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

DOE Bioenergy Technologies Office (BETO) 2015 Project Peer Review

Polyculture Analysis

24 March 2015

Algal Feedstocks

Deborah Newby, Ph.D.

Idaho National Laboratory

Hub --

Sandia National Laboratories

Oak Ridge National Laboratory

Pacific Northwest National Laboratory

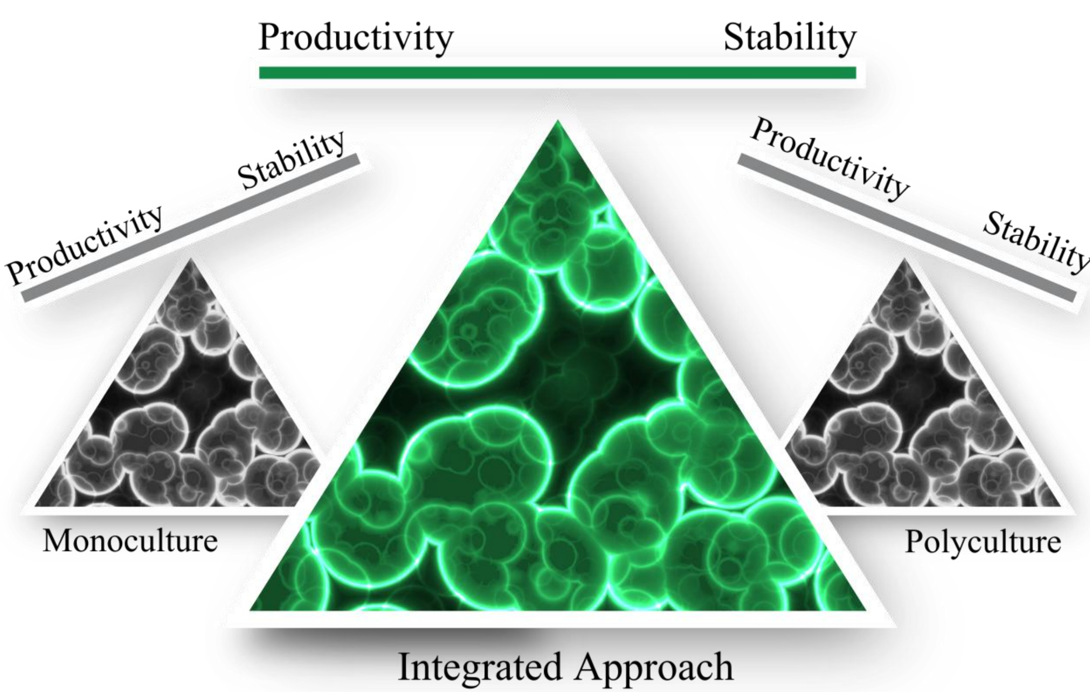
Goal Statement (Hub)

Challenge:

- To contribute to meeting the Renewable Fuel Standard, DOE MYPP has a target of 2,500 gallons per acre per year of algae by 2018 and 5,200 by 2022
- Current (mono-culture based) production levels (13 $g/m^2/day$) are half of the 2022 target due to issues with both <u>productivity</u> and <u>stability</u>

Goal:

- Develop a robust algal production system based on ecological principles
- Apply polyculture-based cultivation to improve annual productivity, culture stability, and reduce overall costs
- Attain reliable annual average production rates of 20-30 $g/m^2/day$ to meet MYPP targets



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Quad Chart Overview (Hub)

Timeline

Polyculture Hub

Project start date: Oct 1, 2014

Project end date: Sept 30, 2017

Percent complete: 15%

Budget

	Total Costs FY 10 –FY 12	FY 13 Costs	FY 14 Costs	Total Planned Funding (FY 15-Project End Date
DOE Funded	N/A	$13K	$433K	$5,275K
INL	N/A	N/A	N/A	$2,150K
ORNL	N/A	N/A	N/A	$975K
PNNL	N/A	N/A	N/A	$300K
SNL	N/A	$13K	$433K	$1,850K

Prior to Hub (FY 13 Costs, FY 14 Costs) — Hub (Total Planned Funding)

Barriers

- AFt-A. Biomass Availability and Cost
- AFt-B. Sustainable Algae Production
- AFt-D. Sustainable Harvesting
- AFt-H. Overall Integration & Scale-up
- AFt-I. Algal Feedstock Preprocessing

Partners

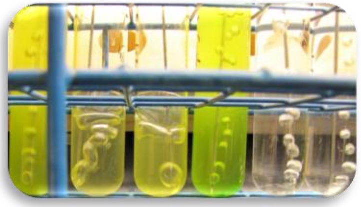
National Laboratories

- INL (41%)
- ORNL (18%)
- SNL (35%)
- PNNL (6%)

Collaborators

- University of California San Diego
- University of Kansas
- Boise State University
- Hydromentia
- Ecological Systems Technology

1 - Project Overview (Hub)



- AOP Merit Review Process
- Leverages previous related work (AOP and LDRD)

Monoculture

- 13 $g/m^2/day$ current production
- Site-specific strain optimization
- Susceptible to pond crashes caused by biological, chemical, or environmental fluctuation
- Limited productive season → low annual yield
- Susceptible to competition from less productive strains

Polyculture

- Based on ecological principles
- Diverse strains that provide stability and better niche utilization
- Natural communities (including blooms) are diverse
- Rapid response to perturbation
- Literature shows potential for increased productivity

- Key challenges that limit the cost effectiveness of algal production
  - low productivity during "off-season" months
  - crop loss due to predators or weedy strains
  - production/scale-up
  - conversion

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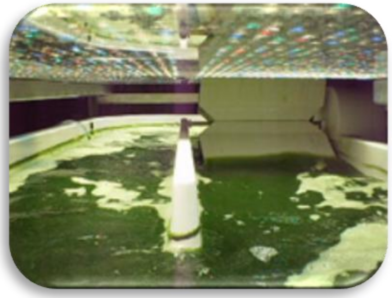
2 – Approach (Technical – Hub)

- **Approach**
  - Develop polycultures around **high performing/production strains** used in monoculture
  - Identify strains with **complementary traits** (e.g., symbioses, accessory pigments, antimicrobial agents) to maximize resource exploitation
  - Target opportunities for **high impact increases** in biomass production (e.g., increasing productive growing season, crop protection strategies to decrease frequency of open cultivation system crashes)
  - Assess performance of polycultures **relative to monocultures** when exposed to different stressors (e.g., thermal stress, grazing pressure, etc.)

- **Critical success factors**
  - Develop consortia with increased annualized productivity as compared to monocultures, considers overyielding and stability (10-20% improvement Go/No Go decision points)
  - Accelerated progress towards MYPP algal production targets
  - Successful scaling from lab to outdoor demonstration
  - Develop industry partnership to further development and implementation



