DOE Bioenergy Technologies Office (BETO) 2019 Project Peer Review

Integrated Pest Management for Early Detection Algal Crop Production EE0007094 (1.3.2.310) – Sun-Setting Project

March 7, 2019
Advanced Algal Systems

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University of California, San Diego

This presentation does not contain any proprietary, confidential, or otherwise restricted information

Overarching Goal

- Develop simple, automated, affordable, and robust technologies for the **early detection and identification** of pathogens, predators, and non-productive competitors in an algal production pond.

Desired Outcomes

- Identify and characterize **molecular signatures** of infestation or infection of a production pond.
- Develop an **automated, mass spectrometry (MS)**-based early detection system that monitors the headspace and culture of production ponds for these molecular signatures to trigger an automated identification system utilizing qPCR augmented with high resolution melt analysis **(qPCR-HRMA)** in order to inform a grower of pond health problems and potential contaminant-specific interventions.
- Demonstrate **improved detection sensitivity and timing** compared to existing technologies across a range of predator and crop organisms.

Relevance

- This technology will inform growers of pond health problems **before** they become a problem, enabling contaminant-specific interventions at an earlier stage than currently available.
- Early interventions will result in reduced biomass losses, smaller interventions to remove contaminants, faster recovery times following interventions, and reduced sterilization and re-inoculation costs.
- Overall, this will increase the stability of algal biomass production and reduce costs and losses due to pond crashes or reduced yield due to contamination, enabling commercial viability of algal biofuels.

Quad Chart Overview

Timeline

- Project start date: 10/01/2015
- Project end date: 03/31/2020
- Percent complete: 95% as of 12/31/18

	Total Costs Pre FY17	FY 17 Costs	FY 18 Costs	Total Planned Funding (FY 19-Project End Date)
DOE Funded	$90,100	$270,603	$218,395	$241,229
Project Cost Share	$29,725	$57,500	$78,000	$44,775

- **Partners:**
  - **Thermo Scientific**

Barriers addressed

- Aft-A. Biomass Availability and Cost
- Aft-B. Sustainable Algae Production
- Aft-E. Algal Biomass Characterization, Quality, and Monitoring

Objective

- Develop simple, automated, affordable, and robust technologies for the **early detection and identification** of pathogens, predators, and non-productive competitors in an algal production pond.

End of Project Goal

- Prototype detection device capable of monitoring >1 molecules indicative of a pond crash with signal to noise ratios ≥ 10 with time of detection less than that of FlowCAM or qPCR

Project Overview
Our Research Questions

- **Q1** - Can MS be used to detect VOCs, particulate matter (PM), or aquatic molecules that signal the early onset of algal degradation?
- **Q2** - If so, can a smaller, simplified, less expensive MS system be developed to make these measurements in the future?
- **Q3** - Can quantitative PCR (qPCR) with High Resolution Melt Analysis (HRMA) be used to identify pathogens or contaminants individually or as classes?
- **Q4** - Can qPCR with HRMA be developed into a simple, inexpensive, automated, single tube technique that is robust and field deployable?
- **Q5** - Will early detection and identification of pathogens and predators in algal production ponds yield enhanced productivity and reduced biomass production costs?

2 - Approach (Management)
Project Team

Dr. Robert Pomeroy, PI

- Assoc. Prof., Dept of Chemistry & Biochemistry
- Member, Cal-CAB and CAICE
- Downstream processing of algae conversion for fuel, fuel additives and polyols, and characterization of molecules at the sea surface microlayer and those directly emitted to gas phase during algal blooms
- Lead investigator, manager
- Responsible for flow of Mass Spectrometry and qPCR HRMA work

Dr. Ryan Simkovsky

- Project Scientist
- Member of Cal-CAB, CAB-Comm, and CEC CILMSF
- Crop protection, mechanisms of biofilm formation, and phage in cyanobacteria
- Responsible for the IMS and LC-MS/MS experiments and the day-to-day operations of the project's objectives

Biweekly group meetings, informal meet ups of the small core team members, teleconferences with UC Davis.

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2 - Approach (Management)

Project Team

Dr. Kimberly Prather - UCSD & SIO

- Distinguished Chair in Atmospheric Chemistry
- Director of CAICE – ATOFMS, CIMS

Dr. Pieter Dorrestein - UCSD

- Associate Professor, SPPS, Pharmacology, Chemistry and Biochemistry
- Director of CMBB & CMSIC - IMS

Dr. Stephanie Fraley - UCSD

- Asst. Prof. Bioengineering
- Developing artificial learning algorithms for qPCR-HRMA identification of crops and contaminants

Dr. Susan Golden - UCSD

- Chancellor's Associates Chair in Molecular Biology
- Member of Cal-CAB, CAB-Comm, CEC CILMSF
- Providing organisms and biological support/infrastructure

Dr. Alissa Kendall - UC Davis

- Assoc. Prof. Civil and Environmental Engineering
- Member of Cal-CAB & CAB-Comm
- Life cycle modeling and Technoeconomic analysis

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2 – Approach (Technical)

- **Task A – Validation**: Project Validation
- **Task B – Baselines**: Demonstrate ability to grow and monitor crop health with or without contamination using current methods to establish baselines of detection & values for TEA/LCA.
- **Task C – Gas Phase**: Develop & evaluate mass spectrometry detection of crop health & death signatures in gas phase – volatiles & aerosol particles
- **Task D – Liquid Phase**: Develop & evaluate mass spectrometry detection of crop health & death signatures in liquid phase – IMS & LC-MS/MS
- **Task E – qPCR+HRMA**: Establish primers & methodology for crop & contaminant identification for automation and rapid analysis.
- **Task F – Integration/Prototype**: Integrate best detection & identification methodologies into a single, automated prototype detection system.

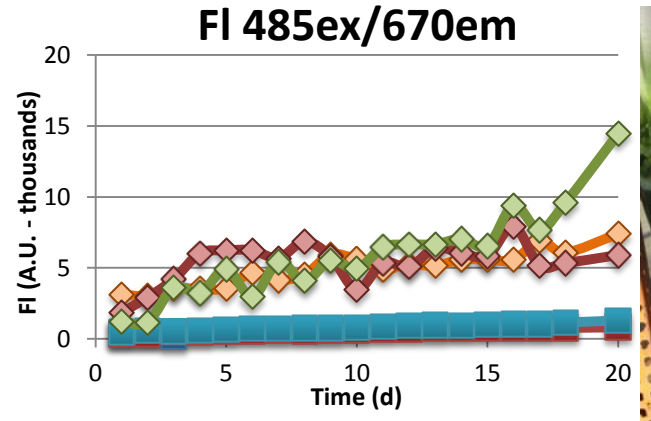
- Evaluate methods at **increasing scales** (flask, mini-MART, polybag, and ponds) on a **diversity** of crops and predators.

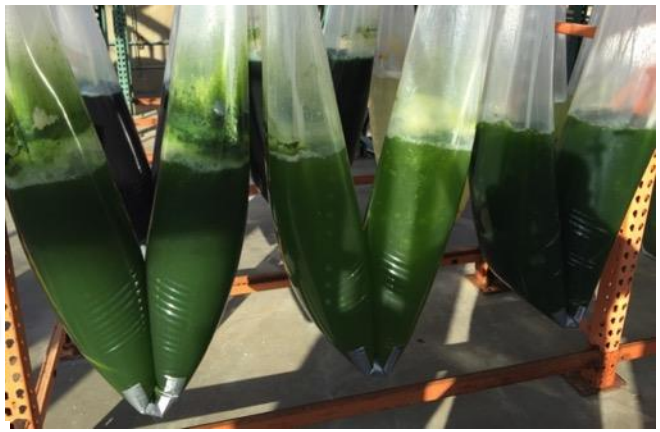
2 – Approach (Technical)

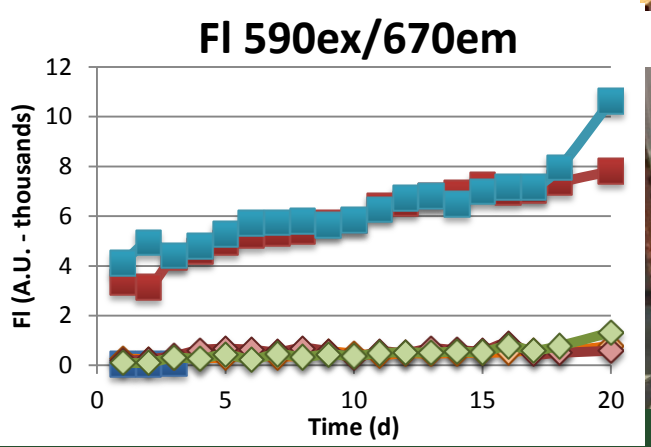
- **Potential Challenges That Were Overcome:**
  - Sampling film states for accurate baselines
  - Ensuring representative & voracious contaminants
  - Identifying molecular signatures
  - Sensitivity of qPCR-HRMA at early time points from diverse cultures
  - qPCR-HRMA of polycultures producing deconvolutable or interpretable results
- **Critical Success Factors & Measures of Success:**
  - Catalog of molecular signatures in gas or liquid phases indicative of contamination or crop health; Associated time of detections/sensitivities relative to current methods.
  - Catalogs of primers and associated HRM curves for diverse crops & contaminants
  - Functioning prototype demonstrated at pond scale
- **Project-wide Go/No-Go at FY17 Q4 (M24) checks for key capabilities necessary to build a functioning prototype (fulfilled & validated M20, FY17 Q3):**
  - Demonstration of scaled growth & analysis, TEA/LCA analysis
  - Molecular candidates in gas or liquid phases
  - Primer sets distinguishing predators & crops via qPCR-HRMA

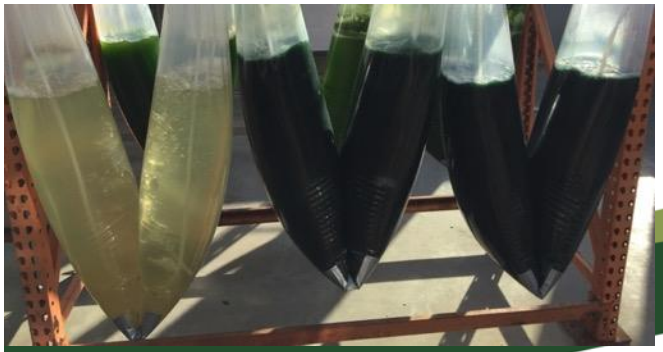
3 – Technical Results – Task B: Growth

- Established baselines yields and kinetics for healthy growth of 5 – 6 strains at flask, polybag, and pond scales.
- Established methods of monitoring for contamination & ongoing establishment of baselines for contamination detection
- Isolated novel voracious predators from pond cultures

