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

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Microalgae

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

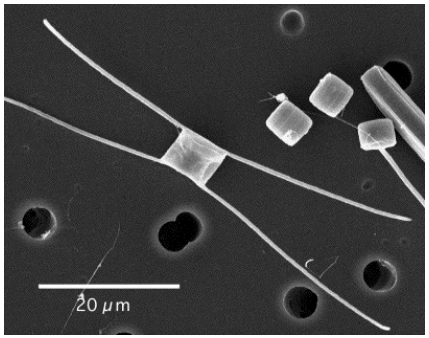


Figure: Microalgae, *Chaetoceros*. Source: sci.sdsu.edu

Figure: Macroalgae, kelp. Source: Sciworthy.com

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

Biofuel

- 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

- 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

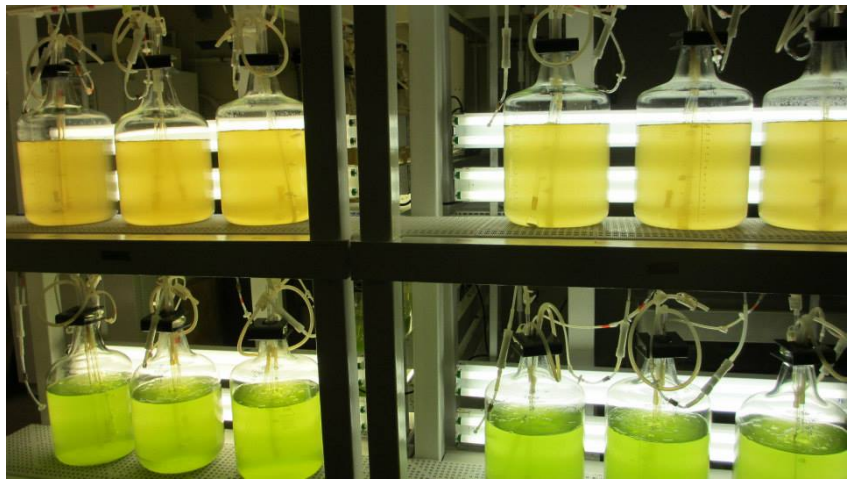


Figure: Algal starter cultures. Source: Horn Point Laboratory

Algal Species Included in the Study

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

Table: Algal species used in the study.

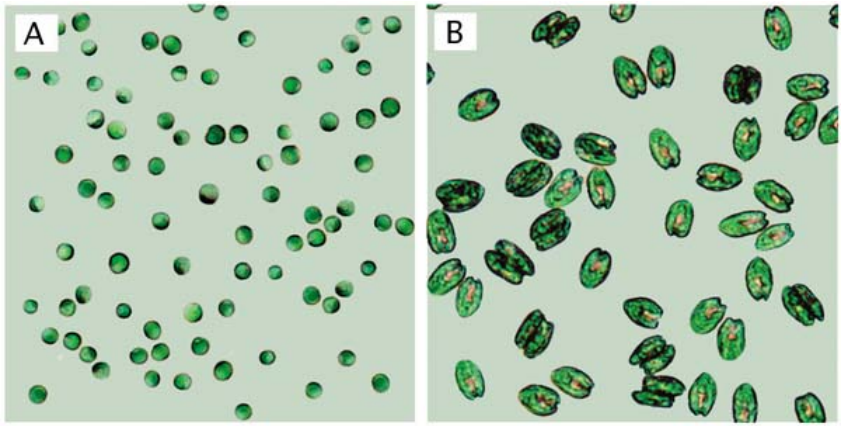


Figure: A. *Isochrysis* sp.. B. *Tetraselmis* sp.. Source: www.fao.org

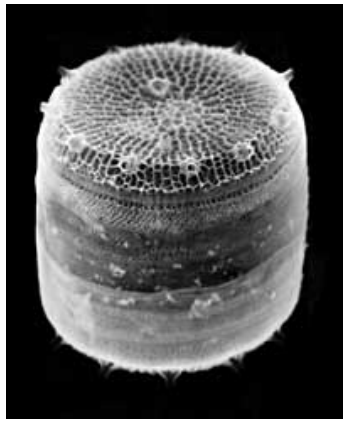


Figure: *Thalassiosira pseudonana* SEM micrograph. Source: www.jgi.doe.gov

Travel

- 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, 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

