ATP3
Algae Testbed
Public-Private Partnership

2015 DOE BETO Algae Platform Review

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Goal Statement

Mission: Establish a **sustainable network of regional testbeds** that empowers **knowledge creation** and **dissemination** within the algal R&D community, **facilitates innovation**, and **accelerates growth** of the nascent algal biofuels and bioproducts industry.

Goals:

- **Increase stakeholder access** to high quality, outdoor cultivation and laboratory facilities
- **Support DOE**'s techno-economic, sustainability, and resource modeling activities
- Help to **close critical knowledge gaps** and inform robust analyses of the **state of technology** for producing algal biofuels and bioproducts

Quad Chart Overview

Timeline

- **Project start date: 2/1/2013**
  - Pre-Award (at risk) 11/12-1/13
- **Project end date: 1/31/2018**
- **Percent complete: 55%**

Budget

Total project cost: $17.05M

DOE Commitment ($15M)

- Open Collaborative Testbeds: $7.3M
  - **DOE share: $5.25M**
  - Contractor share: $2.06M
- High Impact Data: $9.75
  - **DOE share: $9.75M**

	FY 13 Costs	FY 14 Costs	Total Planned Funding (FY 15-Project End Date
DOE Funded	$4,581K	$4,804K	$5,615K
Contributed	$560K	$750K	$750K

Barriers

- **AFt-B** Sustainable Algal Production**:** Existing data on the productivity and environmental effects of biomass feedstock production systems…are not adequate
- **AFt-E** Algal Biomass Characterization, Quality, and Monitoring**:** Physical, chemical, biological, and post-harvest physiological variations in harvested algae are not well researched or understood
- **AFt-C** Productivity and robustness of algae strains against perturbations such as temperature, seasonality, predation, and competition…not well understood.

Partners

ASU (AzCATI) (45%)
National Renewable Energy Laboratory (9%)
Sandia National Laboratories (10%)
Cellana (12%)
Cal-Poly (5%)
Georgia Tech (5%)
Touchstone Research Laboratory (4%)*
UTEX (5%)
Florida Algae (2%)
Commercial Algae Management (2%)
Valicor Renewables (2%)
Open Algae (1%)

* No longer part of ATP^3 as of 8/14

Project Overview: ATP³ national open test bed

The formation of the Algae Testbed Public-Private Partnership **leveraged** the existing resources at AzCATI and our partner sites. The network represents a **collaboration** of industry, laboratory, and educational facilities across nation. ATP³ aims to **convene** algae stakeholders to facilitate opportunities and progress more rapidly to commercialization.

ATP^3's Two Main Objectives

Collaborative Open Testbeds

- Establish **network** of facilities for the algal research community and **increase stakeholder access** to real-world conditions for algal biomass production.
- **Accelerate** applied algae research, development, investment, and commercial applications for biofuel and bioproduct feedstock production.

High Impact Data from Long Term Algal Cultivation Trials

- Design and implement a unified experimental program across different **regional, seasonal, environmental and operational conditions** comparing promising production strains at meaningful scales.
- **Data made widely available** to the TEA/LCA and overall research community allowing for a robust analysis of the state of technology.

Project Management: Work Breakdown Structure

Task 1: Collaborative Open Test Bed

1.1 Operations - Assemble a network of geographically diverse sites to carry out integrated testbed operations.

1.2 Business Development and Marketing - establish a sustainable network of regional testbeds that can continue beyond initial program

1.3 Training and Education - Develop and deploy high quality training and education programs

Task 2: High Impact Data

2.1 Setting Standards - Identify and implement current best practices across our partner sites with a strong focus on continuous improvement

- 2.1.1 Harmonized methods and metrics (Analytical **AND** Production)
- 2.1.2 Data Management (SDMS)
- 2.1.3 Advanced Diagnostics
- 2.1.4 Real Time Monitoring

Task 2: High Impact Data (Cont.)

2.2 Long Term Cultivation Trials - Generating data to support DOE's TEA, LCA, resource modeling and make widely available to the community

2.2.1 and 2.2.2 Unified Field Studies (UFS) and Advanced Field Studies (AFS) – design, validate and implement experimental framework for cultivation trials.

2.2.3 TEA/LCA – update base case to represent current SOT first with ATP^3 UFS/AFS data and later with customer data

2.2.4 Dynamic Modeling – implement and validate existing physics-based computational fluid dynamics model to enhance predictive capability for productivity (strain, location, system, scale)

ATP³ Algae Testbed Public-Private Partnership

Project Approach: Project Timeline

ATP^3 Phase 1:

1. <u>**Months 1-12**</u>: Coordinate mobilization of partnership and initiate work to perform both functions – **Go/No Go for Phase 2 (January 2014).**

   Major Milestones:
   - ATP organization, systems and **processes established**
   - Methodologies **harmonized** across all partner sites
   - Initial cultivation trial and detailed **experimental planning completed**
   - Biomass stocks **available**

Complete

Phase 1 Critical Success Factors:

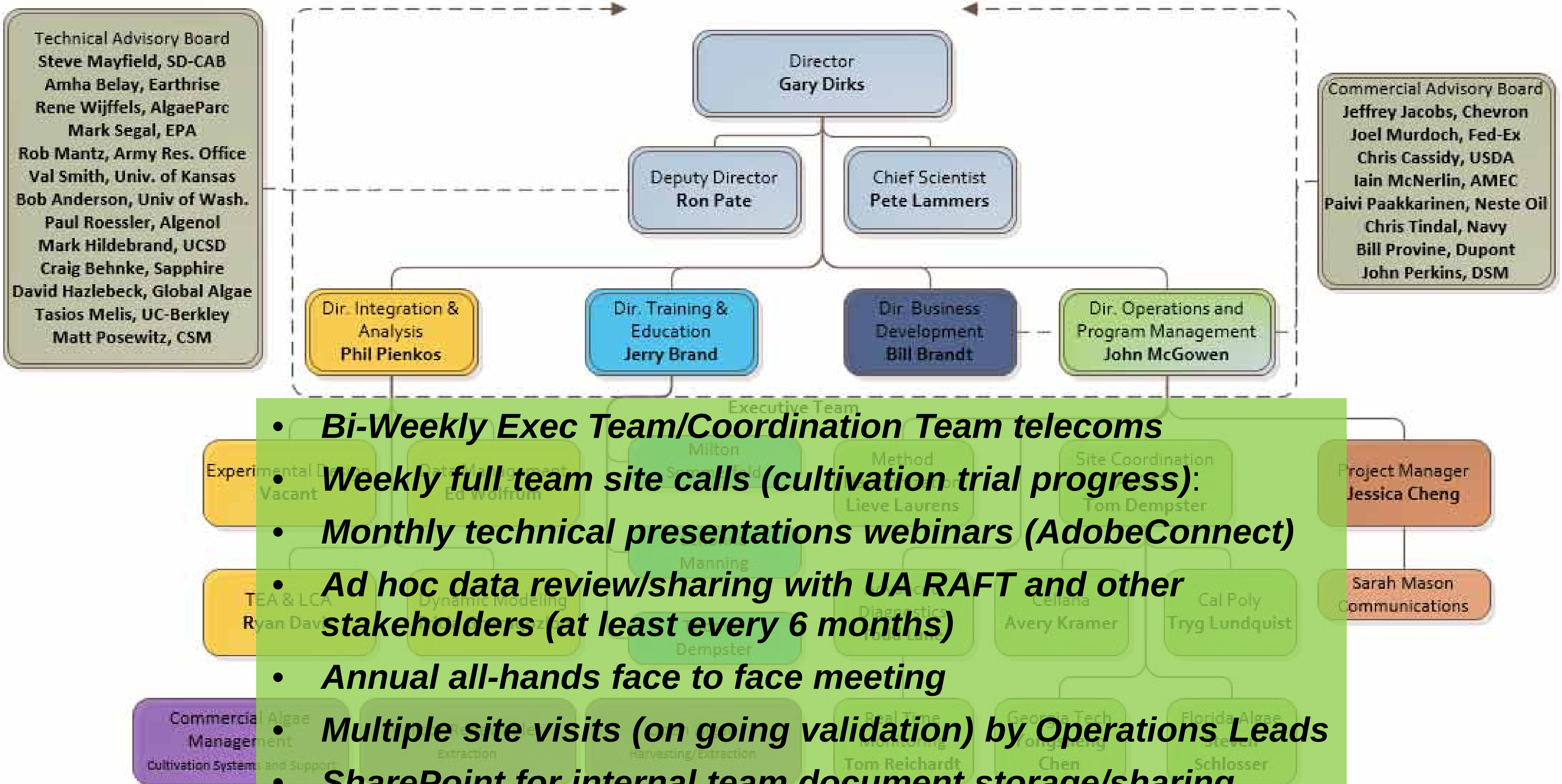
- Harmonized systems and operational protocols verified and validated and experimental framework for Phase 2 defined
- Facilities use agreement boiler plate (MTA/NDA/IP) in place and stakeholder access to test bed network established with customers using 1 or more of the test bed sites
- First call for Scholarship/Innovator complete and projects selected for review
- Biomass stocks available for sale or for support projects

Project Approach: Project Timeline (cont.)

ATP^3 Phases: (Currently in month 25)

2. **<u>Months 13-36</u>**: Long term cultivation trials implementation and building customer base as a user facility – **Go/No Go for Phase 3**
   Major Milestones/Critical Success Factors at end of Phase 2:
   - Cultivation trials complete/data generated
   - Data distribution implemented/data made widely available
   - **State of algal biofuels technology design report** completed
   - Capability of testbed network to **serve stakeholder community** demonstrated
3. **<u>Months 37-60</u>**: Sustainable Testbed Operations.
   Major Milestones/Critical Success Factors:
   - State of algal biofuels technology design report updated with customer data
   - Value network validated and funding secured to **sustain network** in out years

Team Structure and Management Approach

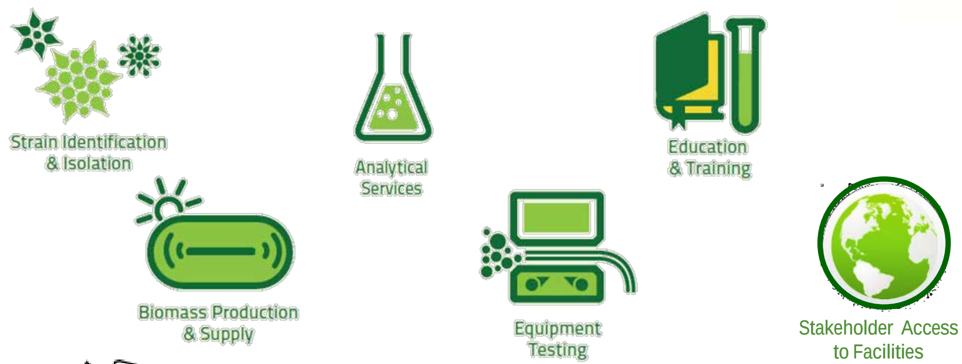


- *Bi-Weekly Exec Team/Coordination Team telecoms*
- *Weekly full team site calls (cultivation trial progress)*:
- *Monthly technical presentations webinars (AdobeConnect)*
- *Ad hoc data review/sharing with UA RAFT and other stakeholders (at least every 6 months)*
- *Annual all-hands face to face meeting*
- *Multiple site visits (on going validation) by Operations Leads*
- *SharePoint for internal team document storage/sharing*
- *Secure data repository w/public data pushed to OpenEI.org*
- *ATP3.org public facing website (LAPS*

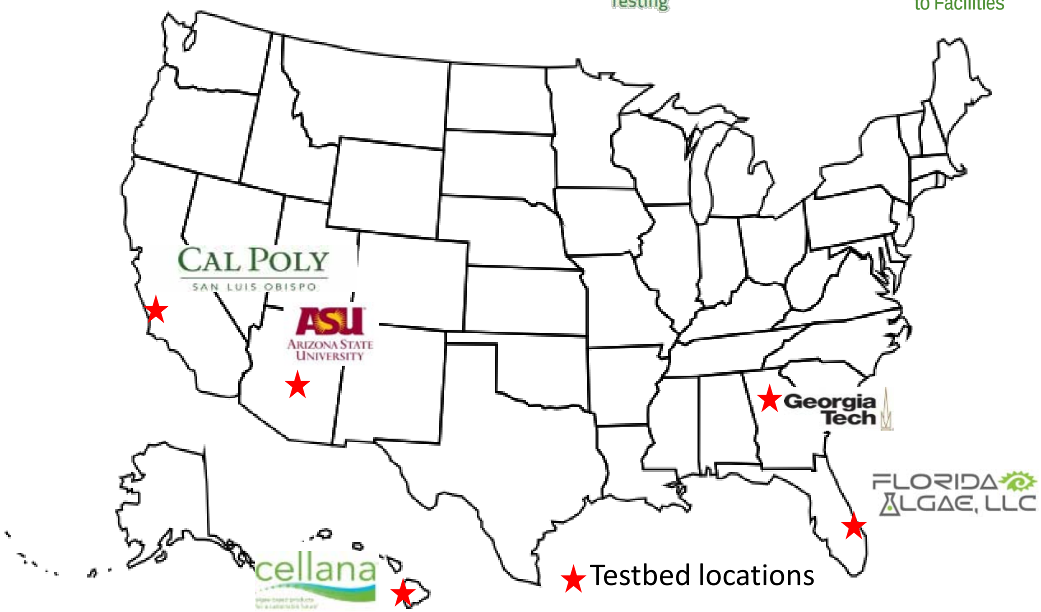
Technical Accomplishments, Progress and Results

Collaborative Open Testbeds

ATP^3 offers access to a wide array of services, capabilities and facilities:



ATP^3 Partner Site	Cultivation Capacity Total (Liters) (unit scale range)		Annual Production Capacity (AFDW)	Yr. Outdoor Operations Began
	Open Pond	Closed PBR		
ASU (AzCATI)	235,000 (200 - 125K)	21,000 (15 – 1500)	1.5 –2.0 MT	2006
Cellana (KDF)	750,000 (200 - 120K)	300,000 (20 – 24K)	12 – 15 MT	2008
Florida Algae	100,000 (500-30K)	200 (indoor only)	1.0 MT	2012
Cal Poly	100,000 (1000- 10K)	1200 (200-1000)	1.0- 1.5 MT	2007
Georgia Tech. (GT)	6000 (500-1000)	200 (indoor only)	< 0.1 MT*	2013
Total	**1,300,000 L**	**320,000L**	**15 – 20 MT**	



Regional testbed facilities for the partnership are physically located in **Arizona, Hawaii, California, Georgia, and Florida.**

ATP^3 Algae Testbed Public-Private Partnership

ATP^3: Testbeds Open for Business

- ***Project Activities***: biomass supply (1kg-100's kg), equipment testing, analytical testing, culture maintenance and consultation services to academia, industry and national labs

- ***Project Categories***: fee-for service activities, sponsored research, and subsidized projects through ATP^3 Support Program

- ***Project Benefits***: access to facilities to drive technology R&D, de-risk and validate technological innovations

Variety of independent and vertically integrated downstream harvesting unit ops

Provide service to ATP³ customers

- Produce algal biomass in the form of slurry, paste and dry powers
- Serve as baseline technologies for the improvement of future harvesting/dewatering and oil extraction processes (Valicor and OpenAlgae platforms)

Support DOE's TEA, sustainability, and resource modeling

- Generate harvesting data for the current harmonized model
- Provide more options to generate data on the selection of harvesting methods
- Provide feedstock for lipid extraction and other downstream product applications

Collaborative Open Testbeds: Site Access and Customer Management

ATP^3 offers a 3-tier fee structure

- Full data confidentiality (fully burdened rate – site specific)
- Open access -willing to share data generated at test bed (discounted)
  - level of discount site specific
- Subsidized Access (Scholarship and Innovators Awards)
  - Proposals for use of the testbed with access to materials, equipment, personnel
  - Travel support available to access sites (primarily for academics)

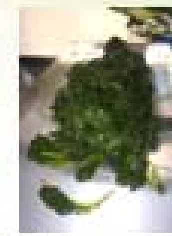
Site Access Plan: Boilerplate IP, MTA, NDA, and facilities use agreement in place for customers

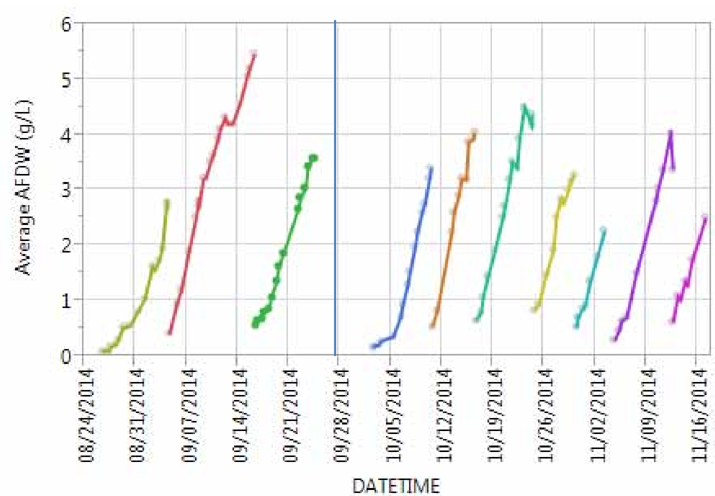
- Simple templates for engagement – one off projects, longer term engagement, and engagement with facility use
- Academic unit approval only for external sales of services – **if no major changes requested, can be approved in ~1 week.**
- Can be applied as pass through to partner sites
  - AzCATI Recharge Center handles agreement and transaction and flows through funding to partner sites – only one party to negotiate with
- AzCATI has already been operating with this testbed framework for >4yrs
- Will transition to consortium based membership model as DOE funding decreases as a means to support transition off direct DOE support for the testbeds

An Example - Toll Biomass Production:
And a framework established for an AFS

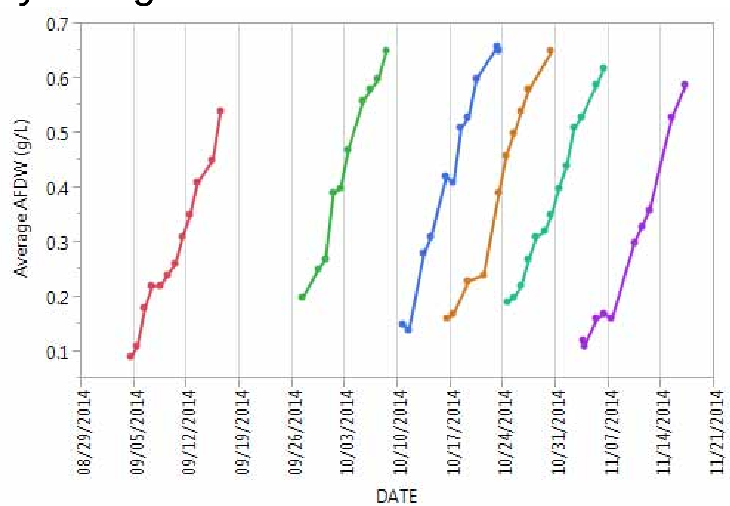
- **Leveraging the Helix^{TM} photobioreactor**
  - Using the Helix as semicontinuous seed platform supporting larger scale cultivation in open pond raceways (2 x 60 m^2) of customer supplied proprietary strain
  - 8 week growth campaign generating 7-8 kg of biomass per batch (media recycle employed)
  - Total project duration 3 months (included indoor scale up)
  - ATP^3 delivered biomass as whole, freeze dried product.
  - Production data generated through harvest
    - centrifuge and MF -->centrifuge process options evaluated (w/GT)
    - can be utilized for TEA/LCA (with permission from client)
  - Experimental framework currently being utilized with other cultivars





Helix Seed Production



60 m^2 cultivation campaign

ATP^3 Offers Support Program

The Goal – encourage and enable small businesses, entrepreneurs and underfunded academic researchers to pursue new approaches to solving technical issues associated with commercialization of algae biofuels, processes, and co-products.

