

Large-scale production of marine algae for biofuels

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Algae Platform Review

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Our primary goal is to evaluate the commercial viability of a fully integrated, *marine* algal-production-to-finished-fuel technology pathway, based on data from scalable outdoor unit operations, that demonstrates:

- (1) by Q4 2013, performance against clear cost goals and technical targets
- (2) by Q4 2014, productivity of 20 g m⁻² d⁻¹ AFDW, or 1,500 gal acre⁻¹ yr⁻¹ processed oil or biofuel intermediate.

Objectives

- Select top strains from >500 novel marine algae screened and characterized
- Harmonize analytical methods among Consortium laboratories
- Demonstrate that “mid-scale” screening (200 L) guides large-scale production
- Produce ton quantities of a single feedstock over 100 daily production cycles
- Demonstrate feedstock processing to biofuel intermediate(s) at scale
- Demonstrate technical and commercial viability of co-products at scale
- Optimize productivity based on nutrient, gas, and water management
- Deliver a Design Report – a comprehensive techno-economic and life-cycle assessment for at least one technology pathway based on demonstrations



Quad Chart Overview

Timeline

Project Start Date: 04/2010
Project Suspended: 08/2011
Project Restart: 05/2013
Project End Date: 12/2014
Percent Complete: 50%

Budget

Total project funding: \$13.4M
- DOE share: \$9.0M
- Contractor share: \$4.4M
Funding received FY12: \$0.0
Funding for FY13: \$9.1M

Barriers Addressed

- **Ft-C Feedstock Genetics & Development:** Enable predictive selection, screening, & characterization of productive marine algae
- **Ft-N Algal Feedstock Processing:** Demonstrate viable technology to deliver biofuel intermediates and co-products
- **Ft-M Overall Integration & Scale-up:** Show that integrated unit operations at scale deliver sustainable production

Partners

Cornell University (Prime)
The University of Southern Mississippi
Duke University
San Francisco State University
University of Hawaii
Sahara Forest Project
Cellana
University of Nordland
Gildeskål Research Station



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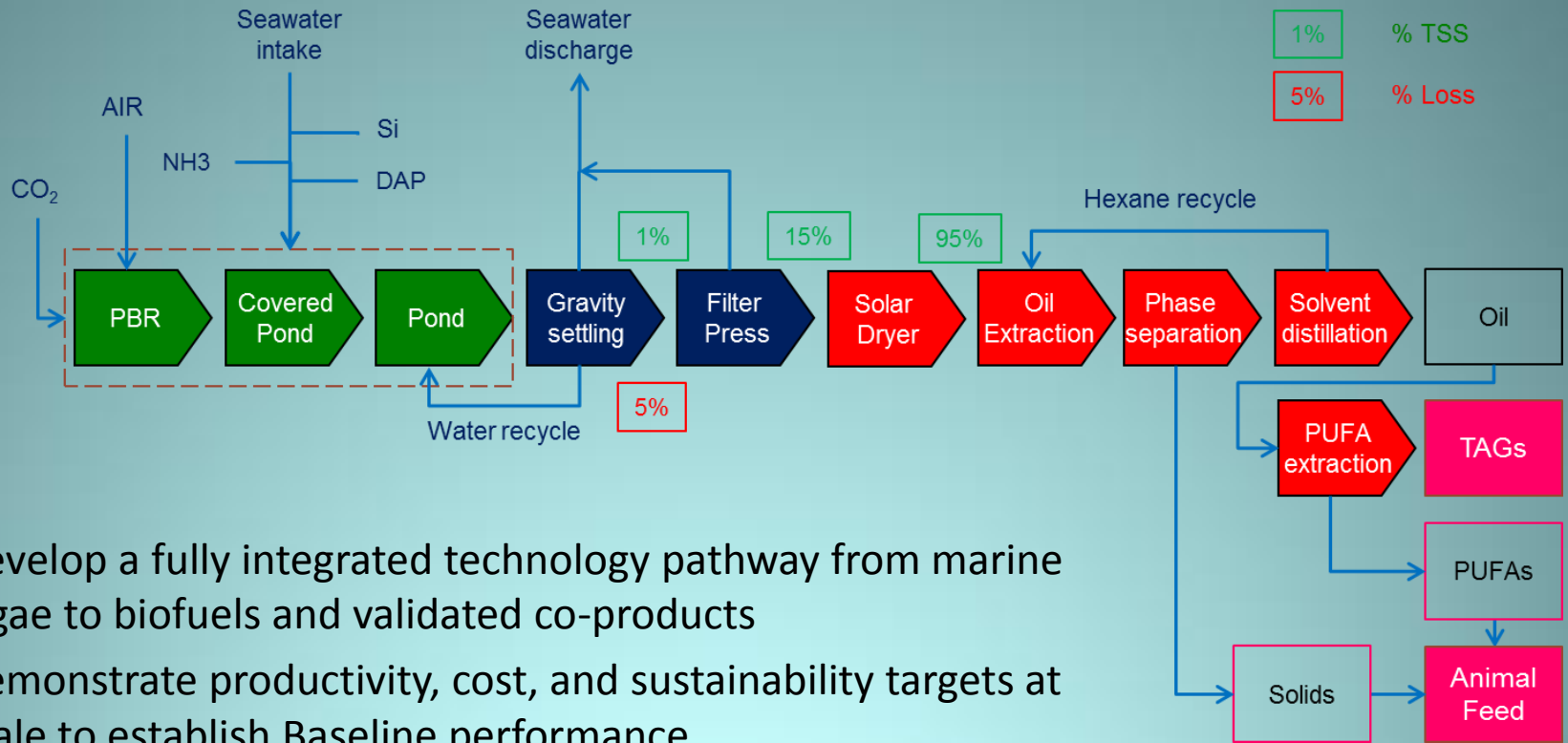
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- Develop a fully integrated technology pathway from marine algae to biofuels and validated co-products
- Demonstrate productivity, cost, and sustainability targets at scale to establish Baseline performance
- Builds on pre-DOE investment of \$100M in R&D Consortium



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Task 1 Strain Development

Improve and screen strains for optimum production characteristics; develop and harmonize innovative measurements

Task 2 Cultivation

Demonstrate improved management of nutrients, gas exchange, and seawater recycling to increase sustained productivity and reduce costs at large scale

Task 3 Harvesting & Dewatering

Demonstrate efficient, low-energy harvesting and dewatering processes at scale

Task 4 Extraction

Toll-process ton quantities to evaluate fuel and animal feed products

Task 5 Co-product Development

Demonstrate nutritional, commercial value of non-fuel co-products in animal feed

Task 6 Integrated Design

Techno-economic and life cycle assessment of a fully integrated technology pathway, based on data from extended, ton-quantity production runs



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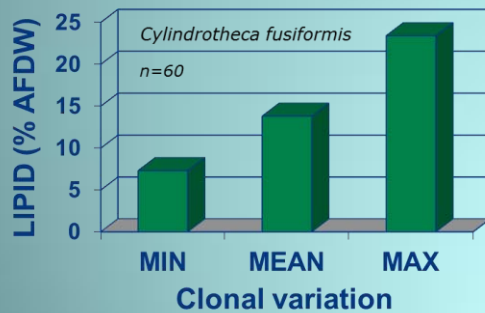
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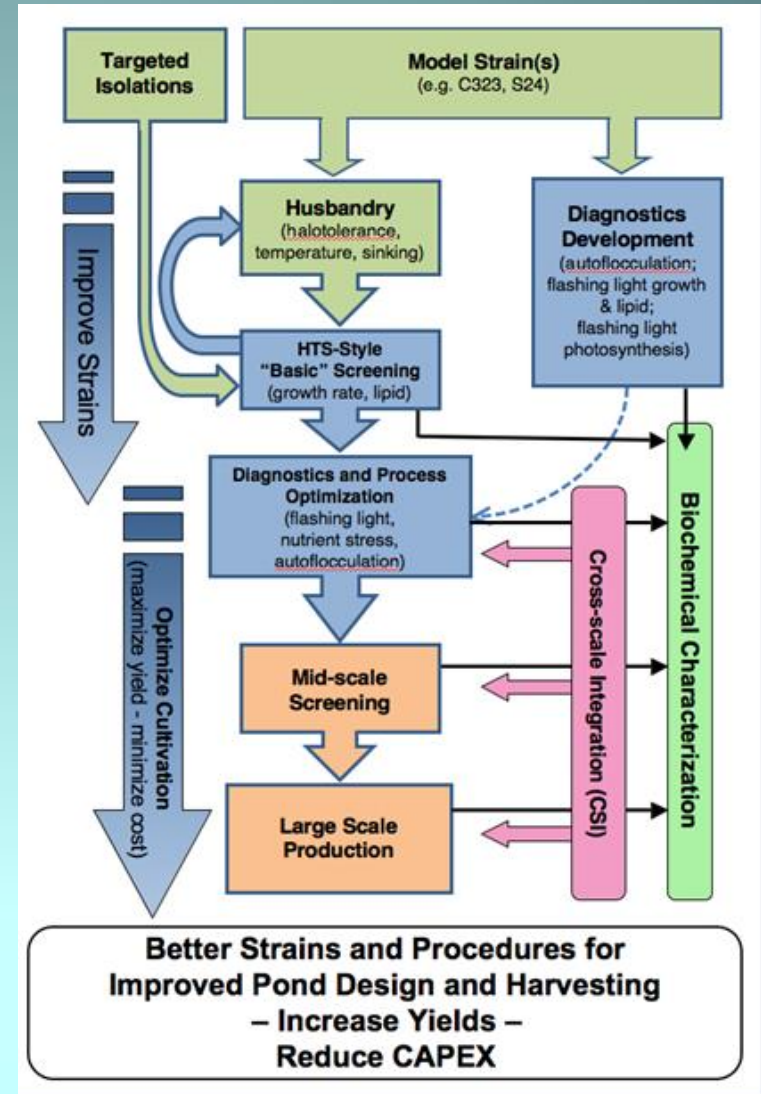
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Objectives

- Select several model strains from >500 novel marine algae screened and characterized
- Improve lipid, growth rate, ash content, halotolerance, and harvestability via husbandry
- Exploit natural variability and apply environmental selection pressure to select phenotypes of interest
- Supply improved strains for mid-scale screening and cultivation teams



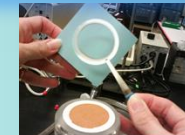
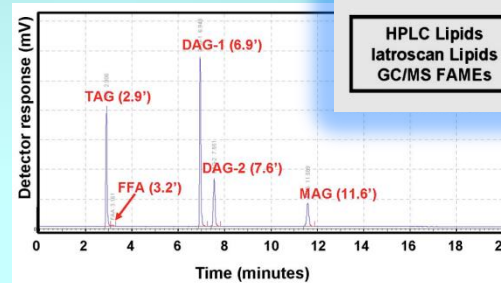
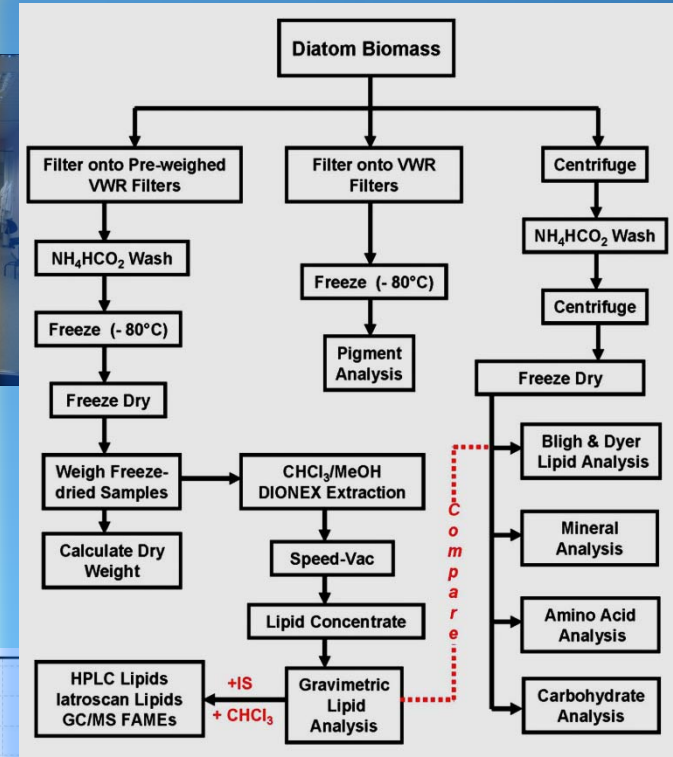
Liang et al. 2005, *J. Appl. Phycol.* 17: 61



