

PPFM &
Algae

Stephen
M. Kelly

Microalgae

Biofuel

Aquaculture

Algal
Species

PPFM

Past
Research

Goals

Identify
PPFM

Algal
Growth

B12 Assay

Nutritional
Analysis

Conclusions

Altering Growth Rates and Nutritional Qualities of Microalgal Feedstock Using Symbiotic Bacteria

Stephen M. Kelly

Advisor: Dr. Mark Holland

Committee: Dr. Mark Frana, Dr. Chris Briand

Department of Biological Sciences,
Salisbury University, Salisbury, MD 21801

sk81638@gulls.salisbury.edu

Microalgae

PPFM &
Algae

Stephen
M. Kelly

Microalgae

Biofuel

Aquaculture

Algal
Species

PPFM

Past
Research

Goals

Identify
PPFM

Algal
Growth

B12 Assay

Nutritional
Analysis

Conclusions

- Unicellular algae
- Economic importance
 - supplements, food additives, chemical extracts
 - aquaculture feedstock
 - biofuel potential

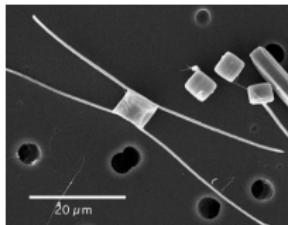


Figure: Microalgae, *Chaetoceros*. Source: sci.sdsu.edu



Figure: *Haematococcus* sp. grown for the food supplement astaxanthin. Source: Cyanotech



Figure: Macroalgae, kelp. Source: Sciworthy.com

Biofuel

PPFM &
Algae

Stephen
M. Kelly

Microalgae

Biofuel

Aquaculture

Algal
Species

PPFM

Past
Research

Goals

Identify
PPFM

Algal
Growth

B12 Assay

Nutritional
Analysis

Conclusions

- Renewable resource
- High oil content
- Need to reduce costs for viability
- Algal monoculture vs. 'synthetic ecology' of various species



Figure: Enclosed algal bioreactors. Source:
enfo.agt.bme.hu



Figure: Open raceway ponds. Source: ethand.net

Aquaculture

PPFM &
Algae

Stephen
M. Kelly

Microalgae

Biofuel

Aquaculture

Algal
Species

PPFM

Past
Research

Goals

Identify
PPFM

Algal
Growth

B12 Assay

Nutritional
Analysis

Conclusions

- Algal feedstock for fin-fish, shrimp, mollusks, etc.
- Required either directly or indirectly in aquatic animal diets
- Algae culture accounts for up to 40% of bivalve hatchery costs
- High oil content
 - carry over to biofuel



Figure: Algal feedstock for oyster larvae. Source:
Horn Point Laboratory



Figure: Algal starter cultures. Source: Horn Point Laboratory

Algal Species Included in the Study

PPFM &
Algae

Stephen
M. Kelly

Microalgae

Biofuel

Aquaculture

Algal
Species

PPFM

Past
Research

Goals

Identify
PPFM

Algal
Growth

B12 Assay

Nutritional
Analysis

Conclusions

	Species	Attributes	Uses
1	<i>Tetraselmis chuii</i>	Chlorophyta - flagellate	Aquaculture feed
2	<i>Chaetoceros mulleri</i>	Ochrophyta - diatom	Aquaculture feed
3	<i>Isochrysis sp.</i>	Haptophyta - flagellate	Aquaculture feed
4	<i>Thalassiosira pseudonana</i>	Ochrophyta - diatom	Aquaculture feed
5	<i>Nannochloropsis oculata</i>	Ochrophyta - flagellate	Potential for biofuel

Table: Algal species used in the study.

