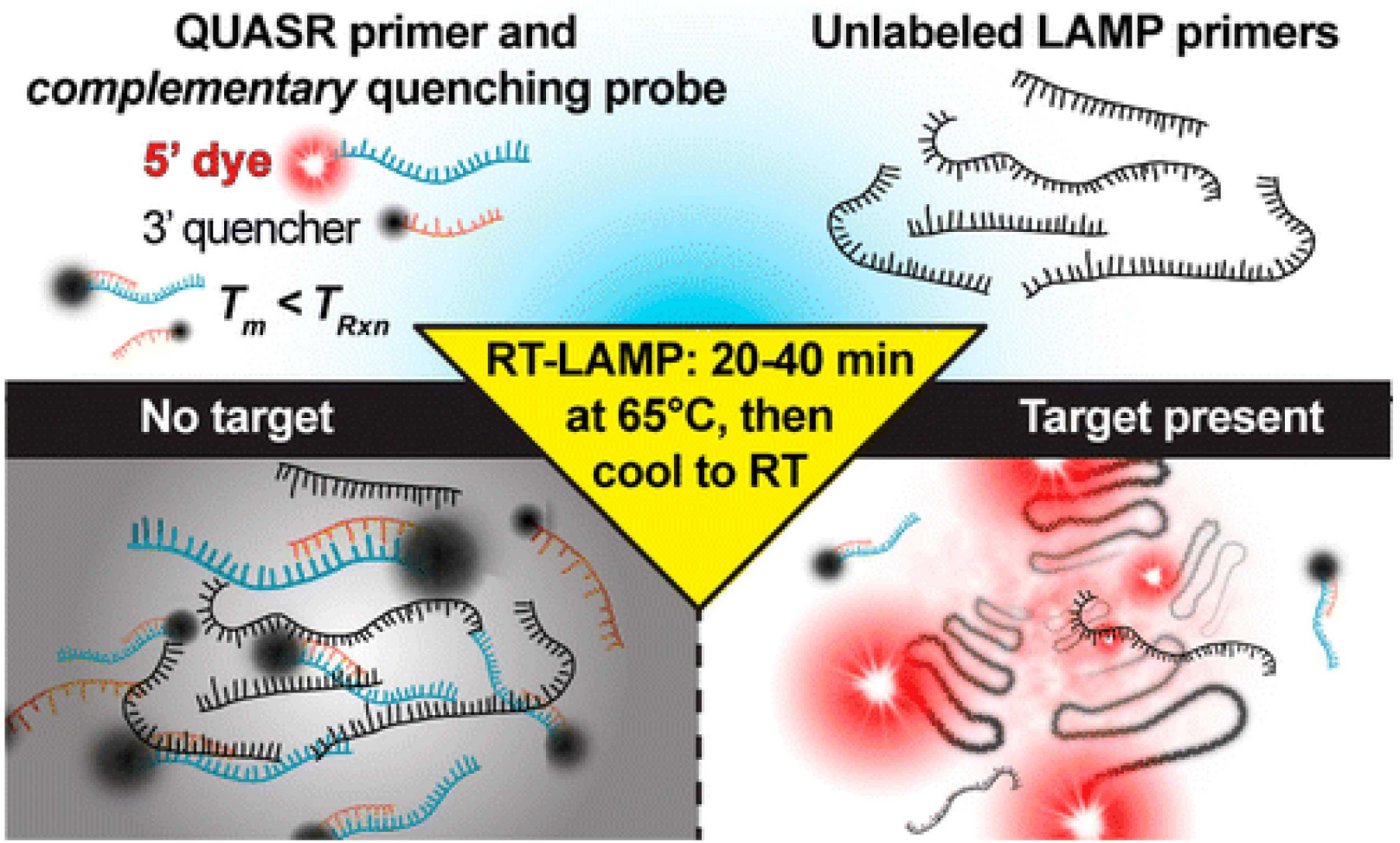


Our success is an encouraging <u>starting point</u> for the discovery of volatile biomarkers of algal wounding.

FUTURE AVOCs work:

- Time course experiments
- Test additional algal strains of interest
- Test additional algal grazers, pathogens, etc
- Test different stress conditions (light, pH, salinity)
- Test different community structures (algae mono- vs. poly cultures, various bacterial cultures, etc)
- Targeted SIM-GCMS analysis for mass algal cultures
- Collect VOCs from mass algal cultures “at scale”, such as outdoor mesocosms or raceways
- Take shorter timepoints to determine earliest detection
- Increase number of biological replicates
- Run longer experiments to determine how long chemical signals persist.