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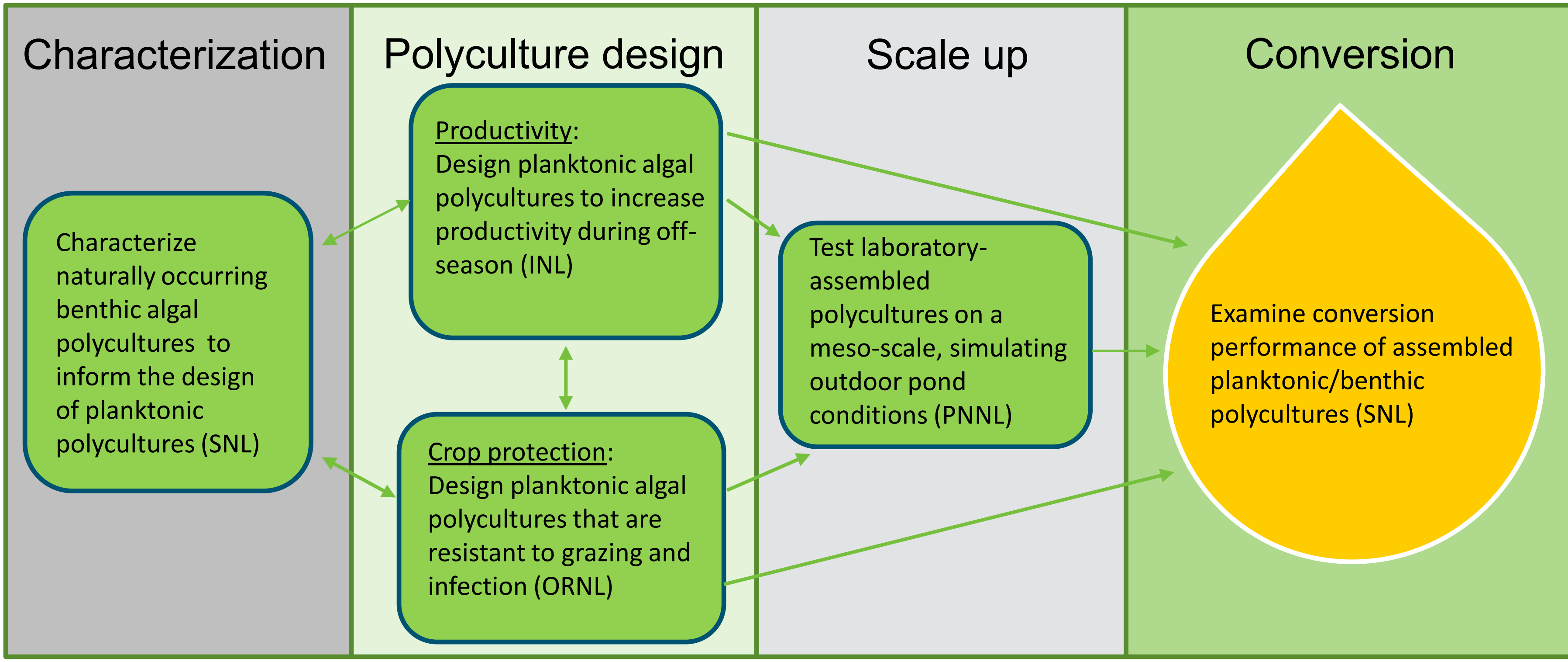
2 – Approach (Technical – Hub)



Risks

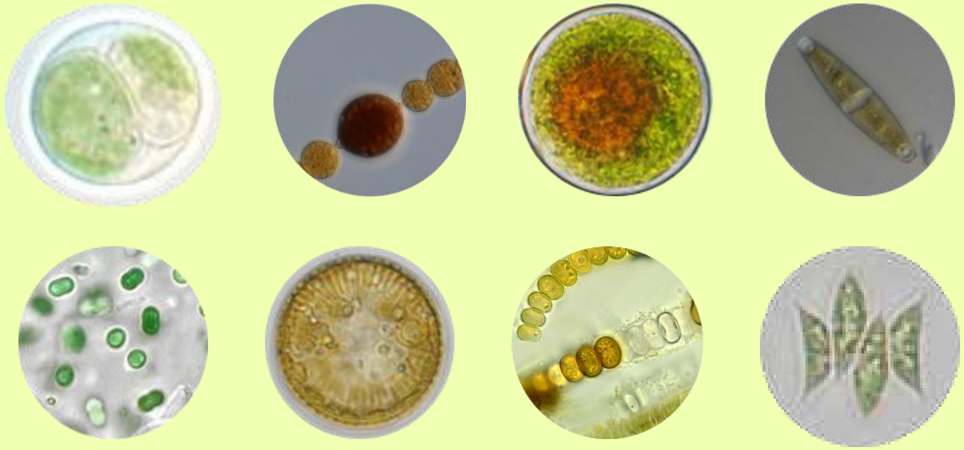
1. Limited polyculture improvement through polyculture
2. Polycultures do not provide significant protection against predation
3. Scaling
4. Timing/seasons

Mitigation

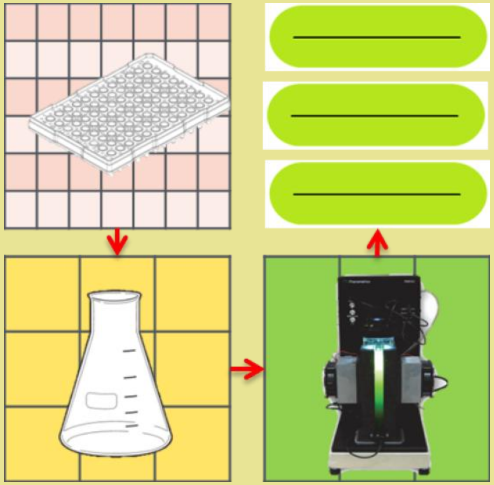
1. Multiple consortia developed, iterative development of consortia
2. Multiple consortia developed, diverse approaches including multi-trophic and natural assemblages
3. Incremental scaling and testing in outdoor ponds as soon as possible
4. Consider multiple potential sites for demonstration

2 – Approach (Technical – INL)