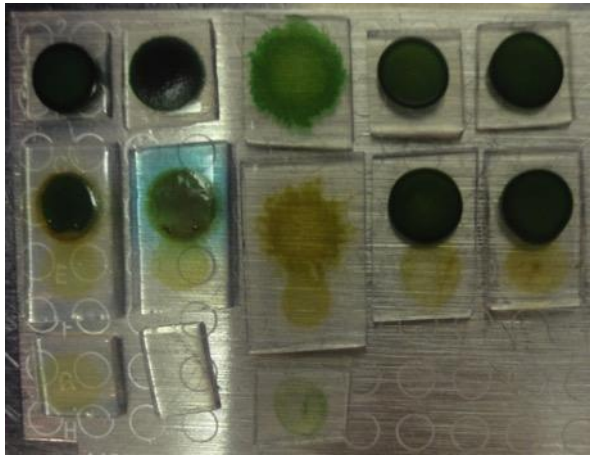




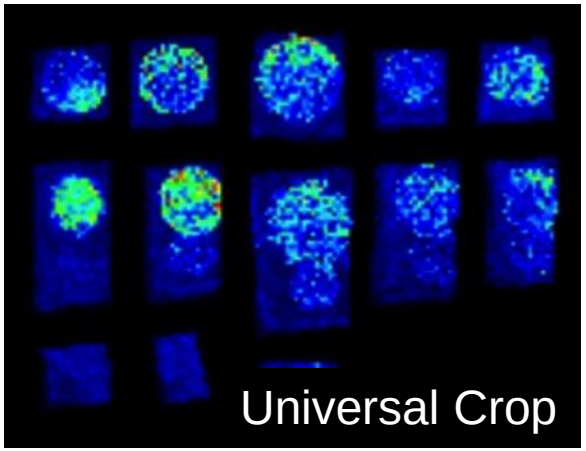


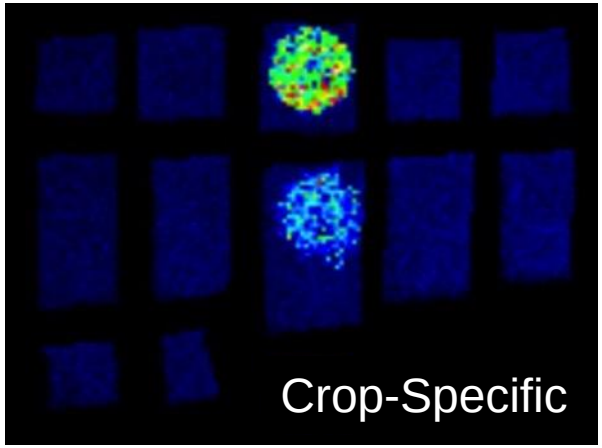


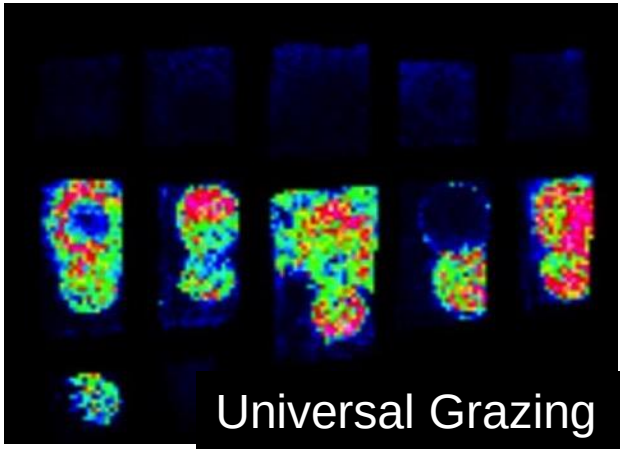
3 – Technical Results – Task D: Liquid/Solid Phase

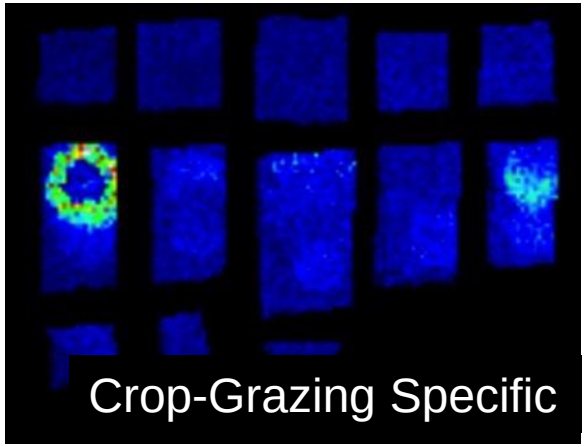


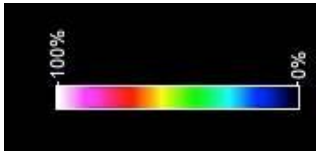
Imaging mass spectrometry (IMS) allowed cataloging of molecular masses associated with diversity of crops, diversity of grazers, or specific interactions of crop and grazer.











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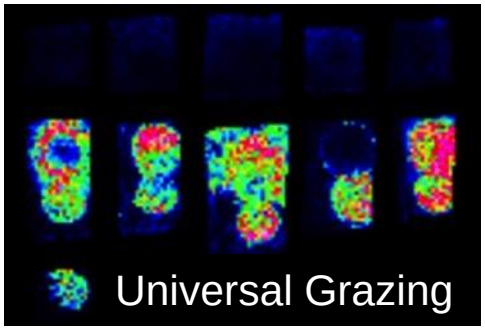
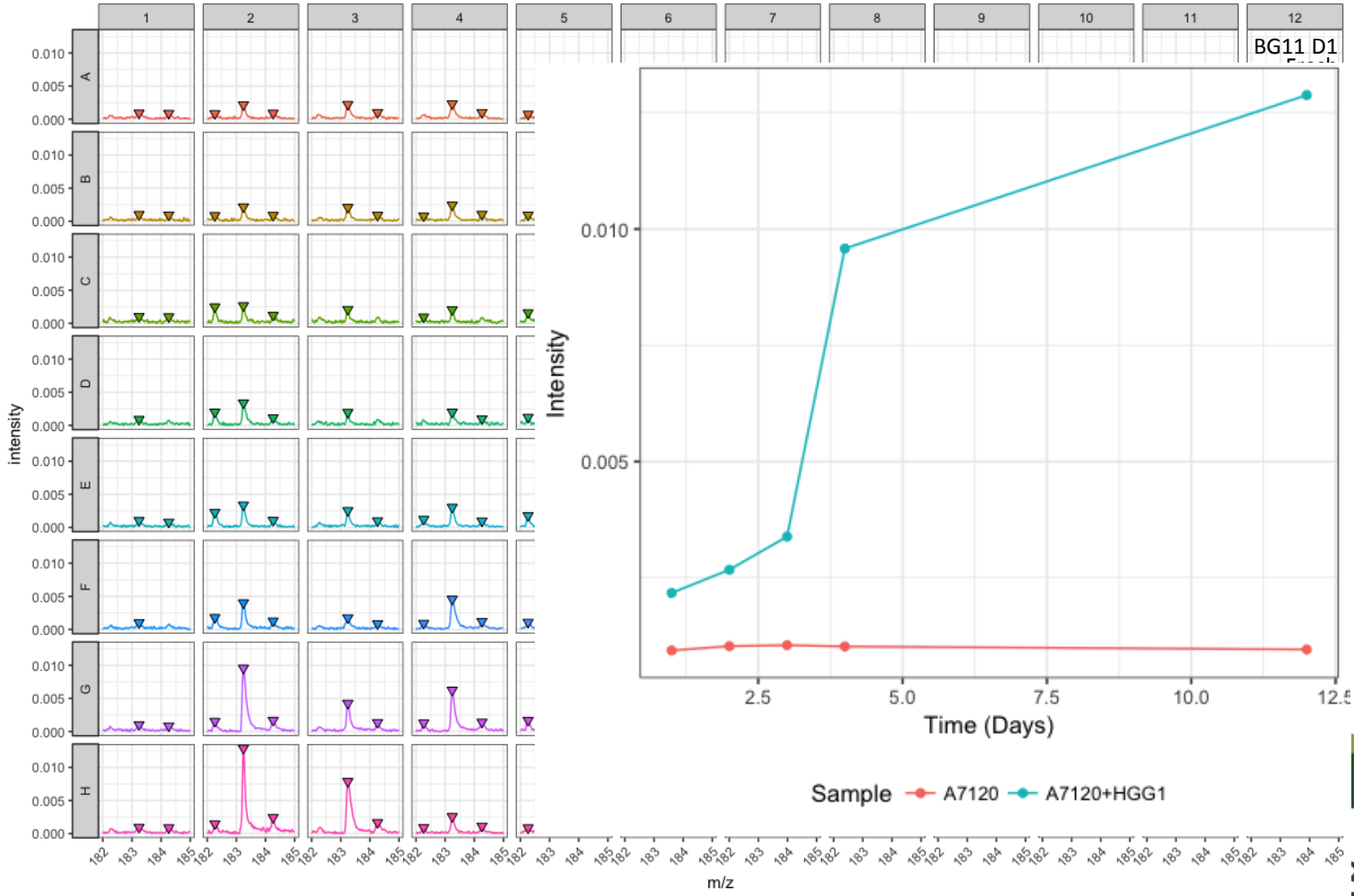
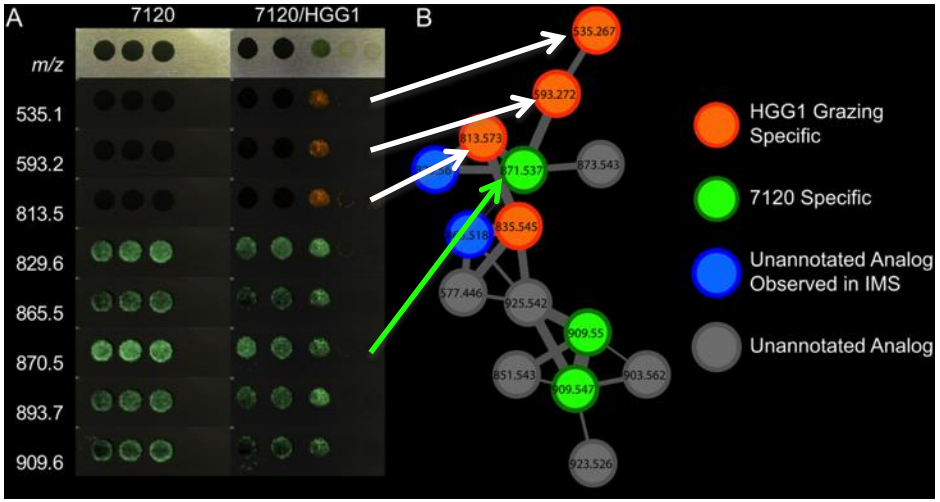