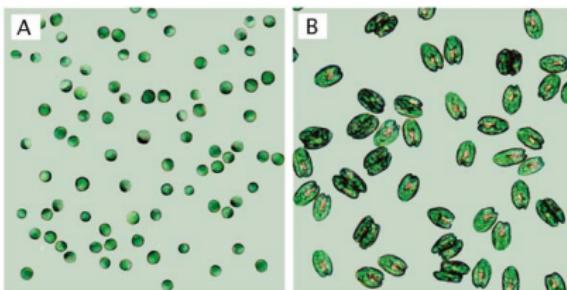


Figure: A. *Isochrysis sp.*. B. *Tetraselmis sp.*.
Source: www.fao.org

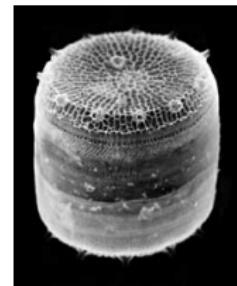


Figure: *Thalassiosira pseudonana* SEM
micrograph. Source: www.jgi.doe.gov

Travel

PPFM &
Algae

Stephen
M. Kelly

Microalgae

Biofuel

Aquaculture

Algal
Species

PPFM

Past
Research

Goals

Identify
PPFM

Algal
Growth

B12 Assay

Nutritional
Analysis

Conclusions

- Horn Point Laboratories, Cambridge, MD
- UTEX Culture Collection of Algae, University of Texas, Austin, TX



Figure: Capitol building at Austin, Texas.



Figure: Horn Point Aquaculture and Restoration Ecology Laboratory. Source:
<http://hatchery.hpl.umces.edu/>



Figure: Austin: Home to massive cycads

Pink Pigmented Facultatively Methylotrophic Bacteria

PPFM &
Algae

Stephen
M. Kelly

Microalgae

Biofuel

Aquaculture

Algal
Species

PPFM

Past
Research

Goals

Identify
PPFM

Algal
Growth

B12 Assay

Nutritional
Analysis

Conclusions

- PPFM, genus *Methylobacterium*
- Ubiquitous plant symbionts
- Able to metabolize Methanol
- Stimulate plant growth, enhance seed germination, produce plant growth regulators
- Can be isolated from water, algae samples

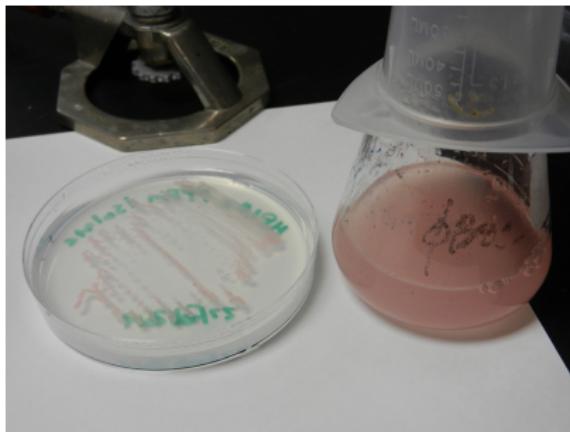


Figure: Solid and liquid PPFM cultures

Why PPFM's?

PPFM &
Algae

Stephen
M. Kelly

Microalgae

Biofuel

Aquaculture

Algal
Species

PPFM

Past
Research

Goals

Identify
PPFM

Algal
Growth

B12 Assay

Nutritional
Analysis

Conclusions

- Avoid genetic modification of algae
- Inexpensive
- Naturally occurring
- Past research suggests potential