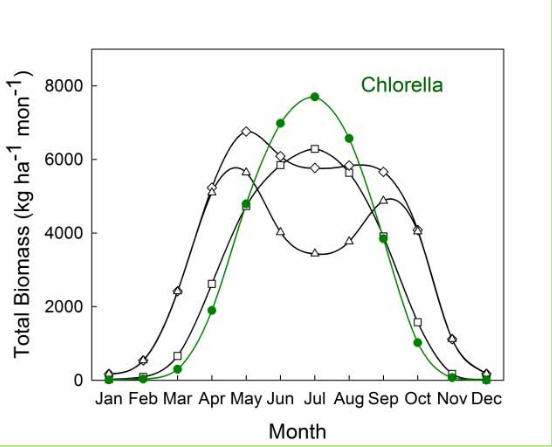
Strain Selection: Trait Complementarity



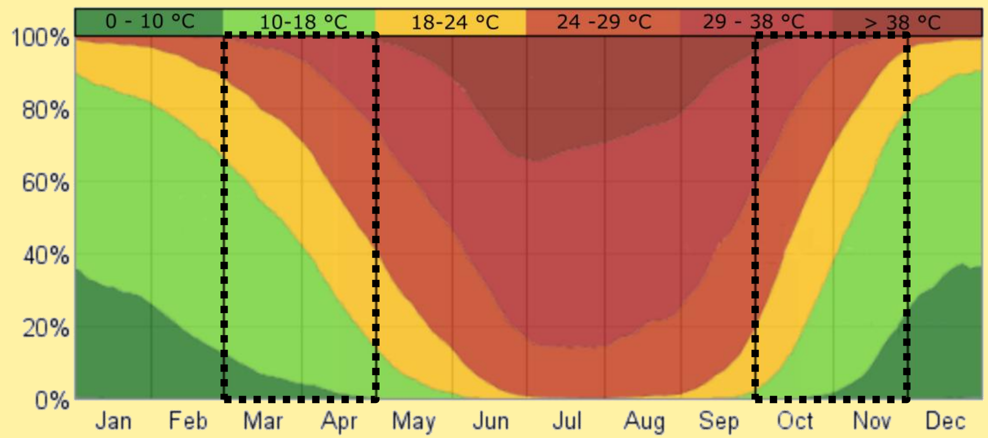
Experimental Plan



Strain Selection: Temp



Glendale, AZ: Temporal Temperature Proportions



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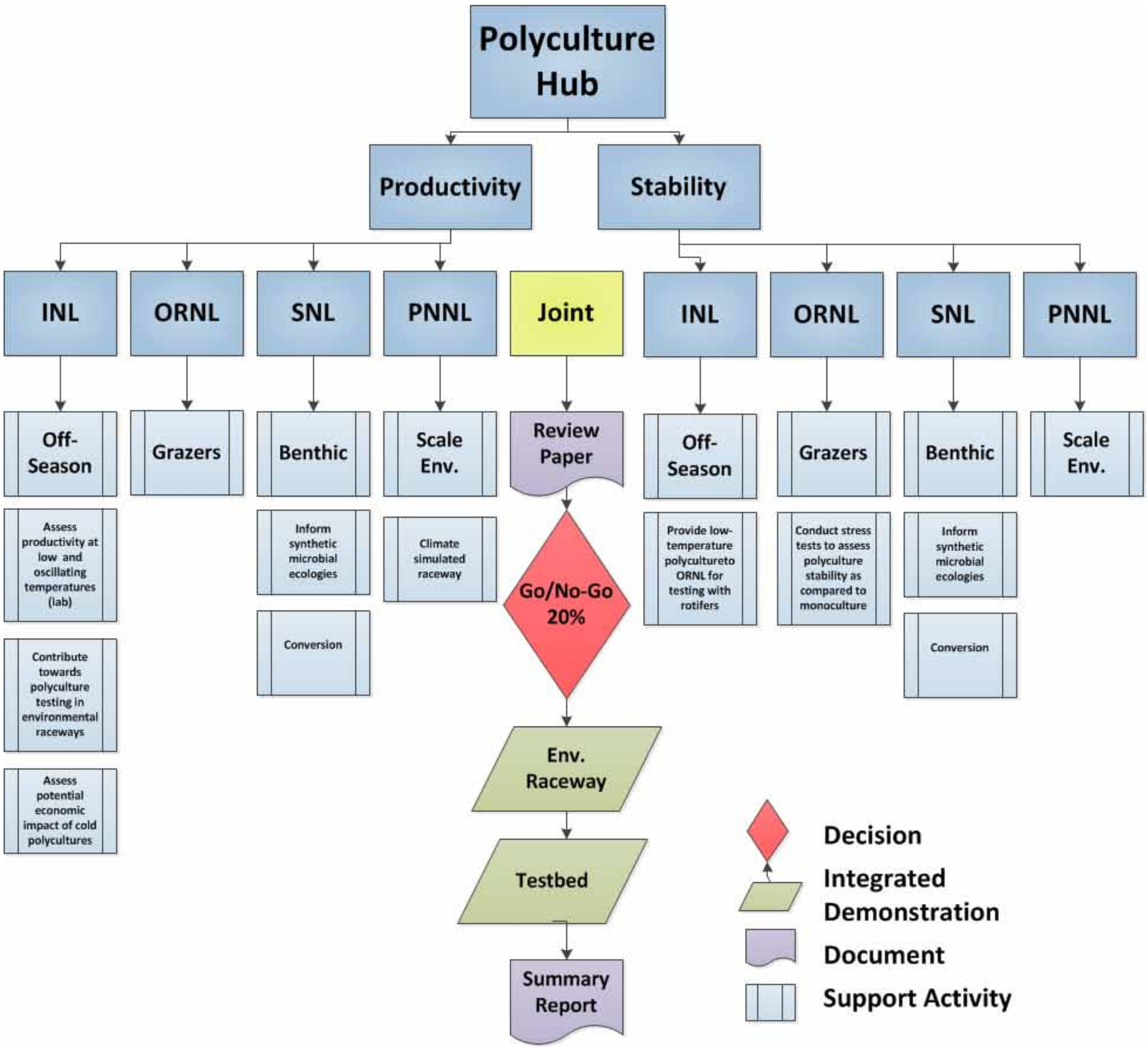
2 – Approach (Management)



Coordination Plan

- Individual laboratory AOPs
- Lab specific milestones
- Joint milestones
- Scaled demonstrations
- Project integration
    - Monthly PI calls
    - Inclusion of collaborators
    - Quarterly HQ calls
    - Quarterly reports to BETO
    - Go To Meetings

3 – Technical Accomplishments (Hub)

New Start

- FY15 Q1 Milestone: Detailed Management Plan was delivered to BETO
  - Detailed Coordination Plan (described previously)
  - Data Management Plan
    - SharePoint Site
    - Use of LAPs and/or other methods/metrics established by the Hub participants
  - Intellectual Property Management Plan
    - IPMP drafted by INL and in review by other laboratories
  - Non-disclosure Agreement
    - NDA drafted by INL and in review

- FY15 Q2 Milestone: Review manuscript to establish baseline for polyculture stability and overyielding

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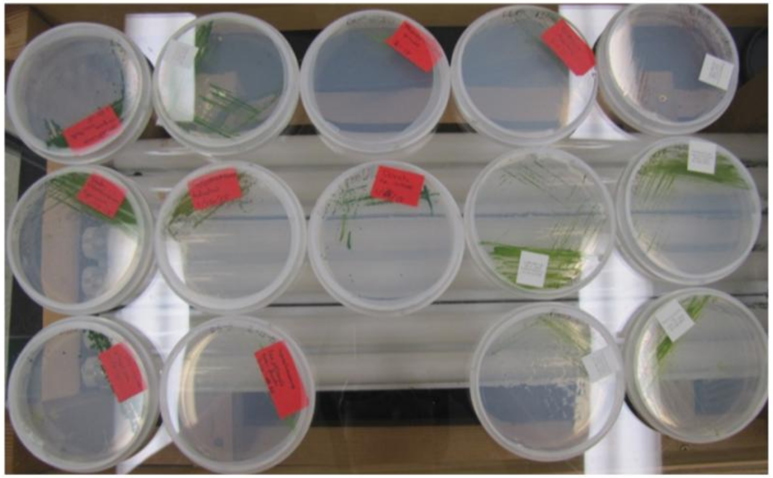
3 – Technical Accomplishments (INL)

- Established a collection of rationally selected cultures
- Acquired and installed nine Phenometrics© reactors
- Obtained hourly environmental conditions

  (e.g., temperature, light intensity, etc.) for multiple target sites
- Developed scripts to replicate site environmental conditions on Phenometric© reactors



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3 – Technical Accomplishments (Hub)

Hub Expertise

	Deborah Newby (INL)	Brad Wahlen (INL)	Michael Huesseman (PNNL)	Teresa Mathews (ORNL)	Ron Pate (SNL)	Todd Lane (SNL)	Ben Wu (SNL)	Anthe George (SNL)	Ryan Davis (SNL)
Algal physiology	◆		◆		◆	◆			
Algal ecology	◆	◆		◆		◆	◆		
Pest control				◆		◆			
Microbial ecology	◆	◆		◆		◆			
Nutrient cycling				◆	◆	◆	◆		◆
Environmental engineering			◆		◆	◆	◆	◆	◆
Molecular biology	◆	◆				◆			
Analytical chemistry		◆		◆			◆	◆	◆
Biochemistry		◆							◆
Microscopy	◆					◆			◆
Conversion					◆		◆	◆	◆

- National Laboratories form Hub Core
- Integrate broader expertise through collaborations (subcontract and informal)
- Routine communication with extended hub facilitates integrated progress towards annual productivity targets
- Ability to shift focus in integrated fashion based on research findings

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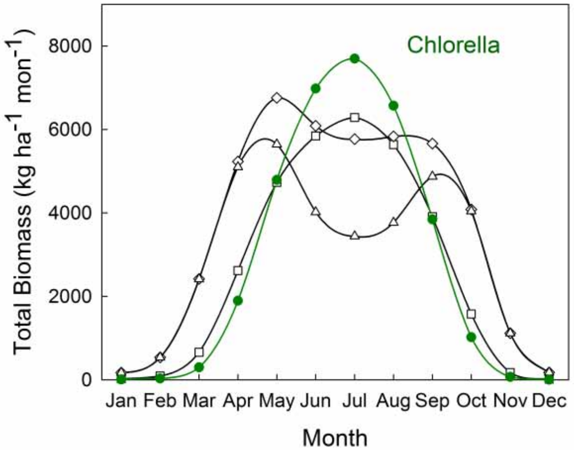
4 – Relevance – Off-season (INL)