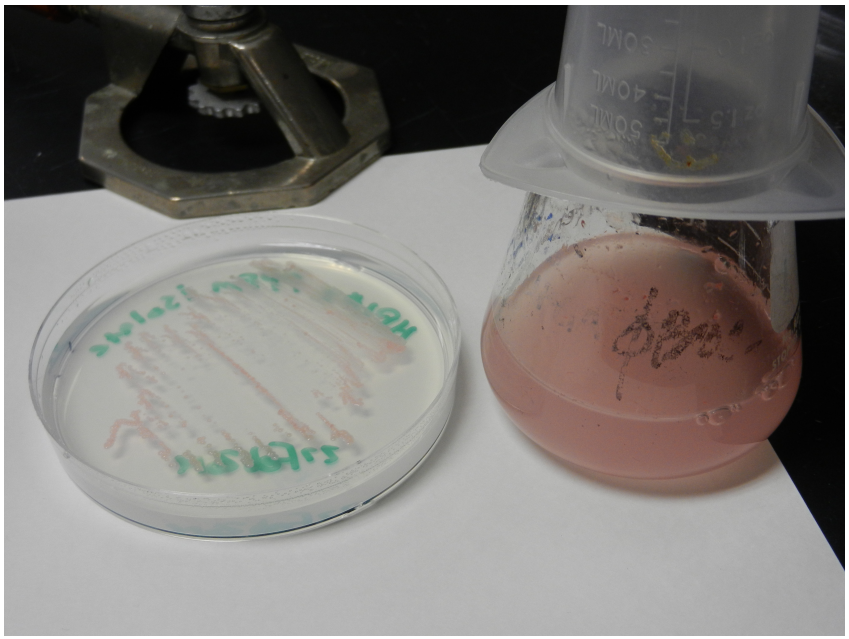


Figure: Solid and liquid PPFM cultures

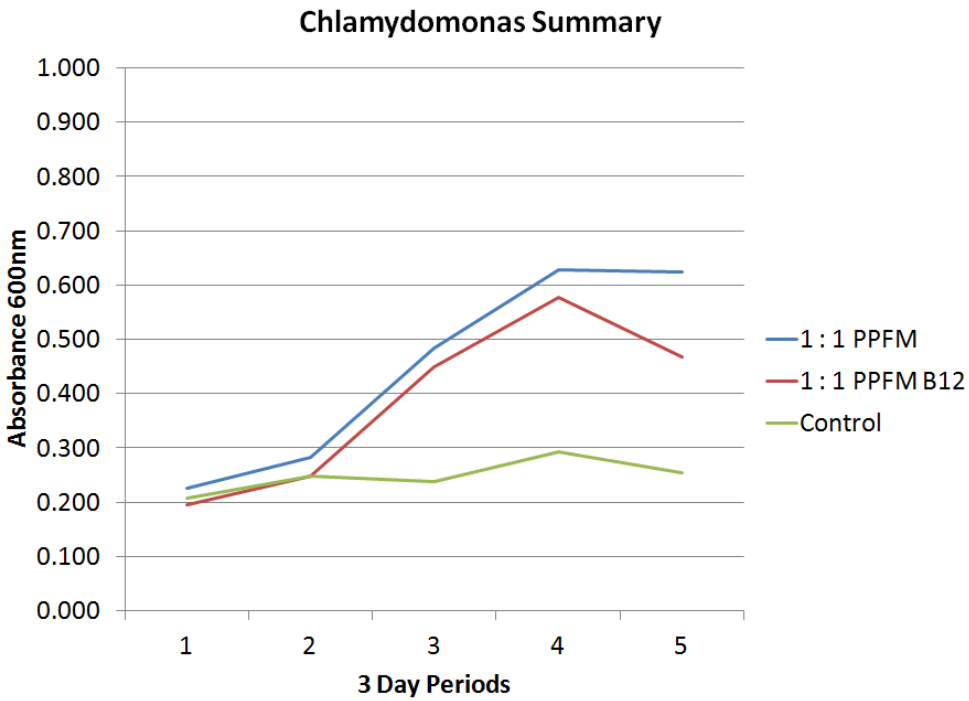
Why PPFM's?