Figure: Rendering of PPFM. Source: newleafsym.com

Past Research

PPFM &
Algae

Stephen
M. Kelly

Microalgae

Biofuel

Aquaculture

Algal
Species

PPFM

Past
Research

Goals

Identify
PPFM

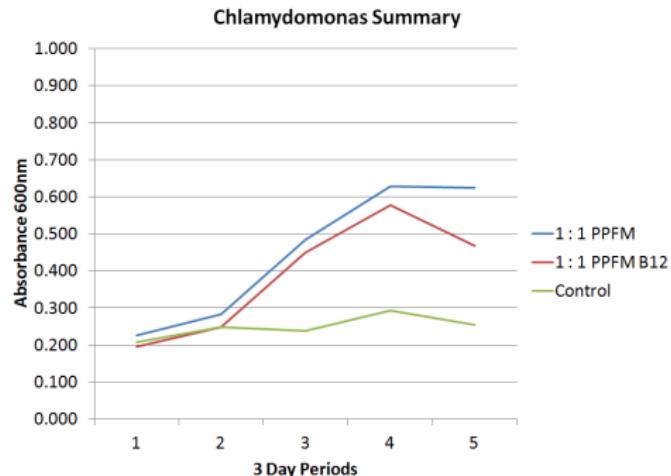
Algal
Growth

B12 Assay

Nutritional
Analysis

Conclusions

- Vitamin B12 overproducing strain of PPFM isolated
- Patented bacterization technology for plants
 - NewLeaf Symbiotics, LLC.
- PPFM supplementation enhanced algal growth in *Chlamydomonas* (unpublished; Chuck Davis, 2010)



Research Goals & Hypotheses

PPFM &
Algae

Stephen
M. Kelly

Microalgae

Biofuel

Aquaculture

Algal
Species

PPFM

Past
Research

Goals

Identify
PPFM

Algal
Growth

B12 Assay

Nutritional
Analysis

Conclusions

- Supplementation with PPFM will benefit microalgae
- Identify PPFM strains
- Test for increased algal growth rate with PPFM supplementation
- Test for increased algal nutritional qualities with PPFM supplementation

PPFM Isolation & Identification

PPFM &
Algae

Stephen
M. Kelly

Microalgae

Biofuel

Aquaculture

Algal
Species

PPFM

Past
Research

Goals

Identify
PPFM

Algal
Growth

B12 Assay

Nutritional
Analysis

Conclusions

- Plate algal sample on AMS + 0.5% MeOH agar
- 16s rDNA amplification & sequencing

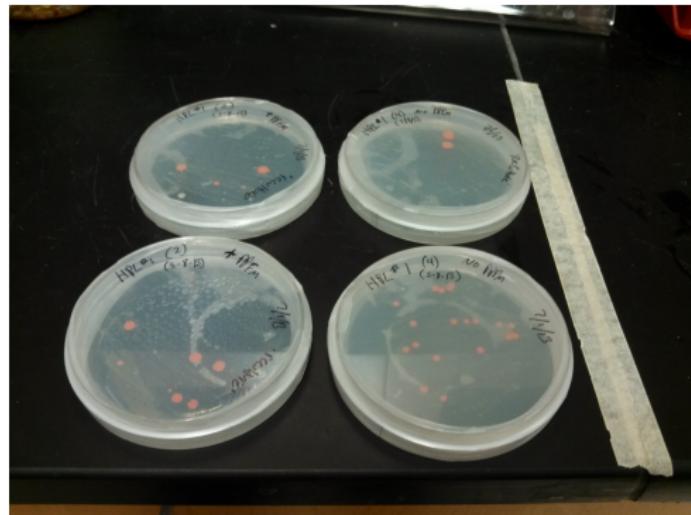


Figure: PPFM colonies forming on plated algae

Algal Growth Analysis

PPFM &
Algae

Stephen
M. Kelly

Microalgae

Biofuel

Aquaculture

Algal
Species

PPFM

Past
Research

Goals

Identify
PPFM

Algal
Growth

B12 Assay

Nutritional
Analysis

Conclusions

- Correlate **chlorophyll autofluorescence** with cell density
- Algae grown \pm PPFM, vitamin B12 over-producing PPFM
- 'Day 7' critical factor, replicate industrial media and conditions