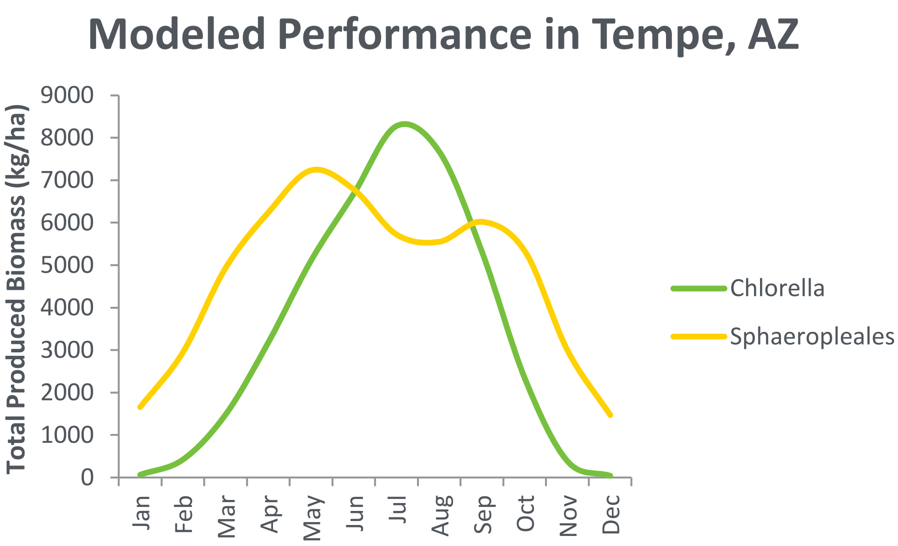
Successful development of off-season polyculture

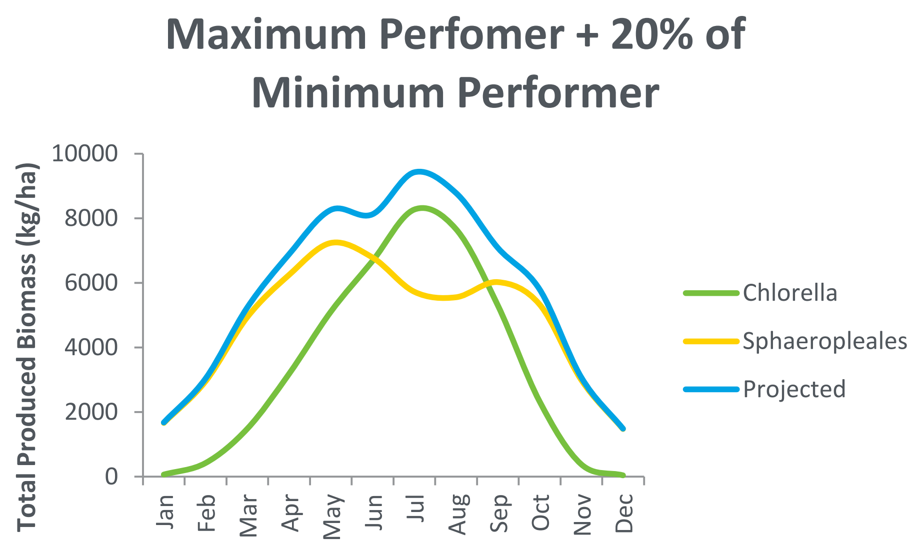
- Contribute to knowledge base for stable, productive polyculture assembly
- Extend productive growing season
- Expand potential sites for algal biofuel farm siting (long-term)

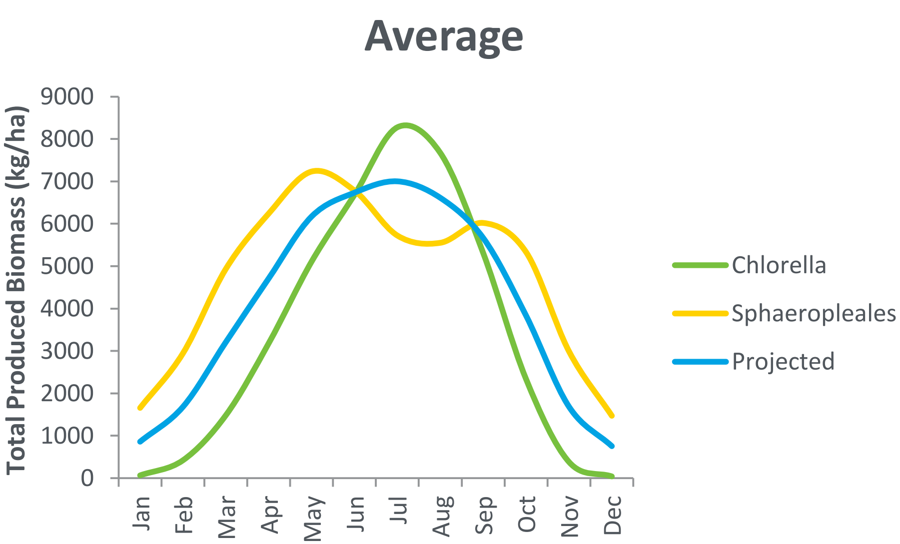


Impact: Lower productivity seasons represent roughly 2/3 of the year, thus even incremental improvements in productivity during "off-season" can significantly advance progress towards annual production target of 20-30 g/m^2/day AFDW

4 – Relevance – Off-season (INL)







- Top Left: Modeled performance of two monocultures
- Bottom Left: Projected polyculture production level if the average of both members is achieved (19% increase over *Chlorella* baseline)
- Top Right: Projected polyculture production level if the minimum performer contributes 20% to the maximum performer (66% increase over *Chlorella* baseline)

4 – Relevance (Hub)

- Several key challenges associated with algal production have not yet been successfully met by monoculture approaches, despite large investments by BETO and others over many years
- Polyculture Hub activities have been designed to address a subset of these key challenges through **integration** of expertise and capabilities
- Integrated polyculture effort and AOP process allows flexibility to adapt and adjust focus based on findings
- Interface with industry on needs
- Routine communication with ATP3

- **Outcomes:**
  - High and reliable biomass productivity, with stable and resilient cultivation that can be done at large scales over long extended (multi-year) periods of time.
  - Polyculture production target: **20 - 30 g/m^2/day annualized biomass productivity**

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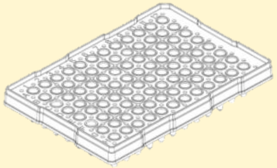
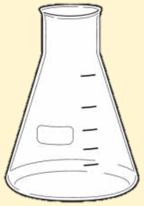
5 – Future Work (INL)





- High-throughput screening of trait-based combinations
- Small-scale shaker flask screening
- Comprehensive examination of promising polycultures using Phenometrics© Photobioreactors
  - Simulation of environmental conditions at target site with replicates
  - Capable of continuous cultivation
  - Evaluate population dynamics throughout experiment
- Field validation of designer consortia performance

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5 – Future Work (INL)

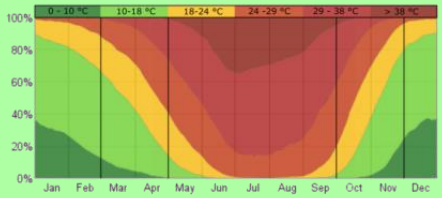
FY15	Q1
	Q2
	Q3
	Q4
FY16	**Q1**
	Q2
	Q3
	Q4
FY17	Q1
	Q2
	Q3
	Q4

