DOE Bioenergy Technologies Office (BETO) 2015 Project Peer Review

Large-Scale Production of Marine Microalgae for Fuel and Feeds

March 24, 2015 Algae Platform Review

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

Goal Statement

• BETO MYPP Goals (3)

Demonstrate

- 1. Performance against clear cost goals and technical targets (Q4 2013)
- 2. Productivity of 1,500 gal/acre/yr algal oil (Q4 2014)
- 3. Productivity of 2,500 gal/acre/yr algal oil (Q4 2018)

DOE Project Goal

Demonstrate a high-value animal feed product

Relevance

Project goals are all top-level DOE MYPP goals

Outcome

A clear pathway to economically competitive, sustainable biofuels

Quad Chart Overview

Timeline

Project Start Date:	04/2010
Project Suspended:	08/2011
Project Restart:	05/2013
Project End Date:	09/2015
Percent Complete:	85%

Barriers

- 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 coproducts
- Ft-M Overall Integration & Scale-up: Show that integrated unit operations at scale deliver sustainable production

Quad Chart Overview

Budget & Partners

		Total Costs FY 10 –FY 12	FY 13 Costs	FY 14 Costs	Total Planned Funding (FY 15-Project End Date	
DOE Funded		\$1,802,522	\$947,782	\$4,240,322	\$2,957,156	
Cost Share	Partner	Period 1 (FY 10 - FY13)		Period 2 (FY 14 – FY 15)		Level (%)
	Cornell University	\$ 80,882		-		30.0
	Cellana	\$ 674,280		\$ 631,564		38.8
	Duke University	\$ 435,092		\$ 110,480		5.6
	GIFAS	-		\$ 77,135		1.7
	Nordland University	-		\$ 455,928		8.0
	Sahara Forest Project (SFP)	-		\$ 574,464		-
	SF State University (SFSU)	\$ 396,381		-		4.7
	University of Hawaii (UH)	\$ 434,132		\$ 228,506		7.3
	Univ of So Mississippi (USM)	\$ 363,327		\$ 27,580		3.0
	Total Cost- Share		\$2,384,094		\$2,105,657	100.0

1 - Project Overview

- The Consortium began in 2008, funded by Shell, which created Cellana, built a 6-acre demonstration facility, and funded 4 years of commercial R&D for \$100 million, ending in 2012
- Original objectives were to demonstrate commercially viable, sustainable biofuels and animal feed co-products from marine algae, and to design a 1,000-ha production facility
- Original objectives are aligned with DOE MYPP goals
- In 2010, our initiative to develop high-value nutritional coproducts was enhanced by funding from USDA and DOE (this project) to
 - increase algae production,
 - expand feed trials at Cornell and University of Nordland, and
 - complete a techno-economic analysis (TEA) and life cycle assessment (LCA) for commercial production

2 – Approach (Technical)

Integrated Process

- Strain development from screening >1,000 new strains to meet product specifications for biofuel and animal feed applications
- Mass culture using an innovative hybrid system of photobioreactors (PBRs) and open ponds to produce ton quantities of two strains for
- 3) Recovery and conversion of algal feedstock to refined biofuels and animal feed ingredients, which were used in
- 4) **Product demonstrations** to assess product efficacy and value, and
- 5) **Design & analysis** of a commercial scale facility based on demonstrated results using a reiterative TEA/LCA process

Unique features: marine algae, PBR technology, co-products **Top challenges**: capital cost, productivity, LCA, EROI* **Critical success factors**: production, processing, product viability

2 – Approach (Management)

Structure

Strain development: Duke University, The U Southern Mississippi, U Hawaii, San Francisco State U, Sahara Forest Project Mass culture, recovery & conversion: Cellana, Shell Product demonstration: U Nordland, GIFAS, Cornell U* Design & analysis, Project management: Cornell University

Process

Communications and Monitoring Progress: monthly conference calls, annual review meetings, web-accessed database and document management systems

Decisions: changes in plan and go/no-go decisions are made by consensus in formal Consortium meetings

*swine and poultry feed studies supported jointly by USDA/DOE

3 – Technical Accomplishments/Results

Strain development

- 1. Developed two novel strains for large scale production
 - Built a collection of >1000 novel strains, screened and characterized at laboratory scale for 17 performance variables, especially specifications for high lipid, high protein productivity
 - *Performance* in large-scale mass culture *matches predictions*

Mass culture

- 1. Capital cost reduction
 - Designed, built and operated for five years a *low-cost* commercial-scale (300 m³) PBR system that delivers continuous high quality inoculum with consistent biochemistry

2. Improved operating capacity

• The "hybrid system" of PBRs and 2-day batch pond cultures avoids crashes common in conventional open ponds, and achieves *demonstrated operating capacity of 350 days/yr*

3 – More Technical Accomplishments/Results

Mass culture (Cont'd)

- 3. Exceeded oil production targets*
 - Sustained production of >3,800 gal/acre/yr algal oil was achieved for two strains
 - Yields exceed two DOE multi-year program plan targets
 - i. Productivity of 1,500 gal/acre/yr algal oil (Q4 2014)
 - ii. Productivity of 2,500 gal/acre/yr algal oil (Q4 2018)

Recovery & conversion

- 1. In ton quantities, successfully extracted the oil fraction for conversion to drop-in biofuels
- 2. In ton quantities, *successfully used the unrefined co-product,* defatted algae meal, *as a feed ingredient* substitute for fishmeal, soy, and corn products to formulate, manufacture, and use animal feeds