Subtask 1.2 Biochemical Characterization

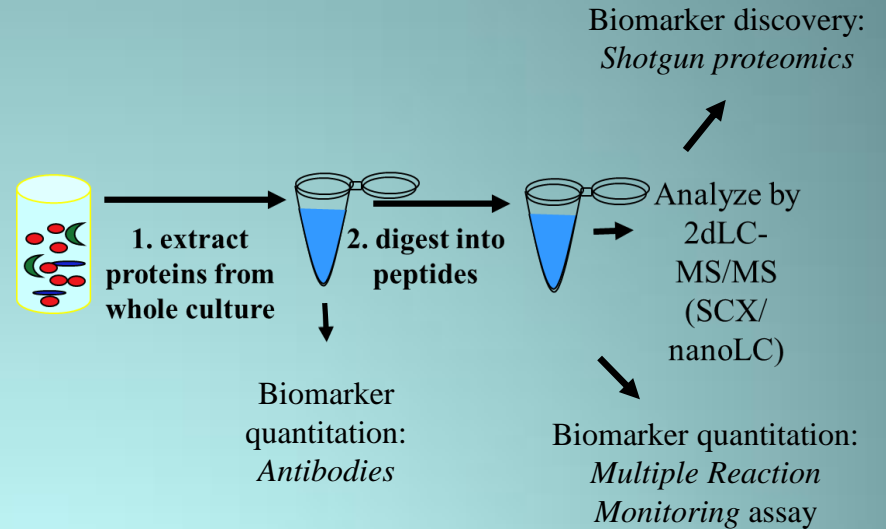
Objectives

- Provide standard methods for biochemical screening at all scales
 - Total lipid
 - Lipid classes
 - Fatty acid methyl ester (FAME)
 - Total protein
 - Total carbohydrate
 - Minerals, Pigments, CHN
- Harmonize methods at all Consortium laboratories using standard reference material to ensure reproducibility and provide a baseline for mass balance
- Develop a micro-gravimetric method for lipid analysis to reduce sample size and accelerate screening



Objectives

- Develop a rapid diagnostic assay that tracks key protein biomarkers of lipid biosynthesis
- Identify genes and pathways via expressed sequence tags (EST) libraries from clades before and during lipid induction
- Discover key biomarker enzymes via shotgun proteomics
- Develop multiple reaction monitoring (MRM) assays
- Apply assays to managing large scale cultivation



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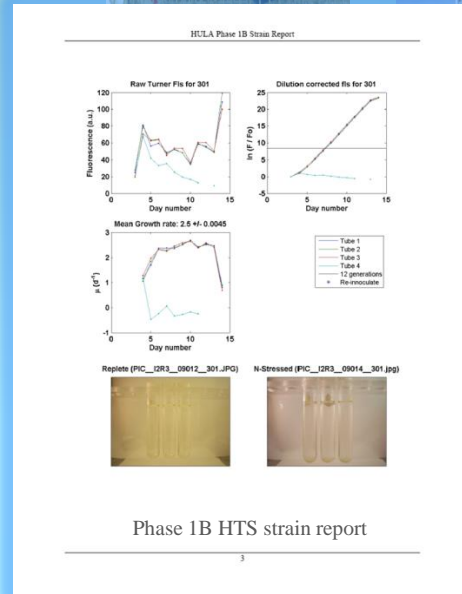
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Objectives

- Demonstrate a screening process that accurately predicts strain performance at large scale
- Use top candidate strains from characterized collection
- Key performance parameters
 - Growth rate (μ : d⁻¹)
 - Lipid yield at harvest ($g\ m^{-2}$)
 - TAG yield at harvest ($g\ m^{-2}$)
 - Sinking rate ($m\ h^{-1}$)
 - Ash content ($g\ g^{-1}$)
 - C:N ($mol^{-1}\ mol^{-1}$)
 - Nile red TAG index ($g\ m^{-2}$)
 - Temperature tolerance
 - Halotolerance
- Benchmark all performance against baseline strain



Objectives

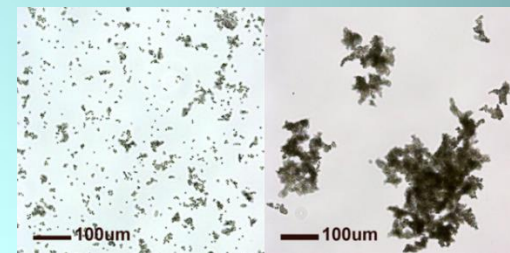
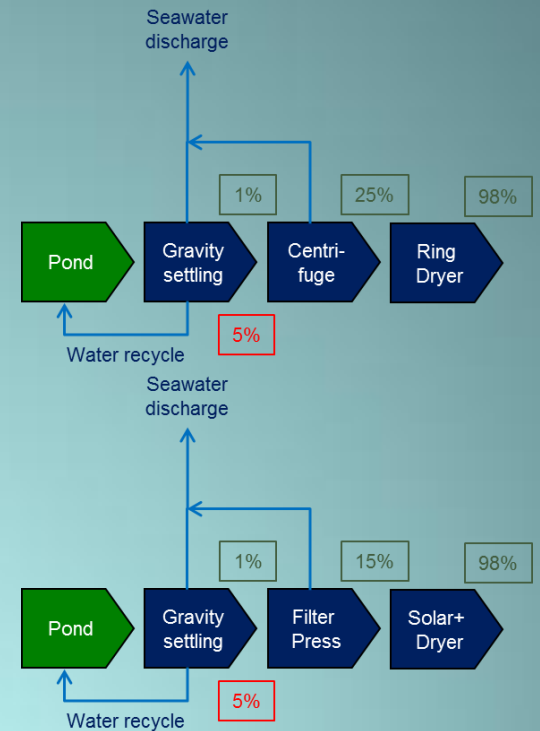
- Sustained production of a Baseline strain for 100 daily production cycles
- Sustained production of *improved strains*, benchmarked against Baseline
- Produce tons (AFDW) to support processing and co-product trials
- Evaluate performance against prime targets to improve yield, reduce cost and energy via:
 - Nutrient management
 - Water management
 - Harvest management
- Key performance targets
 - Productivity $20 \text{ g m}^{-2} \text{ d}^{-1}$ AFDW
 - TAG yield $1,500 \text{ gal acre}^{-1} \text{ yr}^{-1}$
 - Cultivation capex $< \$60 \text{ m}^{-2}$
 - Energy consumption $< 10\%$ fuel energy content



Objectives

- Harvest and dewater tons (AFDW) to support processing and co-product trials
- Demonstrate a gravity-based harvest method using ponds as settlers
- Evaluate low-energy concentration processes
- Key performance targets

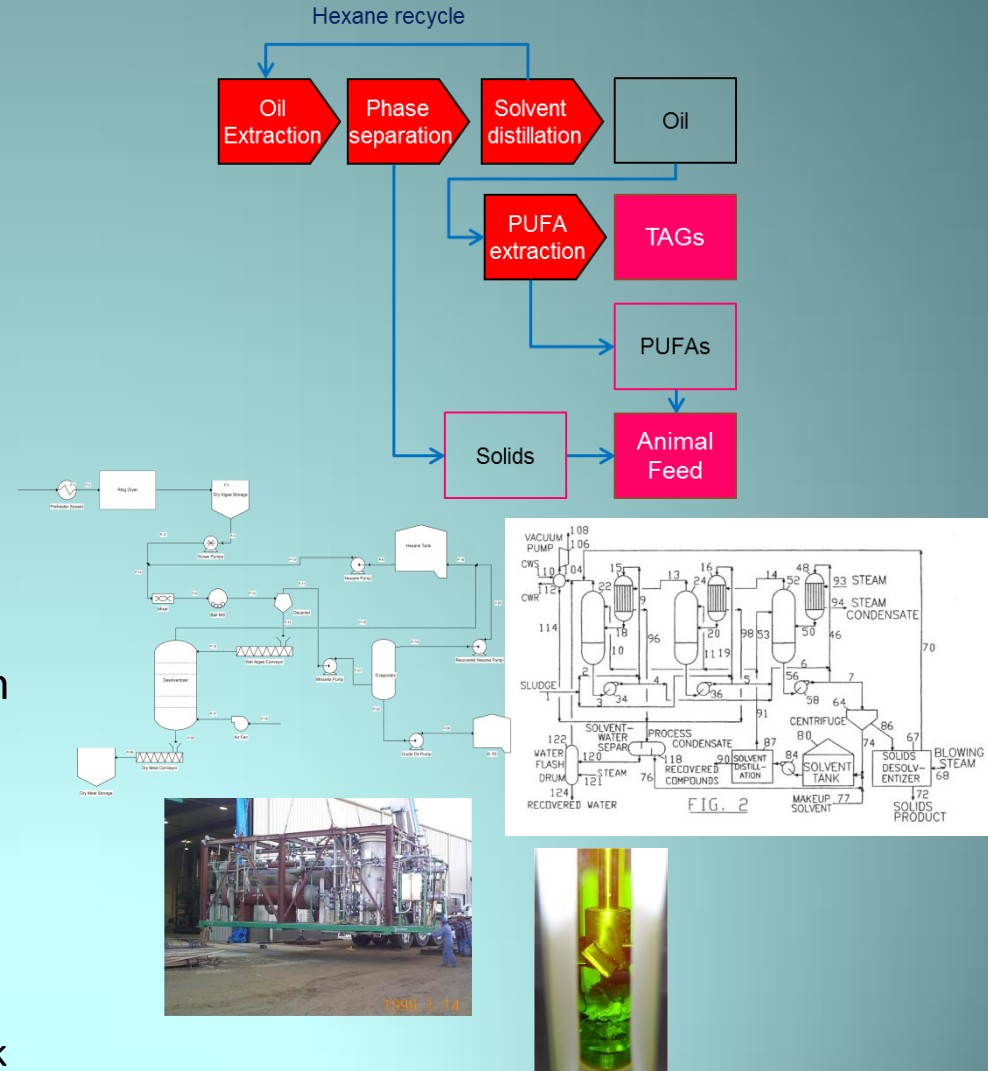
Algae concentration at harvest	0.35 g L ⁻¹
Feedstock after harvest	1% TSS
Feedstock after concentration	15% TSS
Feedstock after drying	98% TSS
Overall efficiency	90%
- Evaluate flocculants for cost-effective dosages that enhance aggregation and settling



Task 4: Extraction

Objectives

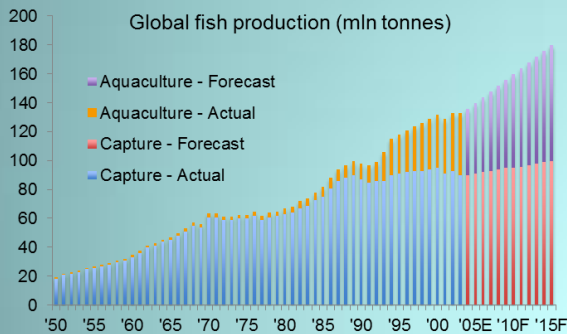
- Toll-process ton quantities (AFDW) of feedstock into a neutral lipid fraction (biofuel intermediate) and the residual defatted biomass
- Supply ton quantities of defatted biomass for co-product formulation and pre-commercial demonstration trials
- Evaluate *dry route*: industry standard hexane extraction, toll-processor
- Evaluate *wet route* alternatives based on key criteria, including
 - Suitable for refining
 - Energy consumption <10% fuel E
 - Capex/opex
 - Overall efficiency
 - LCA rating
 - Bench trials, using 10 to 100 kg feedstock



Task 5: Co-Product Development

Objectives

- Demonstrate commercial value of the defatted biomass co-product in animal feeds
- Formulate feeds for top aquaculture species that produce >30% of world fish production
- Conduct pre-commercial field trials on several different feedstocks

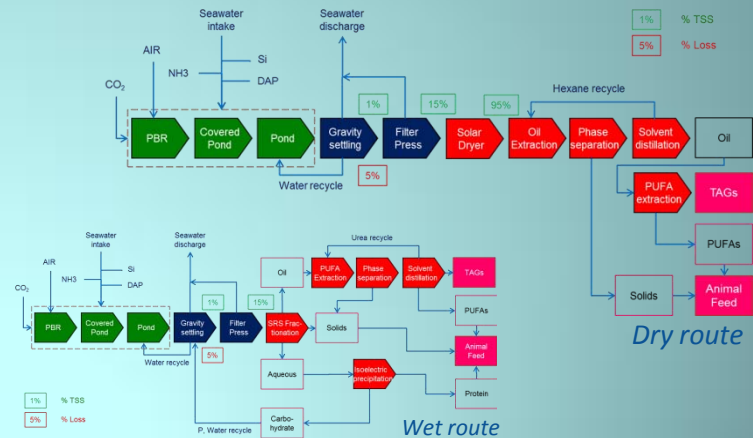
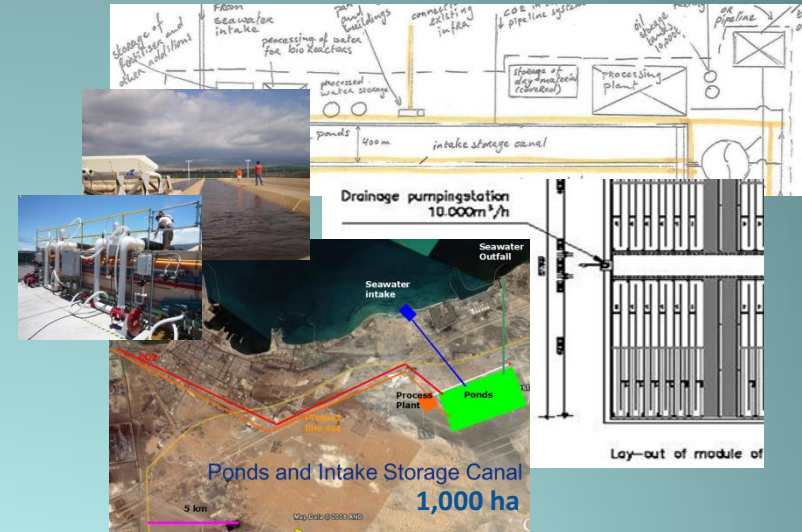