What is it ?

- Access to laboratory, outdoor facilities and resource support for novel projects
- ATP^3 provides subsidized access to testbed facilities, technical expertise, and M&S
- Preference for support will be given to:
  - Short-term projects ranging from 1-3 months (typical project target $10K-$30K)
  - Researchers willing to share data and results widely through publication
  - Projects that leverage on-going activities already occurring (e.g., UFS/AFS)
  - Vetted through internal TAB/Exec team review

Easy to start the process: Visit ATP3.org and fill out an expression of interest form

Initial cohort of support projects includes:

- Novel cultivars for flue gas capture high value products (University of Delaware)
- Carbon management and delivery (LBNL)
- AD with LEA (Cal Poly)
- Feed-forward nutrient control (Penn State)
- VAL for CO_2/O_2 and integrated culture hydraulics and harvest (Searen LLC)
- Rolling acceptance/review of applications

Support may include:

- Biomass (whole, extracted, oil)
- Access to cultivation & downstream equipment (eq. transport, install/removal)
- Access to R&D, production & analytical expertise
- Access to laboratory and office space
- Travel stipends to testbed (currently limited to academic clients)

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Education & Training Workshops

Education & Training

- ATP^3 has hosted 7 quarterly educational workshops
- Over 30 lecture modules
- Over 15 hands-on field site and laboratory activities
- Well attended by broad mix of academic and industrial participants
  - More than 200 participants representing >70 different organizations
  - 35% Foreign participants
- Most recent workshop: Large-Scale Cultivation and Downstream Processing (Nov 3-7th; Mesa, AZ)
- “Principles and Processes: Algae Culture Maintenance, Production and Downstream Processing” planned for May 2015 at NREL and CSM in Golden Colorado

Open Collaborative Testbed: Summary

ATP³ Algae Testbed Public-Private Partnership

Category	CY'12	CY'13	CY'14	CY'15 (projected thru CY 2015)
# Clients	5	18	9	9
# Projects/ transactions	7	19	13	10
Total Testbed Revenue	$48,362	$77,845	$127,893	$165,600 ($250,000)
Average Project Cost	$7,000	$4,100	$9,800	$16,600
Project Type	Biomass Supply (3) Anaytical (1) Consulting/Training (1) Project Demo (1) Equipment Testing (1)	Biomass Supply (4) Anaytical (5) Consulting/Training (2) Project Demo (0) Equipment Testing (1) Cultivation trials (4)	Biomass Supply (3) Anaytical (2) Consulting/Training (2) Process Validation (1) Equipment Testing (2) Cultivation trials (2) Culture Curation	Biomass Supply (3) Anaytical (0) Consulting/Training (1) Process Validation (1) Equipment Testing (2) Cultivation trials (2) Site Access (1)

- The ATP³ testbed network is up and running
- ATP³ completed projects with 30 pay to play customers in last 2+ years
- Streamlined engagement/business processes – can be applied to multiple sites
- **Majority of clients focused on non-fuel applications**
- 5 ongoing/scheduled projects
  - Cultivation trails
  - US and foreign companies paying for direct facilities access
  - Led or teamed on 14 TABB FOA proposals
- Support program underway
  - Great path to scale bench-scale lab results and gain access to facilities. New synergies/new collaborations

- E&T program running well and as planned
- Challenges remain for long term engagement/utilization of the network
  - Market saturation for training workshops
  - Key market segments for use of testbed usually cash poor
  - Access to multiple sites (physical/intellectual bandwidth – not an issue yet with small client base)
  - IP concerns remain a barrier for potential clients

High Impact Data: Long Term Algal Cultivation Trials

ATP[3] sets standards and conducts harmonized, rigorous, and objective long term cultivation trials to provide a realistic assessment of the state of technology for algal based biofuels and bioproducts.

- Our Unified Field Studies (UFS) at the testbed sites along with our Advanced Field Studies (AFS) enable comparison of promising production strains at meaningful scale across variable conditions
- Our Scientific Data Management System and validated, harmonized SOP's for analytical and production processes ensures data integrity across all sites
- Our data from the UFS and AFS will be made publicly available and provide a critical resource to TEA and LCA analysis yielding **high impact, validated data**

High Impact Data: Long Term Algal Cultivation Trials

Task 2: High Impact Data

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Field Studies for High Impact Data

- Unified = All testbed sites performing the same experiment in the same systems with the same protocols and strains simultaneously

Cellana UFS ponds

- Advanced = Sites with various capabilities will test additional production methods and variables to provide data to further enrich the model inputs

Cellana Large Scale Ponds

Setting Standards – Method Harmonization: Production and Analytical

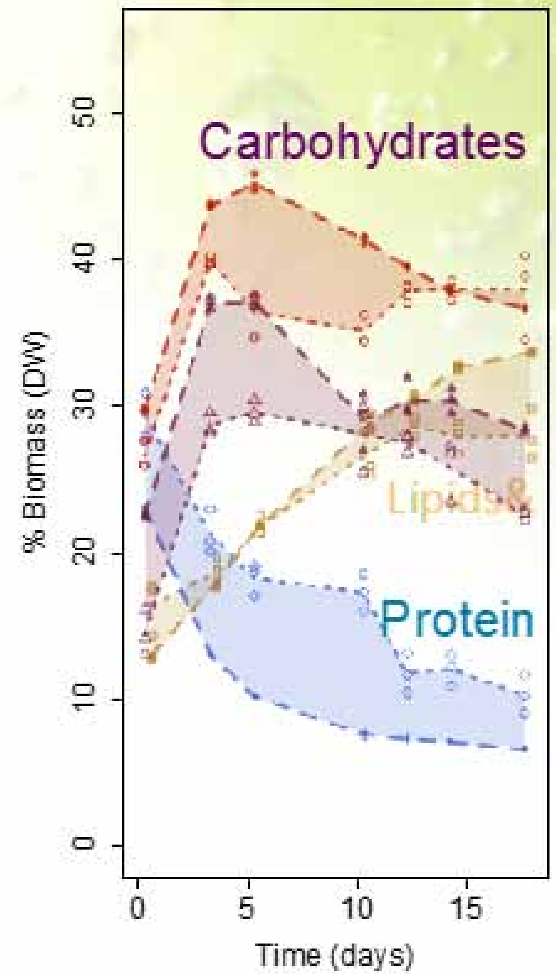
Challenges to multi-site comparisons…

- Analytical method variation (method, operator skill, etc), can make evaluation of and proximate composition across sites challenging. This can have a real impact on TEA, LCA and resource assessment - and thus productivity!
- System and scale variation has the potential to induce unwanted, non-geographical related variability between testbeds as a function of:
  - system design
  - scale of operation
  - source water/nutrients
  - sampling protocols
  - productivity measurement protocols
  - operator skill/training/experience/consistency
  - Other…

Compositional Analysis for Algal Biomass

- Underpinning of productivity calculations, used for LCA, TEA and RA models
- "How hard can it be?"
  - Mass balance closure of 60 – 120%
- Tracking metrics for improvements in costs and productivity need better methods
- Historical methods utilized for algal biomass and process highly variable (12-18% RSD), often not accurate
- Lack of standardized analytical procedures specifically tailored for algal biomass

Fuel	**Yield**
Lipids (kg/ton)	255 ± 51
Green Diesel ($/gallon)	**12.1 ± 2.4**



Anal. Biochem. (452) 86-95 (2014)

Mitigation of Site-to-Site Variance (Systems and Processes)

Harmonized Systems via

- Uniform design of indoor seed cultivation (800 ml columns and 2’x2’ flat panels)
- Uniform design of mini-pond system
- Uniform (and automated) water quality monitoring on production units (YSI)
- Uniform light intensity measurements through adoption of same - LiCor LI190 PAR Quantum Sensor (integrated into YSI units)

Harmonized Processes via

- Rigorous verification and validation of analytical and production methodologies
  - Biomass productivity - AFDW, OD, Nutrients, etc.
  - Biomass composition – Ash, Total FAME, Total Protein, Total Carbohydrate
  - Rigorous verification and validation (round robin) framework implemented
- Indoor and outdoor cultivation SOP’s (pond cleaning, inoculation, sampling protocols, nutrient adds, transfers/splits), Detailed analytical SOP’s
- Standardized data reporting in version controlled and locked down spreadsheets
- Scientific Data Management System

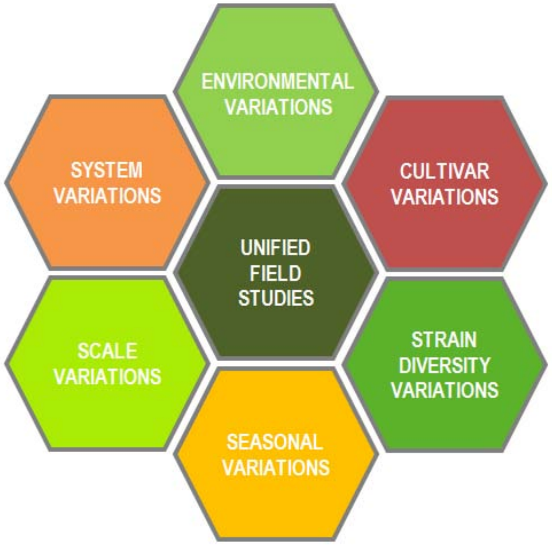
Harmonization Framework 2013 – Anticipate Unified Field Studies (UFS)

Outline

- Round Robin Experiments between participating laboratories using designated ***standard biomass sample*** (reference material, *Nannochloropsis* sp., ~5 kg lyophilized)
- Statistical interpretation and setting QC requirements of measurements for the duration of ATP^3 UFS

Questions

- Which methods should be used for compositional analysis?
- What data format is used for data collection?
- What are the checks/QC in place to make sure analytical and production methods performed ok?



Productivity metrics:

- Volumetric dry weight
- Ash Free dry weight
- Lipids
- Carbohydrates
- Protein

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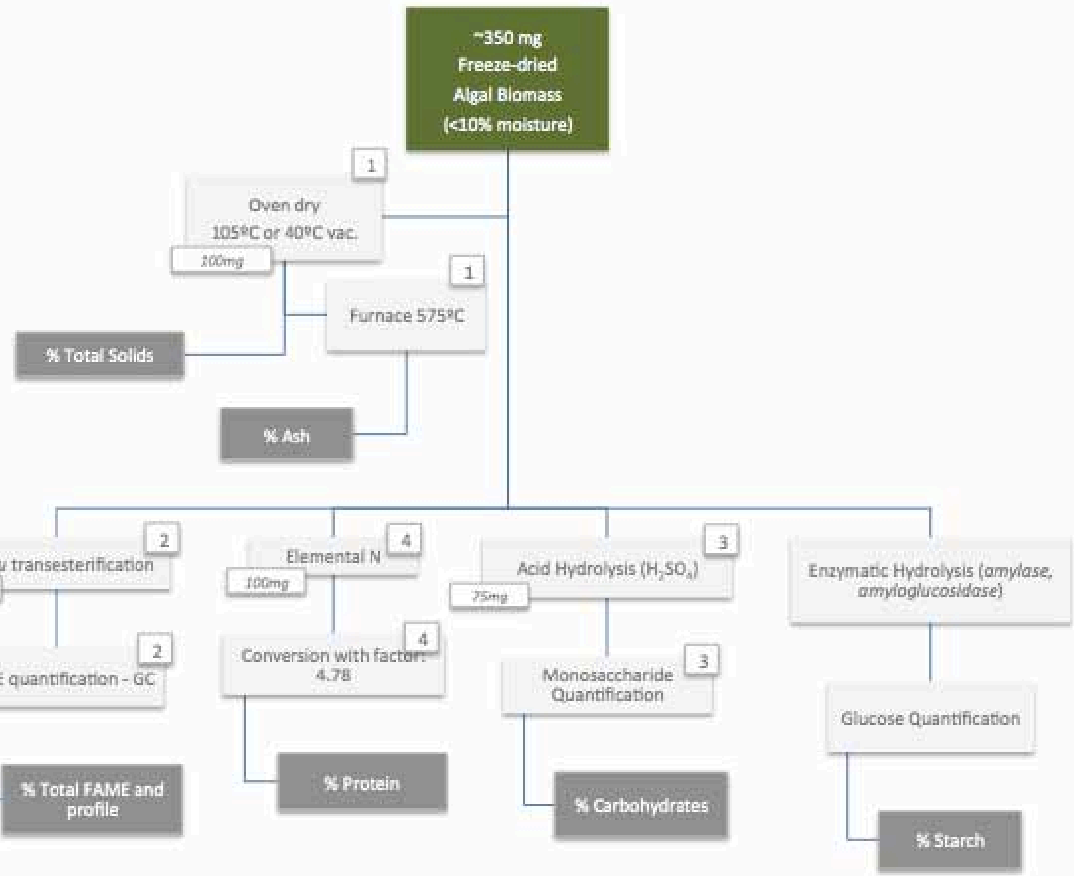


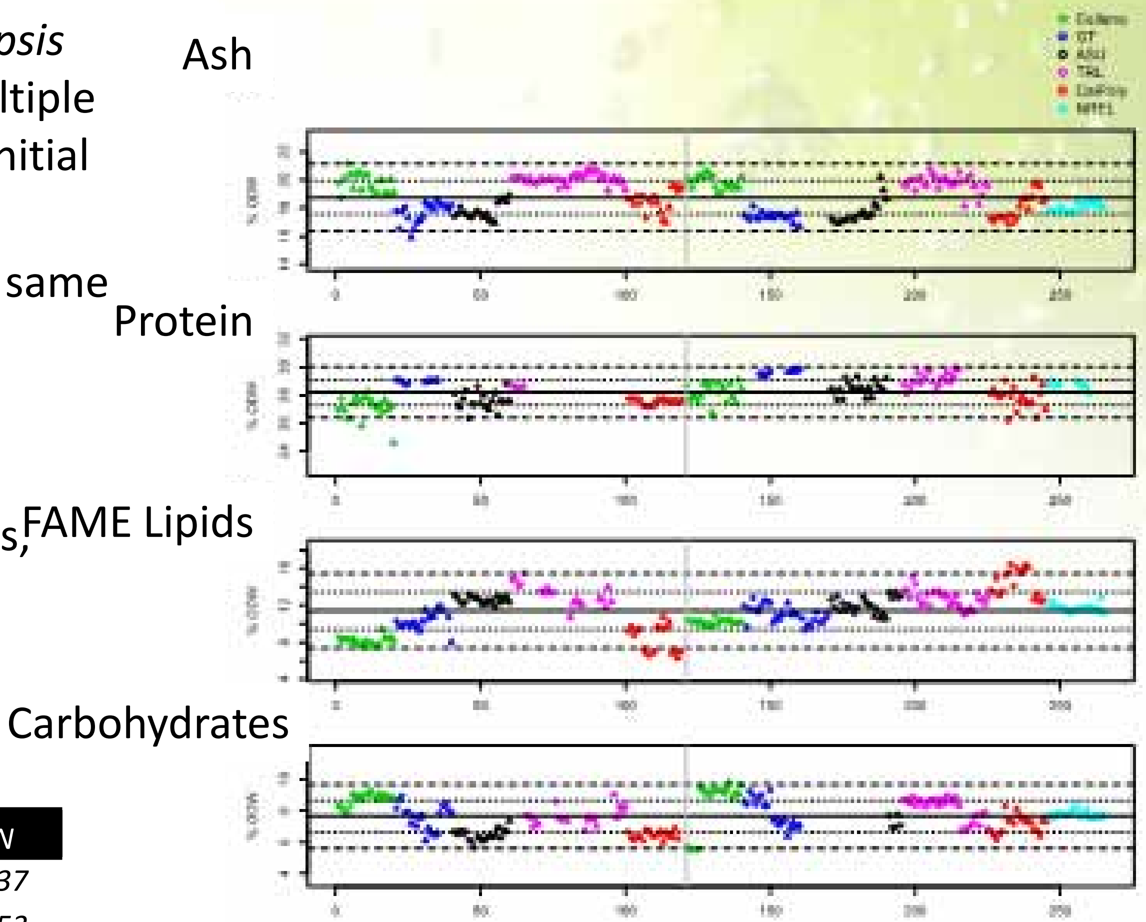
Figure 1: Illustration of summative algal biomass analysis and reference to individual LAPs for analysis details. Procedure and biomass quantities shown for process with in situ transesterification as lipid quantification procedure. Numbers reference individual LAPs identified on page 3

Harmonization Round Robin Data
Two-phase approach Summer 2013

- Reference biomass *Nannochloropsis* sp. material characterized by multiple laboratories, representative for initial production trials
- Total of ~1000 measurements of same biomass sample

(Thank You Site Analysts!)