Key Objective: Provide rapid detection of positive and negative members of the pond microbiome



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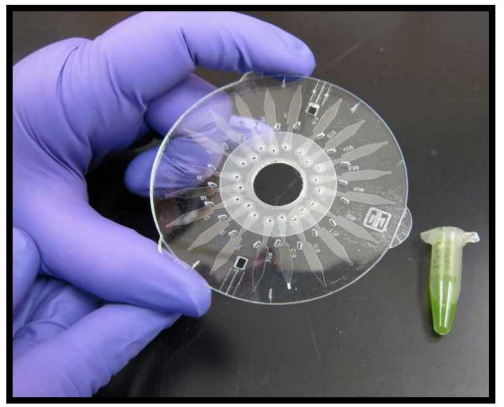
Pond-side Capability



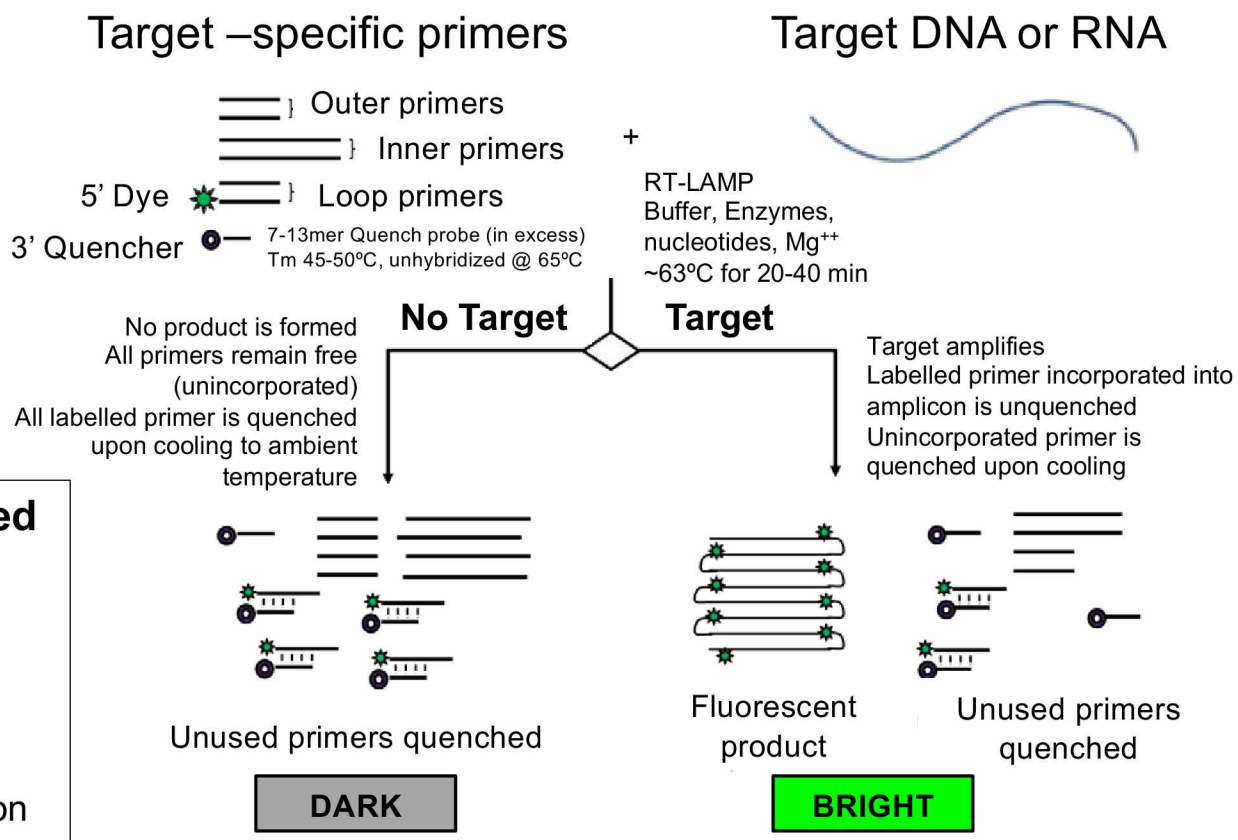
Fluorescence detector

PonDx QUASR Assays

Quenching of Unincorporated Amplification Signal Reporters

Sandia Technology: Analytical Chemistry 2016, 88, 3562-3568

- Six primers allow for enhanced target specific detection
- Assays are fast < 40 min
- Amplification of target allows for very sensitive detection
- Can be multiplexed if using a plate reader
- Can be monitored using the pond-side diagnostic equipment: PonDx