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Objectives

- Establish Baseline economics and resource impact of a fully integrated, algae-to-fuel technology process lineup
- Integrated Design of unit operations to enhance efficiencies, reduce cost, energy use
- Techno-economic analysis to value-engineer and assess comprehensive financial projections
- Life-cycle assessment to quantify overall resource impact, with emphasis on pertinent indices, e.g. RFS
- Validate Baseline from sustained production runs and processing of a single strain – unit operations demonstrated at pilot scale
- Develop alternate pathways: new unit operations, validate with improved strains, compare to Baseline



Major Milestones

Milestone 1: Harmonize measurements of key biochemical parameters

Milestone 2: Sustain production of a single strain for 100 production cycles

Milestone 3: Produce ton quantities (AFDW) of algal biomass feedstock for downstream process development and co-product demonstration

Milestone 4: Assess nutritional value of co-product feeds from several algae in pre-commercial field trials

Milestone 5: Establish Baseline techno-economics and life cycle assessment of an integrated process lineup, validated at production scale

Milestone 6: Quantify improved performance against Baseline with new strains and alternative unit operations

Milestone 7: Deliver a Design Report to compare technology pathways, substantiate progress on production targets, and quantify remaining barriers



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Management Process

Governing agreements: MOU, NDA

Structure: All Consortium members represented by PIs on leadership team

Decision-making process: Consensus

Communications

- Consortium website and database
- Structured reporting: monthly conference calls + progress summaries; quarterly reports; annual leadership team meetings
- Shared data, technical reports, presentations, manuscripts

Resumes Q2 2013....



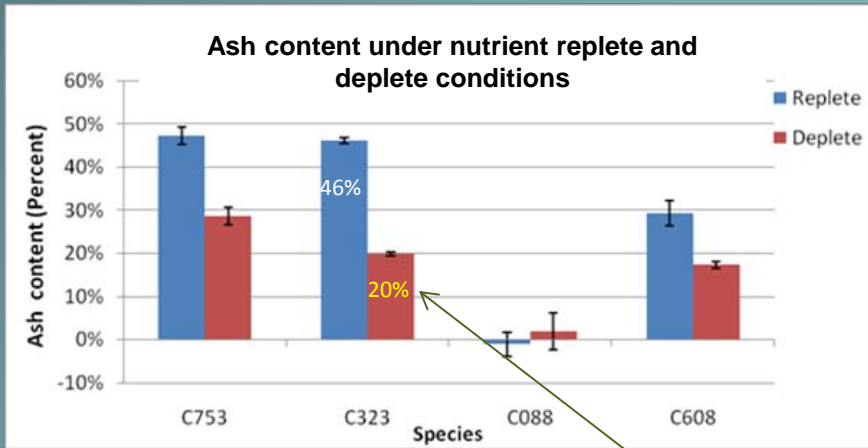
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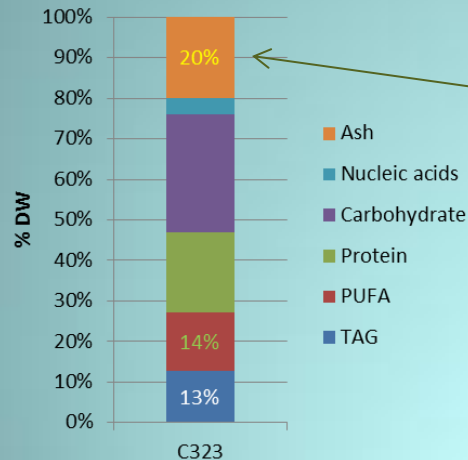
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Mid-scale screening, error bars ± 1 SD; $n=3$ (Pickell 2010)



Large-scale production of C323, optimized for low ash; $n=26$ (Bai et al 2013)

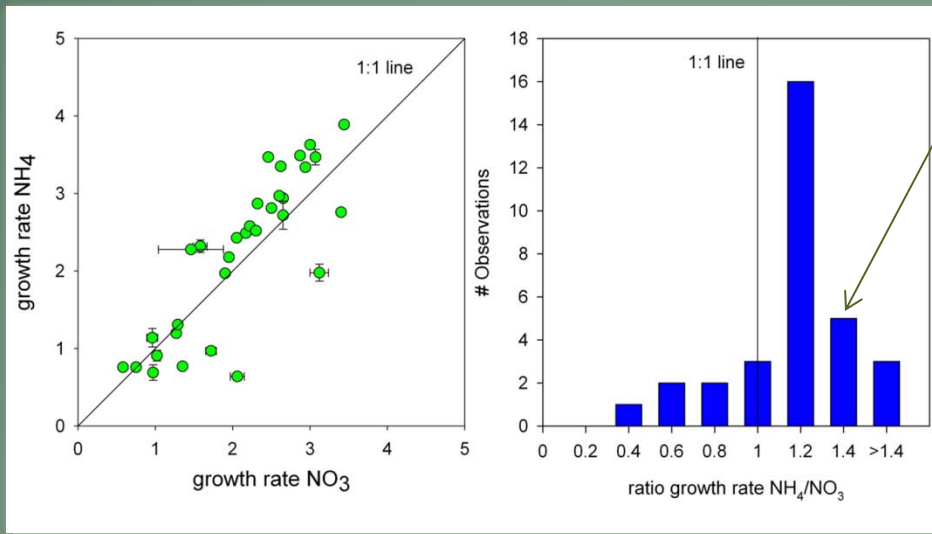
Expected outcome: Demonstrated improvements in characteristics of the Baseline strain that result in higher productivity, lower cost and energy use.

Progress: Production characteristics of the Baseline strain, *Staurosira* sp. (C323), are improved by optimizing nutrient and water management practices.

Reduced Ash content: 46% to 20%

- Lower process volume
- Lower cost
- Lower energy use
- Higher value co-product
- Validated at large scale





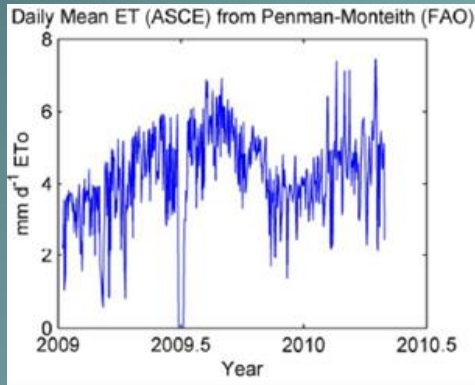
Improved Productivity 40%

NH_4 vs NO_3

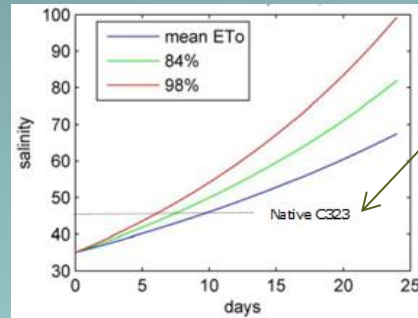
- Improves productivity up to 40%
- Improves N-depletion rate because there is no intracellular pool of NO_3
- Increases lipid and TAG yield because of faster N-depletion
- Lower cost
- Increased HSE risk due to handling
 - Common in agriculture
 - Requires validation for algae culture

Nutrient composition effect on productivity:

Comparison of growth rate (d^{-1}) for top candidate strains grown in NH_4 - vs NO_3 -based nutrient media; $n=32$ (Johnson et al 2010)



Mean evaporation rates at Cellana, 1+yr cycle, cause a mean salinity increase to 45 PSU (Johnson 2010)



Halotolerance of C323, Baseline strain, *Staurosira* sp., allows sustained productivity up to 45 PSU (Johnson 2010)

Reduced Seawater Use 75% Halotolerance

- Target: 10-d water recycle
- Effect on culture: +50% salinity
- Result: Sustained productivity C323
- Impacts: Lower capex, energy use
- Demonstration: C323 + improved strains, Sahara Forest Project, Qatar
- Integration with SFP technology enables freshwater production

Impacts on energy and cost

Task/Operation	GWh per 100 ha per year	
	2013	2015
Water transport		
Intake pumping station	0.853	0.171
Distribution flow from canals to PBRs	0.226	0.045
UV sterilization	0.165	0.033

Reduced energy use for water transport system

Task	Capital Cost (\$ per m ²)		
	2010	Base 2013	Target 2015
Site preparation	22.7	5.7	4.5
PBRs	7.3	6.8	6.1
Ponds	36.2	24.2	15.8
Water transport	22.4	22.4	5.6

Reduced capital costs for water transport system



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Subtask 1.2 Biochemical Characterization

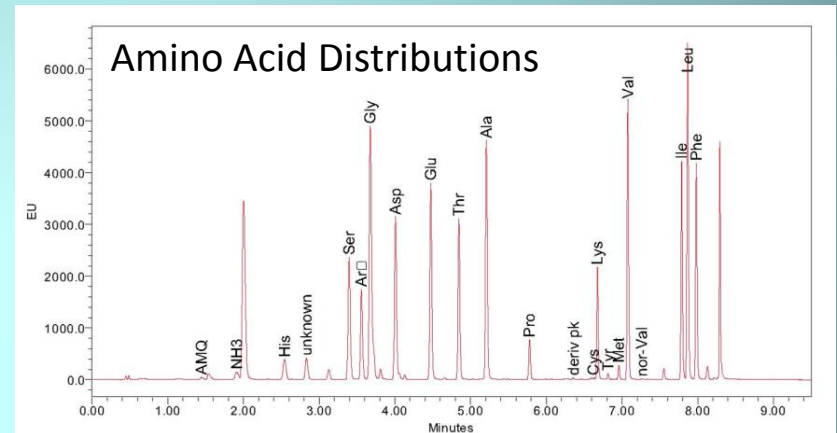
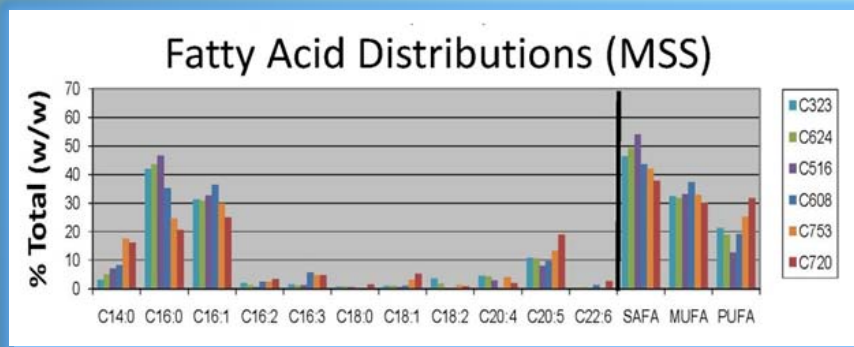
Analyte	Technique / method	Method implementation status						Reference Material	Status
		UH	KPF	WTC	STCT	STCA			
ΣLipids	Dionex ASE CHCl3/MeOH	X	X	X	X	X	C323 dried algae	RR completed, method being finalized	
Ash	High-temp combustion	X	X	X	X	X	C323 dried algae	RR completed, method finalized	
CHN	CHN Elemental analyzer		X	X	X	X	C323 dried algae	RR completed, method finalized	
Moisture + Volatile	Moisture analyzer		X	X	X	X	C323 dried algae	RR completed, method finalized	
Lipid classes	HPLC/ELSD	X	X	X	X	X	CH60 oil	Further development to address quantification issues	
FAME EN14105 mod	GC/FID		X	X	X	X	CH60 oil	Modified for improved quantification	
FAME total FA	GC/MS	X	X				CH60 oil	Shell to implement when need arise	

Harmonization among Consortium laboratories, 2011 status at UH (University of Hawaii), KPF (Cellana) and three Shell labs

Expected outcome: Harmonized, standard methods for measuring key biochemical performance parameters

Progress:

- Harmonization complete
- Reference material established
- New method developed for micro-gravimetric measurement of lipids: reduces sample size to <20 mg AFDW



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Subtask 1.3 Metabolic Pathways

Expected outcome: New assays developed and applied to optimize lipid production

Progress:

- None. Subcontract initiated one month before project suspension (Jul 2011)
- Expect to complete on new schedule (2013 - 2014)



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Subtask 1.4 Screening

Laboratory screening quantifies TAG yield ($\mu\text{g L}^{-1}$) and sinking index (SI) for N-stressed cultures grown on NO_3 (blue) or NH_4 (green). (Johnson et al 2010)

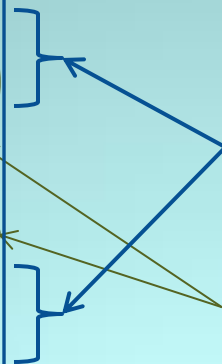
Expected outcome: Lab screening results quantify the best candidates. Mid-scale screening results guide the management of large-scale cultures.