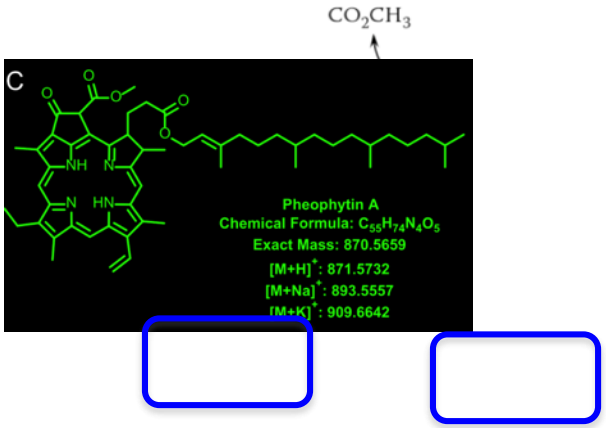
A7120
A7120 + HGG1
A7120 + Ciliate
A7120 + Rotifer
S7942
S7942 + *Tetrahymena*
Chlorella
Chlorella + *Colpoda*
Chlamydomonas
Chlamy + *Colpoda*
Chlamy + Rotifer
Media Controls & peptide standard

Day 1 Fresh
Day 1 Frozen
Day 2 Fresh
Day 2 Frozen
Day 3 Fresh
Day 3 Frozen
Day 4 Fresh
Day 12 Fresh



3 – Technical Results – Task D: Liquid/Solid Phase





- IMS cataloging of molecules, combined with LC-MS/MS fragmentation analysis on liquid cultures and molecular networking identified a cluster of molecules related to chlorophyll break-down products as being grazing indicators.
- These results predicted VOCs detected in Task C.

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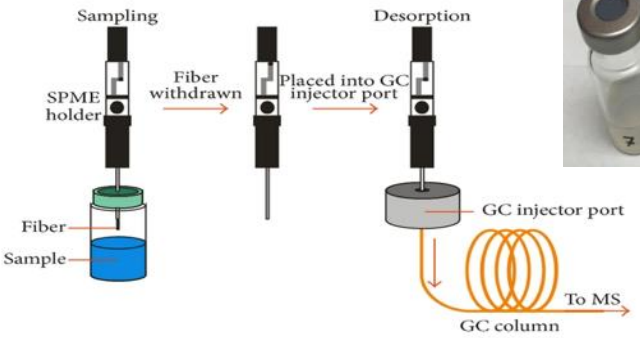
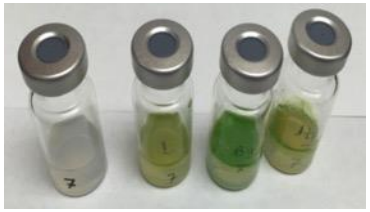
3 – Technical Results – Task C: Gas Phase