* Results are based on actual yields from two strains, each produced continuously for 6 months at demonstration scale

3 – More Technical Accomplishments/Results

Product demonstration

- 1. Biofuels
 - Met specifications for diesel, gasoline, and kerosene in gallon quantities produced by Shell
- 2. Aquafeeds
 - *Pre-commercial feed trials* on farmed salmon, carp, and shrimp in Europe and Asia (University of Nordland [UiN])
 - Demonstrated efficacy validates equivalency to fishmeal, valued at >\$1,500/MT

3. Animal feeds

- *Pre-commercial feed trials* on swine and poultry using thousands of animals in dozens of trials (Cornell)
- Demonstrated efficacy validates equivalency to high-grade soy and corn ingredients, valued at \$600/MT
- Unexpected results add value: trials demonstrated (i) high iron content effective against anemia in swine and (ii) enhanced omega-3 fatty acid content in broiler hen meat

3 – More Technical Accomplishments/Results

Design report – TEA/LCA

- 1. Commercial facility design
 - Complete engineering plans for a 100-ha facility
 - Applied TEA/LCA as a design tool to compare 20 different production and process pathways based on actual production and process data at large-scale

2. Commercialization analysis

- Demonstrated economic feasibility of delivering
 - a fuel price of \$2.76 to \$8.96 per gallon gasoline equivalent (gge) and
 - an EROI of 1.4

for a fuel that meets the Renewable Fuel Standard for advanced biofuels

- 3. Meets the Q4 (2013) goal of the BETO MYPP
 - by demonstrating "performance against clear cost goals and technical targets"

3 – Published Results*

Key Publications – Strain Development, Mass Culture

Bioprospecting for Oil Producing Marine Microalgae: Evaluation of Oil and Biomass Production of >1000 Novel Strains from Milligrams to Tons. *in preparation* (2015) [Johnson, ZI, RR Bidigare, SL Brown, WP Cochlan, JJ Cullen, S Loftus, DG Redalje, ME Huntley]

Demonstrated Large-Scale Production of Marine Microalgae for Fuels and Feed. *Algal Research*, 58 pp, *in review* (2015) [Huntley, M, ZI Johnson, SL Brown, DL Sills, L Gerber, I Archibald, SC Machesky, J Granados, CM Beal, CH Greene]

Air-water Fluxes of N₂O and CH₄ during Microalgae Cultivation (Staurosira sp) in an Open Raceway Pond. Environmental Science & Technology, 46(19): 10842-10848 [S Ferron, DT Ho, ZI Johnson and ME Huntley]

*27 peer-reviewed publications: 13 published, 3 in press/in review, 11 in preparation

3 – Published Results*

Key Publications – Co-Product Demonstrations

Marine Microalgae from Biorefinery as a Potential Feed Protein Source for Atlantic Salmon, Common Carp and Whiteleg Shrimp. Aquaculture Nutrition, 18(5): 521-531 (2012) [V Kiron, W Phromkunthong, ME Huntley, I Archibald and G DeScheemaker]

Effect of Dietary Defatted Diatom Biomass on Egg Production and Quality of Laying Hens. *Journal of Animal Science and Biotechnology* 5:3. (2014) [Leng, X, K Hsu, RE Austic, and XG Lei]

Potential of Defatted Microalgae from the Biofuel Industry as an Ingredient to Replace Corn and Soybean Meal in Swine and Poultry Diets. Journal of Animal Science 92:1306-14 (2014) [Gatrell, S, KK Lum, JG Kim, and XG Lei]

*27 peer-reviewed publications: 13 published, 3 in press/in review, 11 in preparation

3 – Published Results*

Key Publications – Techno-Economics and Environmental Impact

Algal Biofuel Production for Fuels and Feed in a 100-ha Facility: a Comprehensive Techno-Economic Analysis and Life Cycle Assessment. Algal Research, 111 pp. in review (2015) [Beal, CM, L Gerber, DL Sills, ME Huntley, S Machesky, MJ Walsh, JW Tester, I Archibald, J Granados and CH Greene]

Quantitative Uncertainty Analysis of Life Cycle Assessment for Algal Biofuel Production. Environmental Science & Technology, 47, 687–694 (2014) [DL Sills, V Paramita, MJ Franke, MC Johnson, TM Akabas, CH Greene, JW Tester]

Global Impacts of Land Use Intensification Through Microalgal Cultivation. *in preparation* (2015)[Archibald I, LN Gerber, MJ Walsh, ME Huntley, CH Greene]

*27 peer-reviewed publications: 13 published, 3 in press/in review, 11 in preparation

4 – Relevance

• To the BETO MYPP

- Results meet or exceed 3 algae platform goals of the BETO MYPP
 - -150% > 2014 oil production target, 40% > 2018 oil production target, and performance against clear cost and technical targets

Impacts on science and the bioenergy industry

- High value animal feed co-products improve overall revenues
- Costs are reduced; operating capacity is increased
- *Productivity* of algal oil at large scale is greater than any reported
- Global impacts of large-scale production
 - No impact on *freshwater resources*
 - Simultaneous supply of *fuel and feed* global scales match
 - Reduced atmospheric CO₂ and a reversal of ocean acidification are achieved at global scale
 - Enormous land-use change impacts result from intensified cultivation of feed co-products replacing corn and soy

5 – Future Work

This project is substantially complete. Future work consists primarily of publishing results from completed studies.