Progress:

- Example: Baseline C323 (*Staurosira* sp)

TAGs

Strain	$\mu\text{g TAGs / L}$	SI
C273	14022 \pm 156	0.99
C273	7070 \pm 296	0.99
C323	6167 \pm 559	0.98
Ch21	5847 \pm 151	0.58
C003	5740 \pm 474	0.98
Ch22	5368 \pm 129	0.42
Ch22	4672 \pm 664	0.42
Ch22	4507 \pm 90	0.42
MT16	4477 \pm 773	0.58
Ch20	4235 \pm 435	0.28



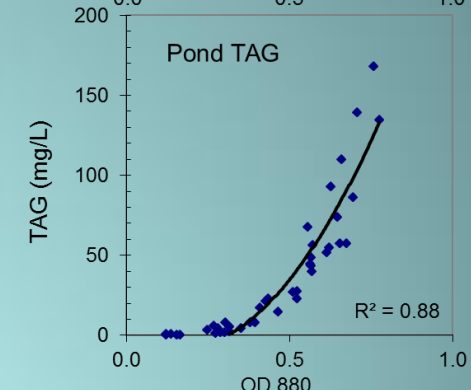
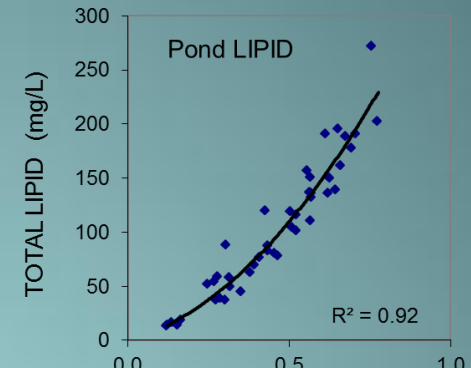
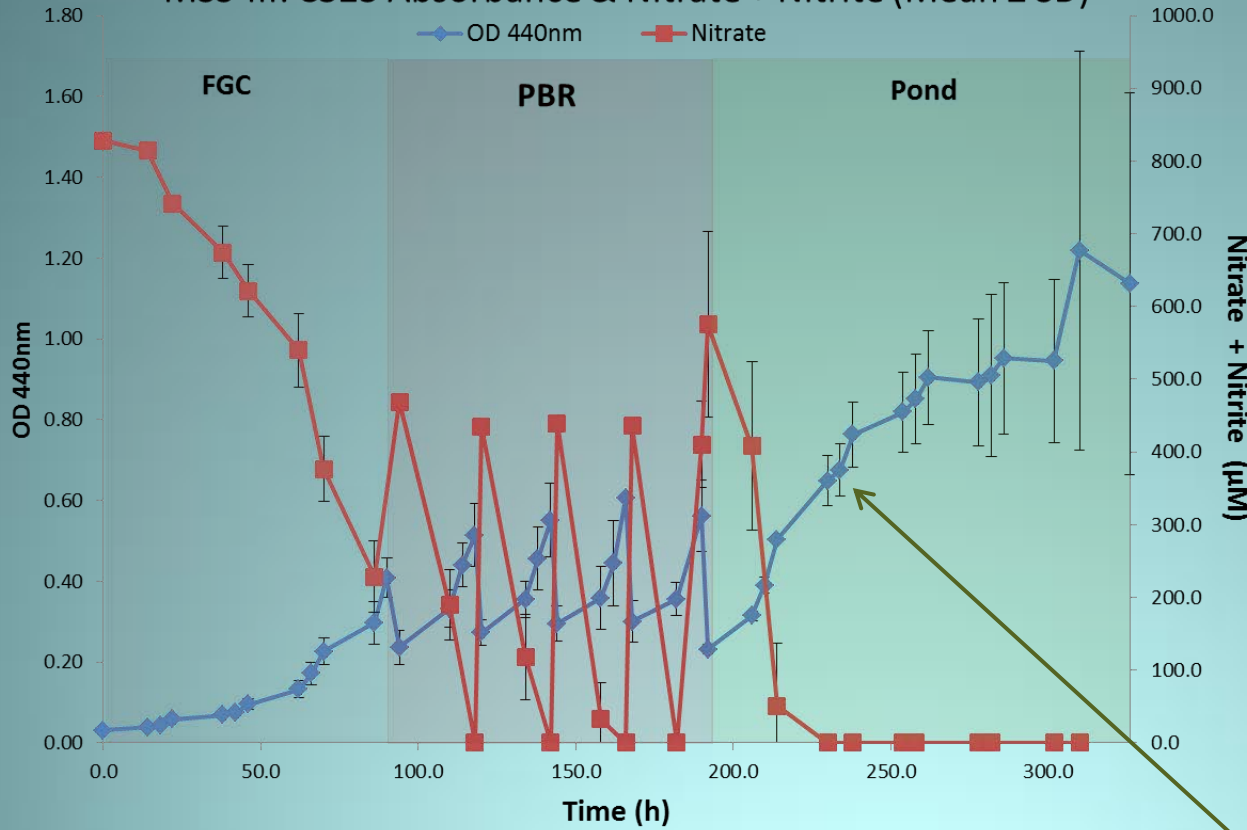
Yield on NH_4 is greater than on NO_3 .

High sinking index (SI) favors harvesting

High Throughput Screening (HTS)



MSS-III: C323 Absorbance & Nitrate + Nitrite (Mean ± SD)



OD tracks Lipid, TAG in 2-day ponds

Full Growth Curve:
allows acclimation.
Standard medium: f/2

PBR simulation:
~50% daily dilution

Production Pond simulation:
5-day batch culture to quantify best yield

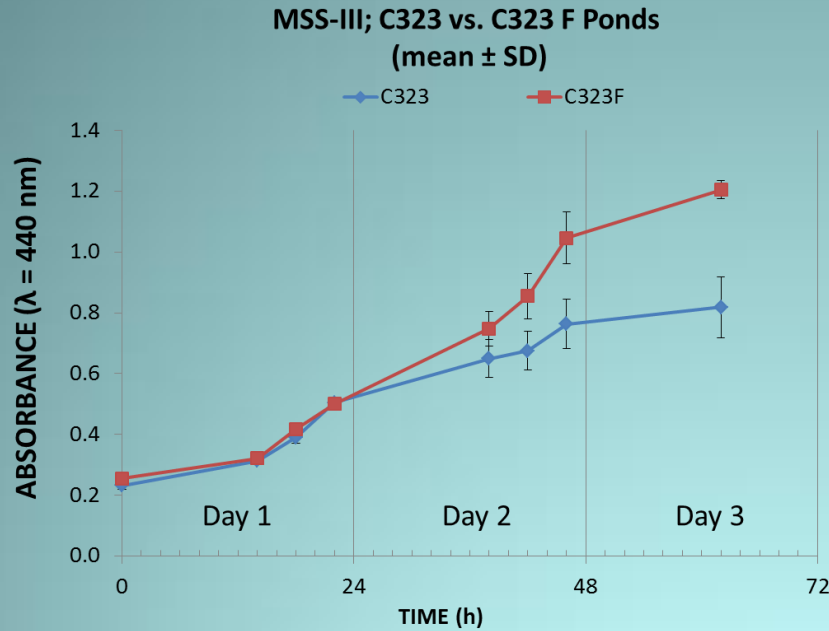
Pond cycle: 2 days

- Highest TAG yield
- N depleted

Mid-Scale Screening (MSS)

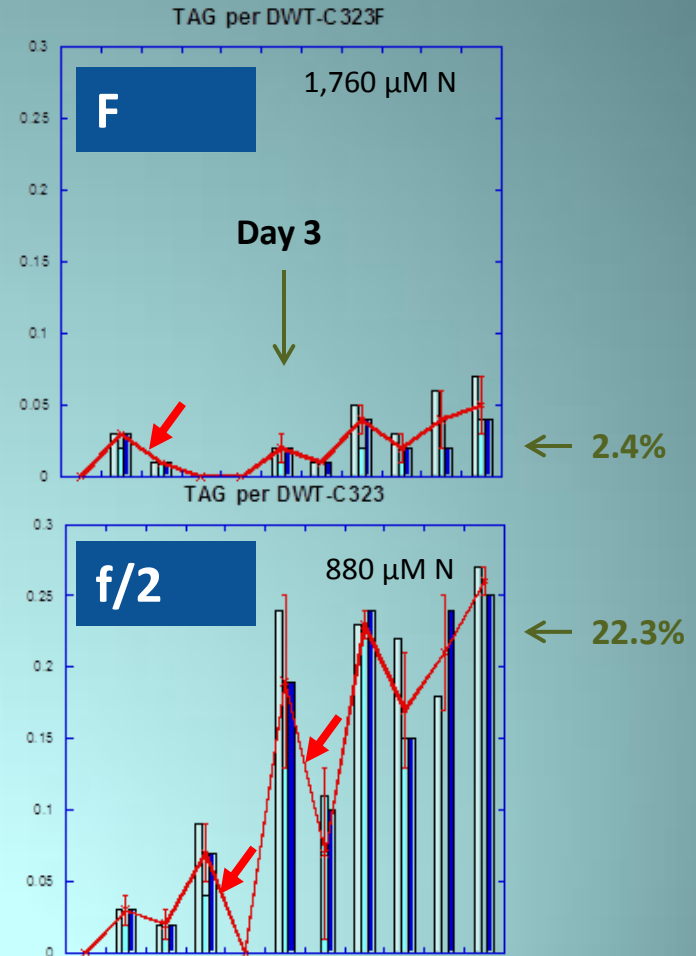


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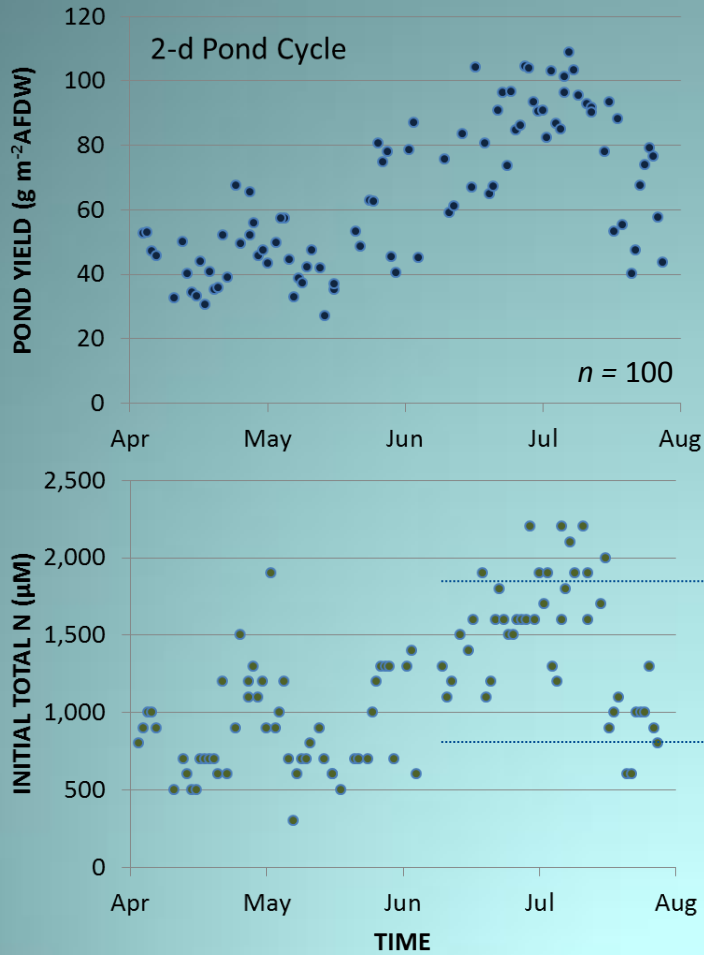


Increased nutrient loading results in higher pond biomass, but lower TAG yield

Nightly TAG loss ↘



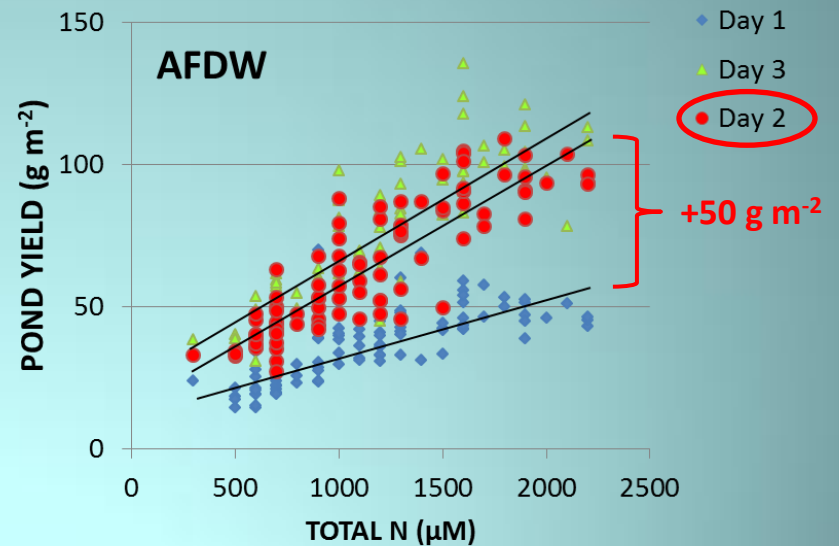
YIELD vs N



Nitrogen loading increases yield in C323 pond production, as predicted by MSS

Expected outcome: Show sustained production of Baseline strain for 100 daily production cycles. Validated 2-day pond cycle. Approach or exceed AFDW productivity and TAG yield targets.