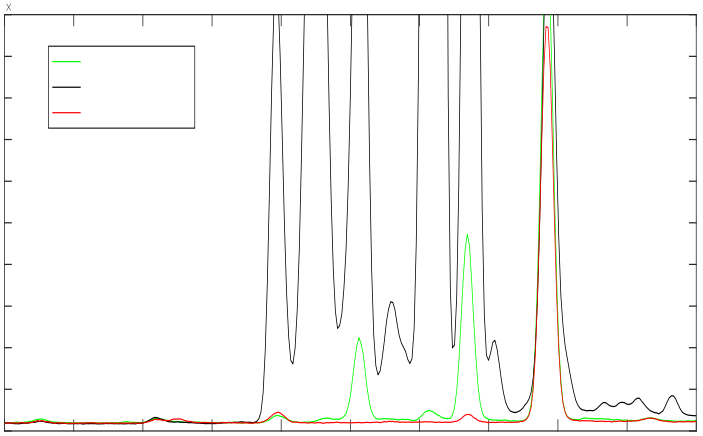
Solid-Phase Microextraction (SPME) Gas



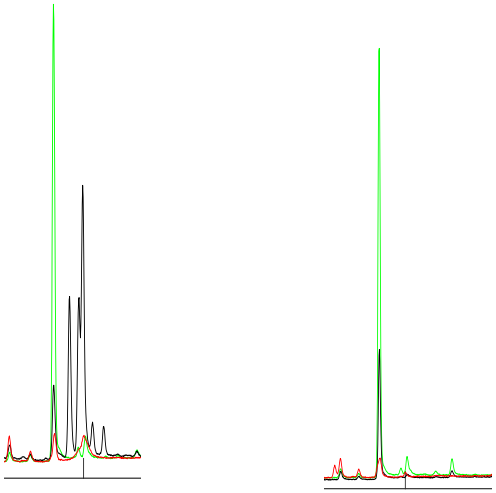


Novel Grazing Signatures

S7942 + HGG1



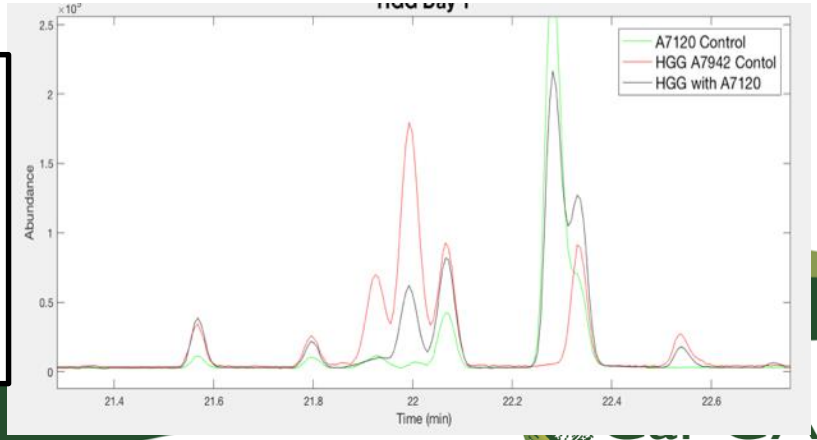
Phytol Detection



S7942 + HGG1

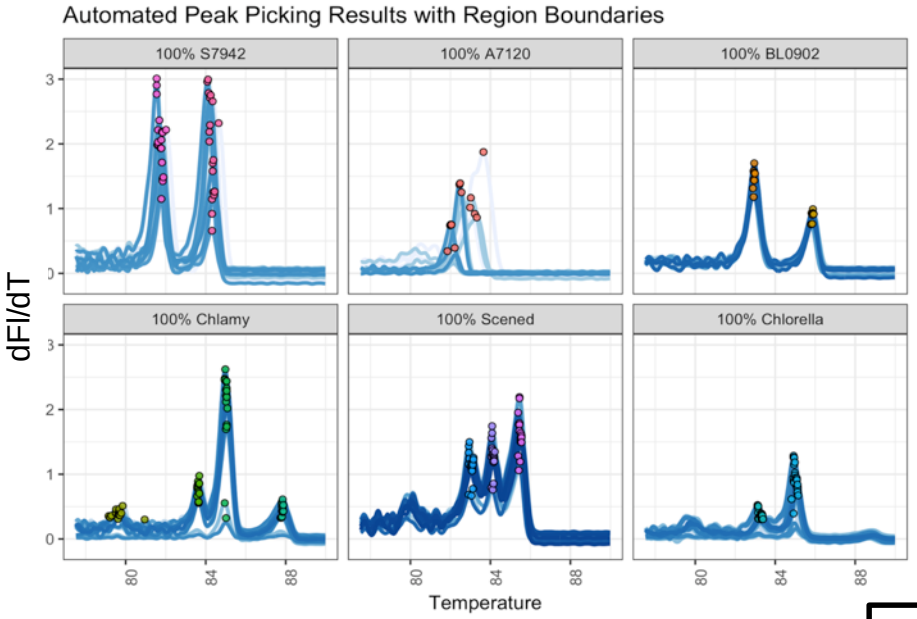
S7942 + LPG1

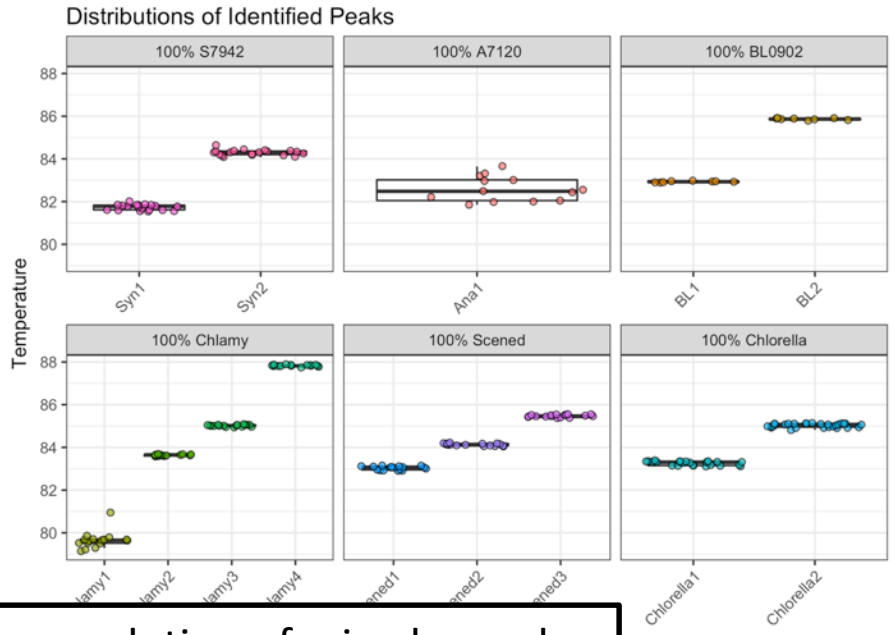
A7120 + HGG1

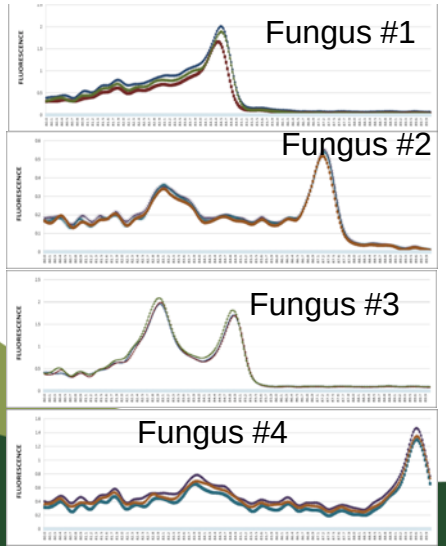


3 – Technical Results – Task E: qPCR-HRMA

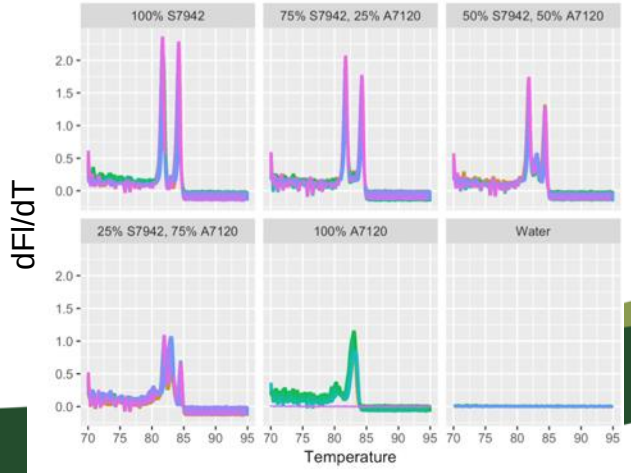
Primer sets designed & tested to produce reproducible, species-specific melt profiles.

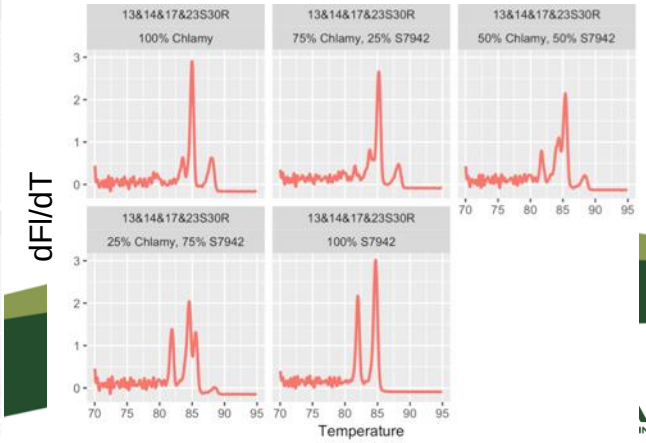




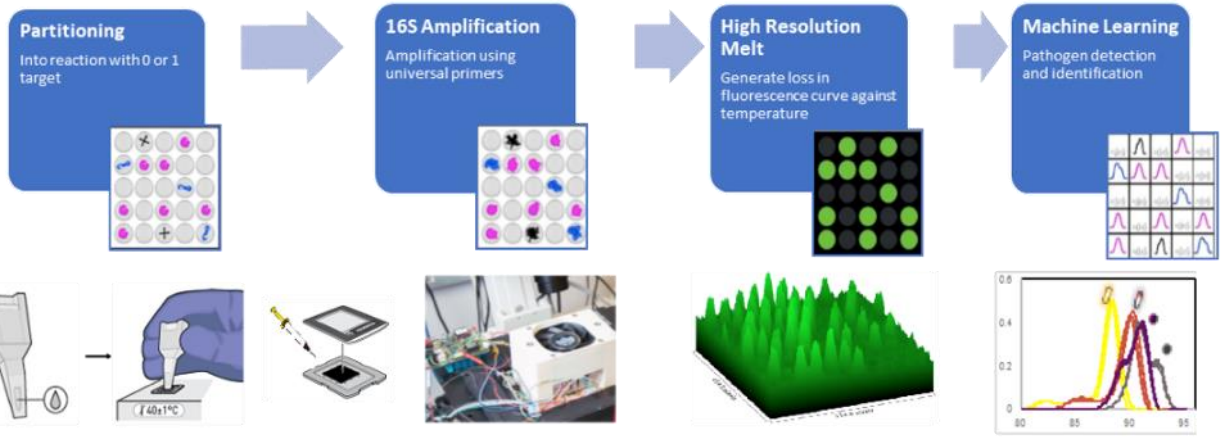


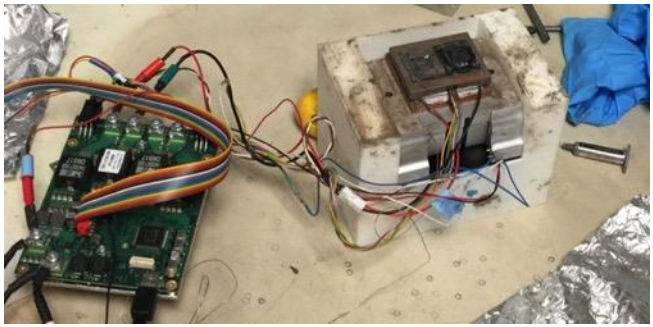
Deconvolution of mixed samples



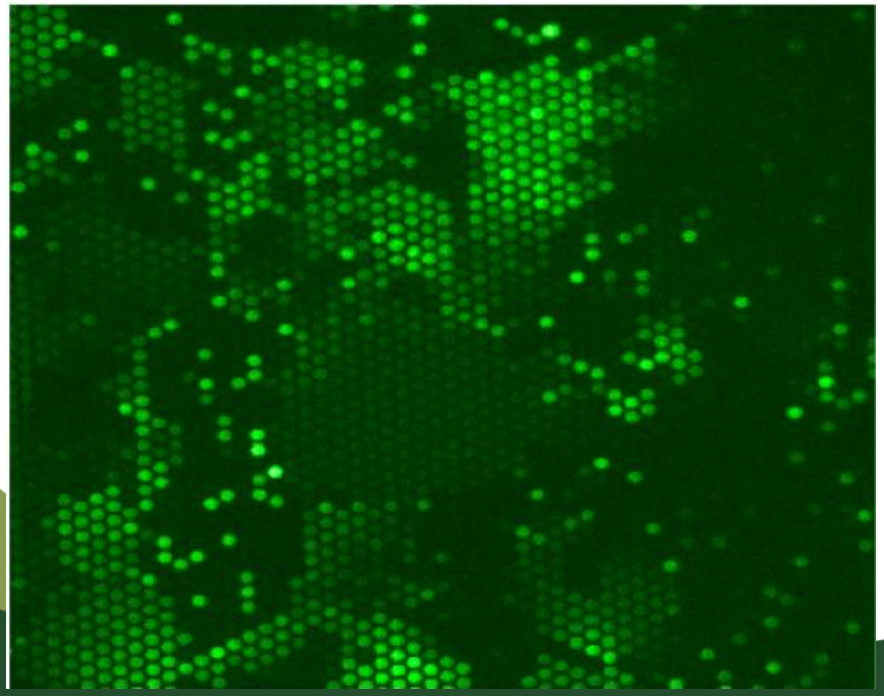


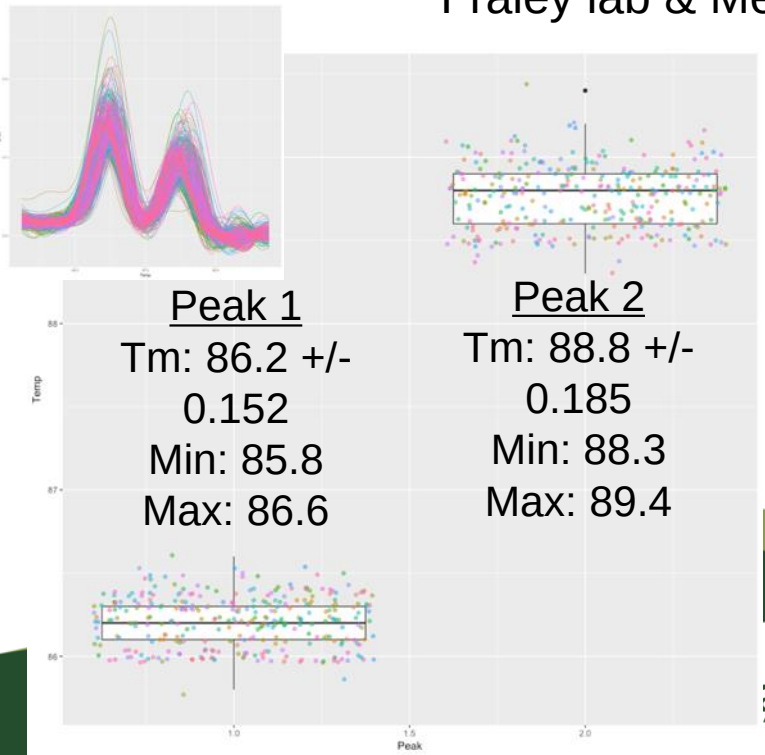
3 – Technical Results – Task E/F: U-dHRM





U-dHRM developed by Fraley lab & Melio, Inc.