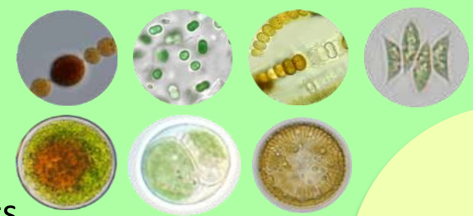
- Demonstrate a 10% improvement in lab biomass productivity compared to monoculture baseline to show the viability of the low-temperature polyculture approach
- Assess performance under *fluctuating* temperature conditions of 2-3 polycultures that demonstrated promising productivity at low temperature
- Validate the merit of the approach through demonstrating the potential for a 20% increase in annualized biomass productivity in polycultures relative to monocultures in preparation for FY17 outdoor testing. (Joint: INL, ORNL, PNNL, SNL)
- Demonstrate at least 10% improvement in biomass productivity compared to monoculture baseline through a polyculture under *fluctuating* temperature conditions
- Contribute to the integrated design of a single consortium that can both increase production and improve the resilience

5 – Future Work (Hub)

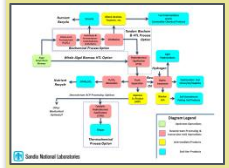
INL

- Tailored polycultures for increased off-season productivity
- Phenometrics photobioreactor comparisons of polycuture vs monoculture
- Population dynamics





SNL

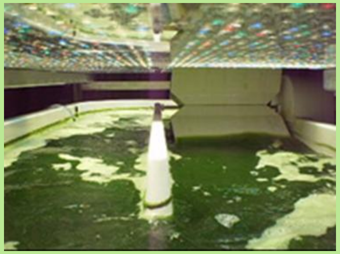


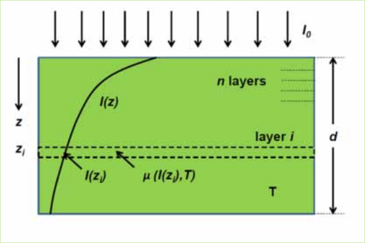
- Polyculture algal turf biomass productivity & quality improvement
- Biochemical & HTL processing and conversion of polyculture biomass
- Probiotic performance enhancement of polyculture
- Comparative performance testing of polyculture vs. monoculture
- Meso-scale pond testing/monitoring

HUB

PNNL

- Evaluate polycultures in climate-simulation ponds
- Predictive modeling of polycultures





ORNL

- Stable polycultures resistant to pond crashes
- Meso-scale pond testing
- Predictive modelling of long term grazer:algae population dynamics

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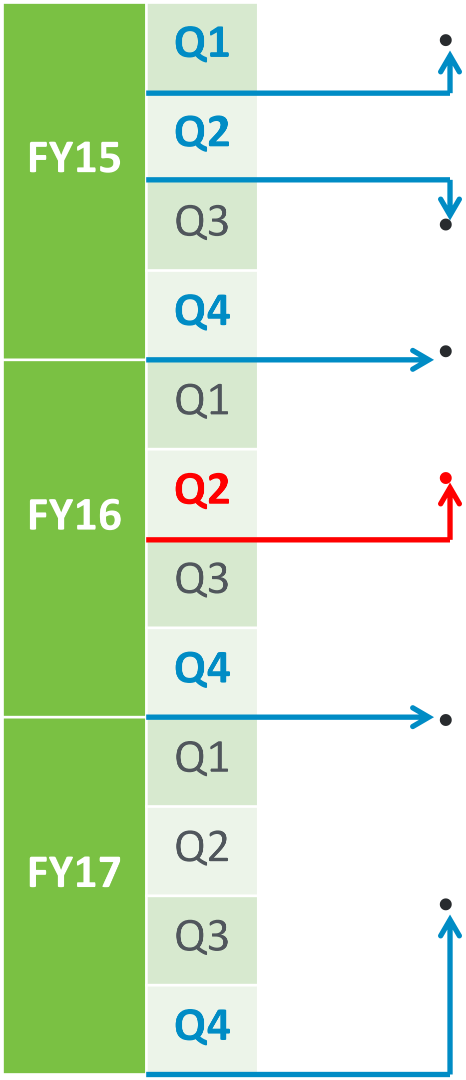
5 – Future Work (Hub)



- Deliver a detailed management plan to BETO showing coordination of efforts among the labs designed to ensure significant advancement towards MYPP goals using algal polyculture
- Submission of review paper to BETO that establishes a baseline for the state of the art for algal polyculture overyielding and resilience
- Demonstrate meso-scale polyculture performance through complementary approaches
- Validate the merit of the approach through demonstrating the potential for a 20% increase in annualized biomass productivity in polycultures relative to monocultures in preparation for FY17 outdoor testing
- Contribute to the development of operation testing protocols and methods for outdoor operations at either ATP3 or RAFT to compare polyculture to monocultures.
- Aim to achieve a target of 20 g/m^2/day annualized biomass productivity based on extrapolation of data generated from test-bed operation (ATP3 or RAFT)

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Summary

- The goal of the proposed project is to develop polyculture-based cultivation approaches to gain improvements in annual productivity, culture stability, and reduction of the overall costs associated with algal cultivation that .
  - Polyculture takes advantage of ecological principles to enhance stability, annual productivity, and resource utilization relative to monoculture.
  - The integrated hub has developed a detailed management plan to coordinate research among its members and maximize their collective progress.
  - The Hub structure will facilitate timely assimilation of new knowledge in a way that is not achievable through disparate projects funded through a competitive process, facilitating integrated progress towards the MYPP goals.
  - MYPP targets will be met by polyculture through high and reliable biomass productivity, with stable and resilient cultivation that can be maintained at commercial scale.

- **The Polyculture Hub will focus on the delivery of field-verified polycultures that resist predation, maintain high productivity during off-season, and are readily converted to drop-in fuels.**

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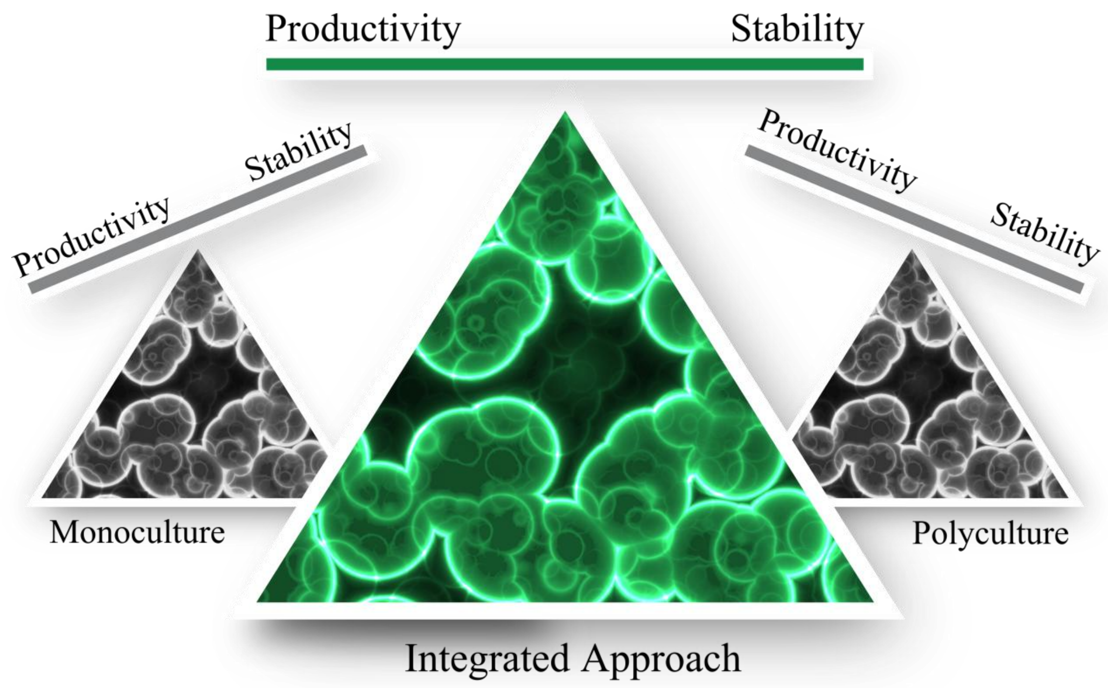
Responses to Previous Reviewers' Comments

- New start for the overall Hub
- Response to Review comments will be included in presentations from SNL, ORNL, and PNNL for research that extends from previously reviewed work

Questions?