- Precision targets <10% RSD met between replicate measurements, except for Carbohydrates



	mean	sd	RSD	*N*
Ash	**18.8**	**1.2**	**6.4**	*237*
Protein	**28.2**	**0.8**	**2.7**	*153*
FAME Lipids	**11.6**	**1.2**	**10.6**	*191*
Carbohydrates	**7.7**	**1**	**12.5**	*198*

Inoculum Production Process

Initial culture density ~0.1-0.2 g/L
Average growth rate ~0.3 g/L/day
Growth period is 9-10 days
Final culture density ~**3.5 g/L**

1 to 1 transfer

Column to panel

Initial culture density ~0.2 g/L
Average growth rate ~0.15-0.2 g/L/day
Growth period is 9-10 days
Final culture density ~**2.0 g/L**

12-2’x2’ panels yield provide biomass for a minimum target of **0.05 g/L** (AFDW) initial mini-pond concentration (sites have 14 available)

Standardization of processes and systems is key to executing meaningful multi-site cultivation trials

UFS Strains/Cultivar: Primary

UFS Strains

- *Nannochloropsis oceanica* (KA32), supplied by Cellana
  - Distributed to all sites fall, 2013. Utilized in *"UFSBaseline"* validation for P1 Go/No Go
  - Not expected to be high productivity but known stable and robust cultivar especially for inland sites – key for establishing baseline seasonal/regional performance
- *Chlorella vulgaris, (*LRB-AZ-1201) supplied by ASU
  - Distributed to sites June, 2014, deployed to field Summer 2014
  - Known to be less robust (open pond), but available and importable to HI, high performer in PBR's
- Representative cultivars for fuel and high value production (feed, omega-3's)
  - Substantial historical data for both strains (faster project startup)
  - Unencumbered with little restrictions on strain/biomass distribution to third parties
- Additional strains to be used for AFS (and as part of UFS in 2015)
- Overall UFS/AFS Experimental Plan was reviewed and approved as part of Go/No Go Jan '14
  - Each season has a specific experimental plan documented with all details including any updates to SOP's etc. Reviewed with team prior to start of experimentation.
  - Significant review and update to plan for year 2 (Spring 2015 through Winter 2015) based on first year results (TAB members engaged for comments) – additional tweaks ongoing based on results to date and availability of additional well characterized strains from other DOE funded efforts

2014 Seasons: Unified Field Studies

	Spring													Summer													Fall													Winter												
2014/2015 Unified Field Study	M				A				M					J				J				A					S				O				N					D				J				F				
Experimental Outline	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	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52
Seed production/pond prep																																																				
Nannochloropsis Only																																																				
Strain switch																																																				
Chlorella Only																																																				
Data sharing/reports																																																				
One strain in panels, Other strain in columns																																																				

- Prime Goal for year 1 UFS trials: Establish Seasonal/Regional Baseline for primary cultivars
- Season = 12 weeks
  - Target of 10-11 weeks dedicated to cultivation in ponds
  - Seasons ran two strains in series
  - 2 weeks for initial grow out, and a target of min of 3 weeks of cultivation per strain after growout. Run under conditions where N:P and C_i not limited
  - <1 week to turn ponds for next strain
  - Repeat

2014 Seasons: Unified Field Studies

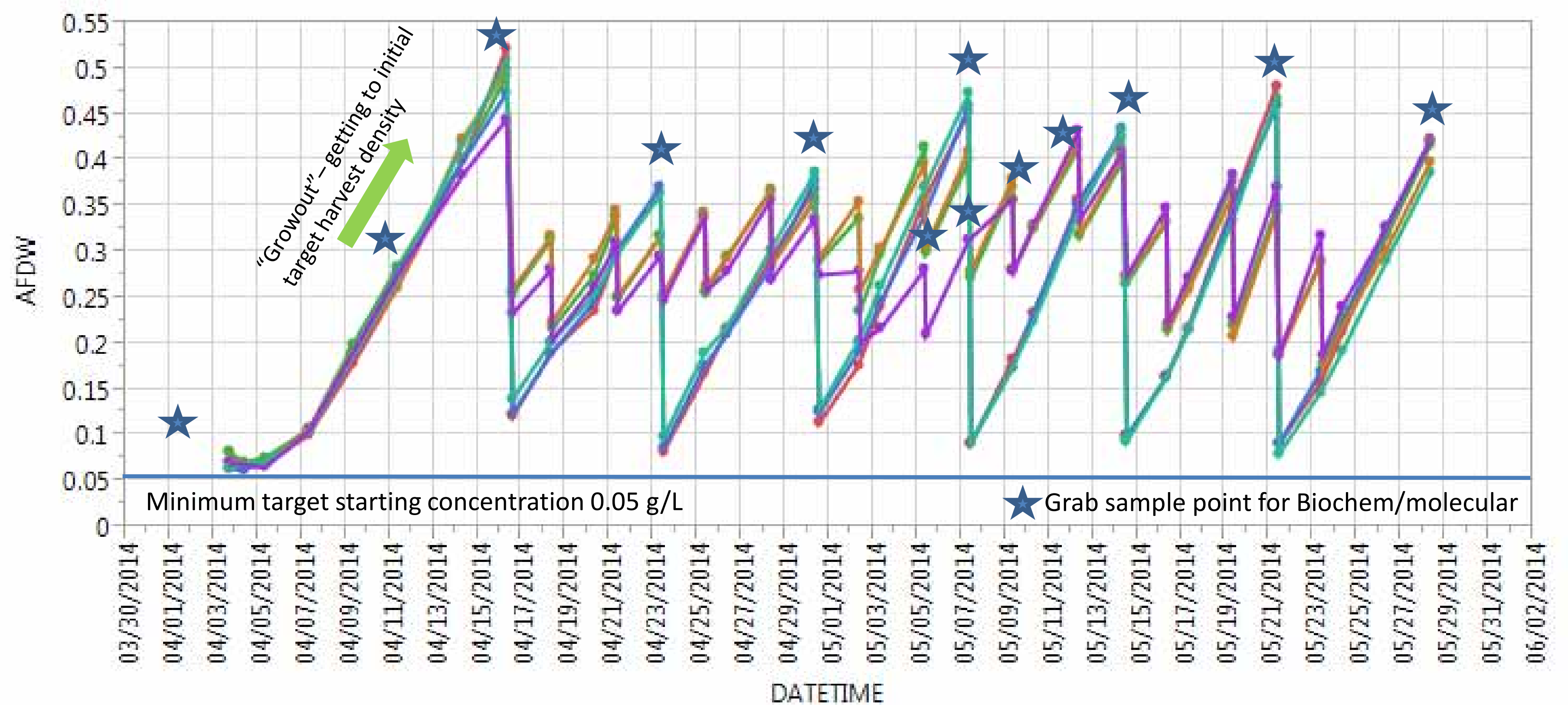
- Spring – 2 different harvest frequencies but same average dilution rate (75% 1x week, 25% 3x/week) - ~0.1/day
  - Spring run was mainly set up as a practice to firm up harvesting logistics at the sites
- Summer – 3 different dilution rates (site specific), 2 harvesting frequencies (1x/wk and 3x/wk)
  - Empirically driven set points for dilution rates
  - Need to push towards more model based setpoints
- Fall – pulled back to only two dilution rates
  - Dilution rate confounded with harvest frequency
  - D = 0.1 (75% 1x/wk); D = 0.2/day (50% 3x/wk)
- Winter - two dilution rates
  - KA32: D = 0.1 (75% 1x/wk); D = 0.2/day (50% 3x/wk) or less do to low productivities for KA32
  - Chlorella - shift to 3x/wk and two different dilution rates (preview of operational baseline for 2015)
- Carryover condition season to season for Yr 1 UFS runs was the 75% 1x week (D = ~0.1/day)

Standard Experimental Conditions and Sampling

Factors	Set Point
Aqueous N (µM)	2200 (136.4 mg/l)
pH (Nanno)	7.9
pH (Chlorella)	7.9
Depth (cm)	25
PW speed (Hz)	20
Inoculum (g L^{-1})	0.05

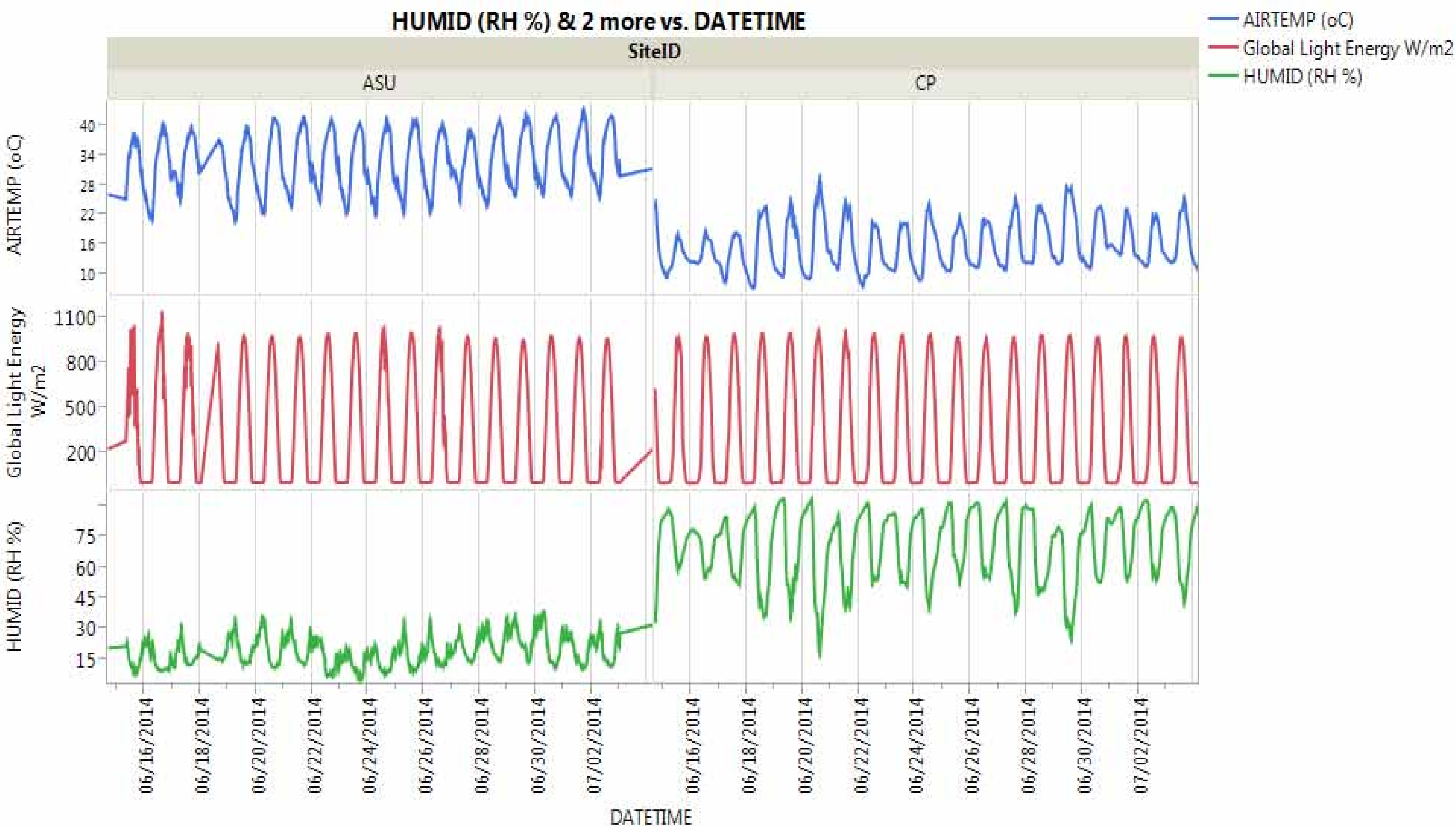
Samples	Schedule
OD@ 750nm	Dawn +60 min, M – F
DW	Dawn +60 min M, W, F, T0, TF
AFDW	dawn +60 min M, W, F, T0, TF
Mass balance (lipid/FAME, carbs, starch, protein). Parameter must have an AFDW associated with all samples	T0 (inoculation or directly post-dilution), TF (prior to a harvest or dilution occurring) – samples MUST be taken within 1 hour of AFDW/OD or a new AFDW sample is required
Nutrients	Dawn +60 min M, W, F, TF
Weather data	Real time (hourly)
In-situ sensors	Real time (15 minute intervals)
Microscopic exam	dawn +60 min M, W, F
Genetic Analysis, qPCR	Weekly, upon pond health decrease,
Manual checks (pH, temp, salinity, depth)	Daily; AM and PM
% Shading	Monthly; AM, Mid, PM
Water chemistry	Monthly ICPMS testing

Standard Experimental Conditions and Sampling

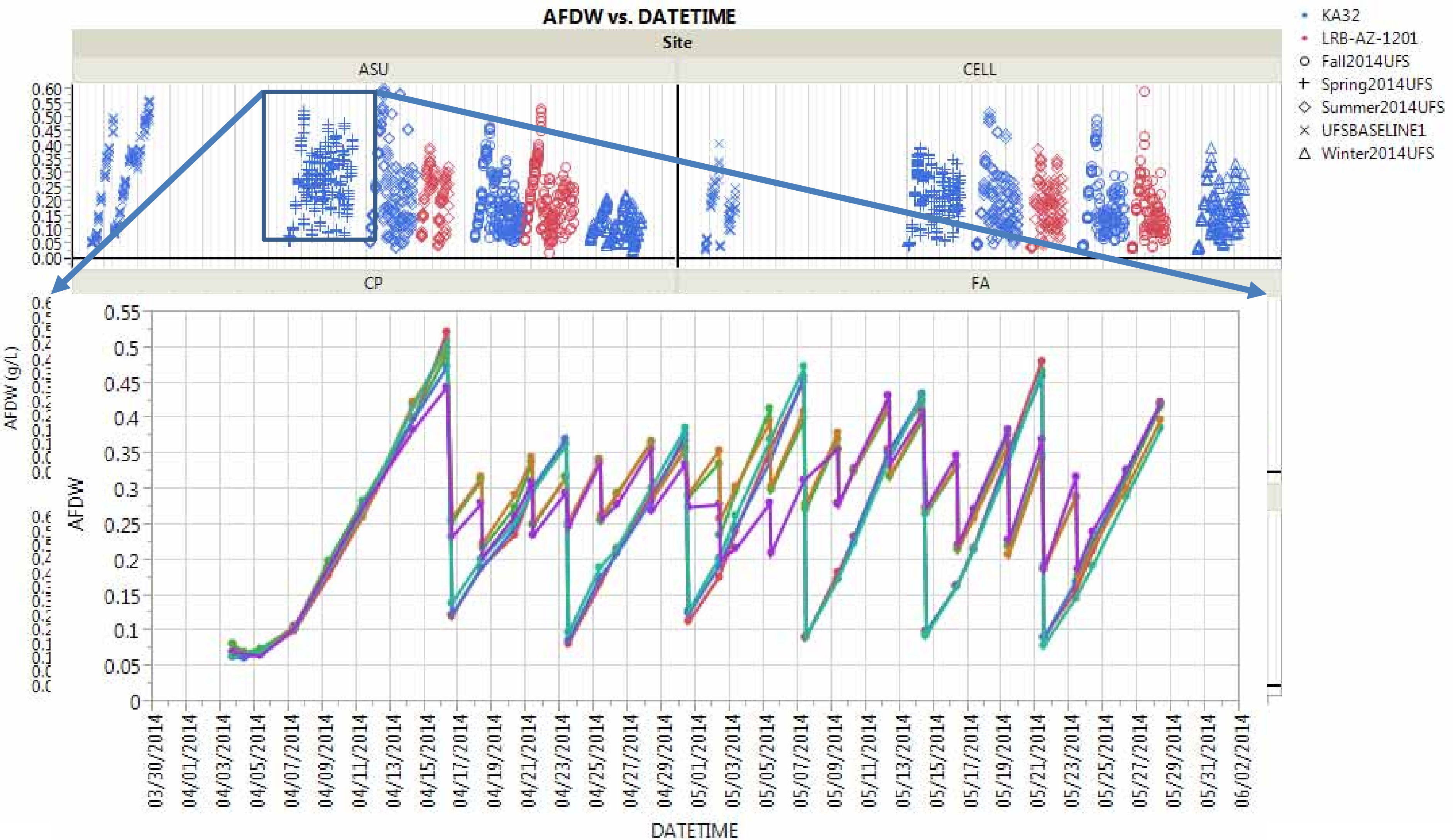


Standardized Production Spreadsheets for capturing grab sample (discrete) as well as continuous (weather, YSI) data for eventual upload to SDMS. Operational summary info/meta data and empirical observations captured. Continuous improvement based on site feedback to spreadsheets to enable more streamlined and realtime QA/QC of data.

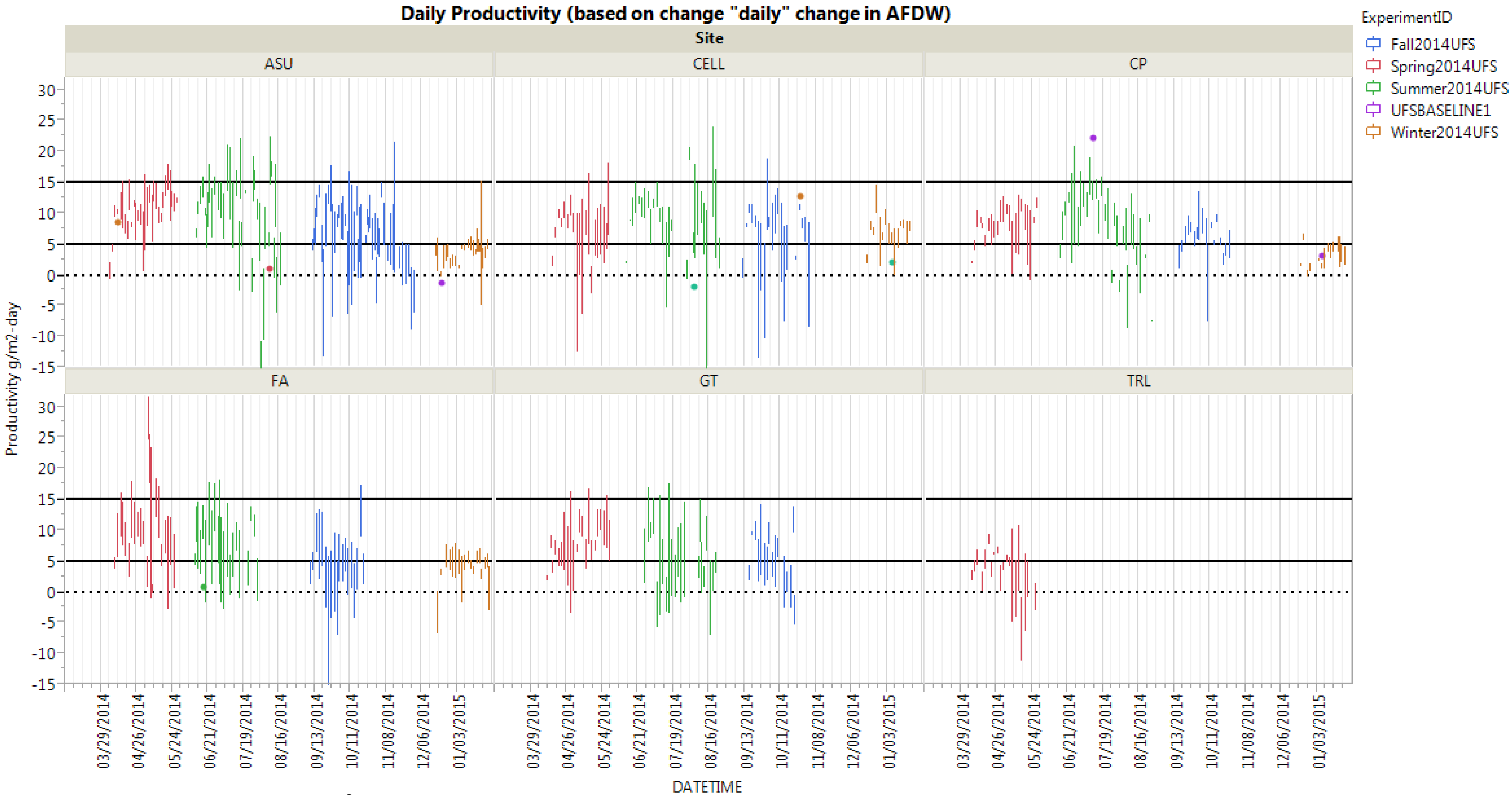
Standard Experimental Conditions and Sampling



Year 1 of the UFS – What did it look like?