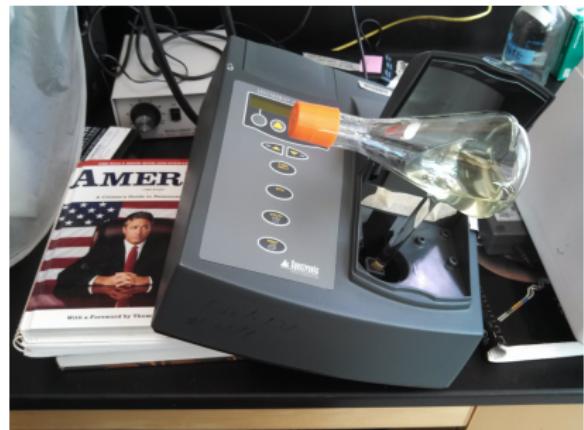
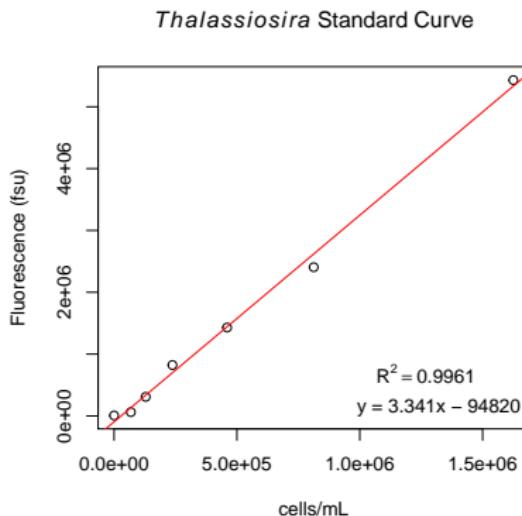