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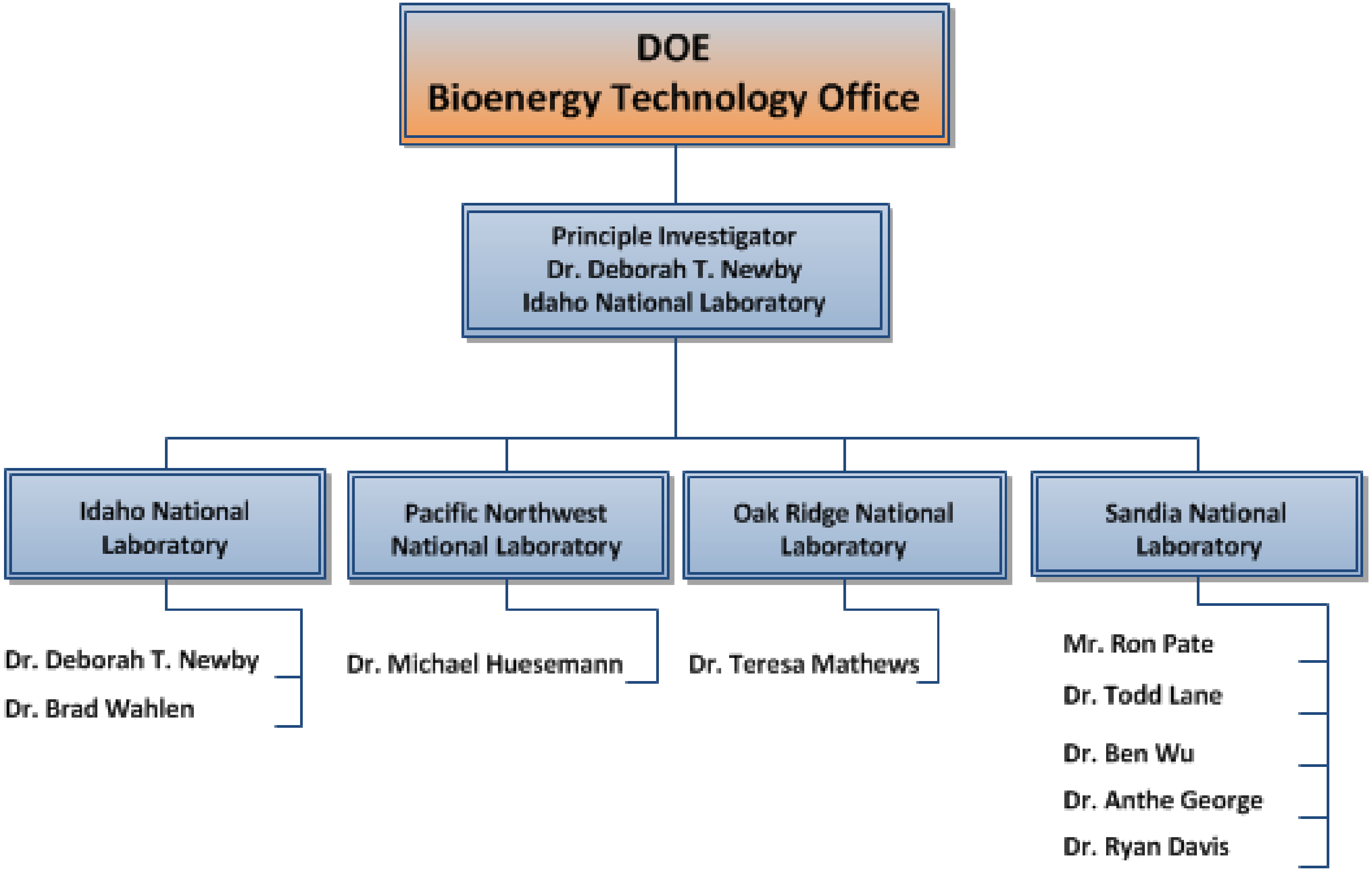
Additional Slides

Publications, Patents, Presentations, Awards, and Commercialization

- Publication in preparation: Review manuscript to be submitted to BETO that establishes a baseline for the state of the art for algal polyculture overyielding and resilience (FY15 Q2).
- Collaboration discussion in process with industry partner, initial focus centers on predators

Additional Slides



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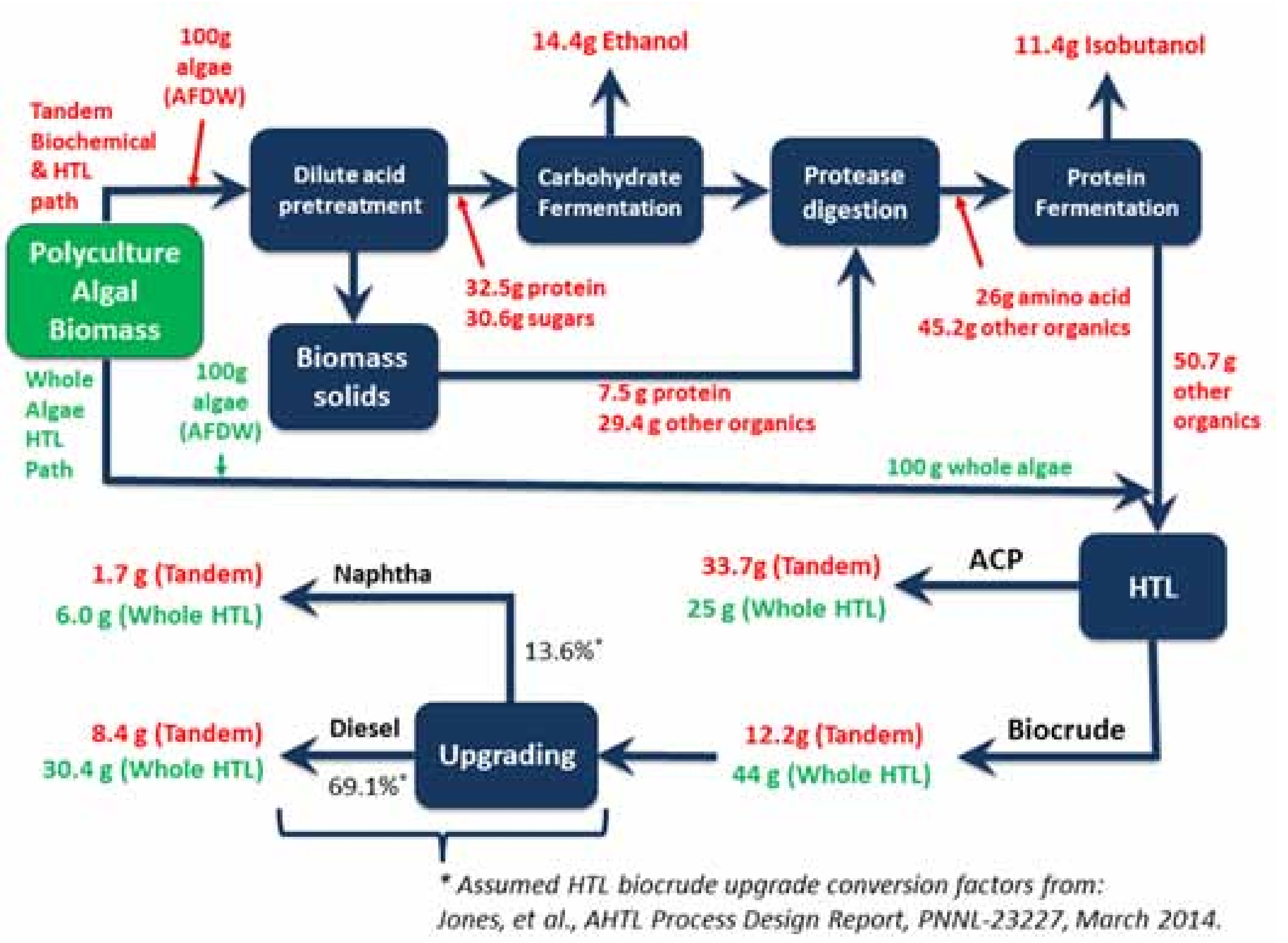
4 – Relevance (Hub)