Daily Biomass Productivity: g/m²-day



Daily Productivity (g/m2-day)

Based on change in total biomass in pond between AFDW data points (1-2 days typically) (area 4.2 m^2, volume 1025L at 25 cm)

UFS Data Reporting

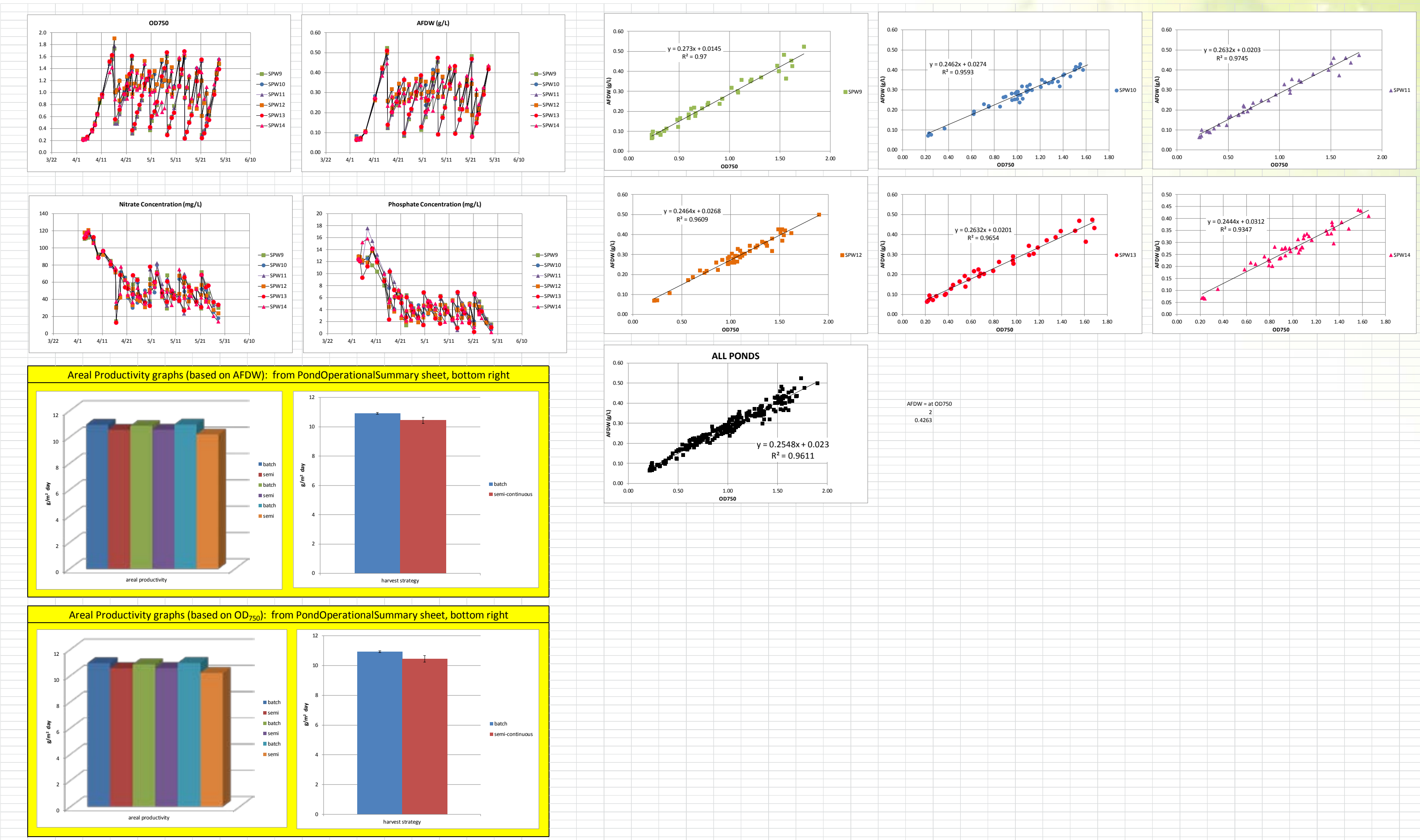
Improve spreadsheets to provide useful feedback

Response to pond operators needs to see data as it is collected

- On-the-fly data graphing for each pond
  - OD_{750}
  - AFDW
  - OD_{750} vs AFDW correlation
  - Nitrate and Phosphate levels
  - Ongoing harvest productivity
- Allows some data QC as data is entered
  - Outliers/errors easy to spot immediately
- Also may allow early trend spotting—perhaps for decision making in pond operations once we go into AFS

UFS Data Reporting

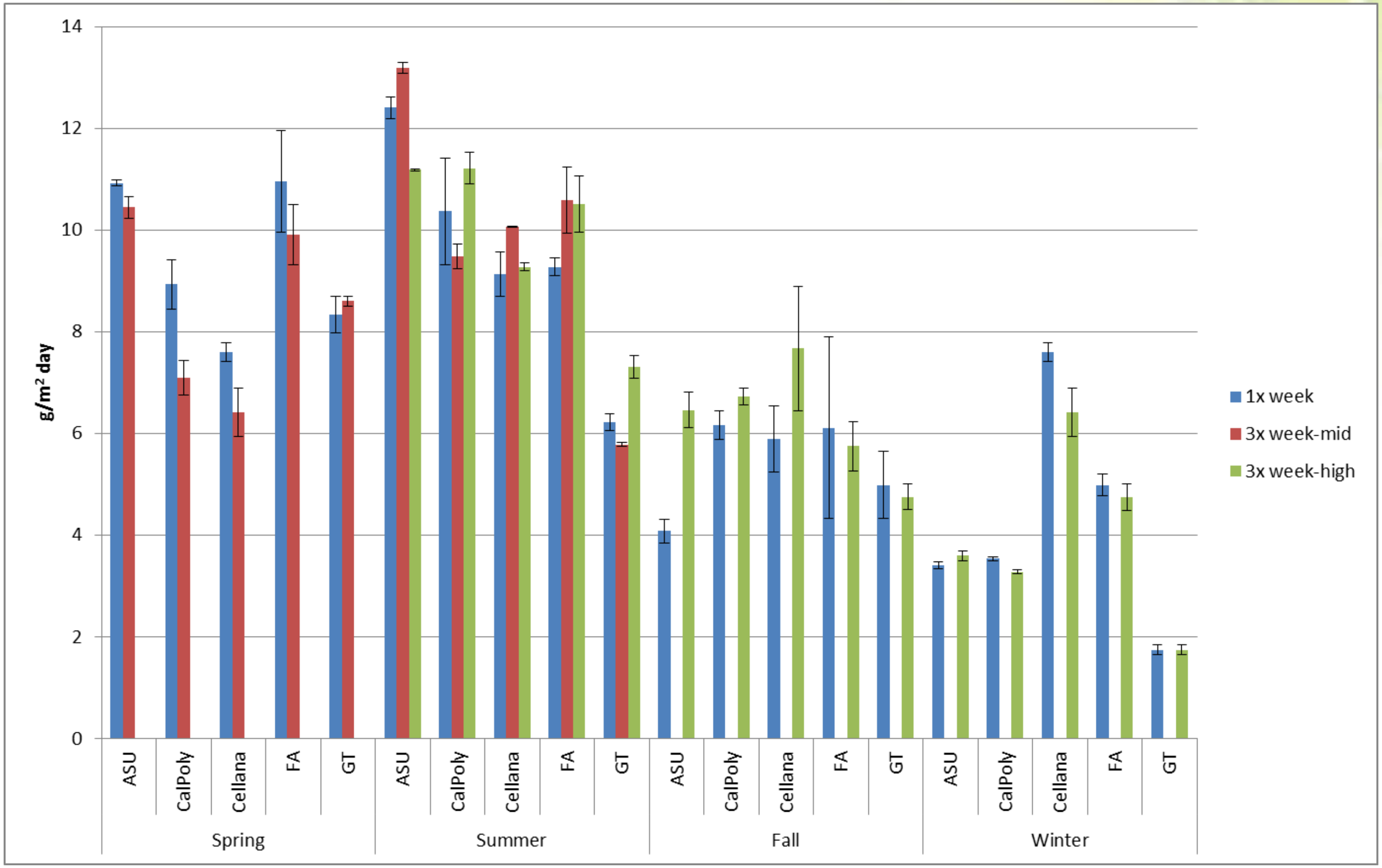
Real-time graphing displayed on GRAPHS tab



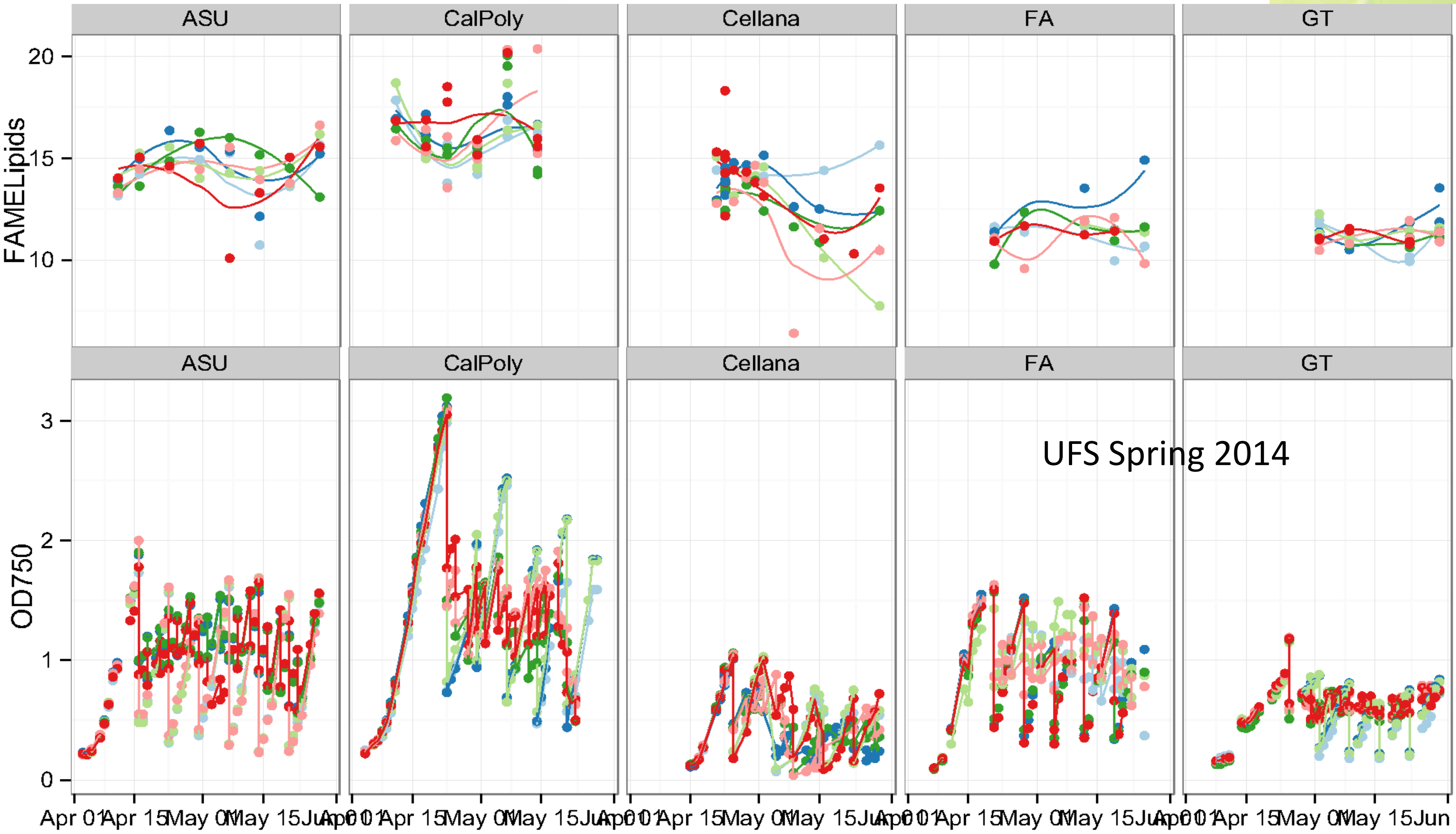
Harvested Biomass Productivity

Productivities from all sites for an entire year of UFS production runs

Nannochloropsis Oceanica (KA32)



Compositional Analysis During UFS: Total FAME and EPA



Harvested Biomass Productivity

Productivity Assessments

- Seasonal variation was clearly the largest determinant of productivity
- Ranged from 7 to 13 g/m^2 day for Spring/Summer, to <2 to 7.5 g/m^2 day for Fall/Winter
- Particularly difficult winter for GT/FA with multiple excessive rain events and freezing temperatures
  - GT had only 1 harvest for Winter Nanno run
  - Too cold to run Chlorella
- Seasonal differentials spring/summer to fall/winter lower than the >5 assumed in harmonized baseline

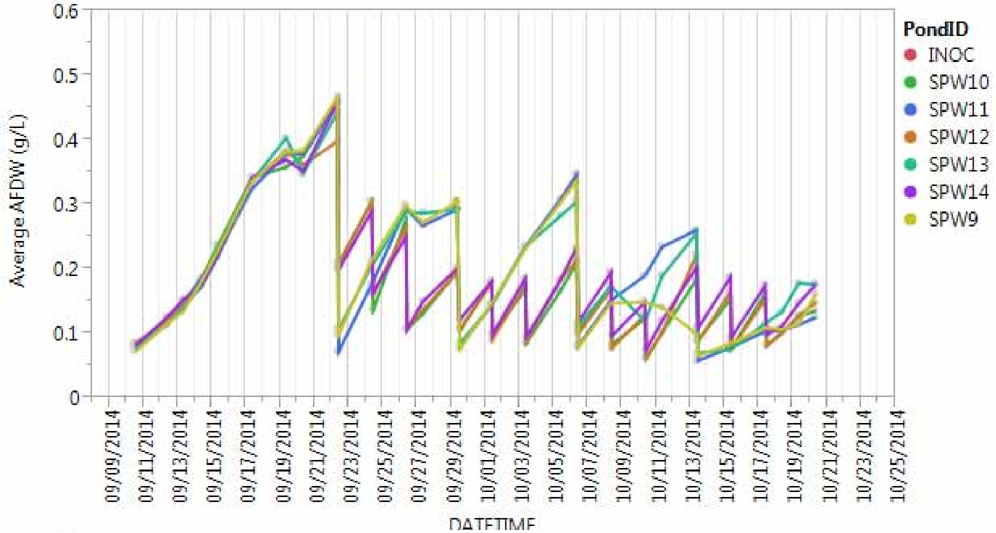
	seasonal ratio
ASU	3.3
CalPoly	3.0
Cellana	1.7
FA	2.2
GT	4.5

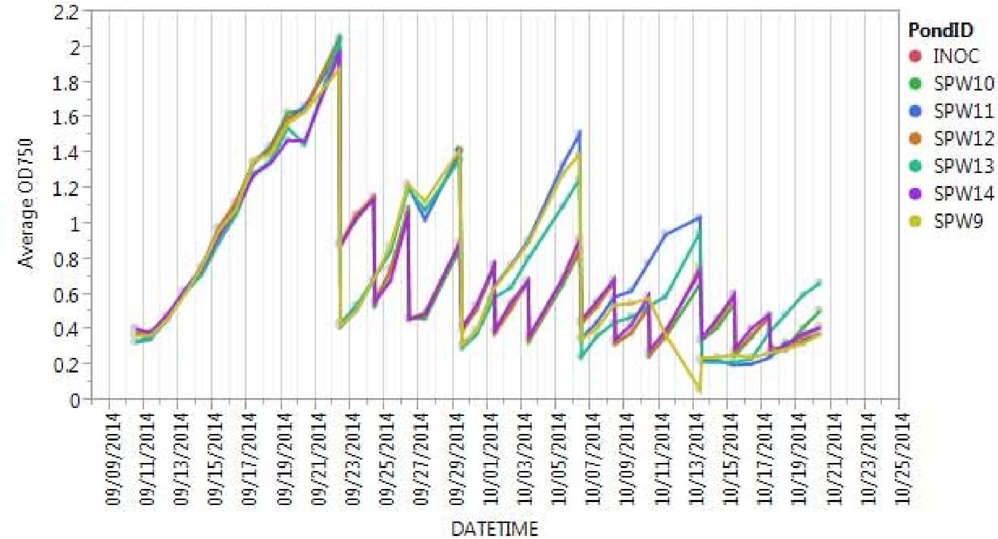
Harvested Biomass Productivity

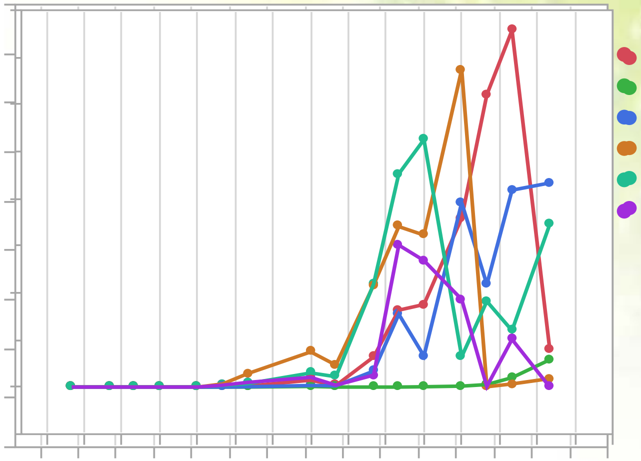
Techno-Economic Modeling (look ahead)

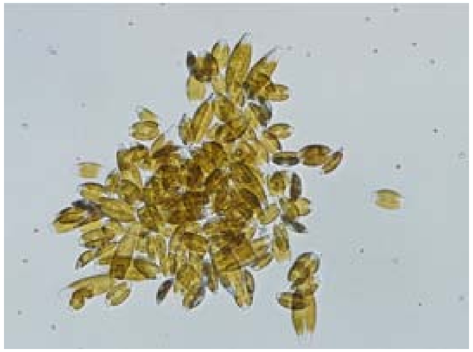
- Formal TEA modeling will be conducted in Q4 FY15 to consider full year's productivity performance at all sites
- Results of Q4 TEA modeling will be leveraged to establish state of technology assessment for algal biomass production
  - SOT benchmarks will be incorporated into NREL's milestone work and are critical metrics for BETO in updating MYPP projections
  - TEA models are strongly dependent on biomass productivity, composition, seasonal variability; FY15 SOT will represent the first formal benchmark to better answer "where are we today" for these parameters relative to future targets for cost viability
- Also maintaining active communication with external modeling stakeholders (ANL – LCA, PNNL – Resource Assessment, TEA) to ensure pertinent data are being collected to meet the needs of other models