Figure: Research supported by Jon Stewart

Results: *Thalassiosira*

PPFM &
Algae

Stephen
M. Kelly

Microalgae

Biofuel

Aquaculture

Algal
Species

PPFM

Past
Research

Goals

Identify
PPFM

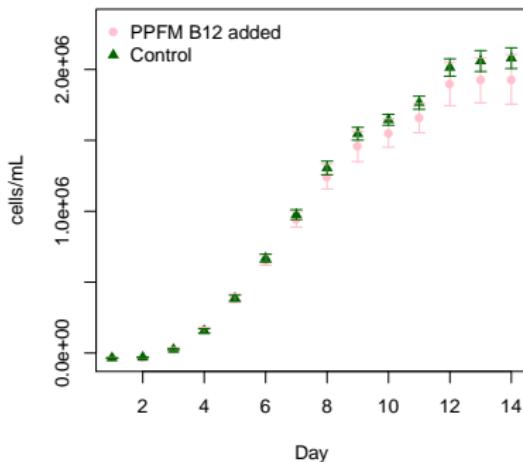
Algal
Growth

B12 Assay

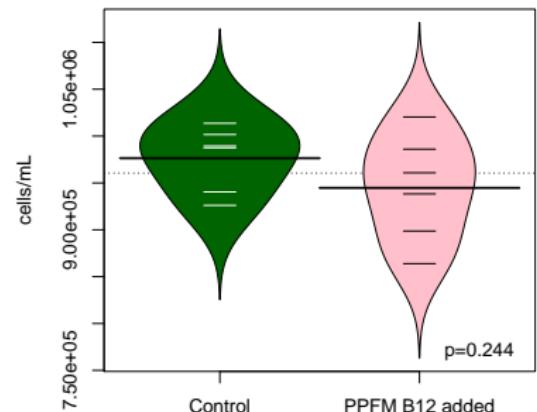
Nutritional
Analysis

Conclusions

Thalassiosira Growth Curve



Thalassiosira: Day 7



Results: *Tetraselmis*

PPFM &
Algae

Stephen
M. Kelly

Microalgae

Biofuel

Aquaculture

Algal
Species

PPFM

Past
Research

Goals

Identify
PPFM

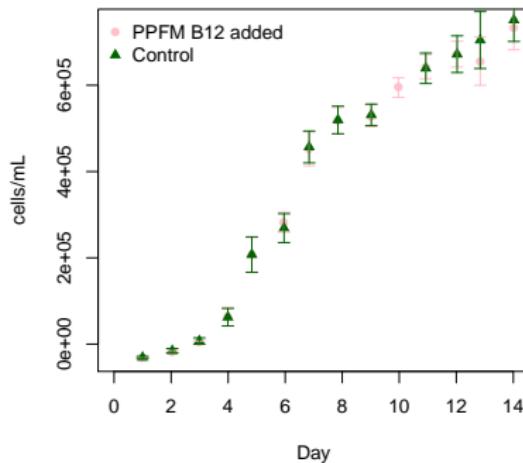
Algal
Growth

B12 Assay

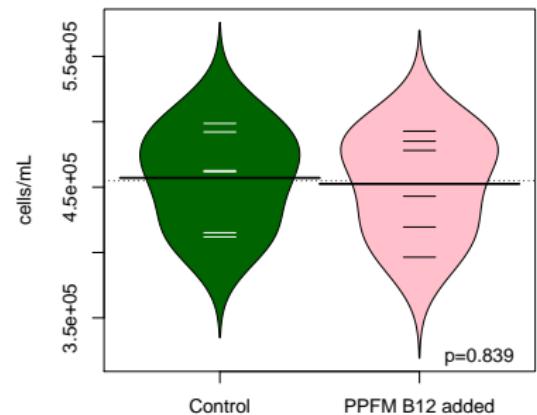
Nutritional
Analysis

Conclusions

Tetraselmis Growth Curve



Tetraselmis: Day 7



Results: Mixed Culture

PPFM &
Algae

Stephen
M. Kelly

Microalgae

Biofuel

Aquaculture

Algal
Species

PPFM

Past
Research

Goals

Identify
PPFM

Algal
Growth

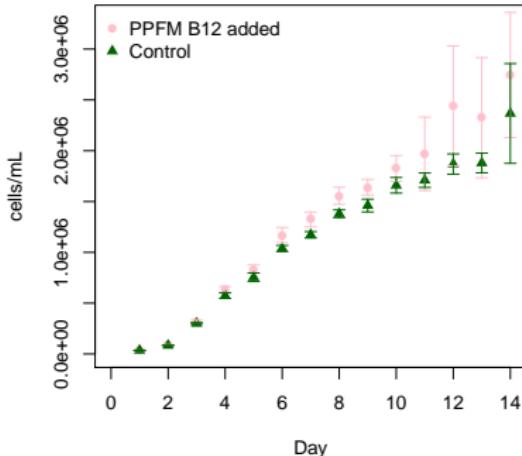
B12 Assay

Nutritional
Analysis

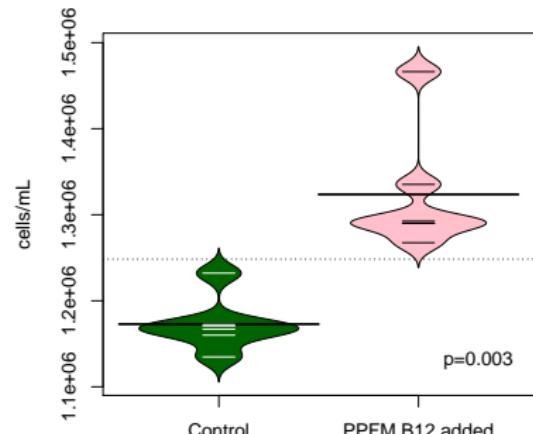
Conclusions

- Four aquaculture species grown together
 - *Tetraselmis, Thalassiosira, Isochrysis, Chaetoceros*