- Biomass assumptions (100g basis)
  - 8g lipid
  - 39g protein
  - 34g carbohydrate
  - 19g other organics

Fuel Production per Processing Pathway		Polyculture Algal Biomass Annual Average Daily Productivity [g m^{-2} d^{-1}] (AFDW)			
		15	20	25	30
Annual Biomass Production [kg/acre] (AFDW)		22166	29555	36943	44332
Tandem Biochemical & HTL Processing & Conversion					
Annual Fuel Production by Type [GGE/acre]	Ethanol:	702	937	1171	1405
	Isobutanol:	709	945	1182	1418
	Naphtha:	135	180	225	270
	Diesel:	701	935	1169	1403
Total Annual Fuel [GGE/acre]		2248	2997	3746	4495
Whole Algal Biomass HTL Processing & Conversion					
Annual Fuel Production by Type [GGE/acre]	Naphtha:	487	649	811	973
	Diesel:	2529	3373	4216	5059
Total Annual Fuel [GGE/acre]		3016	4022	5027	6032

Impact



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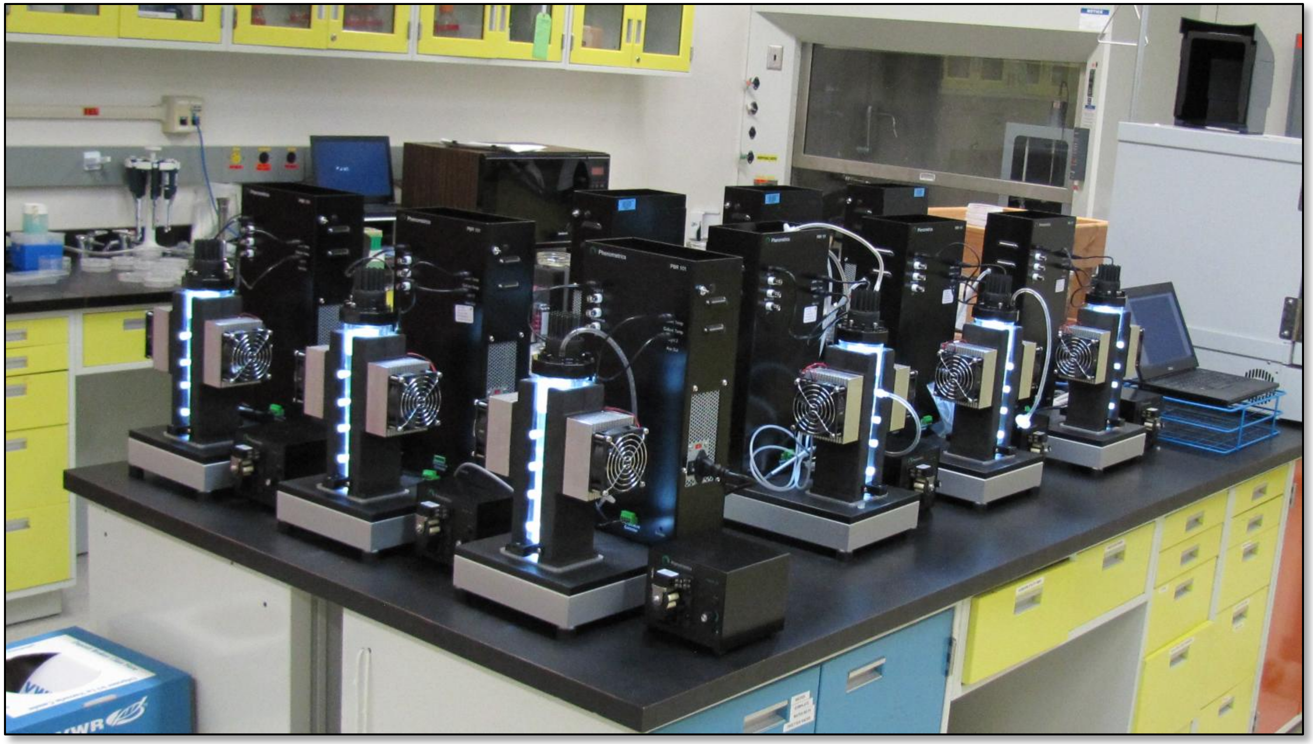
Risk Table

Risk Name:	Risk Description:	Response Plan:	Risk Response: • Accept • Avoid • Transfer • Mitigate
Designer polycultures provide limited improvement	Overyielding has been reported in literature from polycultures but the data to date is inconclusive	Modifications of consortia based on initial results Multiple approaches to designer consortia including natural assemblages Multiple assemblages will be investigated	Accept and mitigate
Polycultures do not provide significant protection against predation	Cell size may not be the most important control on grazing potential	Diversified approaches including multi-trophic levels, and natural assemblages Multiple assemblages will be investigated	Accept and mitigate
Scaling	As with monocultures, solution applicability at larger scales is a major challenge	Scaling will be a challenge both for monoculture and polyculture work. Some proof of principle work will need to be done in the laboratory, but because we hypothesize that polycultures will be more resilient than monocultures to various stressors, the goal is to transition to outdoor ponds as quickly as possible in order to address BETO's goals for productivity. Both PNNL and ORNL have indoor raceway ponds at the mesocosm scale which can simulate outdoor conditions, and work will move from bench scale to mesocosm scale, and eventually to outdoor pond scale in outyears. Multiple assemblages will be investigated	Accept and mitigate
Timing/seasons	Pond crash or seasons could force delays of 6 months to a year for outdoor testing	Leverage ATP3 consortium ponds that are in geographically and climatologically distinct sites, some with long growing seasons e.g., Cellana	Accept and mitigate

Lab-Scale simulation of site conditions

- Phenometrics© Photobioreactors
  - Utilize sinusoidal temperature and light fluctuations to simulate growth conditions experienced at potential test sites during “off-seasons”