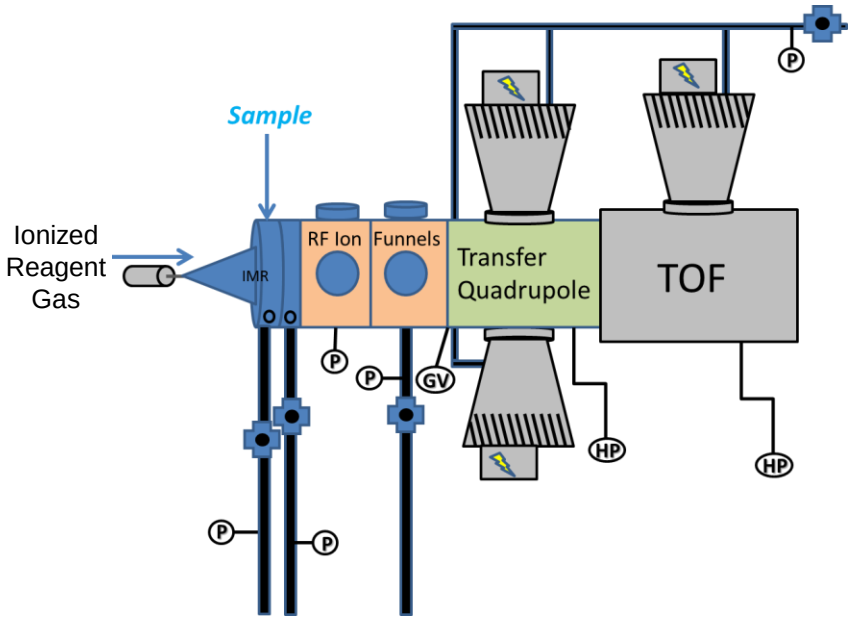


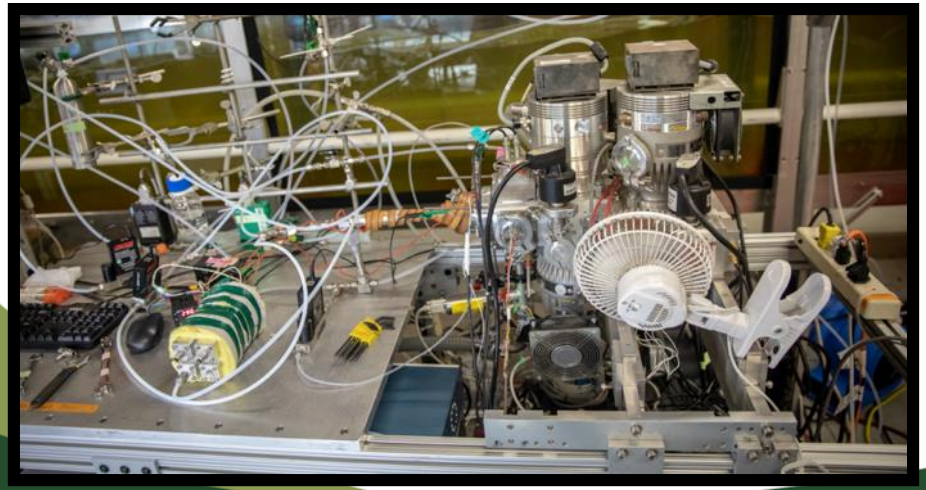


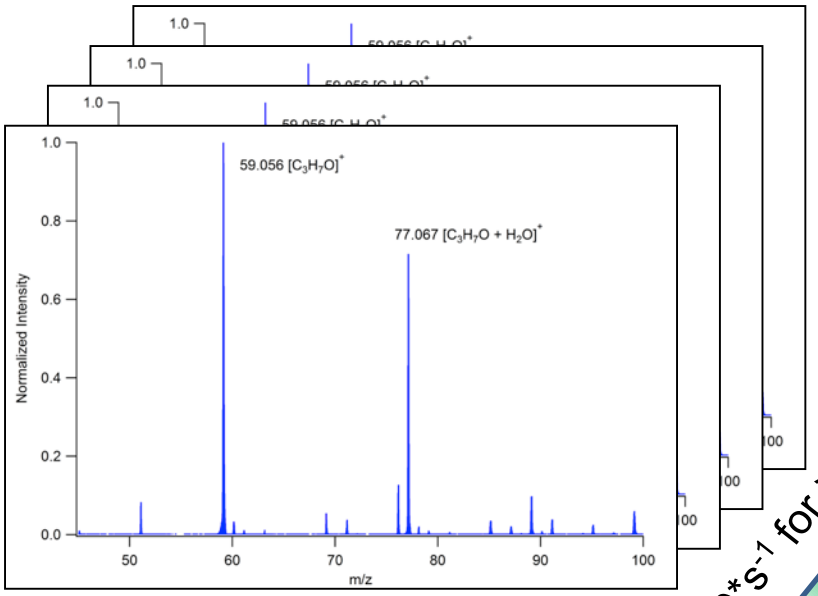
Peak 1
Tm: 86.2 +/- 0.152
Min: 85.8
Max: 86.6