Mixed Culture Growth Curve



Mixed Culture: Day 7



Results: B12 Deficient Media

PPFM &
Algae

Stephen
M. Kelly

Microalgae

Biofuel

Aquaculture

Algal
Species

PPFM

Past
Research

Goals

Identify
PPFM

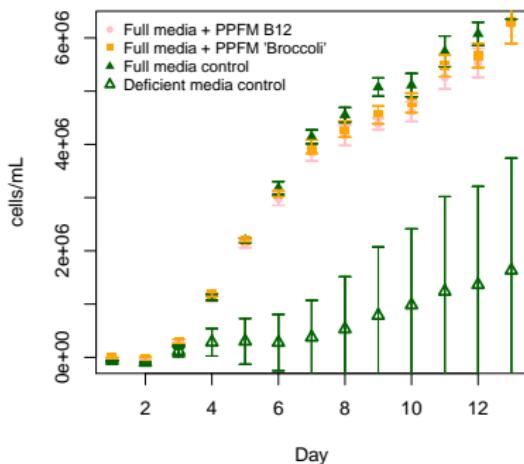
Algal
Growth

B12 Assay

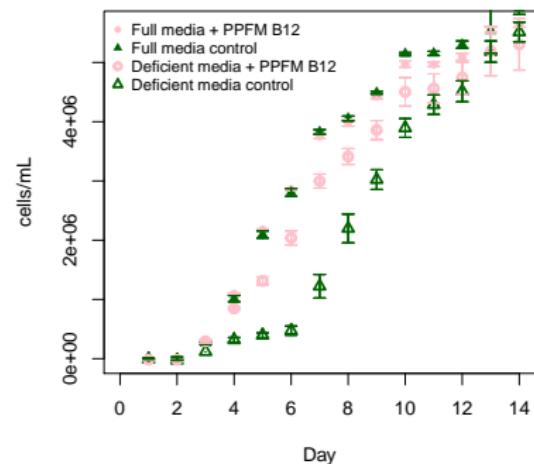
Nutritional
Analysis

Conclusions

Isochrysis Growth Curve



Isochrysis Growth Curve



Results: B12 Deficient Media cont.