Challenges for KA32



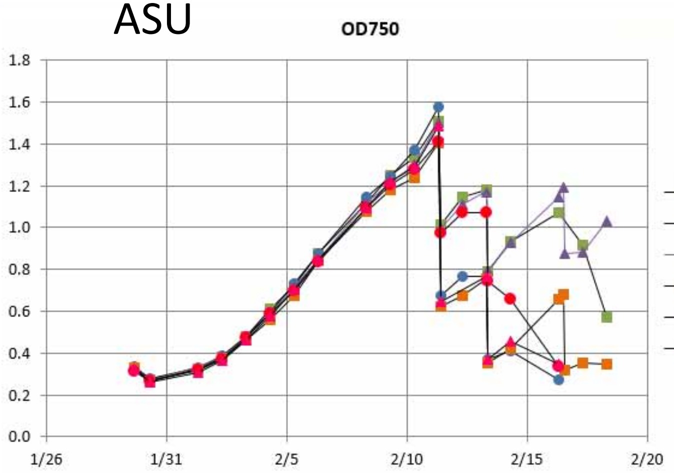




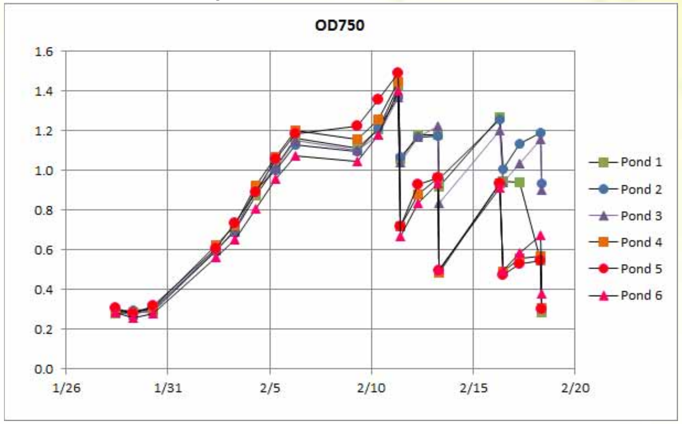


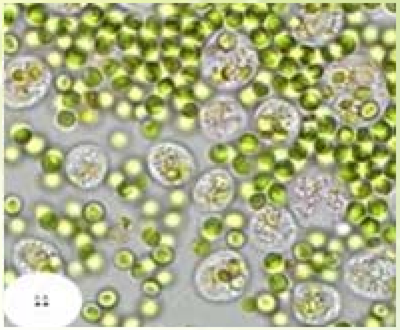
Challenges for LRB-AZ-1201

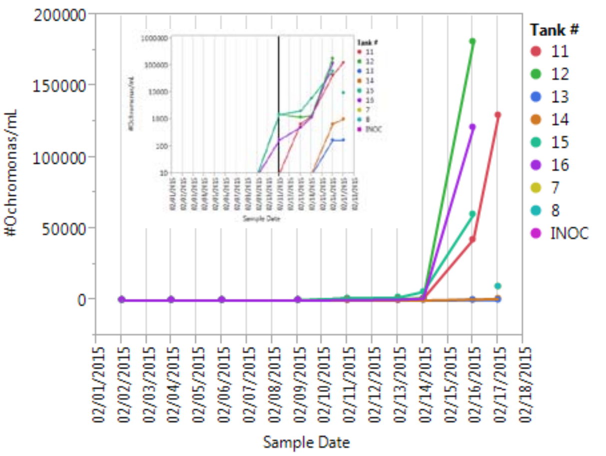
ASU

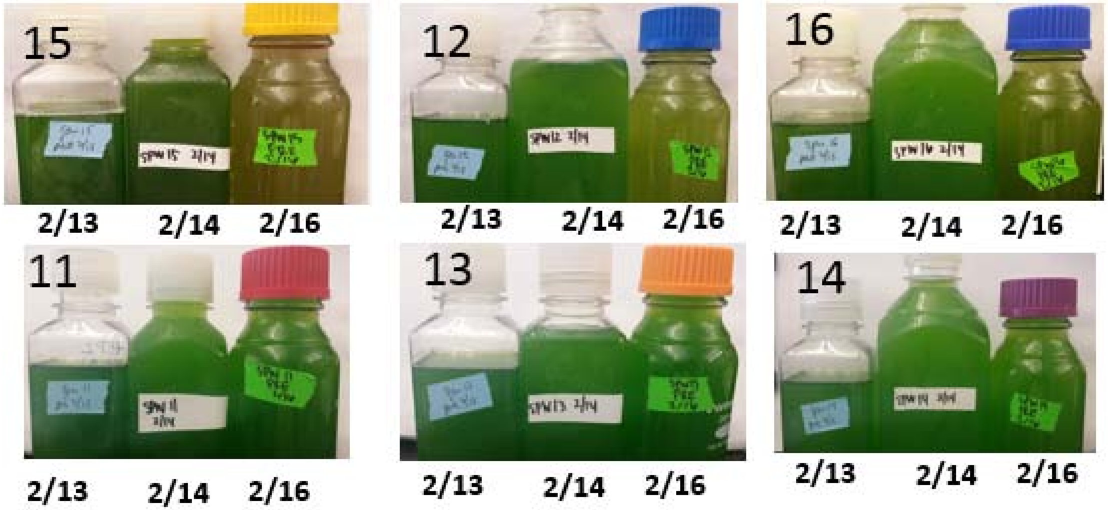


Cal Poly



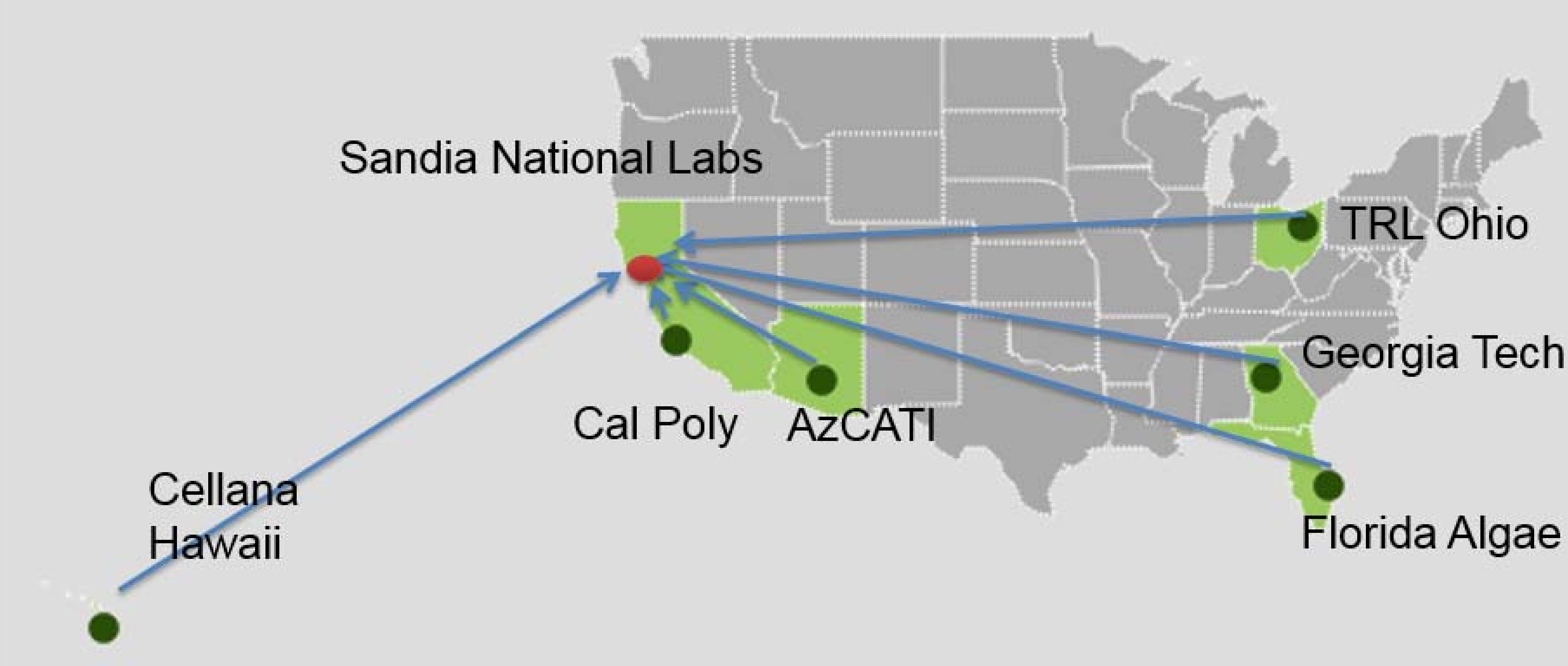






First counts for pond 12, 15 and 16 on 2/11
First counts for Pond 11 on 2/13
First counts for Pond 13, 14 on 2/16

Advanced Diagnostics:
Metagenomics of ATP³ Pond Samples

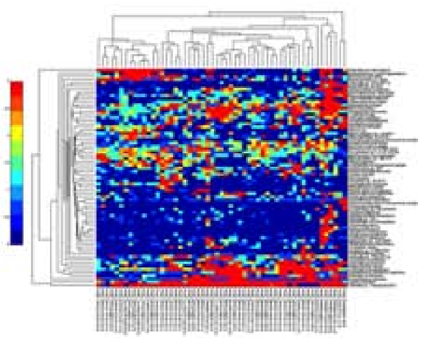


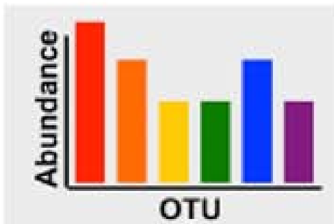
Sample acquisition

- Harvesting by centrifugation: 50 mL samples
- Resuspension in RNALater
- Storage at 4 overnight followed by -20
- Shipment on wet ice.

16S/18S Metagenomics workflow

Microbial community sample → Extract DNA → PCR amplification indexing and library preparation → 16S based sequencing → Analysis pipeline

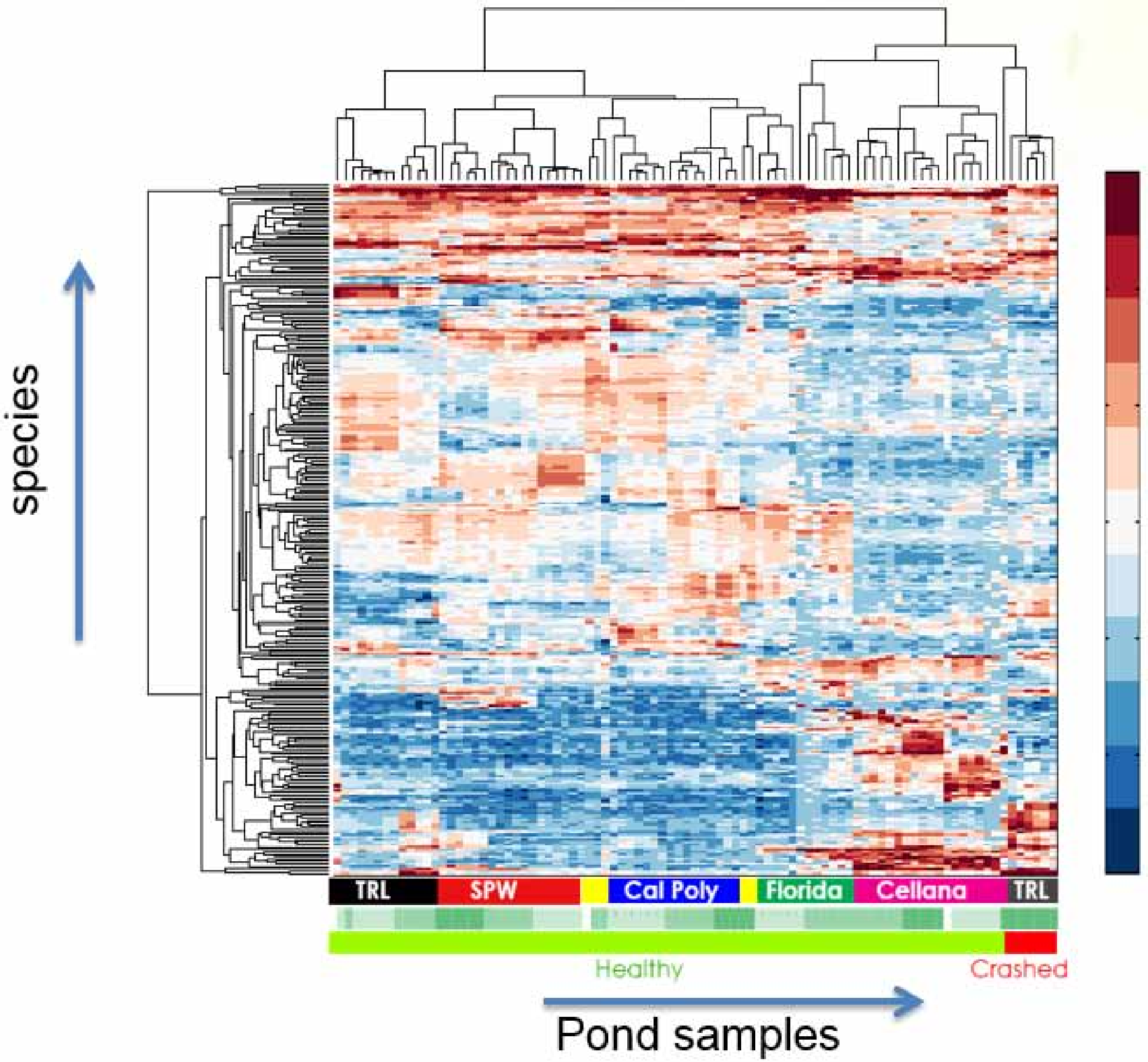




Relative abundance of OTUs in community

Quality filtering → Dereplication and finding DE novo OTUs → Use database Green Genes and Silva to identify OTUs → Community composition of organisms

16S Analysis of spring samples



- Meta Summary for spring data from all sites
- Heatmap was made from clustering the classified OTU abundances for both species and samples.
- The Ponds from the same site cluster together, as do healthy vs. crashing ponds

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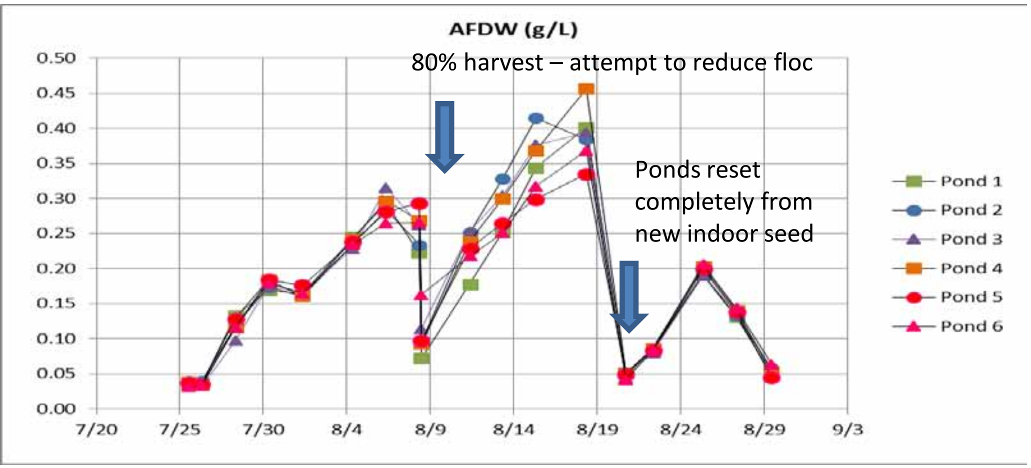
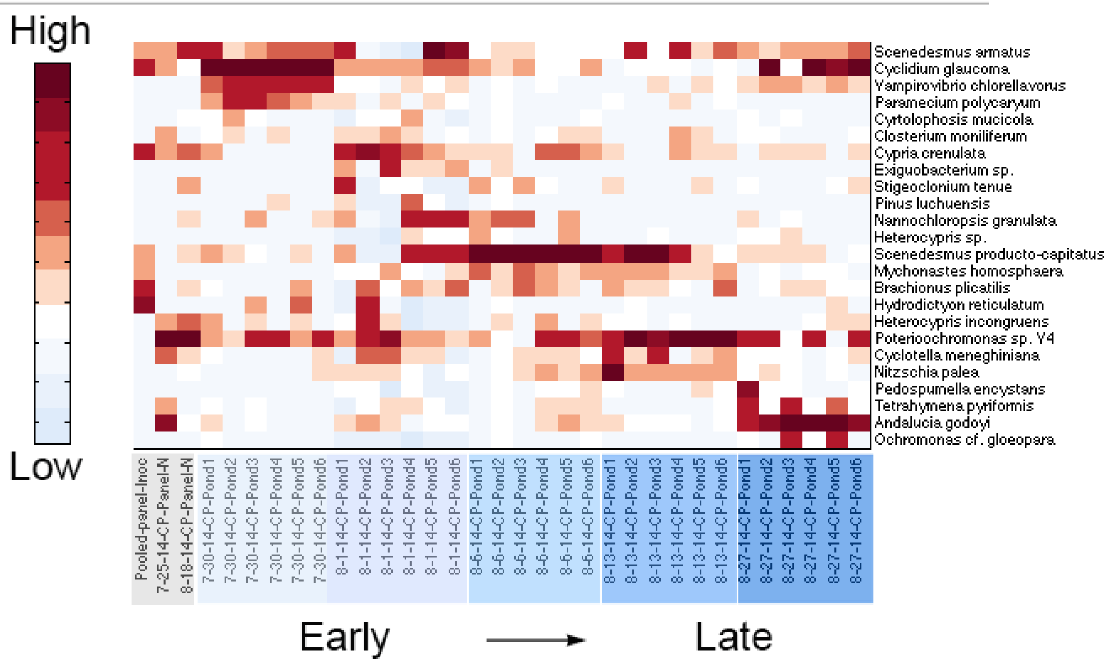
Genetic Identification of Eukaryotic Contaminants





Suboptimal pond performance as indicated by AFDW and O.D. and physical observation - ponds flocculating

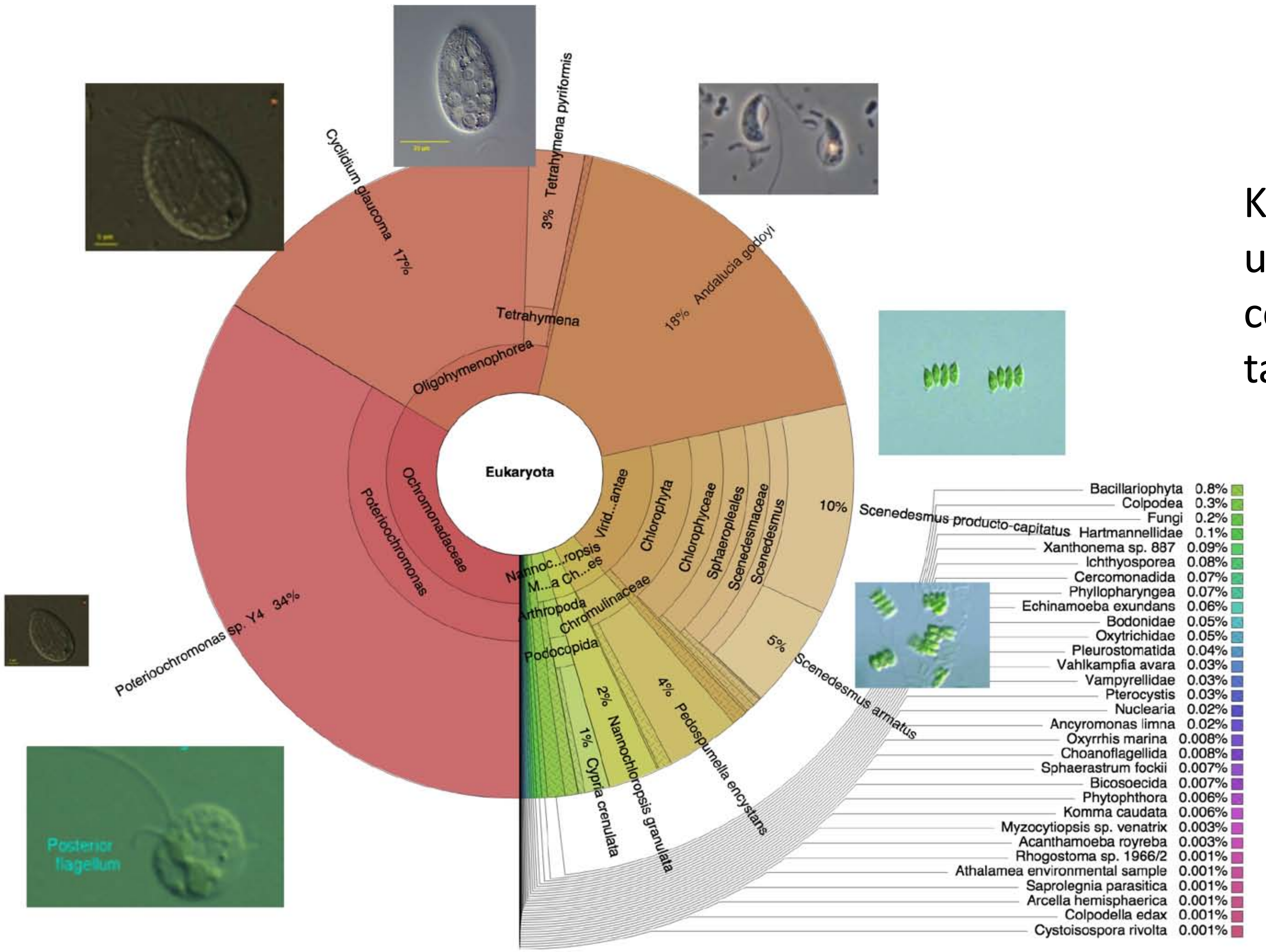
Contaminants were observed by microscopy.