Publish, publish, publish. The Consortium's 27 peer-reviewed publications to date include 3 manuscripts accepted for publication and 11 manuscripts in preparation. More are expected.

Maintain public access to Consortium results. The Consortium will maintain its website – *www.algaeconsortium.com* – for one year beyond project end to allow continued use of the project database and document management system for producing further publications. *Algaeconsortium.com* will continue to provide the public with access to peer-reviewed articles and other information regarding the Consortium until September 2016.

Summary

Overview This Consortium has demonstrated a fully integrated process for the production of biofuels and high-value bioproducts at precommercial scale

Approach Demonstrate the fully integrated process. Apply reiterative TEA/LCA to improve design and performance.

Technical Accomplishments/Progress/Results

- Yield >3,800 gal/acre/yr algal oil tons produced
- Successful conversion to biofuels and feed ingredient co-products
- High-value feed ingredients demonstrated
- Low energy, low cost system design
- Fuel price \$2.76 to \$8.96 per gge, EROI>1.4, meets RFS

Relevance Results exceed BETO MYPP oil production goals for 2014 and 2018. Improved technology and methods reduce cost of production, co-products increase revenues. Global impacts are significant.

Future work Project substantially complete. Manuscripts now in press/in preparation bring peer-reviewed publications to a total of 27.

Thank you

Additional Slides

Responses to Previous Reviewers' Comments

Key Comments and Responses from 2013 Peer Review – 4 areas

1. Project Approach

Project goals not aligned with BETO program goals. Project goals are focused on BETO program goals and have met or exceeded three major goals. (Slides 2, 9, 11)

Capital costs of the hybrid system are too high to be economically viable for fuel production. See Huntley et al and Beal et al (abstracts on Slides 21, 22).

This appears to be a process... with an extremely minor, if any, fuel co-product. Ibid

Energy input is significantly greater than fuel energy produced. Our analysis yields an EROI >1.4 (Beal et al, in review)

...the team has no ideas for how to make the process more economical or more energy efficient. Our reiterative TEA/LCA process was used as a design tool to improve economics and energy efficiency (Beal et al). We quantified 10 alternate pathways for two different types of algae.

2. Technical Progress: Accomplishments

...the energy input is significantly exceeding the energy value of the fuel. EROI >1.4 (Beal et al) No assessment of overall average productivity. >23 g/m2/d See Beal et al (Slide 22) Integrated design is not focused on a relevant process for biofuels. This process design produced more algal oil than ever reported at large scale. See Huntley et al and Beal et al

Responses to Previous Reviewers' Comments - 2

Comments and Responses from 2013 Peer Review – 4 key areas

3. Relevance

System design is not relevant to biofuels and the team does not have a plan to make it relevant. This system design produced algal oil at a rate that exceeded BETO MYPP targets four (4) years ahead of schedule (Huntley et al). For dimensions of economic and environmental relevance, see Beal et al.

Strain does not produce significant lipid quantities, so co-product work is not relevant for biofuels. Average was >38% lipid over six months each for two strains produced. See Huntley et al

4. Critical Success Factors

Success of this consortium will be dependent on its ability to produce significant quantities of biomass. We produced >4 MT ash-free dry weight of two strains (Huntley et al)

Does not include economic viability for biofuels. We completed a TEA/LCA study of 20 alternate production process scenarios (Beal et al)

Does not include energy efficiency of the process. See Beal et al. EROI>1.4.

Does not include need for co-product value to use biofuel relevant strains. See Beal et al.

Co-product should focus on establishing market value, not just demonstrating viability. See Beal et al. *Does not include protein and lipid productivity as key aspect of yield.* Lipid yields are the highest ever reported at large scale. Protein yields are >50x greater than agricultural crops. See Huntley et al.

In Review / In Press – with Abstract

27. Demonstrated Large-Scale Production of Marine Microalgae for Fuels and Feed. *Algal Research*, 58 pp *in review* (2015) [Huntley, M, ZI Johnson, SL Brown, DL Sills, L Gerber, I Archibald, SC Machesky, J Granados, CM Beal and CH Greene]

We present the results from sustained tonne-quantity production of two novel strains of marine microalgae, the diatom Staurosira and the chlorophyte Desmodesmus, cultivated in a hybrid system of 25-m³ photobioreactors and 400-m² open ponds at a large-scale demonstration facility, and then apply those results to evaluate the performance of a 100-ha Base Case commercial facility assuming it were built today. Nitrogen fertilization of 2-d batch cultures in open ponds led to the greatest yields - from both species - of ~75 MT ha⁻¹ yr⁻¹ biomass, and ~30 MT ha⁻¹ yr⁻¹ lipid, which are unprecedented in large scale open pond systems. The process described here uses only seawater, discharges no nitrogen or phosphorus in any form, and consumes CO₂ at 78% efficiency. We estimate the capital cost of a 111-ha Base Case facility at \$67 million in Hawaii, where actual production was performed, and \$59 million on the Gulf Coast of Texas. We find that large-diameter, large-volume PBRs are an economical means to maintain a continuous supply of consistent inoculum for very short-period batch cultures in open ponds, and thus avoid biological system crashes that otherwise arise in longer-term pond cultures. We recommend certain improvements in cultivation methods that could realistically lead to yields of 100 MT ha⁻¹ yr⁻¹ biomass and >50,000 L ha⁻¹ yr⁻¹ algal oil. Comprehensive technoeconomics and life cycle assessment of 20 end-to-end production lineups, based on the cultivation results in this paper, are presented in a companion paper by Beal et al