Peak 2
Tm: 88.8 +/- 0.185
Min: 88.3
Max: 89.4

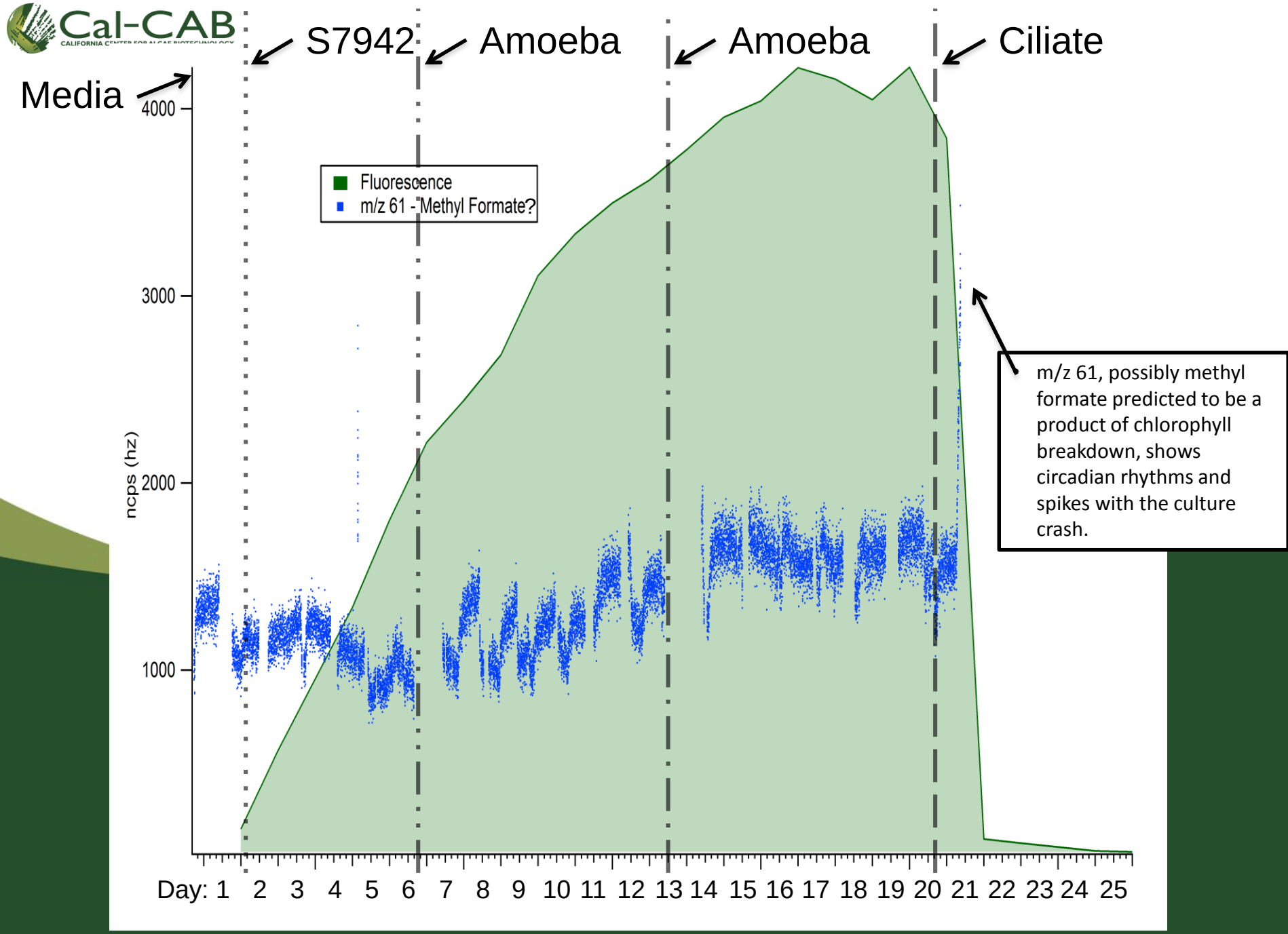
3 – Technical Results – Task C/F: CIMS







65,000*s^{-1} for >2 months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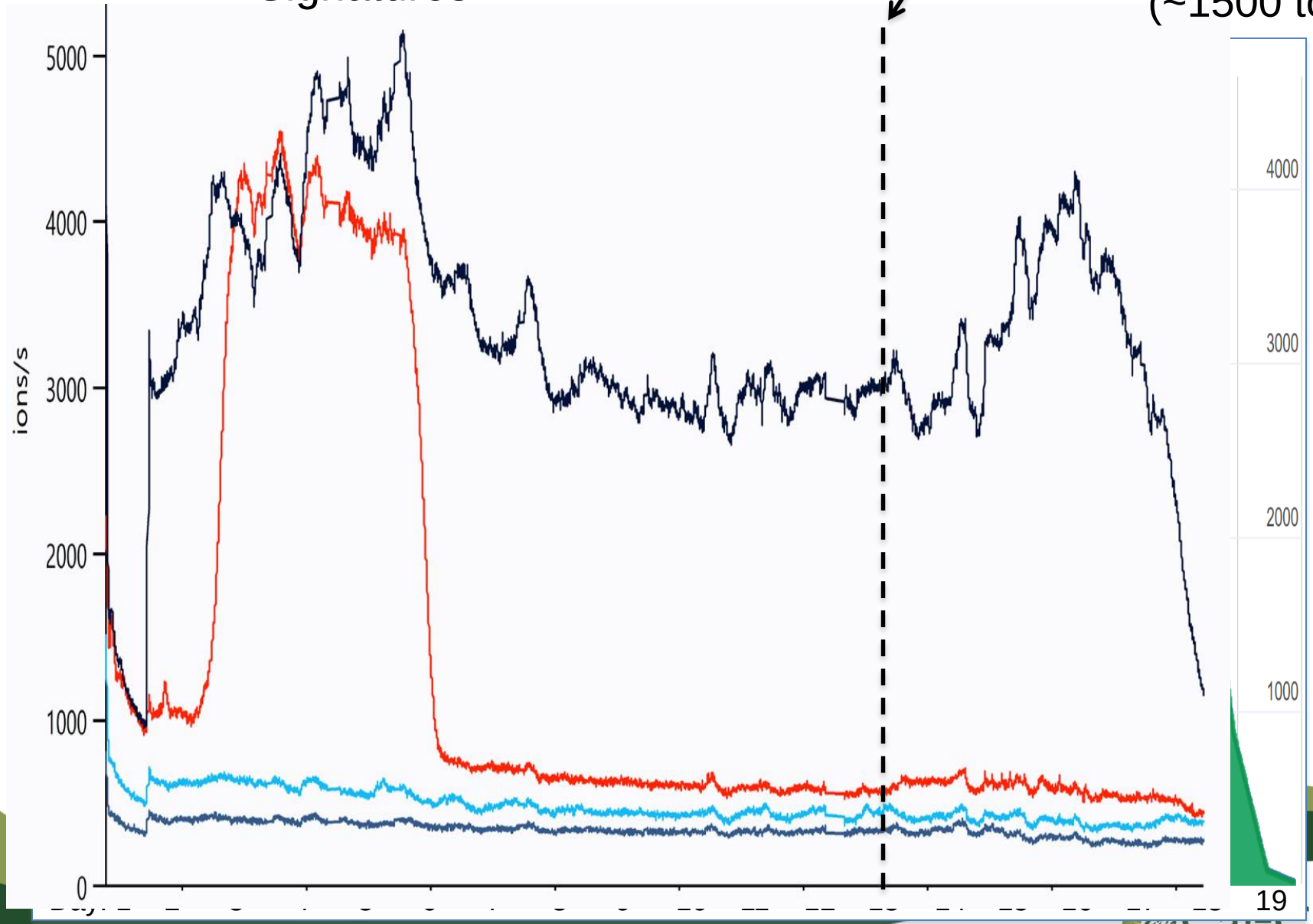
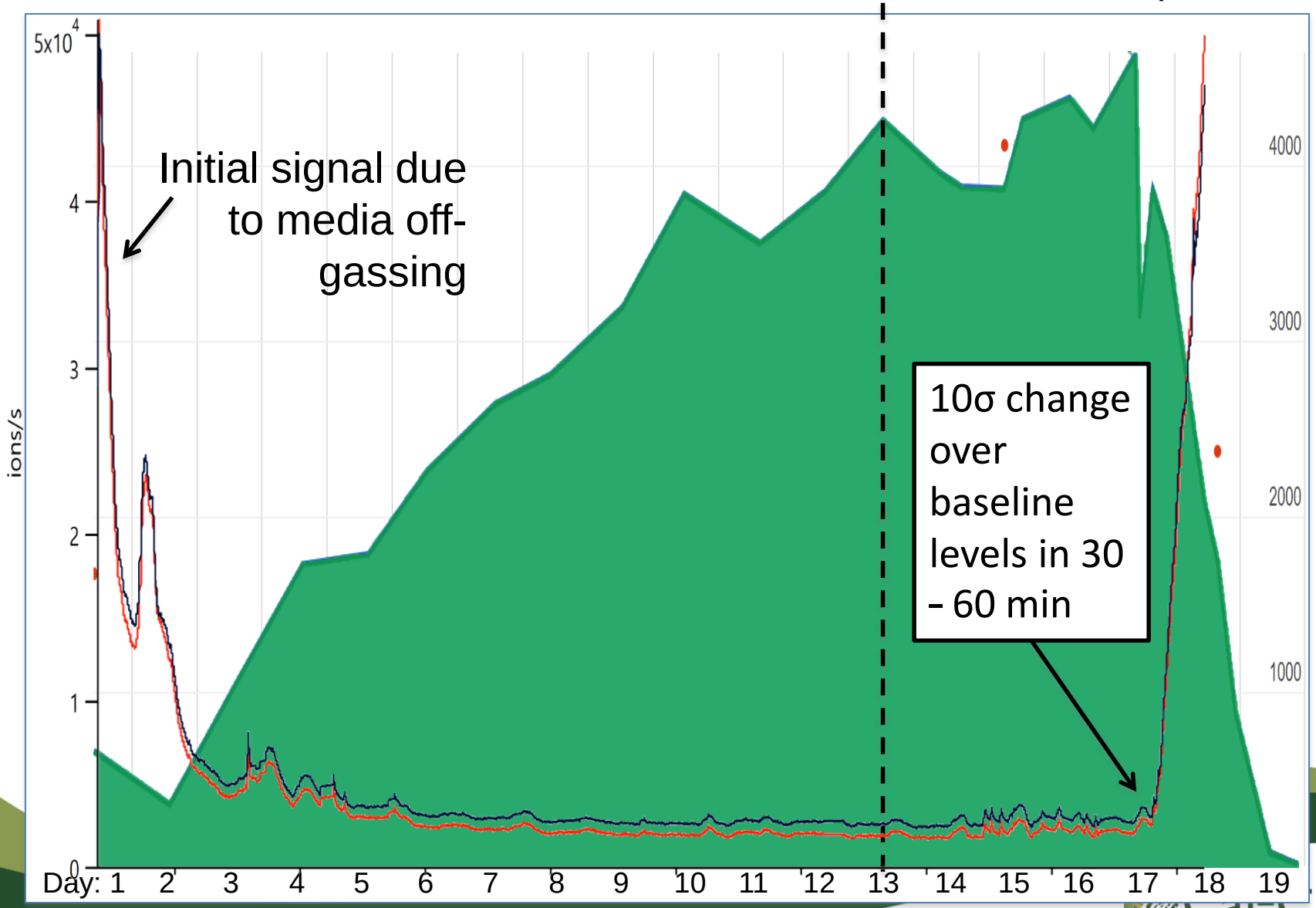
Media
S7942
Amoeba
Amoeba
Ciliate
Fluorescence
m/z 61 - Methyl Formate?
ncps (hz)
4000
3000
2000
1000
Day: 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
m/z 61, possibly methyl formate predicted to be a product of chlorophyll breakdown, shows circadian rhythms and spikes with the culture crash.

Healthy Growth Signatures

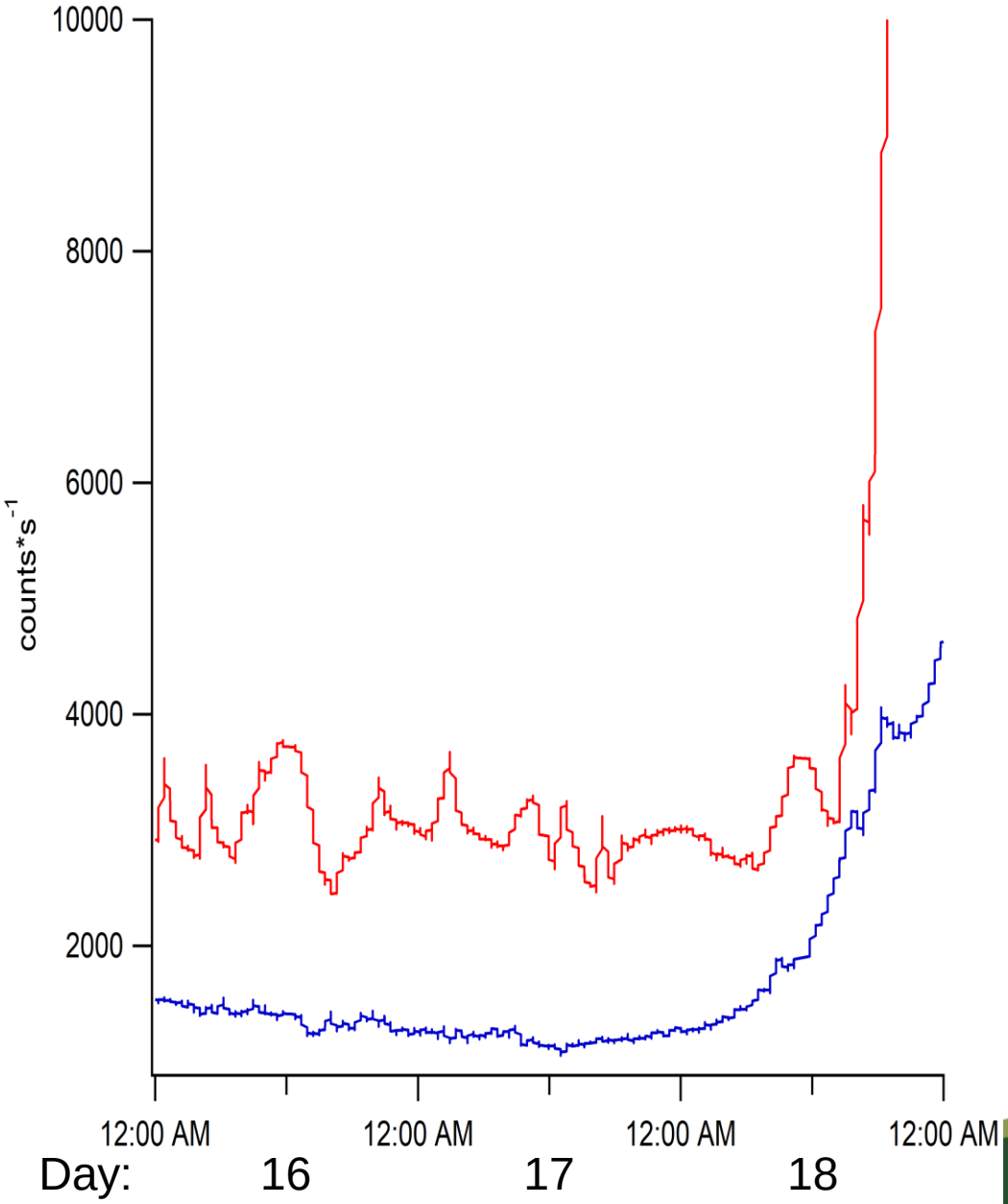
Tetrahymena predator added at 0.1 cells/ml (~1500 total)