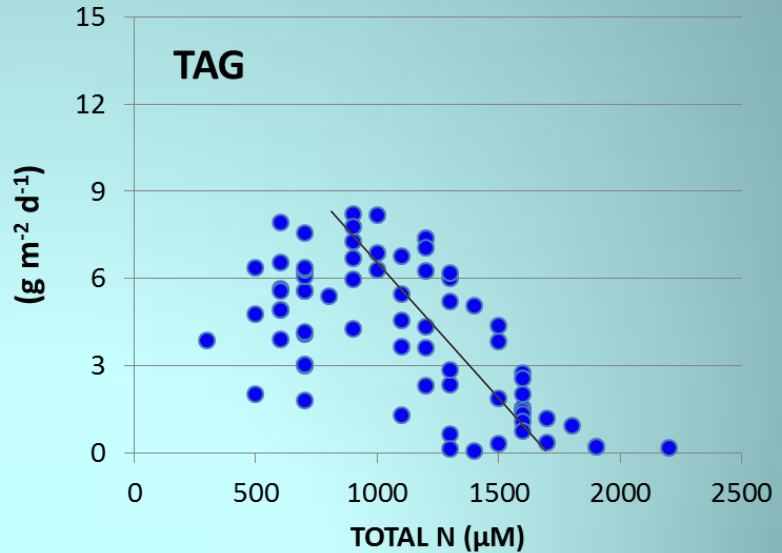
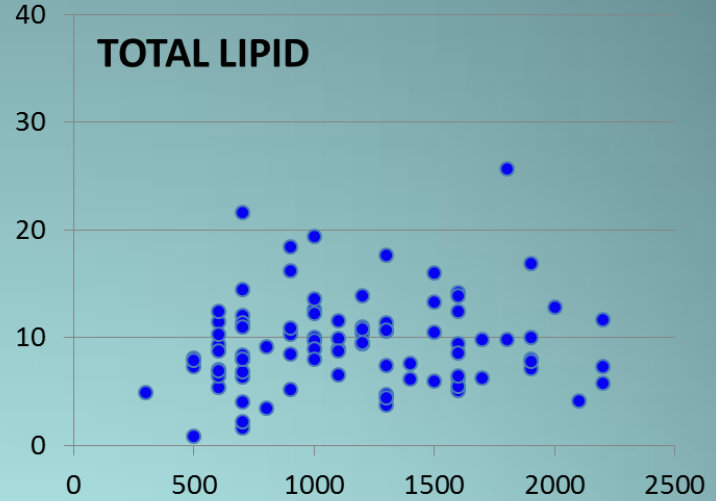
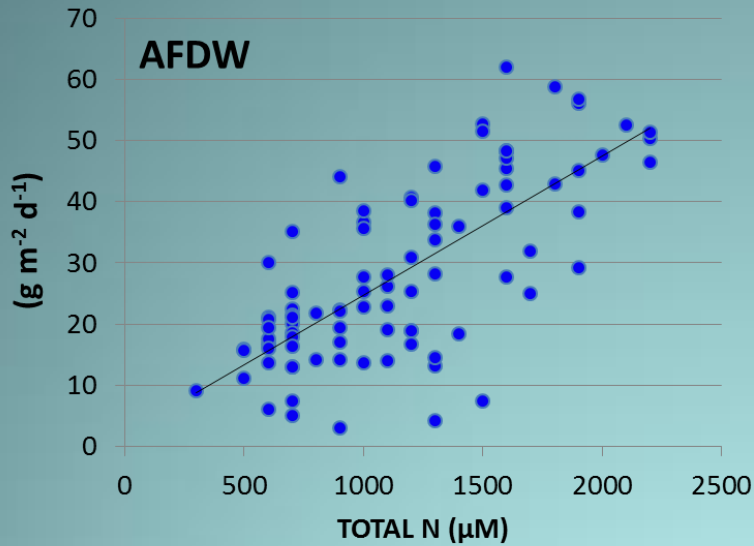
Progress: Baseline C323 (*Staurosira*)



2-day pond cycle is most productive, exceeds 2014 goal: $>20 \text{ g m}^{-2} \text{ d}^{-1} \text{ AFDW}$



PRODUCTIVITY



Nitrogen loading effect on productivity in day 2 ponds: AFDW increases, total lipid is unaffected, and TAG decreases.



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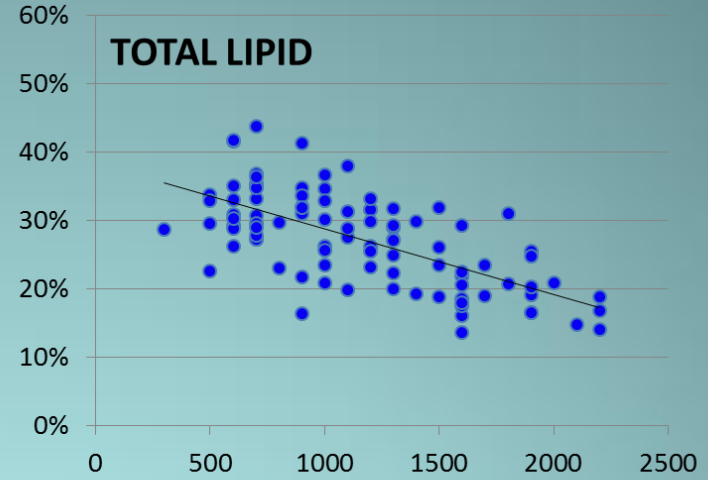
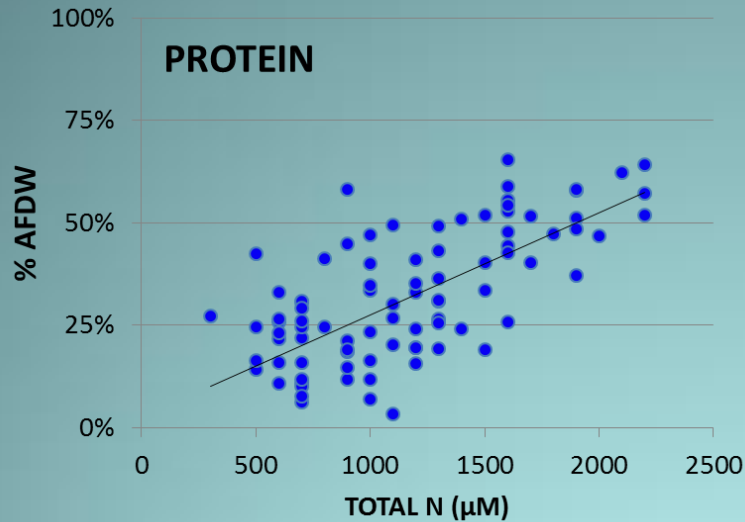


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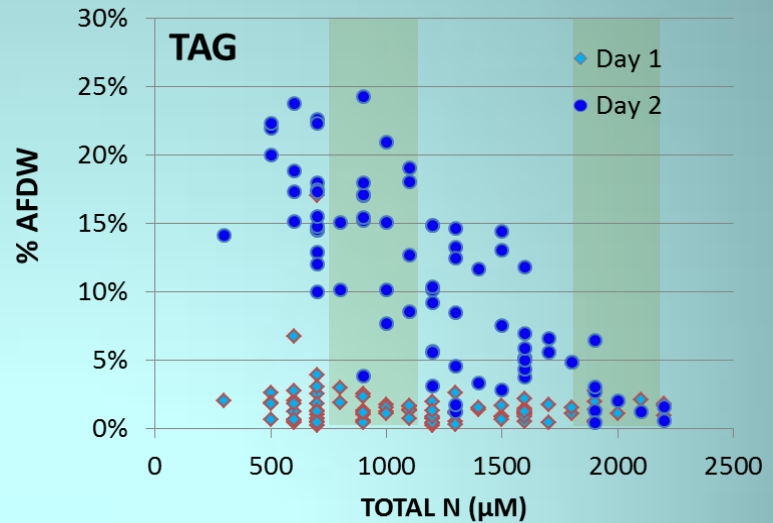


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% CONTENT



Nitrogen loading effect on feedstock content: protein content is enhanced; lipid and TAG decrease.



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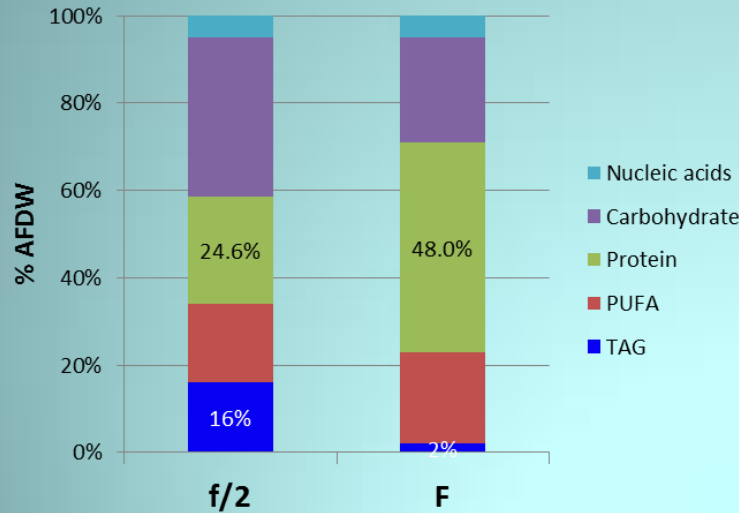
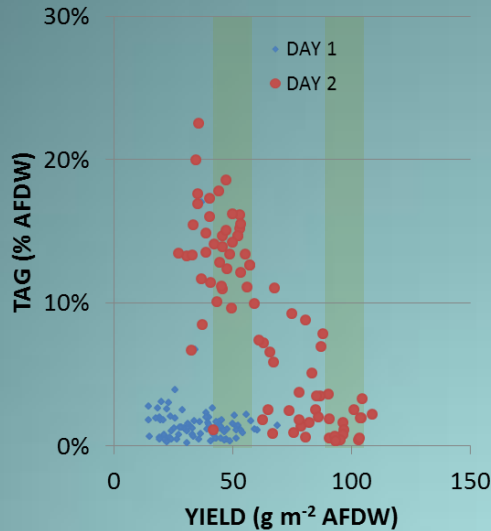


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YIELD



	Yield		units
	f/2	F	
TAG	1,365	320	gal acre ⁻¹ yr ⁻¹
LIPID	2,901	3,359	gal acre ⁻¹ yr ⁻¹
PROTEIN	6.9	25.3	MT acre ⁻¹ yr ⁻¹
AFDW	28.1	52.7	MT acre ⁻¹ yr ⁻¹

Effect of N loading on yield:

TAG yield at is greatest at low N. At high N, protein yield triples. Dissolved N in process water is undetectable in both cases.