In Review / In Press – with Abstract

26. Algal Biofuel Production for Fuels and Feed in a 100-ha Facility: a Comprehensive Techno-Economic Analysis and Life Cycle Assessment. Algal Research, 111 pp. in review (2015) [Beal, CM, L Gerber, DL Sills, ME Huntley, S Machesky, MJ Walsh, JW Tester, I Archibald, J Granados and CH Greene]

This techno-economic analysis/life-cycle assessment is based on actual production by the Cornell Marine Algal Biofuels Consortium with biomass productivity > 23 g/m²-d. Ten distinct cases are presented for two locations, Texas and Hawaii, based on a 100-ha production facility with end-to-end processing that yields fungible co-products including biocrude, animal feed, and ethanol. Several processing technologies were evaluated: centrifugation and solvent extraction (POS Biosciences), thermochemical conversion (Valicor), hydrothermal liquefaction (PNNL), catalytic hydrothermal gasification (Genifuel), combined heat and power, wet extraction (OpenAlgae), and fermentation. The facility design was optimized by co-location with waste CO_2 , a terraced design for gravity flow, using renewable energy, and low cost materials. The case studies are used to determine the impact of design choices on the energy balance, operating and labor costs, capital costs, water depletion potential, as well as the human health, ecosystem quality, non-renewable resources, and climate change environmental indicators. The most promising cases would be economically competitive at market prices around \$2/L for crude oil and \$1,400/MT for animal feed, and also provide major environmental benefits and freshwater savings. As global demands for fuels and protein continue rising, these results are important steps towards economical production at an industrial scale.

In Review / In Press – with Abstract

25. Bias in ordinary least squares when the independent variable X is uncontrolled: Chlorophyll a and photosynthetic rates at Station ALOHA. *Limnology and Oceanography, in press* (2015) [Laws, E. A., R. R. Bidigare and D. M. Karl]

It is well known that ordinary least squares (OLS) provides a biased estimate of the slope of the functional relationship between an independent variable X and a dependent variable Y if there are errors in X and if X is merely measured but not controlled. The errors in X typically include both measurement errors and natural variability. When Y is a function of more than one independent variable, the effect of the independent variables in addition to X on an OLS treatment of X versus Y is mathematically indistinguishable from the effect of errors in X. To argue that an OLS is unbiased when X is uncontrolled amounts to arguing that all the scatter in the data is due to errors in Y. A more likely scenario is that Y is a function of more than one independent variable, in which case repeated measurements of X will provide no clue as to the magnitude of the natural variability that impacts the OLS. We illustrate this conundrum by considering the relationship between chlorophyll a concentrations and photosynthetic rates at the Hawaii Ocean Time---series Station ALOHA, where variability of the productivity indices almost completely obscures the relationship between chlorophyll a concentrations and photosynthetic rates if the data are analyzed by OLS

In Preparation – with Abstract

24. A Global Life Cycle Assessment Framework for Evaluating the Potential Large-scale Impacts of Emerging Bioenergy Technologies. (2015) [Gerber L.N., M.J. Walsh, I. Archibald, M.E. Huntley, C.H. Greene] (In preparation for *Environmental Science & Technology*)

Life Cycle Assessment (LCA) is a well-established methodology that has been extensively used for anticipating the adverse environmental effects caused by biofuels and bioenergy technologies. However, traditional LCA does not allow for assessing accurately the overall potential impacts on the agricultural and energy systems of an emerging feedstock or technology that will be implemented at global scale in the future. The aspects that are difficult to address by the conventional method include: 1) indirect effects, such as land use change, 2) the interaction of the technology with the other components of the system, such as the competition with other feedstocks, and 3) the spatial and temporal variability of life cycle inventory data or model parameters, such as the electricity mix or the improvement of technologies over time. This paper aims at presenting a novel LCA framework addressing all of these aspects for the evaluation of global-scale impacts of emerging bioenergy technologies on agricultural and energy systems. It is illustrated by an application case study about large-scale development of micro-algae cultivation for the combined production of biofuel and feed as a commodity to replace soybean. The case study shows that the method allows for capturing the dynamic interactions between the emerging technology and the other components of the system in a regionalized way and over time, while providing synthetic indicators of environmental performance that can be used to orientate policies and decision-making.

In Preparation – with Abstract

23. Global Life Cycle Analysis Demonstrates Potential Benefits of Algal Co-production. (2015) [Walsh M.J., L. Gerber, I. Archibald, M. E. Huntley, C.H. Greene]

Using forecasts of future energy and agricultural demands, system dynamics to assess land use change patterns, and life cycle analysis, the impacts of several energy and biofuel scenarios are examined for the years 2016-2050. Preliminary results indicate that an algal technological platform can substantially reduce greenhouse gas emissions and other critical indicators, primarily though altering land use change patterns. The high yields of oil from algae grown on marginal lands reduce the need to produce terrestrial biofuel crops, and consequentially the agricultural and pristine land required for production. Additionally, the co-production of protein for animal feed or food products from algae helps offset land-intensive agricultural crops such as soy, further reducing land requirements. GHG emissions and other indicators are optimized when algal production coupled with other renewable energy technologies (wind, solar), especially in regions that currently have carbon intensive electricity mixes. Impact on water, fertilizer and pesticide usages are also examined.

22. Global Impacts of Land Use Intensification Through Microalgal Cultivation. (2015) [Archibald

I., Gerber L.N., Walsh M.J., Huntley M.E., Greene C.H.]