Heat map of read abundances
In Illumina sequencing library
- Rough estimate of species abundance
- Not dependent on any *a priori* knowledge

Caveats:
- several potential sources of bias can distort abundances
- Presence does not imply causality

Identified conntamination by ciliates (*Cyclidium glaucoma*), chrysophyte (*Poterioochromonas sp*) and flagellate (*Andalucia godoyi*).
Scenedesmus and a few diatoms

Phylogenetic Breakdown of Eukaryotic Contaminants



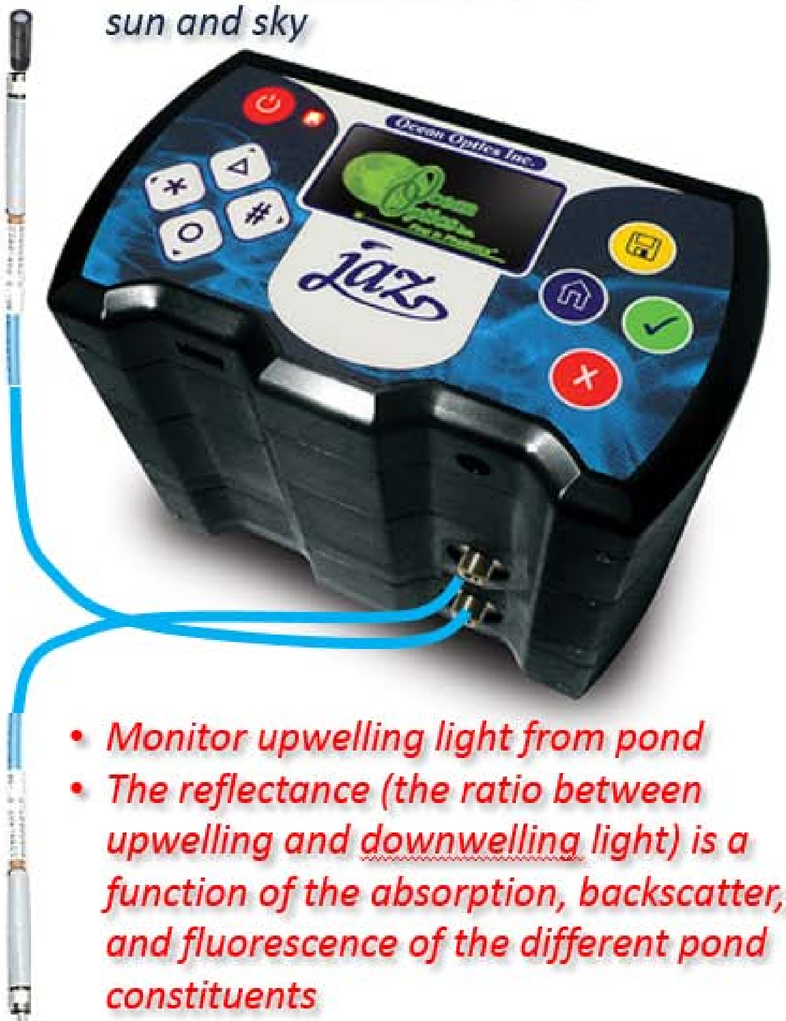
Krona chart provide a user interface to examine community structure and taxonomic breakdown.

Advanced Diagnostics Summary

- Workflow for sequencing high throughput 16S and 18S metagenomic samples established
- New bioinformatics software pipeline which is
  - fast and efficient to carry out the analysis and producing user friendly outputs
  - pipelines used and developed:
    - MAGPie (Metagenomics and Amplicon Sequencing Pipeline)
    - & RapTOR (Rapid Threat Organism Recognition)
- In process - developing and improving our data-mining tools for in-depth analysis of these datasets
- Progressing through summer and fall samples now
- Data sets will be made available with the cultivation data

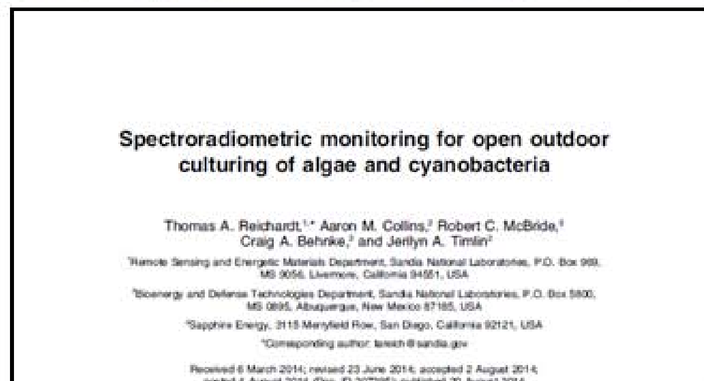
Realtime Monitoring:
Implementing Sandia National Labs' Spectroradiometric Monitoring Approach

- *Monitor downwelling light from the sun and sky*



- *Monitor upwelling light from pond*
- *The reflectance (the ratio between upwelling and downwelling light) is a function of the absorption, backscatter, and fluorescence of the different pond constituents*

- *A detailed reflectance model has been developed for interpretation of wavelength-resolved data*



Spectroradiometric monitoring for open outdoor culturing of algae and cyanobacteria

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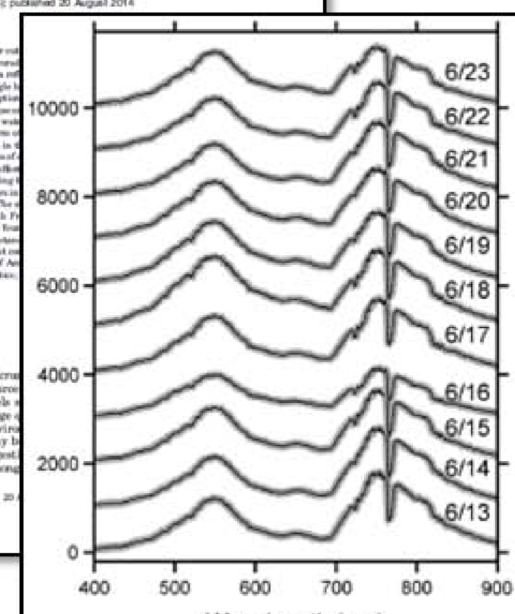
1. Introduction



ATP^3 Deployment: Monitoring Six Mini-Raceway Ponds Simultaneously

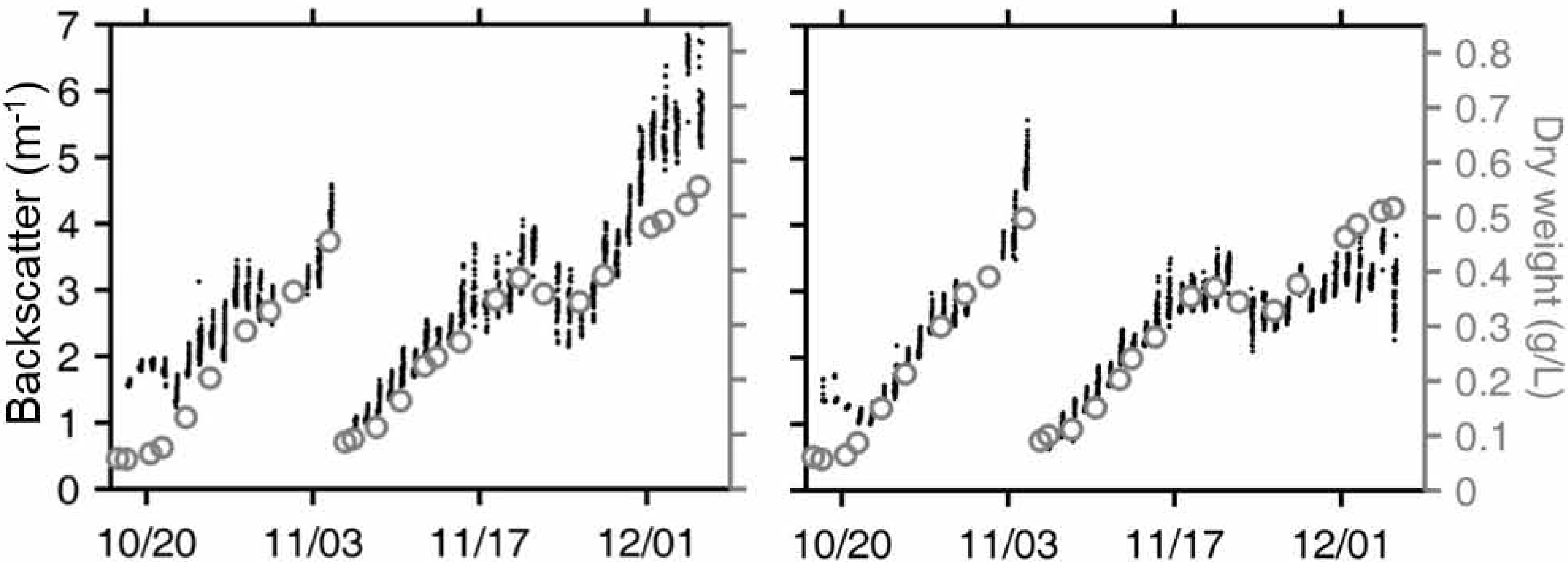
- *Spectrally resolved (400-1000 nm @ 0.3-nm resolution) measurements acquired on 6 ponds at 5-min intervals*
- *Instrument accessible via internet*

Monitoring downwelling irradiance

Instrument housed inside temperature-controlled enclosure

Monitoring upwelling radiance from all six mini-raceway ponds

Analysis of ATP^3 Data for Two Mini-Raceway Ponds



Culture backscatter scales with dry weight measurements

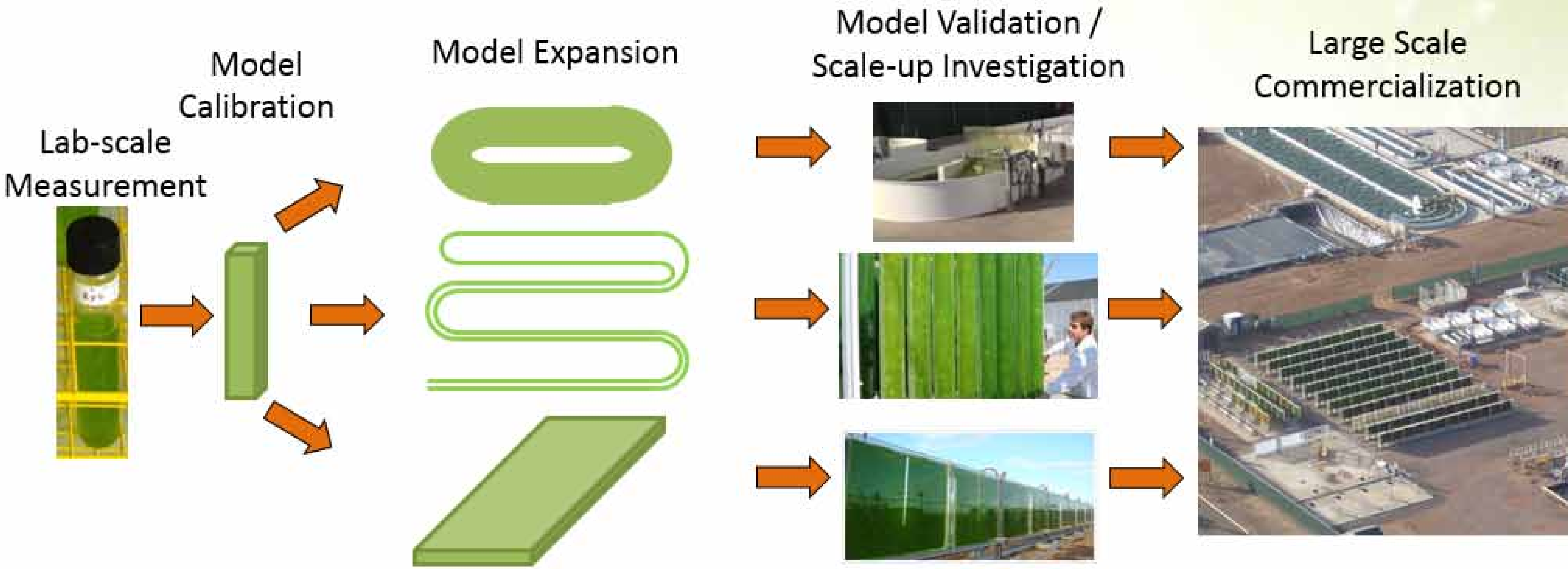
Backscatter could serve as a proxy for biomass, contamination detection

But challenges persist both early and late in the growth cycle

Additional project resources allocated to continue to advance this technique

Physics Based Dynamic Modeling: We Need Models!

- We need to be able to optimize algae growth and lipid production in large commercial scale systems
- It is too time consuming and expensive to test various solutions on a commercial scale
- A computational model facilitates faster and cheaper optimization



Lab-scale measurement and model images

Algae cultivation systems

Algae Growth Model Overview

Predict algae production based on:

- Algae strain
- Light intensity (depth dependent)
- Temperature
- Nutrient concentration (N, P, and CO_2)
- pH
- Salinity
- Respiration

Governing Equation:

- Biomass concentration, B
- Production rate, P
- Basal metabolic rate, B_M
- Predation rate, P_R
- Biomass source or sink, B_L
- Maximum instantaneous production rate, P_{max}
- Productivity Limitation functions, $f_{1\text{-}5}$

$$\frac{\partial}{\partial t} B(\mathbf{x},t) = \left(P - B_M - P_R\right) B(\mathbf{x},t) + \frac{B_L}{V}$$

$$P = P_{\max} \cdot \left[f_1(v)\, f_2(I)\, f_3(T)\, f_4(S)\, f_5(pH) \right]$$

$$f_3(T) = \begin{cases} \mathrm{e}^{-k_{T1}\left(T - T_{opt,1}\right)^2} & T \le T_{opt,1} \\ 1 & T_{opt,1} < T \le T_{opt,2} \\ \mathrm{e}^{-k_{T2}\left(T - T_{opt,2}\right)^2} & T > T_{opt,2} \end{cases}$$

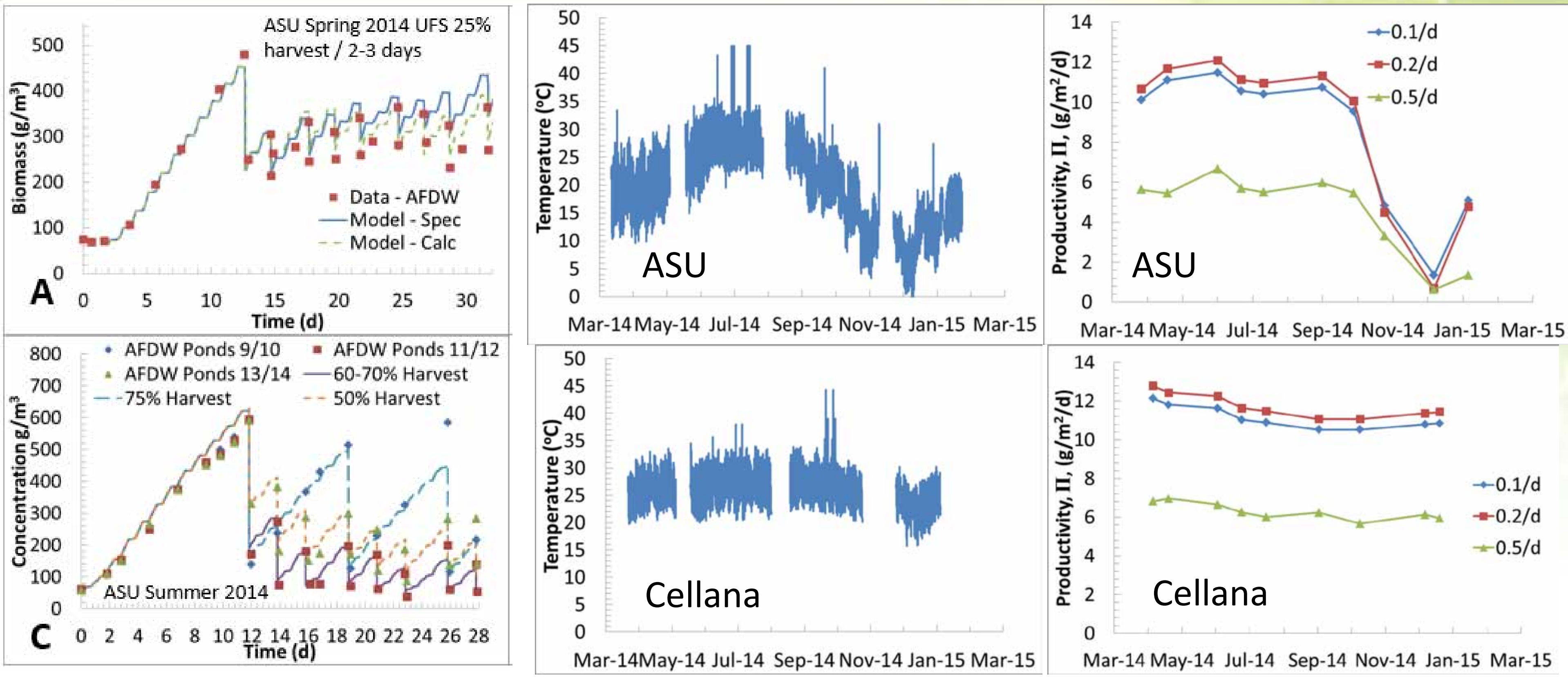
$$f_2(I) = \frac{e}{Kess \cdot \Delta z} \left(e^{-\alpha_B} - e^{-\alpha_T} \right)$$

$$\alpha_B = \frac{I}{I_{opt}} e^{-Kess\left(H_T + \Delta z\right)}, \quad \alpha_T = \frac{I}{I_{opt}} e^{-Kess \cdot H_T}$$

$$Kess = K_{e,b} + K_{e,B} B$$

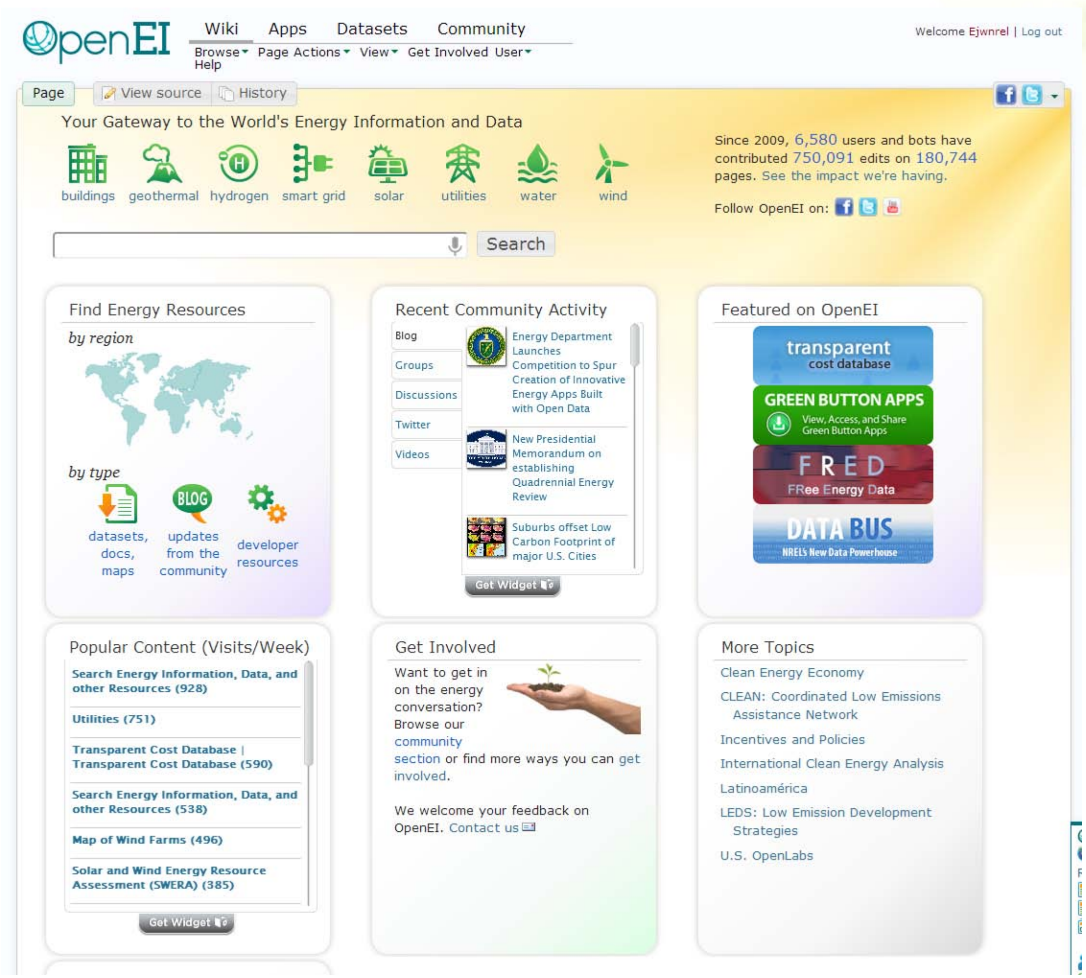
Gharagozloo, et al, J. Appl. Phycol. 2014