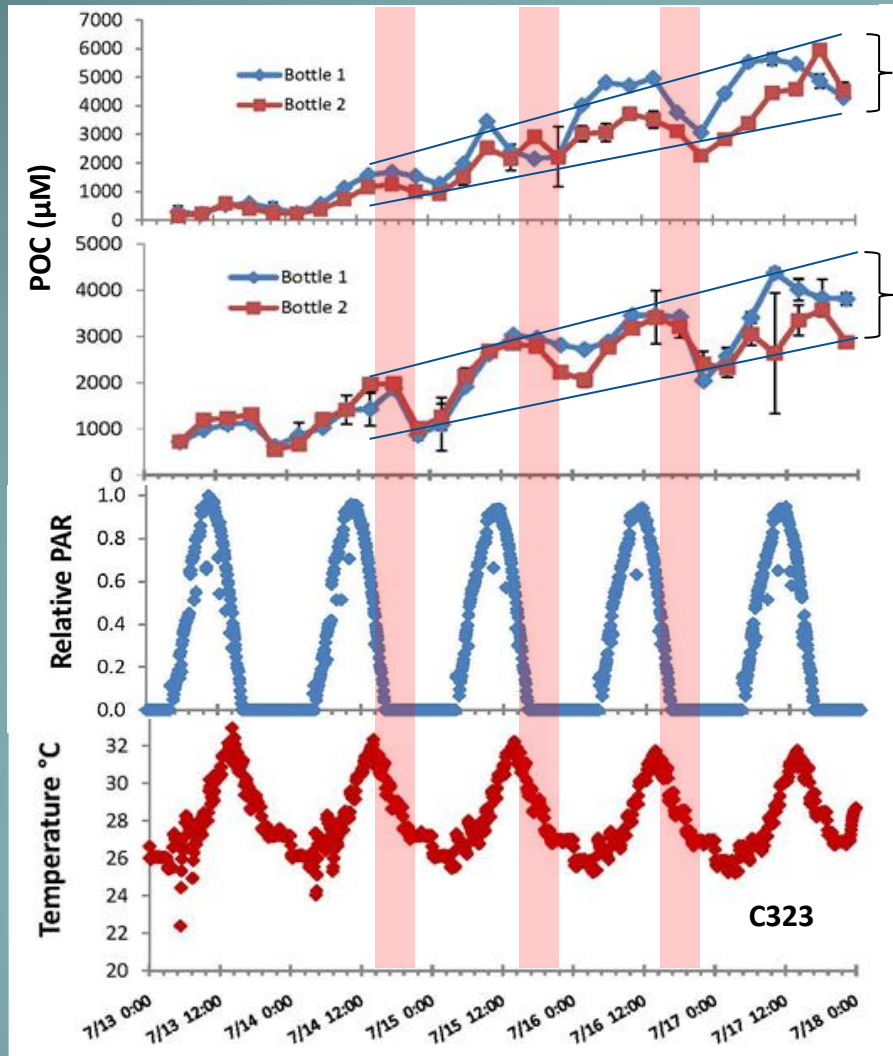
2014 target: **1,500 gal acre⁻¹ yr⁻¹**
 C323: **1,365 gal acre⁻¹ yr⁻¹**



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Task 2 Cultivation



Carbon loss can be 30-40% daily.
 Doubles CO₂ use (g CO₂ g ADFW⁻¹)
 from 2.0 to 3.8 at 25% day⁻¹

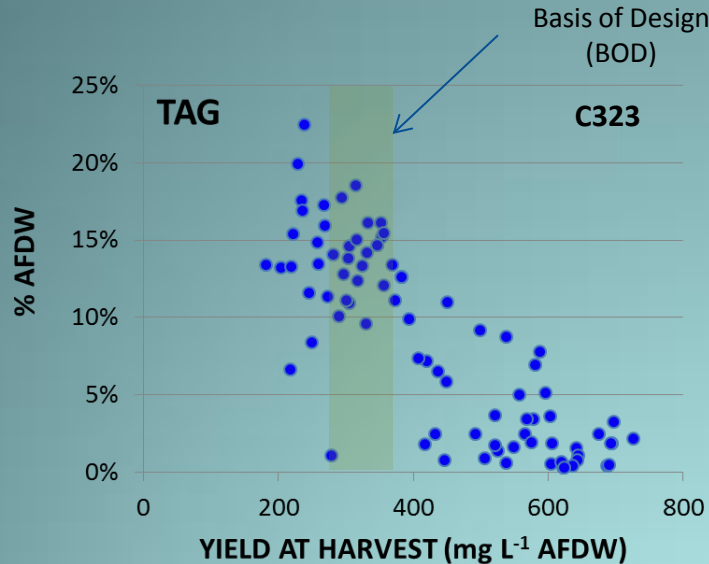
Harvesting at the right time

- *Increases* revenues,
 - *Decreases* capex, opex, energy use
- Requires highly resolved real time measurements, not fully developed for large scale cultivation

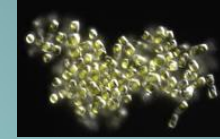
C323: Shaded bars show periods of maximum loss over a 3-day grow-out period, in covered + production ponds, simulated in the laboratory (Johnson & Ringuet 2010)



Task 3 Harvesting



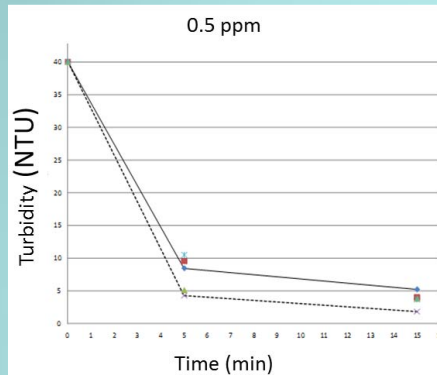
Expected outcome: Harvest tons of algae using a demonstrated low-energy, gravity-based method, without flocculants.



Progress: Baseline C323

- Long-term harvest of two strains using ponds as settlers – no flocculants
- Harvest meets key performance targets

Concentration at harvest	350 mg L ⁻¹
Feedstock after harvest	>1% TSS
Feedstock after concentr'n	>15% TSS
Overall efficiency	95%*
- Tons delivered



Other strains: some polymer flocculants have been evaluated; may be cost-effective.

* Required to meet local discharge regulations



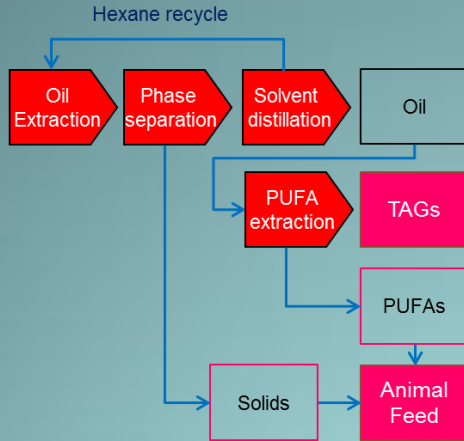
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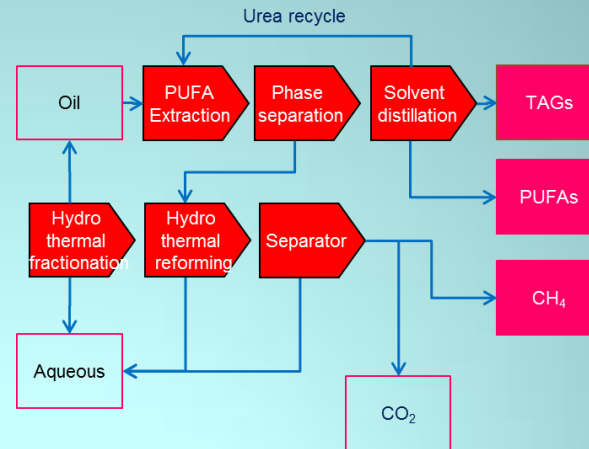
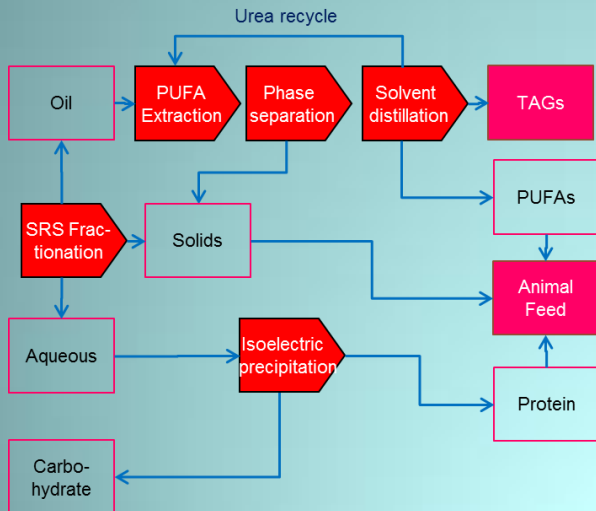


Expected outcome: Deliver tons of algae processed by *dry route* hexane extraction. Demonstrate a viable *wet route*.

Progress:

- Tons delivered: 2 strains, Baseline C323
- Pilot and lab demonstrations of wet route

A. Dry route to fuel and feed



C. Wet route to fuels

B. Wet route to fuel and feed



Task 5 Co-product Development

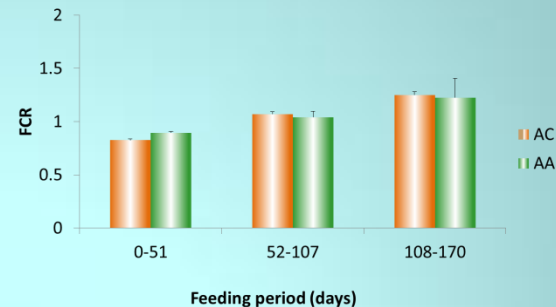
Test algae Fish species	Protein replacement levels		
	Salmon	Carp	Shrimp
C323 - L3	5%	25%	25%
C323 - H3	10%	40%	40%
C88 - L8	5%	25%	25%
C88 - H8	10%	40%	40%

Expected outcome: Demonstrated nutritional, commercial value of non-fuel co-products in aquafeeds

Progress:

- Efficacy demonstrated at pilot scale
 - salmon, carp, shrimp, cod
 - poultry, swine (USDA/DOE)
- Pre-commercial trials
 - aborted during suspension
 - will resume and complete mid-2014

Defatted biomass effectively replaces fishmeal protein at highest levels tested in salmon, carp, and Pacific whiteleg shrimp (Kiron et al 2010)



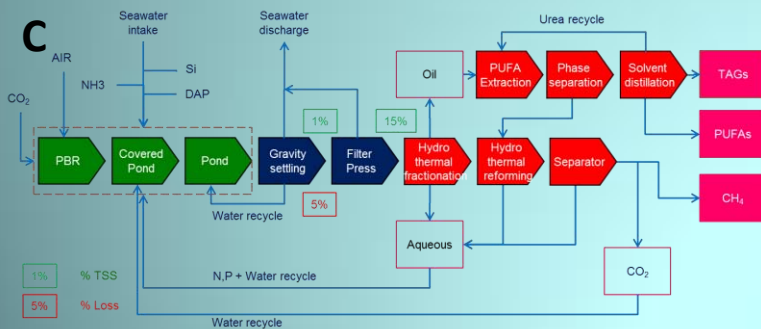
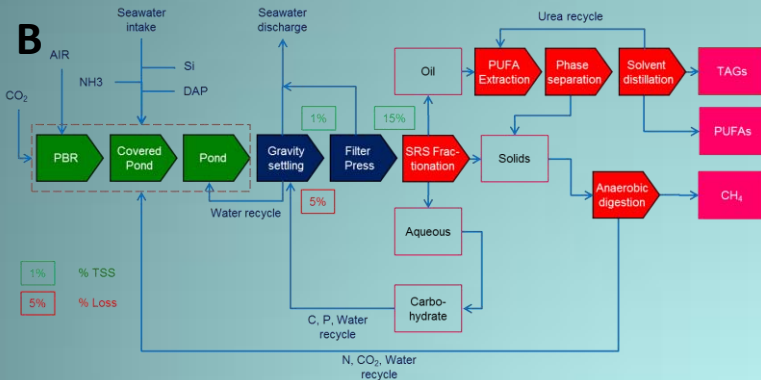
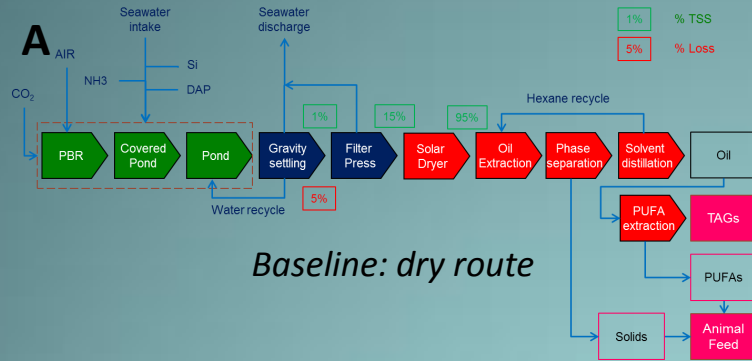
Atlantic cod feed conversion ratio

Atlantic cod fed control (AC) and algae feeds (AA) for 170 days (Bajgai et al 2011)



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Task 6 Integrated Design



Expected outcome: Techno-economic and life cycle assessment of one fully integrated technology pathway, based on data from extended, ton-quantity production runs

Progress:

- Three technology pathways
- Integrated Baseline pathway demonstrated: *dry route* C323
- Basis of Design complete
- Mass balance
- Economics and energy
- Comparative LCA
- Completion estimate: 2013 (Q4)

Updating....