Recent large scale cultivation studies have demonstrated that microalgal cultivation can result in a land use reduction of two orders of magnitude, compared with conventional agriculture, and produce a high protein food component with many potential uses. This paper examines the Greenhouse Gas Reduction that results from the consequent release of land and discusses economic and other consequences. Methods for enabling widespread adoption of the technology are proposed.

In Preparation – with Abstract

21. Growth Characteristics and Transcriptome of a Lipid-Accumulating Marine *Chlorella* **Species.** (2015) [Mansfeldt CB, LV Richter, RE Richardson, WP Cochlan, R Bidigare, and BA Ahner]

Chlorella strain C596 is a salt-water photosynthetic alga capable of accumulating a high proportion of cellular lipids, phenotypes that are economical and industrially useful. In this study, we first characterize several general physiological parameters of the organism such as low salt tolerance and heterotrophic carbon assimilation; we found that the organism grows best at an undiluted salt concentration and can utilize a wide range of carbon substrates in the dark. We then sequenced the RNA pools from *Chlorella* strain C596 during exponential growth and as it transitioned from a nutrient replete to deficient condition. The organism begins to store lipids when the cell becomes phosphate and nitrate limited. Transcripts encoding for enzymes involved in both starch and lipid biosynthesis, among others, were up-regulated as the sample transitioned into a nutrient deficient state. Transcripts for two of the lipid biosynthesis enzymes -triacylglyceride lipase and a diacylglyceride acyltransferasewere also monitored by reverse transcription quantitative polymerase chain reaction (RT-qPCR) which confirmed transcriptome trends. These two transcripts can potentially serve as useful bioindicators of lipid accumulation. Additionally, the seed culture was found to maintain a community of bacteria, potentially influencing the growth characteristics of the organism. Therefore, we sequenced RNA from an additional two cultures displaying distinct growth characteristics likely because of a shift in the bacterial community. The presence of a *Ruegeria* like species was found to be associated with a faster growth rate, suggesting a symbiotic relationship between the *Chlorella* species and specific members of the bacterial community.

In Preparation – with Abstract

20. Enhanced Quantum Yield of Photosynthesis for Marine Microalgae Grown Under Fluctuating Light Conditions: Recommendations for Growth of Dense Cultures in Shallow Pond Systems. [Redalje, DG and ML Stone]

A single strain of *Chaetoceros muelleri* was subjected to a variety of light treatments in order to identify if there was any change in quantum yield as a function of fluctuating light relative to static light with equal total daily light doses. The fluctuating light conditions were similar to those found in natural systems, with frequencies ranging between 0.10 Hz and 2.00 Hz. Quantum yield was determined using two methods: the standard method using ¹⁴C uptake to determine instantaneous quantum yield, and a second method using particulate organic carbon uptake relative to light absorbed over a 24-hour period. The various light treatments resulted in similar trends of quantum yield from the culture, regardless of the method used. Cultures subjected to fluctuating light treatments of 0.5 Hz, 0.67 Hz, and 1.00 Hz yielded higher values than did those subjected to light fluctuating at 0.10 Hz and 2.00 Hz. There also appeared to be some enhancement of the photosynthetic apparatus in this species when exposed to fluctuating light, relative to static light with the same total daily light dose.

In Preparation – with Abstract

19. Effects of Sequence and Severity of Macronutrient Depletion on Neutral Lipid Production in Two Strains of Chlorella 211-18. [Cochlan, WP, J Herndon, CE Ikeda, F-R Buttler and RR Bidigare]

Two strains of the chlorophyte species, *Chlorella 211-218* isolated from coastal Hawaiian waters were cultured under controlled laboratory conditions to quantify neutral lipid (NL) production as a function of macronutrient sufficiency. Although up-regulation of NL is a well-known process triggered by macronutrient (nitrogen, phosphorus, and silicon for diatoms) limitation, there has been little study on the interactive effects between macronutrient depletion and uncoupled photosynthetic growth, and NL production as a function of strain variability. The relative TAG content of lipids increased from <10 to >50% as a function of increased nutrient deficiency, and daily TAG productivity averaged > 40 mg TAG/L/d during the stationary growth phase for both strains. After 4 days of such nutrient-depleted growth, concentrations of total lipids and triacylglycerols exceeded values of 260 and 145 mg/L, respectively. Given the need for all macronutrients to be utilized prior to discharge into the natural environment, it is essential that potential inhibitory influences of inter-nutritional limitation (i.e., nitrate limitation of orthophosphate uptake) be quantified to minimize both cultivation operating expenses and effluent discharge concentrations, particularly when scaled to commercial applications.

In Preparation

18. Effects of nitrogen sources on cell growth and the production of lipid and triglycerides by the green alga Chlorella 211-18. [Cochlan, WP, J Herndon, CE Ikeda, F-R Buttler and RR Bidigare]

In Preparation

- **17. High-throughput Measurement of Phytoplankton Neutral Lipids with a Fluorescent Probe.** [Johnson ZI, A Barnett, RR Bidigare, SL Brown, F Bruyant, JJ Cullen, C Rafuse, DG Redalje, E Rowe, K Stanaway, BAS Van Mooy]
- 16. Bioprospecting for Oil Producing Marine Microalgae: Evaluation of Oil and Biomass Production of >1000 Novel Strains from Milligrams to Tons. [Johnson, ZI, RR Bidigare, SL Brown, WP Cochlan, JJ Cullen, S Loftus, DG Redaljee, ME Huntley]