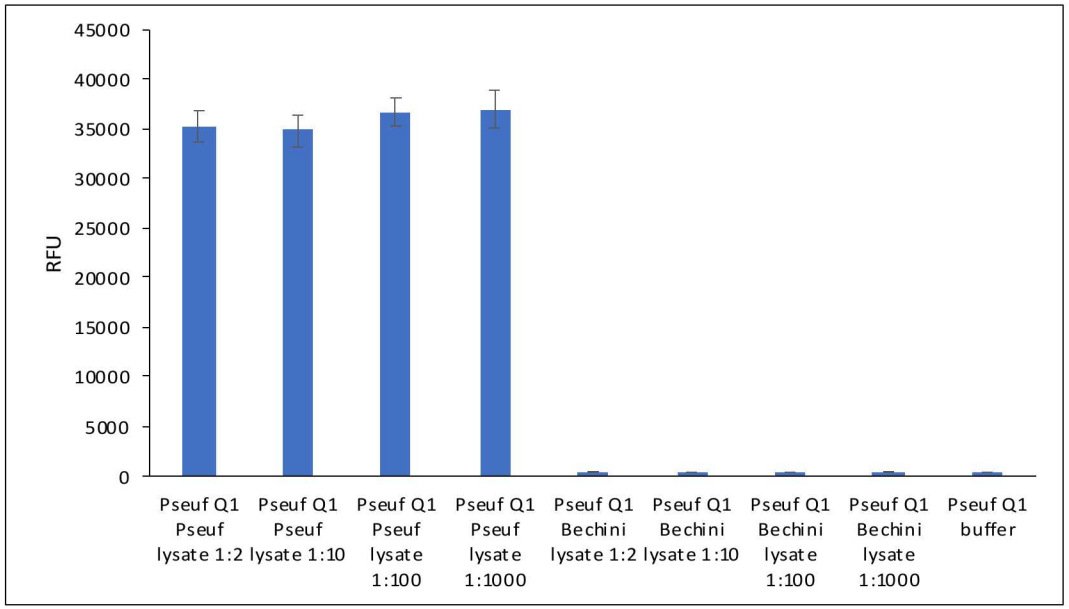
Assay reagents are pre-mixed and provided to GAI staff so that protocol is:

1. 9 µl assay mix + 1 µl pond lysate
2. Heat in plate reader or on PonDx @ 63ºC, 20-40 min
3. Cool to room temperature
4. Detect fluorescence using plate reader or on PonDx box

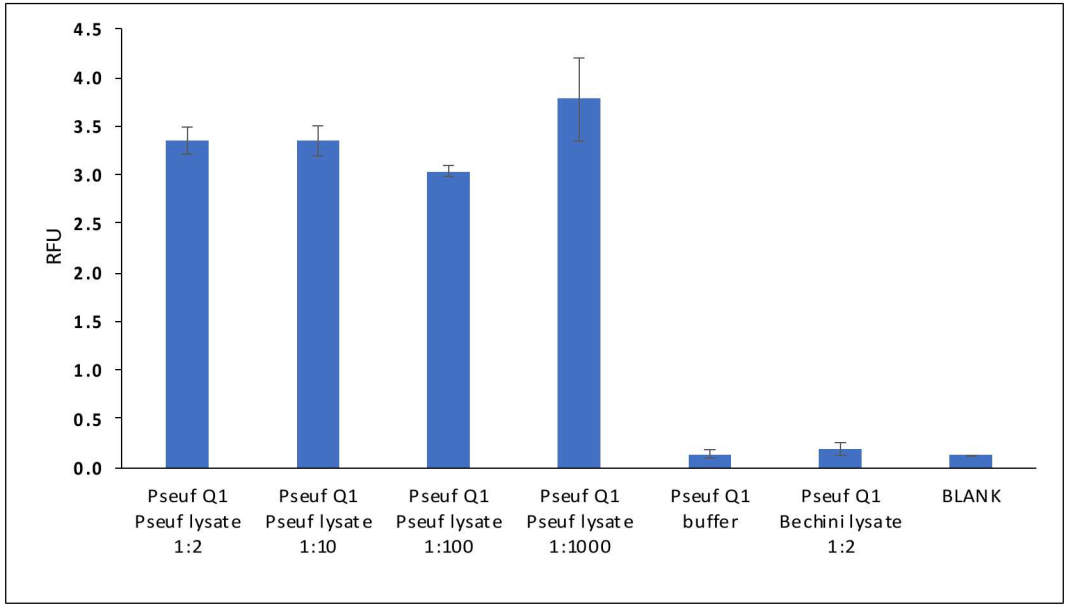
Assays for the detection of algal pond microbiota

Assays can be performed on a plate reader with temperature controls or on the PonDx box

Tecan



PonDx



QUASR Probe designed to detect organism of interest: *Pseufofulvimonas gallinarii*
Dected on Tecan plate reader Ex 532 nm Em 571 nm and PonDx box

Thank you!!

Sandia National Laboratories

Carolyn Fisher
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Pamela Lane
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James Jaryenneh
Matt Moorman
Stephen Anthony
Curtis Mowry
Joshua Whiting

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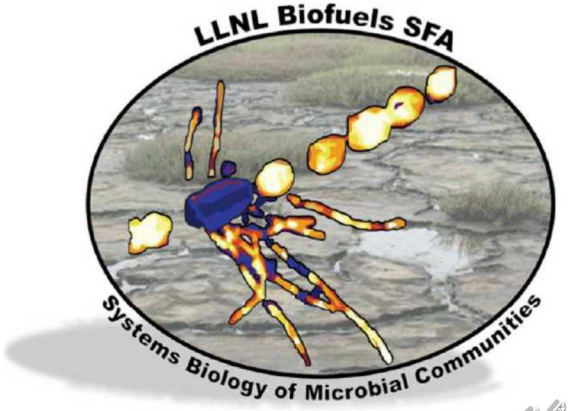

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