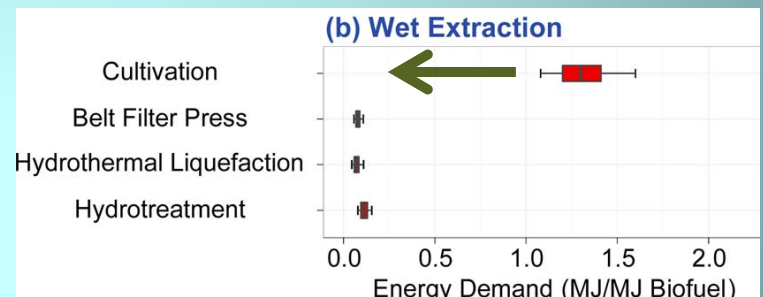


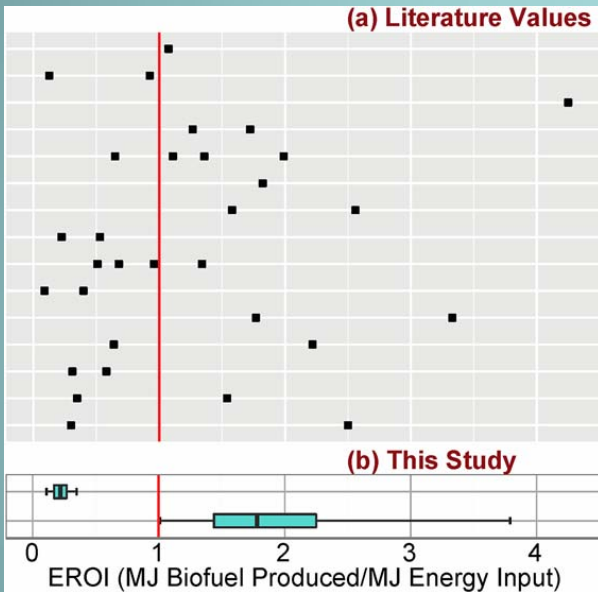
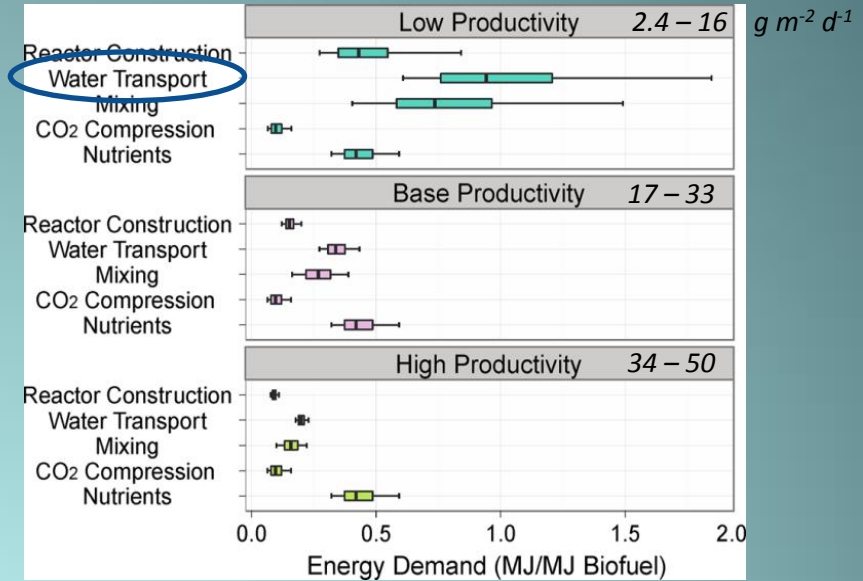
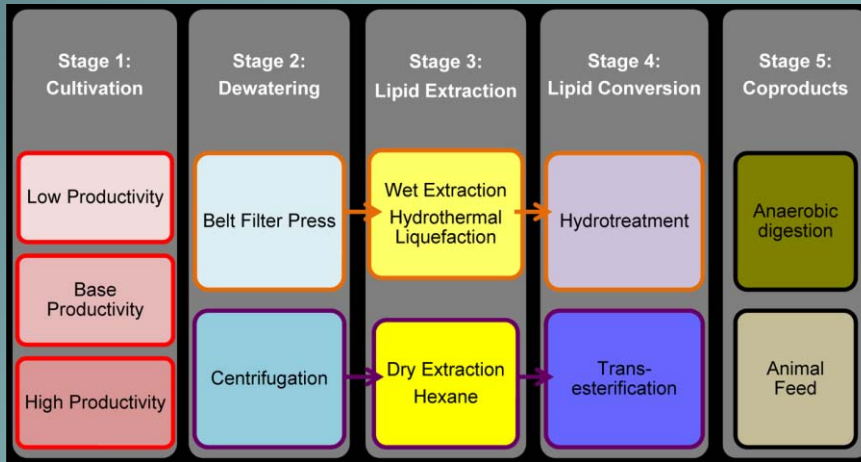
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Task	Capital Cost (\$ per m ²)		
		Base	Target
	2010	2013	2015
Site preparation	22.7	5.7	4.5
PBRs	7.3	6.8	6.1
Ponds	36.2	24.2	15.8
Water transport	22.4	22.4	5.6
CO ₂	4.9	4.9	0.5
Harvesting	15.7	3.9	3.9
Drying	4.7	4.7	0.0
Processing	9.0	9.0	9.0
Total	122.9	81.6	45.4

Capital cost and energy analysis for the baseline Basis of Design, showing improvements since 2010 and further improvements targeted by project end, Q4 2014. New values established by value engineering and pilot demonstration.

Task/Operation:	GWh per 100 ha per year	
	2013	2015
Cultivation		
Water transport		
Intake pumping station	0.853	0.171
Distribution flow from canals to PBRs	0.226	0.045
UV sterilization	0.165	0.033
CO ₂		
Pressurization & transport	0.460	0.046
PBRs & Ponds		
Photobioreactor Air lift	0.520	0.520
Pond circulation	0.924	0.924
Harvesting		
Harvesting	0.030	0.030
Pump harvest to dewatering	0.136	0.136
Concentrating	0.240	0.240
Pump concentrate to processing	0.043	0.043
Total	3.60	2.19





Life cycle assessment for the 2010 Basis of Design quantifies advantages of the wet route, and will be updated for all unit operations across technology pathways.

From DL Sills et al. *Environ. Sci. Technol.* 47, 687 (2013)



BETO Multi-Year Plan

By 2013 (Q4), establish

- Cost goals and technical targets for one algal system
- TEA (Techno-Economic Analysis) for one additional pathway, including co-products

By 2014 (Q4), demonstrate

- $> 20 \text{ g m}^{-2} \text{ d}^{-1}$ AFDW
- $1,500 \text{ gal acre}^{-1} \text{ yr}^{-1}$

This Project

Now established

- Cost goals and technical targets for Baseline algal system

By 2013 (Q4)

- TEA for two additional pathways, including co-products

Now demonstrated:

- $>25 \text{ g m}^{-2} \text{ d}^{-1}$ AFDW
- $1,365 \text{ gal acre}^{-1} \text{ yr}^{-1}$



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Technical & Market Factors

Task

Water:

- Seawater vs freshwater improves resource impacts 1 - 6
- Recycling of seawater improves cost and energy use 1,2,6
- Integrated freshwater production is a bonus 2,6

Yield:

- Nutrients, light, and gas exchange are scientifically managed to improve yield (*crop husbandry*) 1,2,3,6

Economics & life-cycle assessment:

- Demonstrated viability of co-products 5
- Highest level of sustainability 4,6
- Fundable in realistic project-financing market 6



Top remaining challenges

Task

Co-product trials:

- Produce ton quantities of 2 improved strains 2
- Complete pre-commercial feed trials 5

Integrated Design:

- Update basis of design, value engineering, mass balance, TEA and LCA for three pathways 6

Contributions to state-of-the-art

- How to produce 50 g m⁻² day⁻¹ AFDW, or 1,365 gal acre⁻¹ day⁻¹ biofuel intermediate, at scale 1 - 4
- Sustainable production from a “hybrid system”
PBR:Covered Pond:Pond (0.09:0.11:0.80) 1 – 3
- Commercially viable aqua-feed co-products 5,6



Milestone 1: Harmonize measurements of key biochemical parameters

Milestone 2: Sustain production of a single strain for 100 production cycles

Milestone 3: Produce ton quantities (AFDW) of algal biomass feedstock for co-product demonstration

Milestone 4: Assess nutritional value of co-product feeds from several algae in pre-commercial field trials

Milestone 5: Establish Baseline techno-economics and life cycle assessment of an integrated process lineup, validated at production scale

Milestone 6: Quantify improved performance against Baseline with new strains and alternative unit operations

Milestone 7: Deliver a Design Report to compare technology pathways, substantiate progress on production targets, and quantify remaining barriers



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Relevance: Directly addresses BETO primary objectives for 2013 and 2014 – design report and yield, *plus* requested co-product development

Approach: Comparative TEA/LCA of 3+ pathways based on actual production

Technical accomplishments:

Yield: sustained production runs ($n=100$) establish Baseline for first strain: $>25 \text{ g m}^{-2} \text{ day}^{-1}$ AFDW; $1,365 \text{ gal acre}^{-1} \text{ day}^{-1}$ biofuel intermediate.

Co-products: established viability at pilot scale

Future work: Produce and test 2 strains in pre-commercial co-product trials; complete Design Report of comparative integrated pathways

Success factors/challenges: Water, yield, economics

Status: Overall 50% complete, expect delivery on schedule



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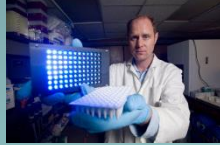
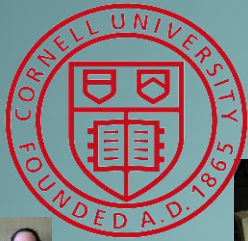


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Productivity

"Results seem to be extrapolated from small-scale experiments."

"How do they propose to achieve [higher productivity], when best-case scenario on sustained microalgae is 20-30 g/m²/day?"

"A major problem was that the presentation gave the impression Cellana was achieving 100-150 MT/ha/yr of algae production."

"Data on productivity and economics lacked sufficient detail to be meaningful"

We believe these questions are best addressed by the data shown in this presentation, based on 100 outdoor pond production runs, sustained for 4 months, of the first strain selected for such large-scale production. The data on *Staurosira* clearly show that yield of 50 g AFDW/m²/day or 150 MT AFDW/ha-yr can be attained with ~2 mMol nitrogen in modified f/2 medium. The data also show that at these high productivity levels, protein yield is significantly increased at the expense of lipids, and especially TAGs.

Large-scale results on the production of *Staurosira* have a strong bearing on economics, emphasizing that any business model for algae biofuel production can allow for a controllable ratio of biofuel to co-products. We believe the data set with *Staurosira* provides a strong foundation for the detailed techno-economic and life-cycle analyses to be concluded this year (2013). We expect to also complete comparable analysis of additional strains produced since 2010.



Aquafeed co-products:

"More attention is needed on the effect of different strains and conflicting goals of lipid extraction on byproducts (e.g. aquaculture feeds)"

"...this young project [should] address how... to produce valuable by-products (e.g. aquaculture feeds while producing biofuels in a sustainable manner"

"The value of whole algae production optimized to aquaculture feed needs to be rigorously compared to the value of biofuel plus lipid-extracted algae meal."

"Specific project work on economics and co-products are not relevant to commercial scale biofuel production"

The first three reviewers' comments accurately underscore a major goal of this project, which is namely to quantify the technical and commercial viability of algae biofuel co-products as aqua-feeds, and to incorporate those empirical findings into comprehensive techno-economic and life-cycle analyses.

We cannot agree with the comment that *"Specific project work on economics and co-products are not relevant to commercial-scale biofuel production."* Any business model for algae biofuel production must evaluate co-products. Furthermore, large-scale production trials in this project were intentionally designed to vary the production ratio of biofuel feedstock to co-products so that corresponding variation in the resulting economics could be understood on the basis of actual performance. Indeed, the ability to vary production of these two commodity products [fuels & feeds] on a time scale of days confers an unusual and unique hedge compared to any other biofuel crops.