In Preparation – with Abstract

15. Defatted Microalgal Biomass as a Protein Ingredient in Feeds of Atlantic Cod. [Viswanath, K, Ø Hagen, B Bajgai, CA Johnsen, ME Huntley]

Biomass obtained as a co-product from the biorefinery of the marine microalga *Staurosira* sp. was evaluated as a replacement for fishmeal protein in the feeds of Atlantic cod (*Gadus morhua*). The algal protein was used to replace 5% of the fishmeal protein. Two animal protein- and two plant protein-based feed groups were tested, each group with the algal product or without it (control). The feeds were offered at apparent satiation levels to triplicate tanks of juvenile Atlantic cod (av. initial wt. 9.64 g) for 170 days. In both plant protein- and animal protein-based feed groups, growth and feed performance indicators of fish that received the control fishmeal feed and those that were fed on the microalgal feed were not significantly different. As for the fish body composition, an effect of algal inclusion was detected only in the body lipid content. Incorporation of microalgal protein in feeds did not affect the fish muscle fibre size distribution or fibre number. Thus, the inclusion of microalgal biomass at the level studied did not influence the performance of juvenile Atlantic cod, demonstrating that the potential of the microalga as a feed ingredient.

In Preparation – with Abstract

14. Marine Microalgae: Climate, Energy, and Food Security from the Sea. [Greene, C.H., I. Archibald, L. Gerber, M.J. Walsh, M.E. Huntley, D. Sills, C. Beal, J. Tester. J. Granados, S.C. Machesky]

The world faces three interrelated crises in energy, food, and climate security. The large-scale, industrial cultivation of marine microalgae can play a major role in averting all three of these crises. Microalgae exhibit rates of primary production that are typically more than an order of magnitude higher than the most productive terrestrial energy crops. Thus, they have the potential to produce an equivalent amount of biofuel and/or food in less than one tenth of the land area. Scaling up production numbers from demonstration-scale cultivation facilities, the current total demand for liquid fuels in the United States can be met by growing microalgae in an area of 250 thousand square kilometers, or about 2.5% of the country's land area. The total global demand for liquid fuels, which is approximately six times that of the United States, can be met by growing microalgae in an area of 1.5 million square kilometers, about 2.2 times the size of Texas.

In contrast to the problems associated with growing first-generation terrestrial energy crops, the industrial production of biofuels from marine microalgae face far fewer environmental challenges. First, the land-use requirements for growing marine microalgae are not only reduced by over an order of magnitude, they also do not compete with those of terrestrial agriculture for arable land. Second, because the cultivation of marine microalgae can be highly efficient in its use of nutrients, only losing those that are actually harvested in the desired products, the issues associated with inefficient fertilizer use and subsequent eutrophication of aquatic and marine ecosystems are irrelevant. Finally, because

In Preparation – with Abstract

14. Greene, C.H. et al (Cont'd)

freshwater is not required, the cultivation of marine microalgae does not have to compete with agriculture or other users for this resource, which often is scarce in many of the non-arable environments that are most suitable for this industry.

The advantages of producing biofuels from marine microalgae instead of terrestrial energy crops go far beyond avoiding the environmental issues associated with land-use change, nutrient efficiency, and freshwater usage. For microalgal biofuels to be cost competitive with fossil fuels, they must be produced with sufficiently valuable co-products. Animal feeds are one type of co-product possessing a global market of appropriate scale and value. However, by mid-century, the protein demand of a global population of 9 billion people will be unsustainable with existing industrial agricultural practices, especially with anticipated future constraints on the use of fossil fuels and fertilizers. The industrial cultivation of marine microalgae can be the basis for a new "green revolution." To gain a sense of its potential, we can once again scale up the production numbers from demonstration-scale cultivation facilities. From the same 250 thousand square kilometers needed to meet the current total liquid fuel demand of the United States, 800 million metric tons of protein is simultaneously produced. This corresponds to 5.7 times the total annual global production of soy protein. From the same 1.5 million square kilometers needed to meet the current total global liquid fuel demand, 4.8 billion metric tons of protein is simultaneously produced. This corresponds to 34 times the total annual global production of soy protein. In addition to these staggering quantitative advantages, it should be noted that the microalgal protein is of higher nutritional guality than soy protein in terms of amino acid composition.

In Preparation – with Abstract

14. Greene, C.H. et al (Cont'd)

In terms of climate security, the industrial cultivation of marine microalgae can lead to the production of true carbon-neutral biofuels. To produce such carbon-neutral fuels, methods must be developed to utilize CO_2 captured directly from the atmosphere. If such direct air capture methods are successfully developed, then this could lead to the production of long-lived, carbon-negative biopetroleum products. The sequestration footprint of direct air capture by marine microalgae required to balance current global anthropogenic CO_2 emissions (1.24 million square kilometers) is comparable in scale to the land area required to meet current total global liquid fuel demand (1.5 million square kilometers). In addition, afforestation and other favorable land-use practices applied to land freed up from agricultural food and biofuel production can also have significant positive mitigation effects on CO_2 emissions.