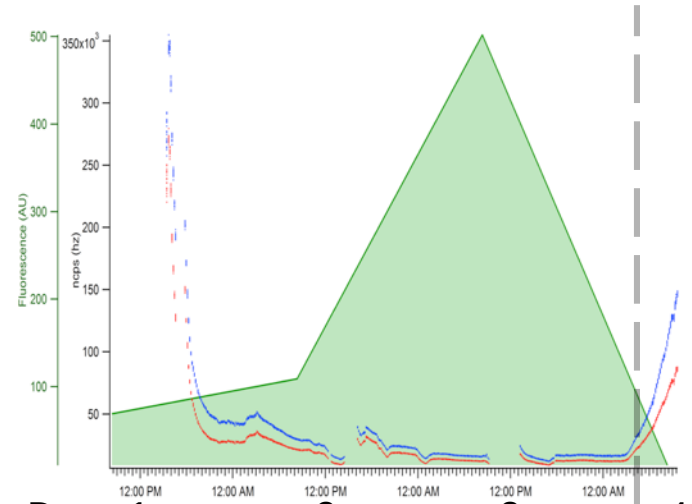


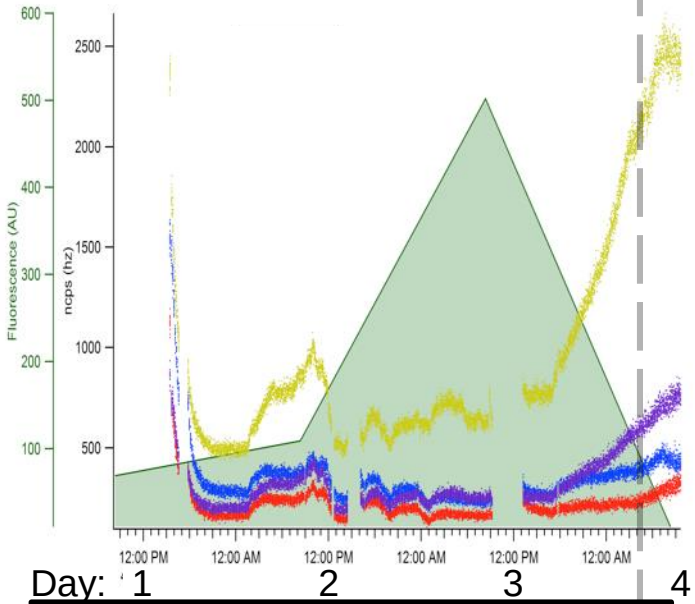
Crop Death Signatures



Earlier Signatures of Cell Death







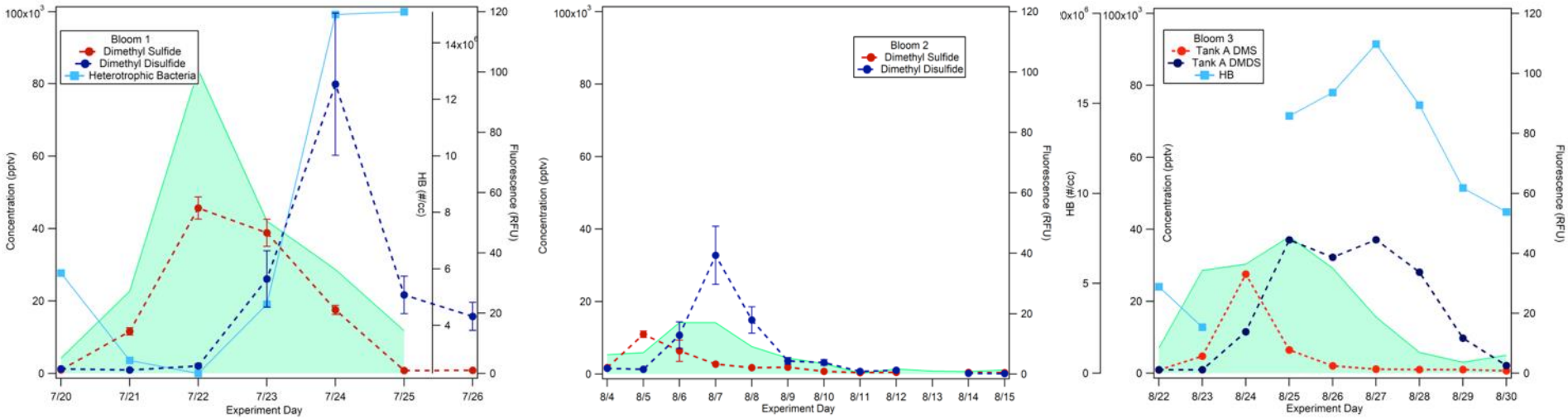
Some early signals only present in non-axenic cultures

Biological Effects on Air Sea Transfer – BEAST 2018 CAICE Summer Project

Along with the 33 m wave flume, 3 MART tanks were monitored for over 2 months continuously using the CIMS.

A video discussion of the experiment can be viewed at:
https://caice.ucsd.edu/news-post/caice-research-highlighted-on-kpbs-npr-midday-edtion/

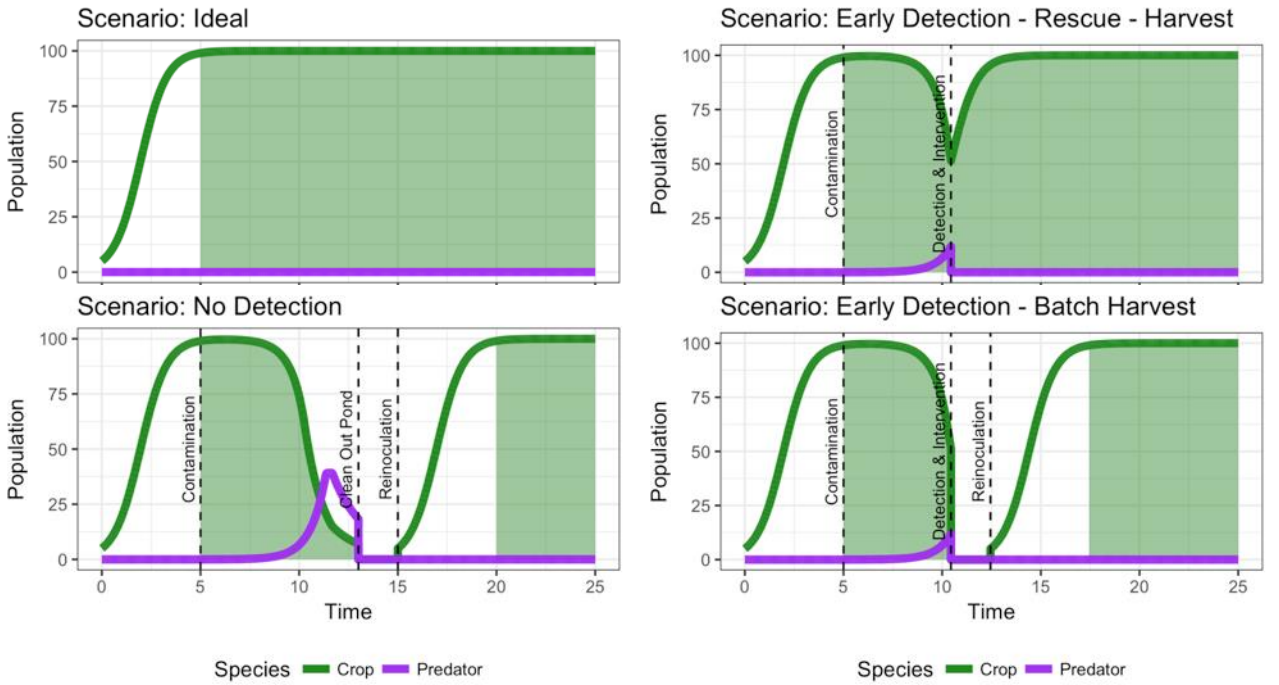
Scaled Axes Show Proportionality of DMS+DMDS to Fluorescence



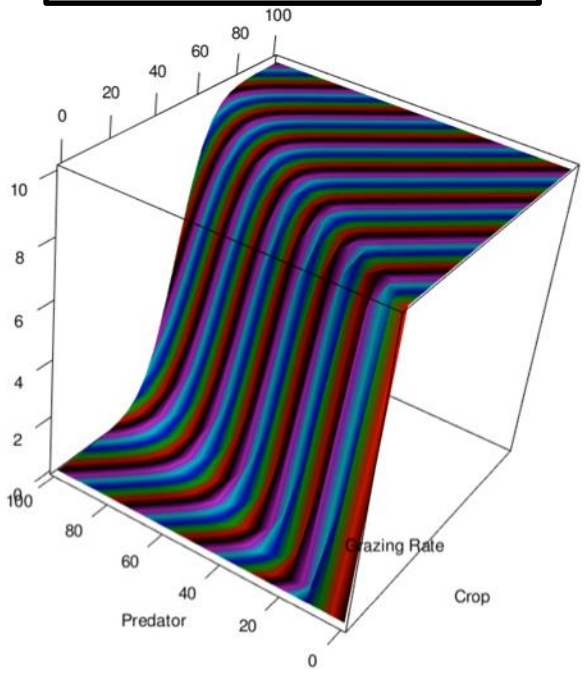
Dimethyl Disulfide tracks with rise of heterotrophic bacteria or the crash of the algal population and peaks after the DMS peak.

3 – Technical Results – Task B/F: Economics

Growth cycle scenarios illustrate example calculations of biomass yields (integrated green area) and impacts of predation and types of interventions



Differential Model of Grazing Rate Based on Predator & Prey Populations



We incorporated the concepts learned from the above scenarios, the above differential model of grazing, and crash data directly from our experiments into an economic model with one primary continuous variable: Time of detection. The model, written as a script in the R programming language, can also take alterations to secondary variables, such as the costs of the detection device, materials for biomass growth, intervention times and costs, etc. This allows for the calculation and production of the above graphs, the costs of production based on changes in the scenario details, and the monitoring system's limits of detection.