PPFM &
Algae

Stephen
M. Kelly

Microalgae

Biofuel

Aquaculture

Algal
Species

PPFM

Past
Research

Goals

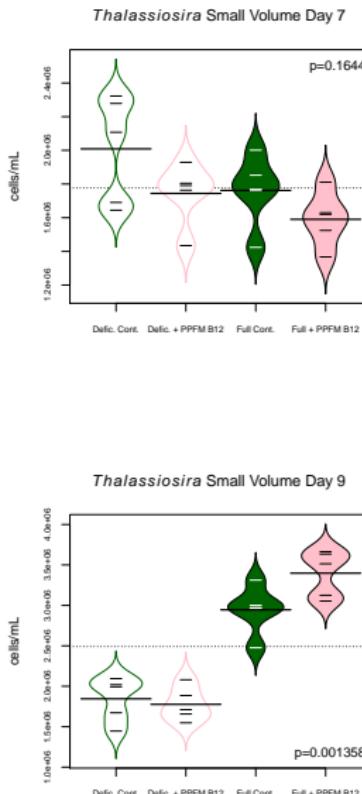
Identify
PPFM

Algal
Growth

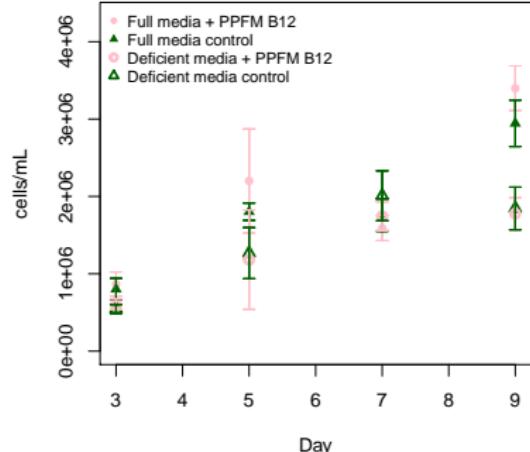
B12 Assay

Nutritional
Analysis

Conclusions



Thalassiosira Small Volume Growth Curve



PPFM Ratio

PPFM &
Algae

Stephen
M. Kelly

Microalgae

Biofuel

Aquaculture

Algal
Species

PPFM

Past
Research

Goals

Identify
PPFM

Algal
Growth

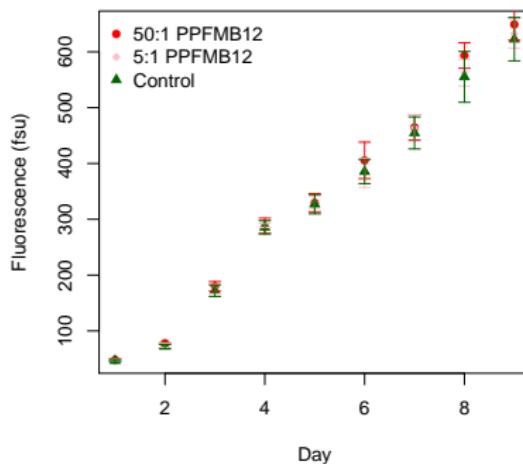
B12 Assay

Nutritional
Analysis

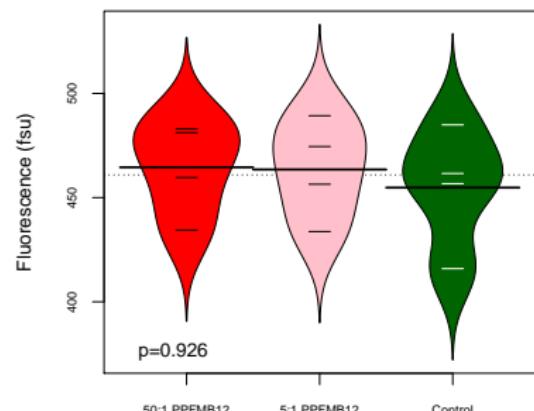
Conclusions

- Test PPFM supplementation at higher level

Mixed Culture Growth Curve



Mixed Culture Day 7



Vitamin B12 Assay

PPFM &
Algae

Stephen
M. Kelly

Microalgae

Biofuel

Aquaculture

Algal
Species

PPFM

Past
Research

Goals

Identify
PPFM

Algal
Growth

B12 Assay

Nutritional
Analysis

Conclusions

- Competitive ELISA assay test

	Species	Type	Processing	Avg. Conc. (ng/mL)	St.Dev. (ng/mL)
	1	Mixed Culture	Control	Homogenate	0.29
	2	Mixed Culture	Control	Supernatant	0.00
	3	Mixed Culture	Experimental	Homogenate	0.06
	4	Mixed Culture	Experimental	Supernatant	0.92
	5	Tetraselmis	Control	Homogenate	0.14
	6	Tetraselmis	Control	Supernatant	0.00
	7	Tetraselmis	Experimental	Homogenate	0.28
	8	Tetraselmis	Experimental	Supernatant	0.00

Table: Adjusted algal data. Concentration in ng/mL, n=3.

	Material	Processing	Concentration (ng/mL)
	1 F/2 media	NA	1.79
	2 PPFM B12	Supernatant	39.37
	3 PPFM B12	Homogenate	41.19
	4 PPFM Broccoli	Supernatant	44.93
	5 PPFM Broccoli	Homogenate	45.87

Table: Data for PPFM and F/2 media samples. Concentration in ng/mL, n=1.

Nutritional Analysis

PPFM &
Algae

Stephen
M. Kelly

Microalgae

Biofuel

Aquaculture

Algal
Species

PPFM

Past
Research

Goals

Identify
PPFM

Algal
Growth

B12 Assay

Nutritional
Analysis

Conclusions

- Analyze algal feedstock for lipid profile, amino acid content
- Requires 2g dry algal biomass, 15L culture
- Mixed algal culture \pm PPFM B12



Nutritional Analysis

PPFM &
Algae

Stephen
M. Kelly

Microalgae

Biofuel

Aquaculture

Algal
Species

PPFM

Past
Research
Goals

Identify
PPFM

Algal
Growth

B12 Assay

Nutritional
Analysis

Conclusions

	Compound	Algae Control	Algae + PPFM B12	% Change
1	Crude Protein	21.80	29.46	35.14
2	Crude Fat	9.07	19.58	115.88

Table: Summary of nutritional analysis results. Increased nutritional qualities are in red. Values represent grams per 100g of sample