In Review / In Press

- Demonstrated Large-Scale Production of Marine Microalgae for Fuels and Feed. *Algal Research*, 58 pp [Huntley, M, ZI Johnson, SL Brown, DL Sills, L Gerber, I Archibald, SC Machesky, J Granados, CM Beal and CH Greene]
- 26. Algal biofuel production for fuels and feed in a 100-ha facility: a comprehensive techno-economic analysis and life cycle assessment. *Algal Research*, 111 pp. [Beal, CM, L Gerber, DL Sills, ME Huntley, S Machesky, MJ Walsh, JW Tester, I Archibald, J Granados and CH Greene]
- 25. Bias in ordinary least squares when the independent variable X is uncontrolled: Chlorophyll a and photosynthetic rates at Station ALOHA. *Limnology and Oceanography* [Laws, E. A., R. R. Bidigare and D. M. Karl]

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- 24. A global Life Cycle Assessment framework for evaluating the potential large-scale impacts of emerging bioenergy technologies. *Environmental Science & Technology* [Gerber L.N., M.J. Walsh, I. Archibald, M.E. Huntley, C.H. Greene]
- 23. Global Life Cycle Analysis Demonstrates Potential Benefits of Algal Co-production. [Walsh M.J., L. Gerber, I. Archibald, M. E. Huntley, C.H. Greene]
- 22. Global Impacts of Land Use Intensification Through Microalgal Cultivation. [Archibald I., Gerber L.N., Walsh M.J., Huntley M.E., Greene C.H.]

In Preparation (cont'd)

- 21. Growth Characteristics and Transcriptome of a Lipid-Accumulating Marine Chlorella Species. (2015) [Mansfeldt CB, LV Richter, RE Richardson, WP Cochlan, R Bidigare, and BA Ahner]
- 20. Enhanced quantum yield of photosynthesis for marine microalgae grown under fluctuating light conditions: Recommendations for growth of dense cultures in shallow pond systems. [Redalje, DG and ML Stone]
- 19. Effects of sequence and severity of macronutrient depletion on neutral lipid production in two strains of *Chlorella* 211-18. [Cochlan, WP, J Herndon, CE Ikeda, F-R Buttler and RR Bidigare]
- 18. Effects of nitrogen sources on cell growth and the production of lipid and triglycerides by the green alga *Chlorella* 211-18. [Cochlan, WP, J Herndon, CE Ikeda, F-R Buttler and RR Bidigare]
- 17. High-throughput measurement of phytoplankton neutral lipids with a fluorescent probe. [Johnson ZI, A Barnett, RR Bidigare, SL Brown, F Bruyant, JJ Cullen, C Rafuse, DG Redalje, E Rowe, K Stanaway, BAS Van Mooy]
- 16. Bioprospecting for oil producing marine microalgae: evaluation of oil and biomass production of >1000 novel strains from milligrams to tons [Johnson, ZI, RR Bidigare, SL Brown, WP Cochlan, JJ Cullen, S Loftus, DG Redalje, ME Huntley]
- Defatted microalgal biomass as a protein ingredient in feeds of Atlantic cod. (2015) [Viswanath, K, Ø Hagen, B Bajgai, CA Johnsen, ME Huntley]
- 14. Marine microalgae: climate, energy, and food security from the sea. [Greene, CH, I Archibald, L Gerber, MJ Walsh, ME Huntley, D Sills, C Beal, J Tester. J Granados, SC Machesky]

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13. Techniques for Quantifying Phytoplankton Biodiversity. *Annual Review of Marine Science* 7. DOI: 10.1146/annurev-marine-010814-015902 [Johnson, ZI and AC Martiny]

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- 12. Potential of defatted microalgae from the biofuel industry as an ingredient to replace corn and soybean meal in swine and poultry diets. *Journal of Animal Science* 92:1306-14. [Gatrell, S, KK Lum, JG Kim, and XG Lei]
- 11. Effect of dietary defatted diatom biomass on egg production and quality of laying hens. *Journal of Animal Science and Biotechnology* 5:3. [Leng, X, K Hsu, RE Austic, and XG Lei]

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- 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]
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- 8. Smartphones: Powerful Tools for Geoscience Education. *EOS, Transactions American Geophysical Union* 94: 433-434. [Johnson ZI and DW Johnston]
- 7. Dual potential of microalgae as a sustainable biofuel feedstock and animal feed. *Journal of Animal Science and Biotechnology* 4:53. [Lum, KK, J Kim, and XG Lei]

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- 6. Air-water fluxes of N₂O and CH₄ during microalgae cultivation (*Staurosira* sp) in an open raceway pond. *Environmental Science & Technology*, 46(19): 10842-10848 [S Ferron, DT Ho, ZI Johnson and ME Huntley]
- 5. 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]
- 4. A suite of microplate reader-based colorimetric methods to quantify ammonium, nitrate, orthophosphate and silicate concentrations for nutrient monitoring *Journal of Environmental Monitoring* **13**: 370-376 [Ringuet, S, L Sassano and ZI Johnson]

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- 3. Can microalgae make pork "greener"? Suis No 102, pages 14-19. [Gatrell, S, J Kim, and XG Lei]
- Potential and limitation of a new defatted diatom microalgal biomass in replacing soybean meal and corn in diets for broiler chickens. *Journal of Agriculture and Food Chemistry*. 61:7341-8. [Austic, RE, A Mustafa, BY Jung, S Gatrell, and XG Lei]