Model Validated for Multiple Locations and Seasons



- Same calibration applied to all cases
- Model to be utilized to inform 2015 experimentation
  - Dilution rate example above right: 30 day simulation based on actual light/pond temps
- Pushing more integration and utilization of cultivation data for other productivity modelling (e.g., PNNL)

Public Access to Data



Using the existing infrastructure and expertise of OpenEI.org provides a rapid, robust, and low-cost solution for making the ATP^3 datasets public. Curated Spring/Summary 2014 data summary posted for KA32, Fall/Winter 2014 to post in Q3 FY'15

UFS Yr1 Production Summary:

- Met objectives for Yr 1 of the UFS with a robust data set for KA32 albeit with a desire for higher productivities.
- *LRB-AZ-1201 r*emains a challenge for outdoor open pond cultivation – new SW/FW cultivars to be introduced by summer 2015 (*Desmodesmus and/or Scenedesmus*).
- Measurement system gaps remain and require additional effort/vigilance
  - YSI maintenance/calibration in particular, N:P measurements across sites
  - Grab samples for BC and Molecular (ensuring sample integrity)
  - Higher throughput, lower cost analysis a significant gap (e.g., near-IR work at NREL – see L. Laurens Peer Review Presentation)
- Major challenge in the "janitorial services" realm of data curation with serious bottlenecks and underperforming tools (spreadsheets). ID'd as a rising project risk in Q1 FY15.
  - More to be done, but significant streamlining and easier tools for realtime QA/QC by the sites as well as the core data team are now in place.
- Engage more directly with other biomass productivity modelling activities (e.g., PNNL)
- We are behind on data release to OpenEI (~1Q behind in data availability) – protocols and data summary reports (thru summer) are available
- Deep dive into data continues with focus on rapid write-ups for publications as well as supporting modeling efforts (e.g., SOT milestone in 2015 (FY15 Q4).

2015 UFS/AFS Planning:

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	Spring 2015													Summer 2015													Fall 2015													Winter 2015												
2015 UFS Experimental Outline	M				A				M					J				J				A					S				O				N					D				J				F				
	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	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52
Seed Production																																																				
Nannochlorposis Oceanica						DLN Rate N type-amt														DLN rate/N type-amt													DLN rate/N type-amt													DLN rate/N type-amt						
Scenedesmus/Desmodesmus														DLN Rate Ntype-amt													DLN Rate Ntype-amt													DLN Rate Ntype-amt												
Chlorella Vulgaris	DLN Rate																																																			
Data analysis (OpenEI post)																																																				

- Prime Goal for year 1 UFS: Establish Seasonal/Regional Baseline for primary cultivars
- Prime goal for year 2 UFS is to improve upon baseline results through evaluation of additional SW/FW cultivars and seeking out site specific, naturally robust strains.
- Season = 13 weeks (target of 11-12 weeks dedicated to cultivation in ponds)
  - Nutrient sourcing and dilution rate key factors with side by side comparisons of cultivars
  - Crashed/invaded ponds deliberately left running to seek out stable mixed cultures/more robust strain
  - Contamination mitigation strategies explored (lab results brought outside, chemical/physical)
  - Will de-emphasize KA32 as ability to significantly improve on baseline results unlikely
- AFS will focus on larger scales at Cellana, ASU, Cal Poly and include nutrient source (waste water), PBR vs. open pond vs. ARID vs. hybrid, media recycle, and harvesting and additional cultivars (e.g., LANL supplied strains at ASU)

Relevance: ATP^{3}'s near term impact

- Taking algal biofuels and co-products development from research to successful commercialization demands:
  - Solid and objective testing to provide **data**
  - **Integrated process equipment** needed to inform and guide research, systems and process improvements
  - Subsequent **analysis to support** strategic technical and investment decisions
- Providing stakeholder access to quality testbeds in real-world outdoor settings, and expertise and related resources, is necessary to generate new tools, datasets and best practices

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Future Work: 2015-2018

ATP^3 Phases:

2. **<u>Months 13-36</u>**: Long term cultivation trials implementation and building customer base as a user facility – **Go/No Go for Phase 3**
   Major Milestones/Critical Success Factors:
   - Complete UFS/AFS Cultivation Trials
   - Data disseminated to the R&D community
   - **State of algal biofuels technology design report** completed
   - Capability of testbed network to **serve stakeholder community** demonstrated (establish consortium model to carry forward into Phase 3)
3. **<u>Months 37-60</u>**: Sustainable Testbed Operations.
   Major Milestones/Critical Success Factors:
   - State of algal biofuels technology design report updated with customer data
   - Value network validated and funding secured to **sustain network** in out years

High Impact Data Summary

- ATP^3 has established validated framework for implementing rigorous, long-term multi-site cultivation trials
- Allows determination of the effects of regional, seasonal, environmental variation that is to be expected for a national (international) deployment of algae cultivation
- We have established baseline performance across multiple seasons and will extend and expand that through CY 2015 and in to CY 2016.
- Experimental program expanding (AFS) in 2015 to include larger scale and other additional capabilities of the partner sites
- Critical validation data source for biomass productivity modeling, TEA, LCA and RA community - will allow for refinement of the current state of technology (SOT) assessments utilized by DOE and the broader industry/investment community

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ATP3 partners

AzCATI Arizona Center for Algae Technology and Innovation

NREL National Renewable Energy Laboratory

Sandia National Laboratories

cellana algae-based products for a sustainable future

Cal Poly San Luis Obispo

Georgia Tech

UTEX The Culture Collection of Algae at The University of Texas at Austin

Valicor Renewables

Florida Algae, LLC

Commercial Algae Management, Inc.

ASU LightWorks

ASU Arizona State University

U.S. Department of Energy

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Pierre Wensel
Linda Boedeker
Sarah Mason
Travis Johnson
Sydney Lines

UTEX
Schonna Manning
Jerry Brand

Valicor Renewables
Kiran Kadam
Brian Goodall

NREL
Phil Pienkos
Lieve Laurens
Ed Wolfrum
David Crocker
Ryan Davis
Stefanie Van Wychen
Eric Knoshaug

Sandia National Labs
Ron Pate
Todd Lane
Patricia Gharagozloo
Thomas Reichardt
Jessica Drewry
Pamela Lane

Cellana
Valerie Harmon
Martin Sabarsky
Emily Knurek
Kate Evans
Peter Prentiss
Reyna Javar
Kari Wolff
Keao Bishop-Yuan
Lynn Griswold
Christina Boyko
Charlie O'Kelley
Kelli Yamane, BS
Radu Mihaila, MS
Egan Rowe

Cal Poly
Tryg Lundquist
Braden Crowe
Eric Nicolai
Garrett Murawsky
Ruth Spierling

Open Algae
Peter Kipp
Hoyt Thompson

Florida Algae
Steven Schlosser
Chris Withstandley
Mary Riddle
Nancy Pham Ho (FIT)

Commercial Algae Management
Albert Vitale
Robert Vitale

Georgia Tech
Yongsheng Chen
Steven Van Ginkel
Thomas Igou
Zixuan Hu
Terry Snell

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Wyatt Western
Mariah Patton
Carlos Luna
Delaney De Hertogh
Shaylin Mcghee
Caden Offield

GT Undergrads
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Jerry Duncan
Frazier Woodruff
Shusuke Doi
Hao Fu
Patricia Penalver-Argueso
Allison Dunbar
Allison Carr

Sichoon Park
Priya Pradeep
Catherine Achukwu
Christine Yi
Asmita Narode
Chunyan Xu
Yuankun Zhao
Kangyan Wu
Sarasija Cheruvu

CP Undergrads
Aydee Melgar
Gulce Ozturk
Kaitlyn Jones
Michael Antoine
Trung K Tran
Jake Bender
Heather Freed
Daniel McBroom
Michele Hendrickson
Brandon Miller

Gerard Nguyen
Deven Diliberto
Jack Sunderland
Dan Averbuj
Ann Marie Sequeira
Lauren Miller
Michele Hendrickson
Emily Wang
Jack Sunderland
Ann Marie Sequeira
Soroush Aboutalebi
Lauren Miller
Samantha Lui
Michele Hendrickson
Gabriella Campos
Will Briles
Letty Thottathil
Marissa License
Sam Cronin

Eric Krikorian
Javier Garcia
Esme Diego
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Nicholas Hardy
Ryan Stump
Anna Klavins
Cris Swain
Jessica Duffey
Ryan Yanda
Taylor Gater
Rick Brundage
Hunter Lawless
Jared Stine
Sophia De Guia
Joshua Tenhet
Samantha Bock
Leland Lam

Supplemental

Presentations and Publications

Presentations:

1. "An Overview of a Successful Algae Testbed Model: Arizona Center for Algae Technology and Innovation (AzCATI) and the Algae Testbed Public-Private Partnership (ATP^3) at Arizona State University". Thomas A. Dempster, Consortium de Recherches et Innovations en Bioprocedes Industriels au Quebec (CRIBIQ), Sherbrooke, Quebec, October 2012.
2. ATP^3 BETO Project Peer Review, Washington DC, May 2013.
3. "Validated Algae Growth Model and Optimization Study." **Patricia Gharagozloo** and Jessica Drewry. 3rd International Algal Biomass, Biofuels and Bioproducts Conference June 2013.
4. "Pulsed Electric Field (PEF) Processing of Microalgae and Related Activities at Arizona Center for Algae Technology and Innovation (AzCATI) at ASU", Thomas A. Dempster, Bioelectrics Symposium 2013; Karlsruhe, Germany; September 2013.
5. "The Algae Testbed Public Private Partnership -ATP^3" Ron Pate. Algae Biomass Organization, Algae Biomass Summit, Orlando, FL, October 2013.
6. "Composition of Algal Biomass for Biofuels and Bioproducts: High Impact Data and Method Harmonization" **L. Laurens**, E. J. Wolfrum, T. Dempster, J. McGowen, P.T. Pienkos, Algae Biomass Organization, Algae Biomass Summit, Orlando, FL, October 2013.
7. "An Experimental Framework for Performing Long Term Cultivation Trials Across Different Regional, Seasonal, Environmental, and Operational Conditions" John A. McGowen, Bio Pacific Rim Summit, San Diego (December 11th, 2013).
8. "Method Harmonization Efforts for Microalgae Production and Biomass Analyses at Arizona State University's Arizona Center for Algae Technology and Innovation (AzCATI) and the Algae Testbed Public-Private Partnership (ATP^3)" Thomas A. Dempster, 28th Congress of the Phycological Society of Southern Africa, Melkbosstrand, South Africa, January 2014.
9. "Algae Testbed Public Private Partnership (ATP^3): Multi-Region, Long-Term Algae Biomass Cultivation Trials" John A. McGowen, Bio World Congress on Industrial Biotechnology, May 2014.
10. "Overview of Innovative Algae Cultivation Modeling, Diagnostics, and Standardized Analytics Available Through DOE's National Algae Testbed Project – ATP^3" **Philip T. Pienkos,** Ron Pate, John McGowen, Todd Lane, Tricia Gharagozloo, Tom Reichardt, and Lieve Laurens, Symposium of Biotechnology for Fuels and Chemicals, Orlando, FL, May 2014.
11. "Modeling of *Nannochloropsis* sp. Growth in Algae Testbed Unified Field Studies" Patricia Gharagozloo, 5th Congress of the International Society for Applied Phycology, Sydney, Australia, June 25, 2014.
12. "Long term cultivation studies a the Algae Testebed Public-Private Partnership: Preliminary data from the Unified Field Studies" **Philp T. Pienkos**, Valerie Harmon, John McGowen, 4th International Conference on Algal Biofuels, Biomass and Bioproducts (ABBB), Sante Fe, NM, June 2014
13. "Spectroradiometric monitoring for biomass measurement and predator detection in *Nannochloropsis* sp. cultures." **T. A. Reichardt**, A. M. Collins, J. A Timlin, T. A. Dempster, and J. A. McGowen, 4th International Conference on Algal Biofuels, Biomass and Bioproducts (ABBB), Sante Fe, NM, June 2014.

Presentations and Publications

Presentations:

14. "Driving towards a common language for characterization of algal biomass for biofuels and bioproducts: High impact data and method harmonization," **L. Laurens**, J. McGowen, T. Dempster, P.T. Pienkos, 4th International Conference on Algal Biofuels, Biomass and Bioproducts (ABBB), Sante Fe, NM, June 2014.
15. "Driving towards a Common Language for Algal Biomass for Biofuels and Bioproducts: High Impact of Data and Method Harmonization **L. Laurens**, J. McGowen, T. Dempster, P.T. Pienkos, Algae Biomass Organization, Algae Biomass Summit, San Diego, CA October 2014.
16. "Algae Testbed Public Private Partnership (ATP3): Education and Training Workshops Offer Extensive Hands-On Learning Opportunities" T. Dempster, M. Sommerfeld, S. Manning, Jerry Brand, Poster Presentation, Algae Biomass Organization, Algae Biomass Summit, San Diego, CA October 2014.
17. "Performance Evaluation of the Helix™ Tubular Glass Photobioreactor for High Quality Inoculum Production" **J. A. McGowen**, T. A. Dempster, T. Rosov, and D. Cardello, Algae Biomass Organization, Algae Biomass Summit, San Diego, CA October 2014.
18. "Long Term Cultivation Studies at the Algae Testbed Public Private Partnership: Spring and Summer Season Data Update from the Unified Field Studies" J. McGowen, T. Dempster, P. Pienkos, V. Harmon, Poster Presentation, Algae Biomass Organization, Algae Biomass Summit, San Diego, CA October 2014.
19. "Algae Testbed Public Private Partnership (ATP3): Opportunities to Engage in Open Collaborative Testbed Network Activities" **T. Dempster**, J. McGowen, Algae Biomass Organization, Algae Biomass Summit, San Diego, CA October 2014.
20. "Progress and Perspectives of Large Scale Algae Biomass Harvesting: A Case Study at the ATP3 Testbed" **X. Zhang**, J. McGowen, Q. Hu, M. Sommerfeld, Algae Biomass Organization, Algae Biomass Summit, San Diego, CA October 2014.
21. "Modeling and Optimization of Nannochloropsis oceanica Growth in Seasonal Algae Testbed Unified Field Studies" P.E. Gharagozloo, J. L. Drewry, and T.A. Dempster, Poster Presentation, Algae Biomass Organization, Algae Biomass Summit, San Diego, CA October 2014.
22. "Long Term Cultivation Studies at the Algae Testbed Public Private Partnership (ATP^3): Spring and Summer Season Data Update from the Unified Field Studies". **J. McGowen**, T. Dempster, P. Pienkos, V. Harmon, 3rd Asia-Oceania Algae Innovation Summit, Daejeon, Korea, November 2014.
23. "Enabling Algal Technology development at the Algae Testbed Public Private Partnership (ATP^3): J. McGowen, T. Dempster, **P. Pienkos**, L. Laurens, 3rd Asia-Oceania Algae Innovation Summit, Daejeon, Korea, November 2014. (Invited Talk)
24. "Algae Testbed Public Private Partnership (ATP^3): Enabling Algal Technology Research and Development." J. McGowen, BIO Pacific Rim Conference, San Diego, CA, December 2014.
25. P. Pienkos chaired a session at the Pacific Rim BIO Conference in December entitled, "Algae Testbeds: Models for Accelerating Commercialization." The panel included representatives of four algae testbeds: AlgaeParc in The Netherlands, the Algae Testbed Public-Private Partnership (ATP^3) and the Regional Algal Feedstock Testbed (RAFT) in the US, and the Algae Industry Incubation Consortium (AIIC) in Japan.