Amino Acid Content

PPFM &
Algae

Stephen
M. Kelly

Microalgae

Biofuel

Aquaculture

Algal
Species

PPFM

Past
Research

Goals

Identify
PPFM

Algal
Growth

B12 Assay

Nutritional
Analysis

Conclusions

	Compound	Algae Control	Algae + PPFM B12	% Change
1	Hydroxylysine	0.15	0.43	186.67
2	Histidine	0.33	0.60	81.82
3	Methionine	0.39	0.68	74.36
4	Lysine	0.79	1.26	59.49
5	Arginine	0.93	1.44	54.84
6	Tyrosine	0.59	0.89	50.85
7	Cysteine	0.20	0.30	50.00
8	Leucine	1.53	2.18	42.48
9	Proline	0.93	1.30	39.78
10	Glycine	1.09	1.52	39.45
11	Serine	0.80	1.11	38.75
12	Glutamic Acid	1.86	2.50	34.41
13	Phenylalanine	1.02	1.37	34.31
14	Aspartic Acid	1.79	2.40	34.08
15	Isoleucine	0.86	1.15	33.72
16	Valine	1.11	1.47	32.43
17	Threonine	1.00	1.28	28.00
18	Alanine	1.66	2.10	26.51
19	Hydroxyproline	0.17	0.21	23.53
20	Tryptophan	0.21	0.22	4.76

Table: Amino Acid profile for mixed algae culture nutritional analysis. Increased nutritional qualities are in red. Values represent grams per 100g of sample

Fatty Acid Content

PPFM &
Algae

Stephen
M. Kelly

Microalgae

Biofuel

Aquaculture

Algal
Species

PPFM

Past
Research

Goals

Identify
PPFM

Algal
Growth

B12 Assay

Nutritional
Analysis

Conclusions

	Compound	Algae Control	Algae + PPFM B12	% Change
Microalgae	1 DHA (22:6n3)	3.19	5.04	57.99
Biofuel	2 Linolenic (18:3n3)	5.14	7.61	48.05
Aquaculture	3 Myristoleic (9c-14:1)	0.23	0.30	30.43
Algal Species	4 Myristic (14:0)	7.67	9.47	23.47
PPFM	5 Linoleic (18:2n6)	3.37	4.14	22.85
Past Research	6 Behenoic (22:0)	0.54	0.63	16.67
Goals	7 10c-17:1	1.53	1.60	4.58
Identify PPFM	8 Oleic (9c-18:1)	19.34	19.15	-0.98
Algal Growth	9 Lignoceric (24:0)	0.16	0.14	-12.50
B12 Assay	10 EPA (20:5n3)	4.58	3.93	-14.19
Nutritional Analysis	11 Stearic (18:0)	0.46	0.39	-15.22
Conclusions	12 C15:0	0.42	0.34	-19.05
	13 C16:0 [Palmitic]	24.19	19.58	-19.06
	14 Palmitoleic (9c-16:1)	15.25	11.95	-21.64
	15 Margaric (17:0)	0.33	0.21	-36.36

Table: Fatty Acid profile for mixed algae culture nutritional analysis. Increased nutritional qualities are in red. Values represent grams per 100g of sample

Conclusions & Future Directions

PPFM &
Algae

Stephen
M. Kelly

Microalgae

Biofuel

Aquaculture

Algal
Species

PPFM

Past
Research

Goals

Identify
PPFM

Algal
Growth

B12 Assay

Nutritional
Analysis

Conclusions

- PPFM species identified
- Potential increase in nutritional qualities independent of growth rate



Figure: Algal bioreactors, Austin, TX

Acknowledgements

PPFM &
Algae

Stephen
M. Kelly

Microalgae

Biofuel

Aquaculture

Algal
Species

PPFM

Past
Research

Goals

Identify
PPFM

Algal
Growth

B12 Assay

Nutritional
Analysis

Conclusions

- Acknowledgements

- Graduate Committee members
- SU Biology Department faculty
- TEDCO funding
- RAP funding
- Horn Point Laboratories
- UTEX Algae Culture Collection



Figure: Technology & Economic Development Corporation of Maryland, Technology Validation Program