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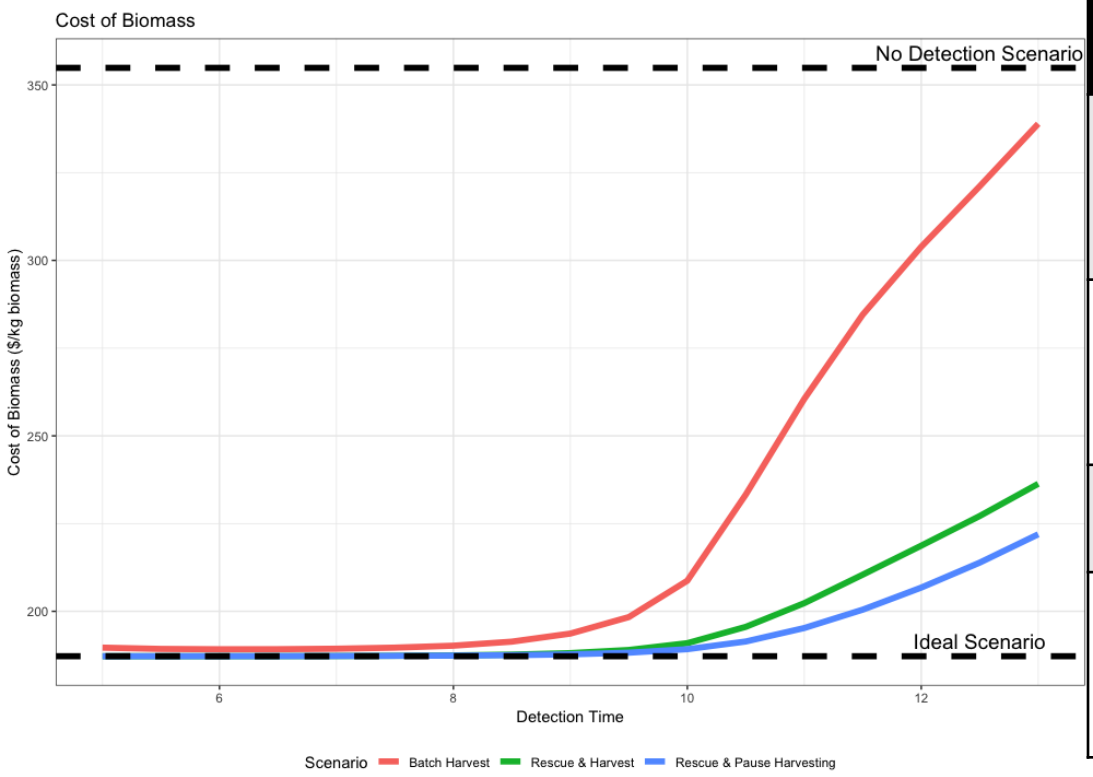
3 – Technical Results – Task B/F: Economics



	Ideal	No Detector	CIMS, Batch Harvest	qPCR, Batch Harvest
Cost of Biomass ($/kg)	187.6	356.9	198.2	232.6
Cost as % of Ideal	100.0	190.2	105.7	124.0
Cost as % of No Detection	52.6	100.0	55.6	65.2
Recovered % Cost by Using a Detector	47.4	0.0	44.4	34.8

Using this model, we can calculate the costs of producing biomass ($/kg) as a function of the time to detection and also compare various detections methods and intervention scenarios relative to either the ideal (no crashes ever) or the worst case scenario (no detector, frequent crashes). Use of the CIMS to inform immediate batch harvesting substantially outperforms other metrics (e.g. qPCR, even when neglecting realistic sampling frequency) with the same intervention scheme and can overall reduced costs associated with losses to near-ideal values.

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4 – Relevance

Overarching Goal

- Develop simple, automated, affordable, and robust technologies for the **early detection and identification** of pathogens, predators, and non-productive competitors in an algal production pond.

Importance

- Early detection systems enable a **more robust** production system with **reduced losses and costs** from catastrophic pond failures and grazing-reduced yields, ultimately allowing **commercial viability**.

Relevance of Project Accomplishments

- Task B: Demonstration of growth & baseline establishments enable comparison with current technology and enable a realisitic and time-dependent economic model.
- Task C: Ability to detect & monitor gas phase molecules indicative of contamination prove principle of detection & sensitivity of system.
- Task D: Catalog of molecular signatures according to crop or contaminant provides IP for technology and enables broad detection capacities.
- Task E: Catalog of qPCR-HRMA compatible primers & resulting curves proves principle of identification in rapid, automated manner.
- Task F: Prototype instrument for detecting contamination at pond scale.

5 – Future Work

- **Task B – Baselines**: Complete
- **Task C – Gas Phase**: Complete
- **Task D – Liquid Phase**: Complete
- **Task E – qPCR+HRMA**: Complete
- **Task F – Integration/Prototype**: Build a cheaper, improved, and more portable detection prototype with an integrated line-switcher and test the system at scale and in the field.

Summary

- We have been investigating mass spectrometry as a platform for an early detection system
- We are cataloging molecules specific to prey-predator interactions, both in the head space & bulk liquid.
- We are refining a Chemical Ionization MS, CIMS, capable of continuous monitoring with out the need for the Gas Chromatography front end.
- We are developing new primer sets to incorporate qPCR HRM as a simple closed tube method of class/species identification.
- Our technoeconomic model shows a substantial recooperation of losses using the CIMS system for early detection and mitigation.
- We are developing IP in the form of chemical marker identification, primer sequences, and new application specific instrumentation.
- Applications & Impacts on other industries: Fermentation (Biofuels, Pharma, Brewing); Aquaculture, Hydroponics, & Field crops; Food Processing Safety & Quality Control

ADDITIONAL SLIDES

Responses to Previous Reviewers' Comments

- One reviewer suggested that an inline, continuously monitoring system may not be necessary This paragraph could also be moved for space for some reason as above, again probably relevance section
  - We believe that the literature and our experiments in monitoring pond crashes with traditional methods or with the CIMS demonstrate that a daily sampling is not sufficient and early detection requires more frequent and rapid assessment. For example, using the CIMS, we have observed exponential increases in signals associated with grazing on time scales of 30 – 60 minutes. The extreme sensitivity of our CIMS system has also allowed us to observe circadian modulation of molecular signals related to the health of the culture; rhythms that are supported by published metabolomics [Diamond S, et al. "*The circadian oscillator in Synechococcus elongatus controls metabolite partitioning during diurnal growth.*" Proceedings of the National Academy of Sciences (2015). Knowledge of these cycles inform threshold values for alerts and can also help to monitor healthy production values. Finally, our economic analysis supports the conclusion that losses are exponentially increasing with time, and thus continuous modeling vastly improves losses over daily sampling.
- One reviewer pointed out the lack of comparison of our work to published work at Sandia National Laboratory.
  - We submitted a letter of support to Sandia researchers for an internal funding opportunity, which they were awarded, and have collaborated and exchanged information via phone conversation, e-mails, and seminars. A primary difference in our approaches is on the instrumentation used, the Sandia group is using fiber or cartridge based accumulation of VOCs over a pond (similar to the SPME data presented here) while our group is using the CIMS as our primary detector. This difference is crucial in terms of applications, because of the advantages in continuous and automated monitoring discussed for the CIMS above, while the fiber-based method averages out signals over the time of collection (usually an hour or more) and requires manual moving of the cartridge from the source (located at Sandia's California site) to the mass spectrometer (located in New Mexico), which further delays signaling of a problem.

Publications, Patents, Presentations, Awards, and Commercialization

Patents

•A disclosure (eDisclosure UD00000743) has been submitted to the technology transfer office (TTO) at UCSD. We are currently in discussion with the UCSD TTO with regards to submission of a provisional patent application versus maintaining information as trade secret.

Publications

•The following manuscripts are nearing submission:

- Mass spectrometry as a tool for characterizing and detecting grazing in algal cultures.
- High Resolution Melt Analysis (HRMA) can identify crops and contaminants of algal growth systems.
- Chemical ionization mass spectrometry: A detection system for algal crop contamination.
- An economic analysis of crop failure and mitigation strategies in algal growth systems.

Presentations

•Simkovsky, R. “Mass spectrometry-based detection of pond infestations.” March 10, 2016. 2016 Food & Fuel for the 21st Century Symposium. University of California San Diego, La Jolla, CA, USA.

•Simkovsky, R. “Developing tools for crop protection in algal biomass production.” April 22, 2016. Marine Biology Seminar. Scripps Institution of Oceanography, La Jolla, CA, USA.

•Pomeroy, R. “Crop Protection Utilizing Integrated Pest Management through Early Detection and Identification of Pathogens and Predators”. October 28, 2016. Howard University, Washington, DC, USA.

•Pomeroy, R. “Integrated Pest Management for Early Detection in Algal Crop Production”. March 9, 2017. DOE BETO 2017 Project Peer Review. Denver, CO, USA.

•Pomeroy, R. "Might as well go Surfing." Aquarium of the Pacific, Long Beach CA, May 2, 2017

Publications, Patents, Presentations, Awards, and Commercialization (Cont.)

Presentations

•Pomeroy, R. "CAICE Innovations". NSF CCI 4th Year Review, La Jolla CA, May 22, 2017- Center for Aerosol Impacts on the Chemistry of the Environment

•Simkovsky, R. “Mass spectrometry-based detection of algal pond infestations” 2nd Annual International Solar Fuels Young conference in La Jolla, CA. July 6, 2017.

•Simkovsky, R. “Mass spectrometry-based detection of algal pond infestations” 2nd Annual International Solar Fuels conference in La Jolla, CA. July 10, 2017.

•Simkovsky, R. “Crop protection for algal-based food and fuel”. Undergraduate and Graduate Student Seminar Course Guest Lecture at California State University Long Beach in Long Beach, CA. September 28, 2017

•Simkovsky, R. “Mass spectrometry-based detection of algal pond infestations” Algae Biomass Summit in Salt Lake City, UT. November 1, 2017.

•Simkovsky, R. “Sniffing out a bad pond: Mass spectrometry-based detection of contamination” Algae Biomass Summit in Houston, TX. October 16, 2018.

•Sauer, J. “Dimethyl Disulfide, an Underappreciated Marine VOC with Potential?” CAICE Phase IIb Launch and Annual Meeting in La Jolla, CA. November 5, 2018.

•Pomeroy, R.; Simkovsky, R.; Sauer, J.; & Manson, J. “Early Detection of Predation of Algal Crops by Chemical Ionization Mass Spectrometry” Poster Presentation at the CAICE Phase IIb Launch and Annual Meeting in La Jolla, CA. November 5, 2018.

•Pomeroy, R. “EOD and Innovation - Overview” CAICE Phase IIb Launch and Annual Meeting in La Jolla, CA. November 6, 2018.