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- 31. Project 2.5.4: Directed High-Throughput Screening. [Redalje, DG, X Chen, C Smith, E Rowe, H Huang, M Tuel, A Boyette, and M Stone], Final Report. 38 pp.
- 30. Determination of working range for macro-scale total lipid analysis [Bai X, E Knurek, S Hamilton, C Hertz], 7 pp
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- 28. PBR wall flow meter calibration check [Thomas-Hall, S. & G Rose] 5 pp.
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- 26. Impact of solar radiation and evaporation on in-pond productivities: [Dorland, R & J Granados] 10 pp.
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- 24. Phase 2 Diel Experiments, High-Throughput Screening (HTS) [Johnson, Z & S Ringuet] 44 pp.
- 23. Summary of Mid-Scale Screening 2010A Round 1 [Pickell, LD, X Bai, HI Forehead & SR Thomas-Hall] 32 pp
- 22. Summary of Mid-Scale Screening 2010A Round 2 [Pickell, LD, X Bai, HI Forehead & SR Thomas-Hall] 35 pp
- 21. Summary of Mid-Scale Screening 2010A Round 3 [Pickell, LD, X Bai, HI Forehead and SR Thomas-Hall], 12 p.
- 20. 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
- 19. Lipid stability test for algae material collected on filters [Bai X, M Workman & C Hertz] 8pp.
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- 15. Pond Production Model Predictions in Support of Cultivation Decisions [Cullen, J] 1 p
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- 12. Protozoan contamination [Forehead, H, C O'Kelly, G Rose, G Foreman, J Herndon, L Pickell & S Thomas-Hall] 3pp.
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- 8. High-Throughput Screening Manual No 2 [Johnson, Z] 72 pp
- 7. 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
- 6. 18S sequencing results for top isolates. [Johnson, Z] 30 pp
- 5. Silica Dissolution in Seawater [Pickell, LD, and R Foreman] 21 pp.
- 4. Summary of Mid-Scale Screening V (MSS-V) [Cochlan, WP, X Bai, HI Forehead, LD Pickell and SR Thomas-Hall] 22 pp.

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

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1. Cellana Pre-Screening Technical Report. (2008) [O'Higgins, L, M Huntley, Z Johnson, D Redalje, C O'Kelly, R Bidigare, J Cullen, W Cochlan, M Lopez, and S Brown] 107 pp.

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- "Effects of sequence and severity of macronutrient depletion on neutral lipid production in two strains of Chlorella 211-18," Ocean Sciences Meeting, Honolulu [Cochlan, W. P., F. Buttler, J. Herndon, C. Ikeda and R. R. Bidigare] – February
- "The effects of salinity on algae biomass production," Presentation Algae Biomass Organization (ABO 2014) [Johnson, ZI and S Blinebry] October
- "Large-scale Production It's all about (more) Nitrogen," plenary session, Algal Biomass Organization Summit, San Diego [Huntley, M] – October
- "Using TEA/LCA as a design tool: Results for a 100-ha economically competitive facility based on actual large-scale production," Algal Biomass Organization Summit, San Diego [Beal, CM] October
- "Large-scale Production Results from N fertilization," Poster for Algal Biomass Organization Summit, San Diego [Huntley, M et al] – October
- "Uncertainties of Environmental Impacts for Algal Biofuels and Feed Production," Poster for Algal Biomass Organization Summit, San Diego [Sills, D, L Gerber, M Huntley, C Greene, J Tester, I Archibald, J Granados, S Machesky, C Beal] – October

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 [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 an effective fishmeal replacement in feeds of hybrid tilapia," AQUA 2012, Prague, Czech Republic [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

"Effects of sequence and severity of macronutrient depletion on neutral lipid production in two strains of *Chlorella vulgaris,*" *First International Conference on Algal Biomass, Biofuels and Bioproducts,* St Louis, USA [Cochlan, WP, J Herndon, & RR Bidigare] – July

"Mid-scale screening of marine phytoplankton for large-scale production of biofuel feedstock," *First International Conference on Algal Biomass, Biofuels and Bioproducts,* St Louis [Pickell, L, H Forehead, S Thomas-Hall, X Bai, & J Obbard] – July

"Harvesting and processing of microalgae biomass," *First International Conference on Algal Biomass, Biofuels and Bioproducts,* St Louis [Thomas-Hall, S, J Obbard, L Pickell, & R Dorland] - July

"Algal autotrophic and heterotrophic Lipid synthesis for biofuel production," IABBB Meeting. St Louis [X Bai, E Knurek, M. Workman, C Hertz, S Hamilton & J Obbard]- June

"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

2011 (Cont'd)

"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 [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 [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, Honolulu [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 [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 [Johnson, Z, R Bidigare, S Brown, F Bruyant, W Cochlan, J Cullen, M Huntley, D Redalje, and G de Scheemaker] – July

2009 (Cont'd)

"Next-generation fuels and food from marine photosynthetic microbes," Pacific Rim Summit on Industrial Biotechnology & Bioenergy, Honolulu [Bidigare, RR, SL Brown, ZI Johnson, CJ O'Kelly, ME Huntley, R Dorland, I Archibald, J Cullen, D Redalje and WP Cochlan] – November

"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 [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 [Thomas-Hall, SR, X Bai, B Paul, T Stone, S Brown, WP Cochlan, Z Johnson, R Bidigare, and M Huntley] – July

SUMMARY 27 peer-reviewed Publications 31 Technical Reports 35 Presentations

Patents, Awards, and Commercialization

No patents have been applied for based on the work supported by DOE.

No special awards have been received.

Commercialization is being led by Cellana, the only commercial partner in the Consortium. Cellana has entered into an offtake agreement for algal oil with Neste Oil, and is pursuing other commercial applications of the technology,

All primary results from this project are being published in the open, peer-reviewed literature. The publications from this project – cited above – provide a comprehensive and detailed analysis of commercialization potential. This information will be available to anyone with access to the open literature.