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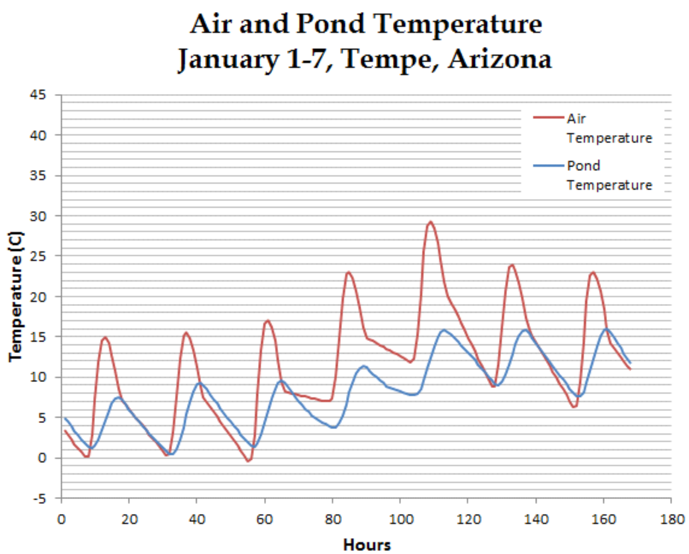
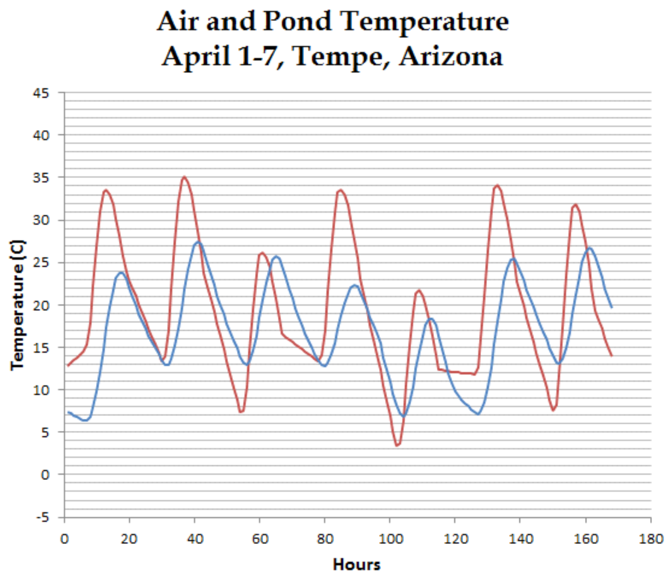
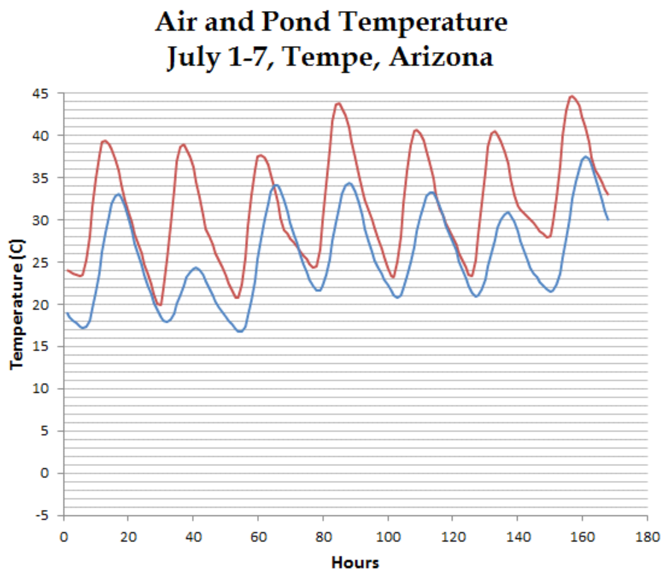
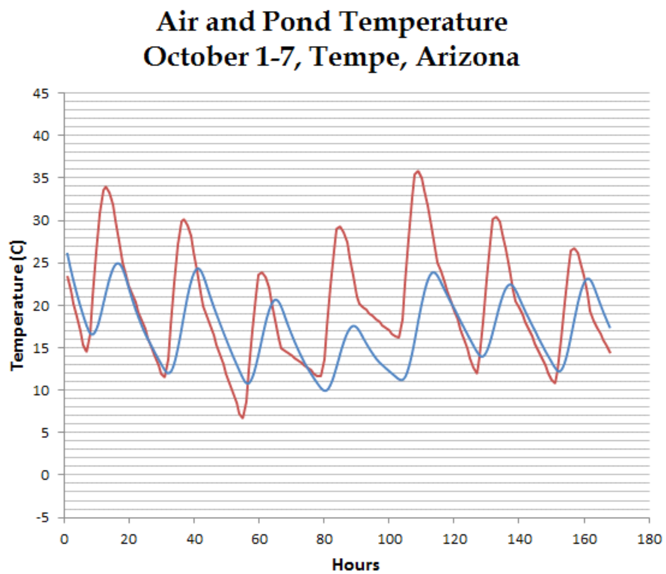
Air and Pond Temperature









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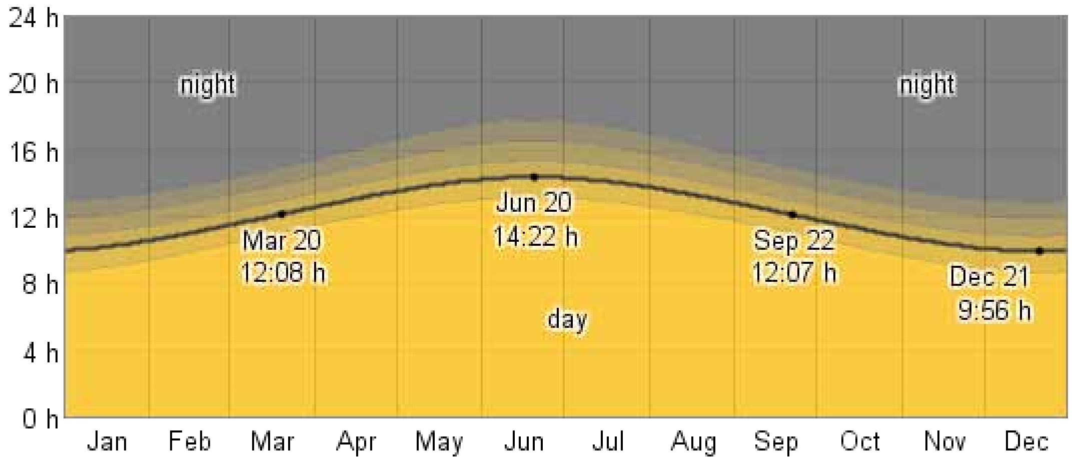
Historical Sunlight

- Daily average hours of sunlight from 1974-2012 at a weather station near Glendale, AZ



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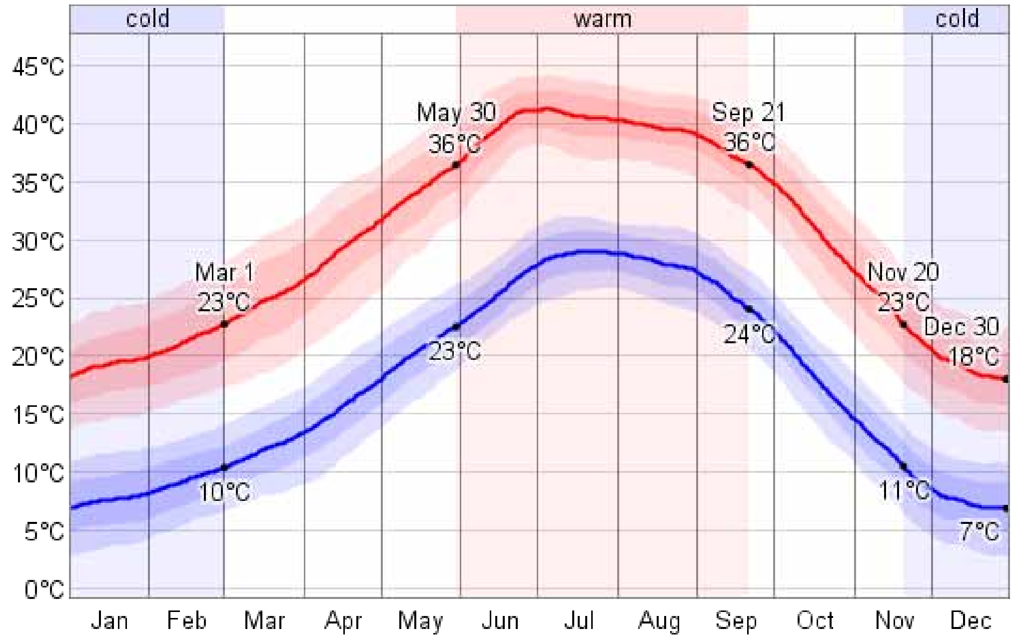
Historical Temperature Data

- Daily average high and low air temperatures from 1974-2012 at a weather station near Glendale, AZ



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