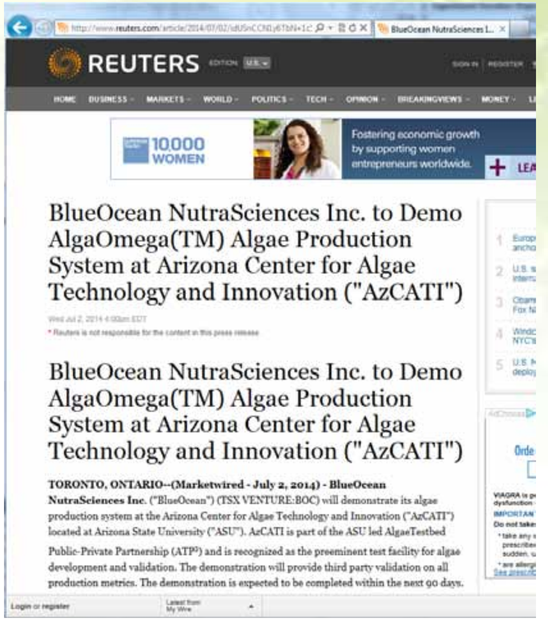
Publications

1. Gharagozloo, P. E., Drewry, J. L., Collins, A. M., Dempster, T. A., Chris V. Choi, C. V., and S. C. James. Analysis and modeling of *Nannochloropsis* growth in lab, pond, and raceway experiments. 2104. *J. Appl. Phyc*. Online edition © Springer Science+Business Media Dordrecht February 2014 DOI 10.1007/s10811-014-0257-y. 12 pp
2. Park, S., Van Ginkel, S.W., Pradeep, P, Igou, T., Yi, C., Snell, T., Chen, Yongsheng. (2014) "The selective use of hypochlorite to prevent pond crashes for algae-biofuel production". *Water Environment Federation*. Accepted.
3. Igou, T., Van Ginkel, S.W., Penalver-Argueso, P., Fu, H., Doi, S., Narode, A., Cheruvu, S., Zhang, Q., Hassan, F., Woodruff, F., Chen, Y. S., (2014), "Effect of Centrifugation on Water Recycling and Algal Growth to Enable Algae Biodiesel Production", Water Environment Research, 86 (12): 2334-2338

Why Engage with ATP^3?

Example Pilot Scale PBR/Process Validation Project: Blue Ocean NutraSciences

- **Leveraging ATP³ expertise, capabilities, and facilities**
  - Blue Ocean working with the AzCATI testbed site to validate current production process technology (strain and PBR system)
  - First phase (6-9 mo)
    - small scale validation of previous work performed for Blue Ocean at another institution
    - 250 gallon scale
    - Includes small scale product production for customer evaluation
  - Second phase
    - Already fully scoped (contingent on successful first phase)
    - Larger scale implementation (3000 gallons) and validation of production process and product specifications
  - Standard boilerplate for NDA and the service agreement were negotiated and in place in ~3 weeks.
  - Full pay to play project
  - Can transition P1-P2 without need for revisiting service agreement


REUTERS

BlueOcean NutraSciences Inc. to Demo AlgaOmega(TM) Algae Production System at Arizona Center for Algae Technology and Innovation ("AzCATI")

BlueOcean NutraSciences Inc. to Demo AlgaOmega(TM) Algae Production System at Arizona Center for Algae Technology and Innovation ("AzCATI")

TORONTO, ONTARIO--(Marketwired - July 2, 2014) - BlueOcean NutraSciences Inc. ("BlueOcean") (TSX VENTURE:BOC) will demonstrate its algae production system at the Arizona Center for Algae Technology and Innovation ("AzCATI") located at Arizona State University ("ASU"). AzCATI is part of the ASU led AlgaeTestbed Public-Private Partnership (ATP³) and is recognized as the preeminent test facility for algae development and validation. The demonstration will provide third party validation on all production metrics. The demonstration is expected to be completed within the next 90 days.

ATP3
Algae Testbed Public-Private Partnership

atp3.org

Nutrient Recycling through the Anaerobic Digestion of *Nannochloropsis sp.*: Preliminary Results

Sonicated vs. Whole-Cell Algae Digestion

Methane Yield (L CH_4 /g VS_{in})

- 25% higher for sonicated feed after 30 days.
- 105% higher after 40 days digestion.

Solubilization of Initial Organic Nitrogen

- 17% for the sonicated algae after 30 days.
- 13% for the whole cell algae after 30 days.

CAL POLY
SAN LUIS OBISPO

Nutrient Recycling through the Anaerobic Digestion of *Nannochloropsis sp.*: Preliminary Results

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New Cohort Projects under review - 2 examples

Metabolism-Based pH Control of Outdoor Pilot-Scale Microalgae Cultivation (Penn State)

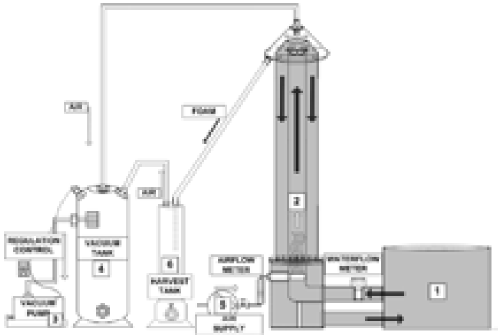
- Translate recently lab demonstrated pH control at low CO_2 buffering by dynamic nitrogen feeding (Wang & Curtis, 2015) control strategy to outdoor setting
- This work will set the stage for a more sophisticated process control algorithms
  - feed-forward
  - model-predictive and
  - adaptive) that are clearly an important component to reducing the costs of algae biomass production

Wang J, Curtis WR. (Accepted for publication Feb 2015) “Proton stoichiometric imbalance during algae photosynthetic growth on various nitrogen sources: Towards metabolic pH control” J. Applied Phycology.

Vacuum AirLift (VAL) technology field demo (Searen LLC)

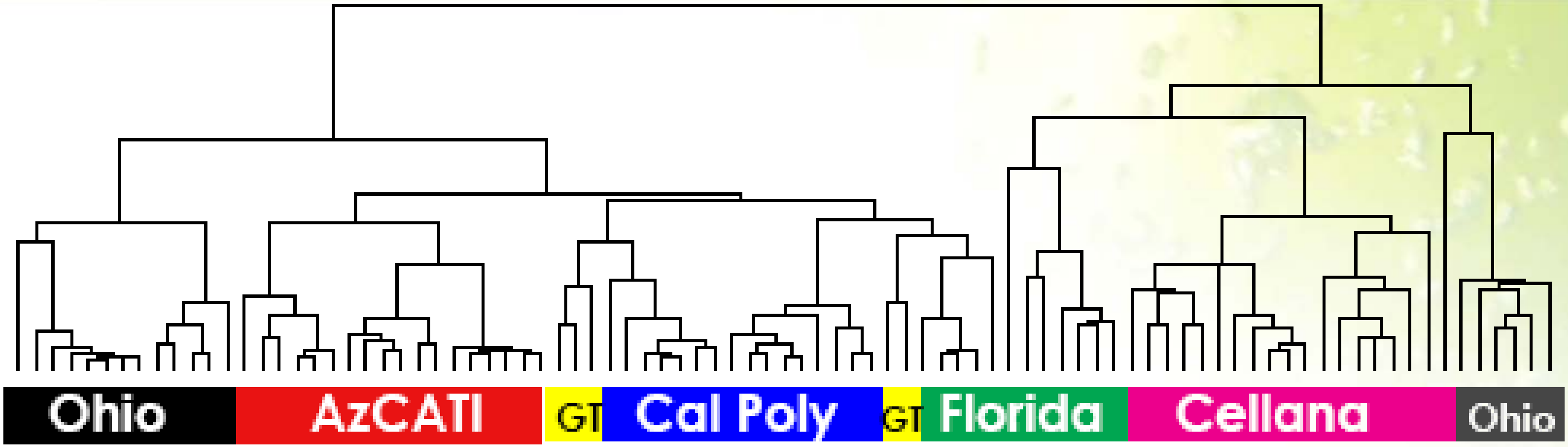
- **Multifunctionality**
  - Particulates removal by flotation
  - O_2 stripping,
  - CO_2 dissolution and
  - water circulation by airlift pump)

Innovative technology (patents (N°07 02308 and N°09 57898)



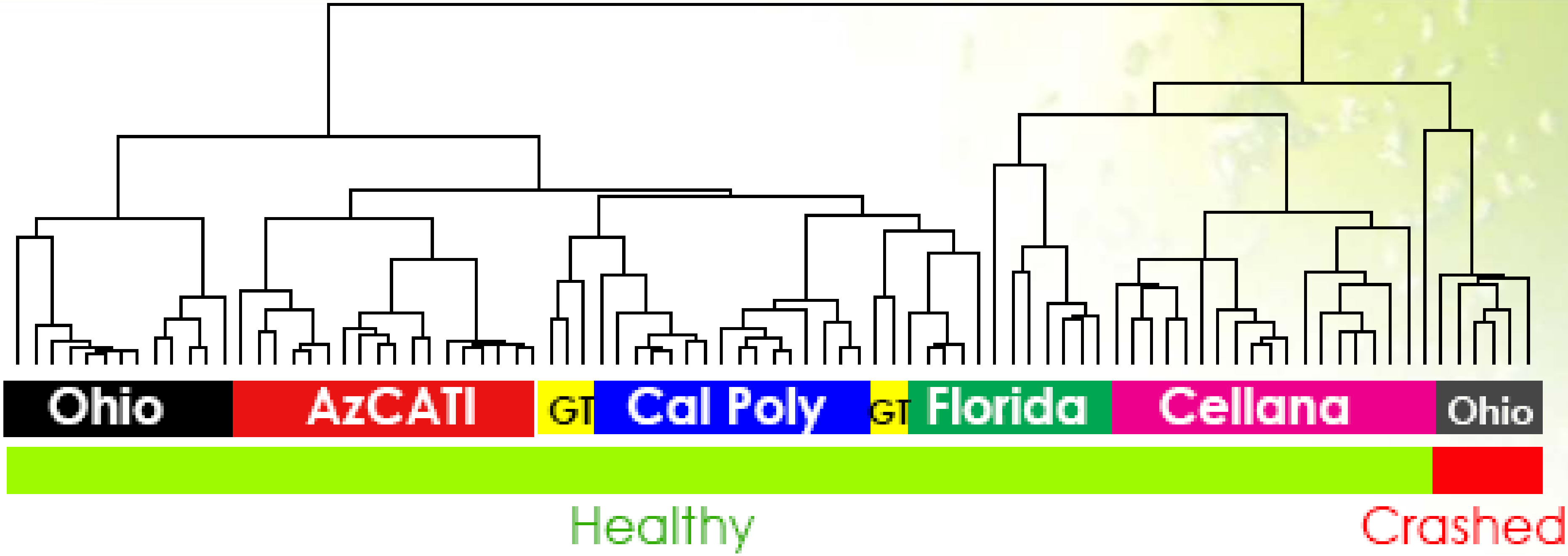
http://dx.doi.org/10.1016/j.biortech.2012.10.056

16S Analysis of spring samples



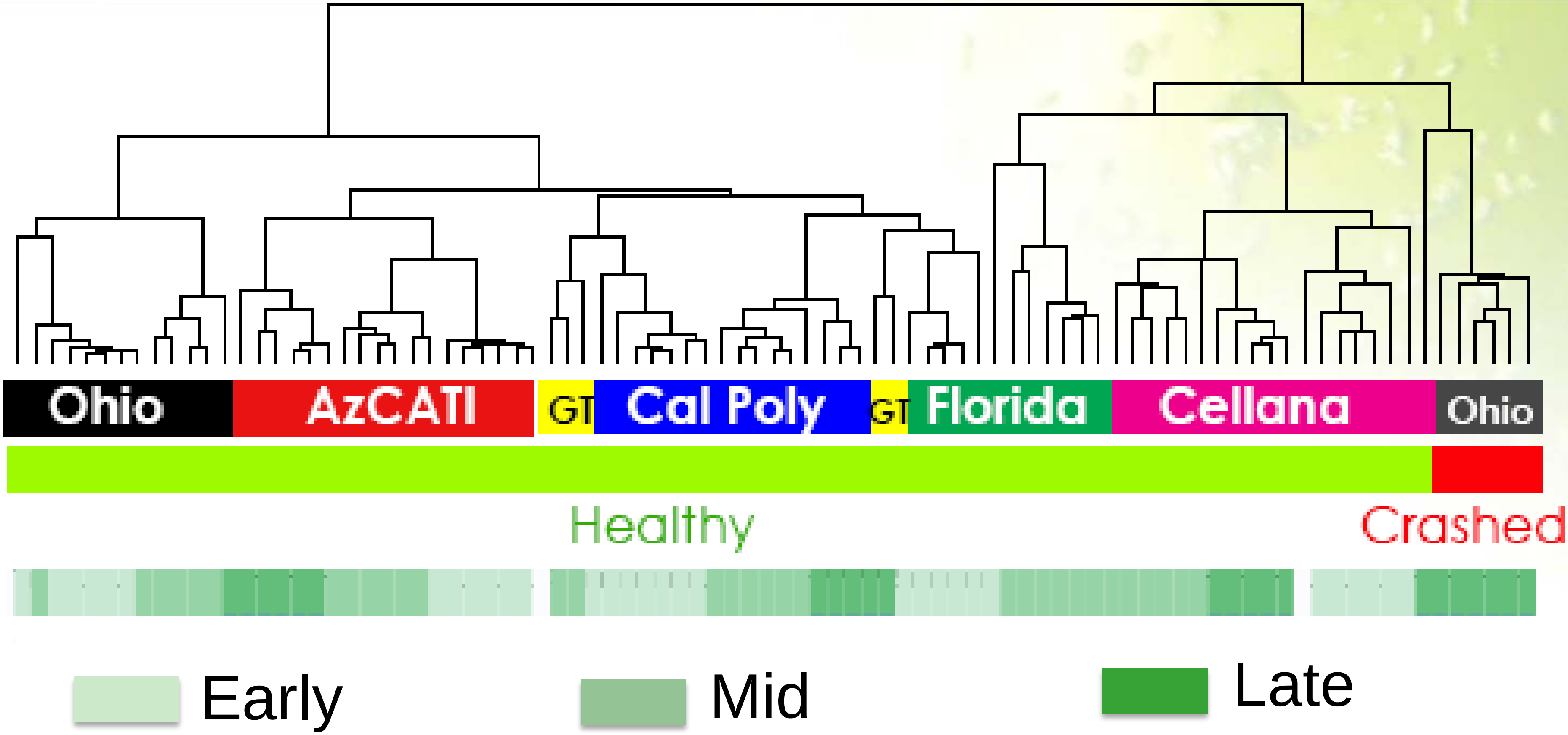
Bacterial communities can identify samples from the same site

16S Analysis of spring samples



Crashed ponds had a different community structure than healthy ponds

16S Analysis of spring samples

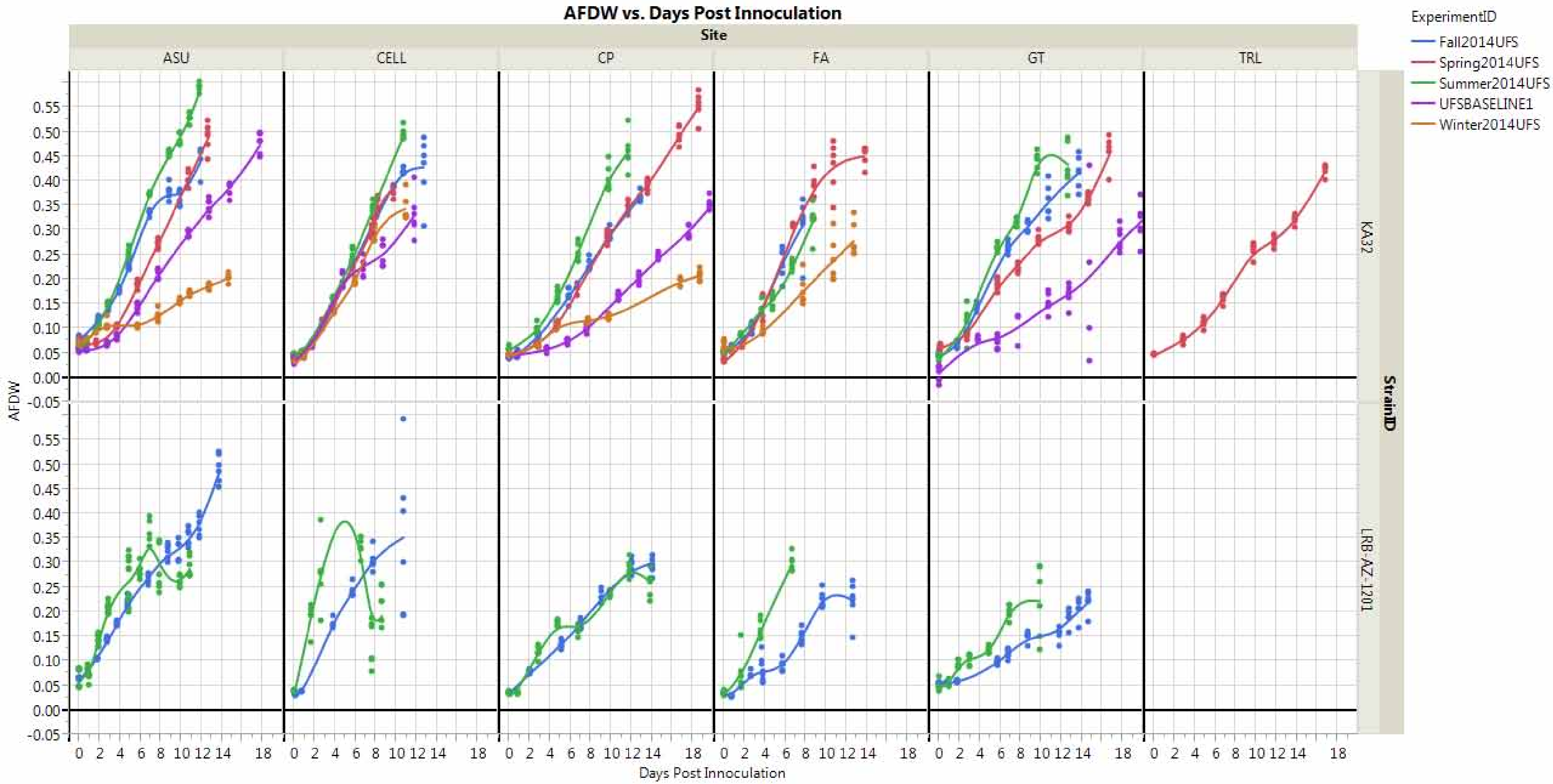


And within same sites the community structure gradually changed with time

Implementation during Unified Field Studies (UFS)

- All samples collected were harvested by centrifugation and freeze dried
- All data collected in **standardized spreadsheets**
- Unique identifiers linking biochemical data to production data (Tracking ID)
- ~5% of the samples were sent to NREL for validation
- Each set of analyses contained at least one set of triplicate QC material

Grow-out Summary and Assessing Pond to Pond Variation:



Sandia Team's Future Vision: Wide-Area Sensing + Real-Time Analysis

…coupled to real-time analysis of the sensor data

- Biomass monitoring
- Contamination detection

Model Has Multiple Fidelities

CFD handled by ANSYS-FLUENT

Levels of Model Fidelity

- Simple single cell fully mixed for light and temperature
- 1D light and/or temperature with depth
- 2D conditions varying with depth and along raceway including flow.
- 3D with flow, varying conditions

Considerations

- Evaporation
- Heat transfer/Radiation
- Fluid dynamics
- Boundary and initial conditions
- Wind
- Settling
- Nutrient and dissolved gas transport

Overview ASU AFS

- Large scale open pond cultivation of strains (1K (4 m^2), 10K (60 m^2), 100K L (500 m^2))

- Flat panel strain and production method comparisons

- Media recycle from downstream processing (DAF/MF/Centrifuge) and impact on productivity

Overview Cal Poly AFS

- Utilization of wastewater media
  - Effect on survival of specific strains
  - Media recycling post biomass removal
  - Semi-continuous cultivation, with enhanced capabilities to look into optimizing dilution rates or hydraulic retention time at specific biomass densities to maximize productivity

Overview Cellana AFS

- Large scale open pond cultivation of strains (80 K L)

- Large scale photo bioreactor (closed system) cultivation (25 K L)

- Harvest efficiency during dewatering and drying