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2013

Fuels and refined co-products from marine microalgae: Comparative techno-economic and sustainability analysis of integrated technology pathways *Biomass and Bioenergy* (In preparation)
[Huntley, M, I Archibald, J Tester, DL Sills, G Foreman, S Machesky & CH Greene]

Quantitative uncertainty analysis of life cycle assessment for Algal biofuel production. *Environmental Science & Technology*, 47, 687–694 [DL Sills, V Paramita, MJ Franke, MC Johnson, TM Akabas, CH Greene, JW Tester]

Carbon allocation under light and nitrogen resource gradients in two model marine phytoplankton *Journal of Phycology* DOI 10.1111/jpy.12060 [Bittar TB, Lin Y, Sassano LR, Wheeler BJ, Brown SL, Cochlan WP and Johnson ZI]

2012

Air-water fluxes of N₂O and CH₄ during microalgae (*Staurosira* sp) cultivation in an open pond. *Environmental Science & Technology*, 46(19): 10842-10848 [S. Ferron, D.T. Ho, Z.I. Johnson and ME Huntley]

Marine microalgae from biorefinery as a potential feed protein source for Atlantic salmon, common carp and whiteleg shrimp. *Aquaculture Nutrition*, 18(5): 521-531. [V. Kiron, W. Phromkunthong, ME Huntley, I. Archibald and G. DeScheemaker]

2010

Geoengineering: The inescapable truth of getting to 350. (2010) *Solutions* 1 (5): 57-66 [Greene, CH, BC Monger and ME Huntley]

A suite of microplate reader-based colorimetric methods to quantify ammonium, nitrate, orthophosphate and silicate concentrations for nutrient monitoring (2010) *Journal of Environmental Monitoring* 13: 370-376
[Ringuet, S, L Sassano and ZI Johnson]



2011

Determination of working range for macro-scale total lipid analysis [Bai X, E Knurek, S Hamilton, C Hertz], 7 pp

Increasing the accuracy of in-pond biomass determination by re-suspending the biomass through pond sweeping [Bai X]

PBR wall flow meter calibration check [Thomas-Hall, S. & G Rose] 5 pp.

Pond calibration study [Thomas-Hall, S], 5 pp

Impact of solar radiation and evaporation on in-pond productivities: [Dorland, R & J Granados] 10 pp.

Increasing the accuracy of in-pond biomass determination by re-suspending the biomass through pond sweeping. [Bai X]

2010

Phase 2 Diel Experiments, High-Throughput Screening (HTS) [Johnson, Z & S Ringuet] 44 pp.

Summary of Mid-Scale Screening 2010A – Round 1 [Pickell, LD, X Bai, HI Forehead & SR Thomas-Hall] 32 pp

Summary of Mid-Scale Screening 2010A – Round 2 [Pickell, LD, X Bai, HI Forehead & SR Thomas-Hall] 35 pp

Summary of Mid-Scale Screening 2010A - Round 3 [Pickell, LD, X Bai, HI Forehead and SR Thomas-Hall], 12 p.

Pre-treatment of lipid samples on VWR glass microfiber filters with a focus on ammonium formate treatment and freeze Dryer drying time [X Bai, J Betts, G Maillet, C Hertz, P Chia, D Onen and WP Cochlan] 8 pp

Lipid stability test for algae material collected on filters [Bai X, M Workman & C Hertz] 8pp.

Optimization of lipid extraction method. [Bai X, E Knurek, S Hamilton, C Hertz] 9pp.

Testing suitability of .35µm cartridge filters for filtering nutrient enriched sea water. [Thomas-Hall, S & H Forehead] 3pp.

Effect of paddlewheel speed, sampling location and sample volume on optical density estimates in ponds [Thomas-Hall, S, R Dorland, T Stone, M Lopez & R Bidigare] 4pp.



2010

Pond Production Model Predictions in Support of Cultivation Decisions [Cullen, J] 1 p

Pond Evaporation [Johnson, Z] 30 pp

Pond Sweeping and flocculation in species C323. [Thomas-Hall, S, H Forehead, X Bai, R Dorland, L Pickell J Obbard] 12pp.

Protozoan contamination [Forehead, H, C O'Kelly, G Rose, G Foreman, J Herndon, L Pickell & S Thomas-Hall] 3pp.

Pond Liner Selection [Foreman, G, R Popov & S Machesky] 5 pp

Harvesting: Re-suspension study [Foreman, G, S Thomas-Hall, A Kramer & S Brown] 9 pp

Out-of-Pond Harvesting Technology: Flocculant-assisted settling and flotation as algae thickening processes [R van Compernelle, D Pickle & S Thomas-Hall] 9 pp

2009

High-Throughput Screening Manual No 2 [Johnson, Z] 72 pp

Determination of FAME distributions for TAG fractions isolated from 8 HTS diatom extracts [RR Bidigare, S Christensen, D Elsey, H Trapido-Rosenthal & C O'Kelly] 17 pp

18S sequencing results for top isolates. [Johnson, Z] 30 pp

Silica Dissolution in Seawater [Pickell, LD, and R Foreman] 21 pp.

Summary of Mid-Scale Screening – V (MSS-V) [Cochlan, WP, X Bai, HI Forehead, LD Pickell and SR Thomas-Hall] 22 pp.

Summary of Mid-Scale Screening – IV (MSS-IV) [Cochlan, WP, X Bai, HI Forehead, LD Pickell and SR Thomas-Hall] 22 pp.

Summary of Mid-Scale Screening – III (MSS-III) [Cochlan, WP, X Bai, HI Forehead, LD Pickell and SR Thomas-Hall] 22 pp

Summary of Mid-Scale Screening –II (MSS-II) [WP Cochlan, L Pickell, X Bai, SR Thomas-Hall, and HI Forehead] 22 pp

Comparative analysis of growth rate calculation interval during Mid-Scale Screening MSS-1. [Pickell, L & WP Cochlan] 10 pp



2009

- Large-Volume Bag Culture Trials at KPF [Vozhdayev, GV] 10 pp
- Summary of Large-Scale Production Shakedown [Cochlan, WP, HI Forehead, LD Pickell, X Bai, and S Thomas-Hall], 17 pp
- Contamination of KPF photobioreactor cultures in December [Forehead, H., B. Paul, , C. Ikeda, , L. Griswold G. Vozhdayev, L. Pickell, J. Herndon, J. Rose, T. Yasin], 7 pp.
- Upstream FCP (First Commercial Plant) BOD (Basis of Design) Premise Document: algae to biofuel. [Zuidgeest, M] 126 pp
- Upstream Design Case Report: FCP algae to biofuel. [Zuidgeest, M] 72 pp
- Target Costing Alternative Development Report: FCP algae to biofuel. [Zuidgeest, M] 168 pp
- FCP CO₂ Transport Key Investigation Phase: Generic effect of CO₂ composition & contaminants [Lee, HT & S Jackson] 41pp
- FCP CO₂ Transport Key Investigation Phase: CO₂ distribution and sparging. [S Jackson & HT Lee] 41 pp
- FCP Upstream and CO₂ Scouting Capital Estimate Report [T Olsen & A Fletcher] 18 pp
- FCP Preliminary assessment of ecological Risks and Regulations [Hein, L and C Aya] 24 pp
- FCP Project: Health, Safety, Environment and Social Performance (HSE-SP) Premises [Hottentot, J & U Nwankwo] 67 pp

2008

- High-Throughput Screening Manual No 1 [Bryant, F] 290 pp
- Pre-screening: a review of 14,350 strains in principal international collections [O'Higgins, L, M Huntley, Z Johnson, DG Redalje, C O'Kelly, RR Bidigare, JJ Cullen, W Cochlan, MDG Lopez & S Brown], 103 pp



2013

“Uncertainty of LCA for algal biofuel production,” *3rd International Conference on Algal Biomass, Biofuels & Bioproducts*, Toronto [[Sills, DL](#)] - June

“Quantum yield for two species of marine phytoplankton grown in semi-continuous culture under fluctuating and static irradiance,” *2013 ASLO Aquatic Sciences Meeting*, New Orleans, Louisiana [[Redalje, DG](#), M Stone, and X Chen] – February

2012

“A need for Qatari leadership in securing a sustainable world for future generations,” *United Nations COP 18 Conference, Bellona Foundation Side Event*, Doha, Qatar [[CH Greene](#)] - December

“Microalgae from biorefinery can be an effective fishmeal replacement in feeds of hybrid tilapia,” *AQUA 2012*, Prague, Czechoslovakia [[V Kiron](#), M Huntley, W Phromkunthong] - September

“Marine microalga as a protein ingredient in feeds of Atlantic cod,” *XV International Symposium on Fish Nutrition and Feeding*, Molde, Norway [[V Kiron](#), Ø Hagen, B Bajgai & M Huntley] – June

“Pumping algae! An alternative energy future,” *Algae & Energy in the Northeast: Advancing knowledge, research and innovation*, University of Vermont, Burlington. [[DG Redalje](#), JJ Cullen, ZI Johnson, M Huntley, and G de Scheemaker] - March

2011

“A quantitative model of microalgal photosynthesis linking laboratory-scale diagnostics to large scale production in ponds,” *4th Congress of the International Society for Applied Phycology*, Halifax, Canada [[J Cullen](#), RF Davis, CT Jones & HL MacIntyre] – July

“A quantitative model of microalgal productivity, from light absorption to biochemical composition,” *First International Conference on Algal Biomass, Biofuels and Bioproducts*, St Louis [[J Cullen](#), RF Davis, CT Jones and HL MacIntyre] – July



2011

- “Effects of sequence and severity of macronutrient depletion on neutral lipid production in two strains of *Chlorella vulgaris*,” *ABBB Meeting*, St Louis, USA [Cochlan, WP, J Herndon, and RR Bidigare] – July
- “Algal autotrophic and heterotrophic Lipid synthesis for biofuel production,” *IABBB Meeting*. St Louis USA [X Bai, E Knurek, M. Workman, C Hertz, S Hamilton & J Obbard]- July
- “Sustainable high-value animal feed co-products from algae biofuel production,” *Algae Biofuels Symposium*, San Diego Center for Algal Biotechnology, San Diego [M Huntley, I Archibald, V Kiron & X Lei] - April
- “Algae for a sustainable future,” *International Symposium of Biotechnology Innovation and Development*, Chongqing, China [J Obbard & X. Bai] – April
- “Algal biofuel production via autotrophic and mixotrophic growth,” *BIT’s 1st Annual World Congress of Marine Biotechnology*, Dalian, China [X Bai, E Knurek, M Workman, C Hertz, S Hamilton & J Obbard] - April
- “Lipid enhancement through autotrophic and heterotrophic growth,” *Keystone Symposium on Biofuels*, Singapore [X Bai, E Knurek, M Workman, S Hamilton, C Hertz, B Bernhardt, N Goes, M Rangelova, T Stone and J Obbard] – March
- “Respiration in large-scale cultures of microalgae,” *Keystone Symposium on Biofuels*, Singapore [H Forehead, B Paul, L Griswold, J Johnson and A Morrow] – March
- “Phytoplankton: good or bad as a potential source of fuel,” *Sea Lion Bowl National Competition*, San Francisco, CA [WP Cochlan] - February
- “Marine Microalgae: Bioprospecting beyond biofuels,” *5th International Bioprospecting Conference*, Tromsø, Norway [V Kiron, M Huntley, W Phromkunthong, I Archibald & G deScheemaker] - February
- “Muscle development and growth of juvenile Atlantic cod (*Gadus morhua*) fed marine micro alga as an alternative protein source,” *Sats på Torsk*, Bergen, Norway (Feb 2011) [B Bajgai, Ø Hagen, C.Solberg, E Sirnes, G deScheemaker, I Archibald, M Huntley and V Kiron] - February



2010

- “The Cellana pathway to algae-based drop-in fuels,” *Biotechnology Industry Organization (BIO) Pacific Rim meeting*, Honolulu [M Huntley] - December
- “Marine algae as biorefineries: the billion-dollar bet on a new industry, observed from the front lines,” *University of Hawaii Oceanography Departmental Seminar* [M Huntley] - November
- “Marine Microalgae: A ‘green’ alternative protein source in aquatic feeds,” *Aquaculture Europe*, Porto, Portugal [Kiron, V, W Phromkunthong, M Huntley, I Archibald & G de Scheemaker] - October
- “Algae to fuel and feeds: the Cellana pathway,” *Asia-Pacific Clean Energy Summit*, Honolulu [M Huntley] - September

2009

- “Next-generation fuels and food from marine photosynthetic microbes,” *SETAC North America 30th Annual Meeting*, New Orleans, LA, [Bidigare, RR, SL Brown, ZI Johnson, CJ O’Kelly, ME Huntley, R Dorland, I Archibald, J Cullen, D Redalje and WP Cochlan] – November
- “Isolation and characterization of marine phytoplankton as a next generation biofuel,” *Joint Meeting of the American Society of Plant Biologists and Phycological Society of America*, Honolulu, HI [Johnson, Z, R Bidigare, S Brown, F Bruyant, W Cochlan, J Cullen, M Huntley, D Redalje, and G de Scheemaker] – July
- “Mid-scale screening of marine phytoplankton for large scale production of biofuels,” *Joint Meeting of the American Society of Plant Biologists and Phycological Society of America*, Honolulu, HI. [Pickell, L, M Pollard, J Herndon, WP Cochlan, and M Huntley] - July
- “Analysis of lipid accumulation in microalgae,” *Joint Meeting of the American Society of Plant Biologists and Phycological Society of America*, Honolulu, HI. [Thomas-Hall, SR, X Bai, B Paul, T Stone, S Brown, WP Cochlan, Z Johnson, R Bidigare, and M Huntley]