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

Figure: Rendering of PPFM. Source: newleafsym.com

Past Research

- 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

- 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

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

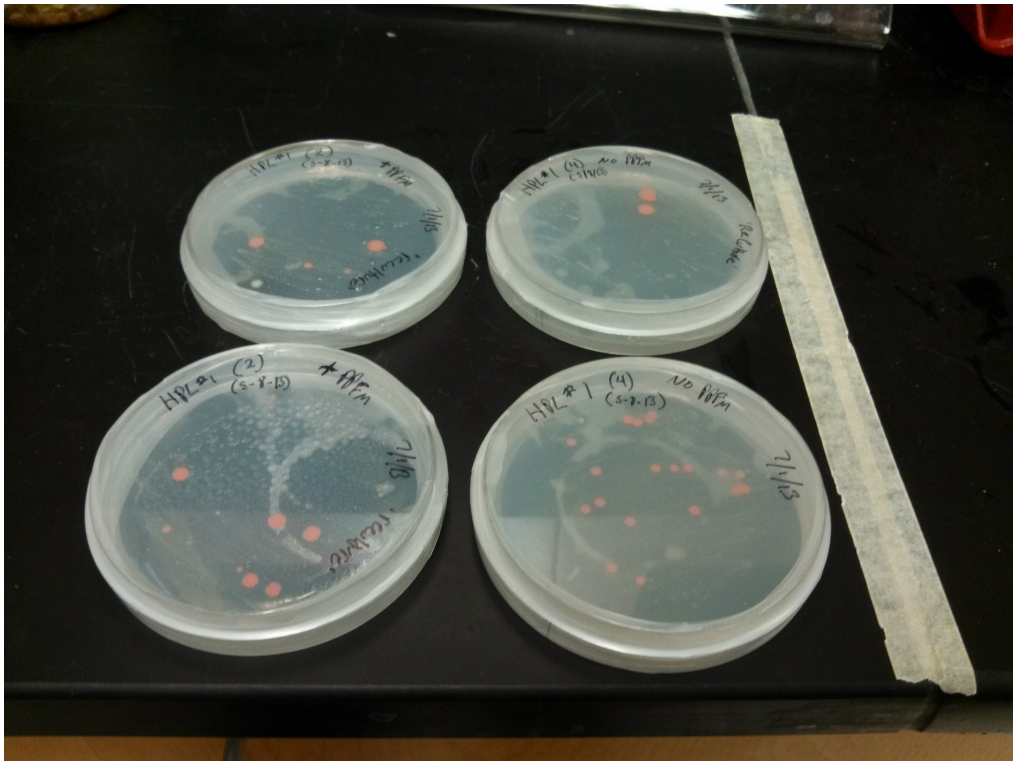


Figure: PPFM colonies forming on plated algae

Algal Growth Analysis

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

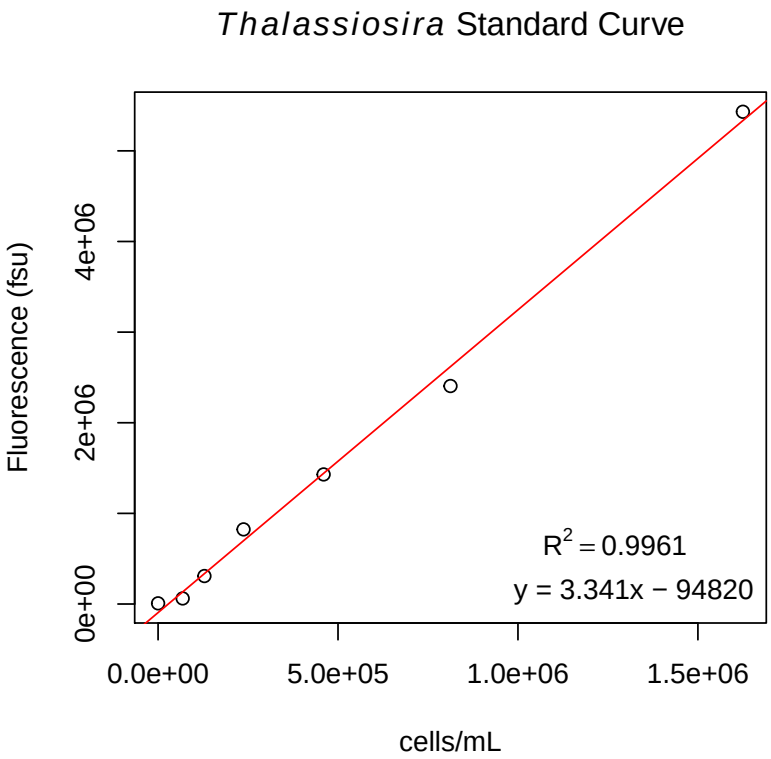
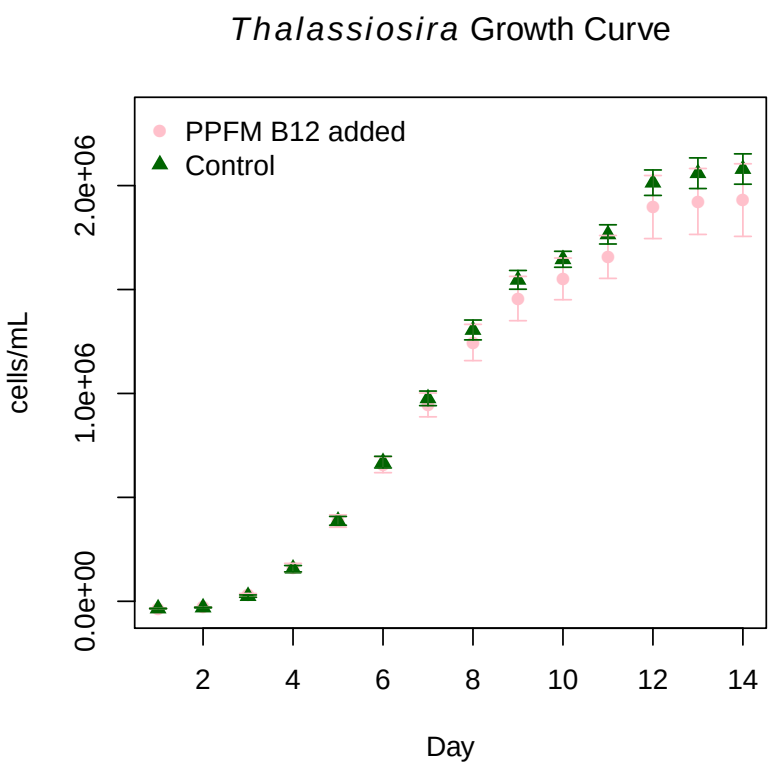
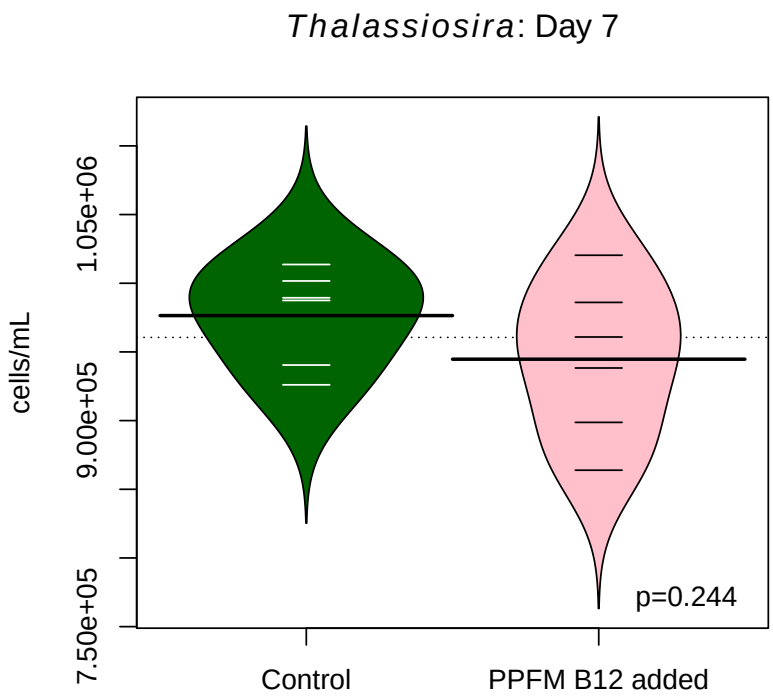


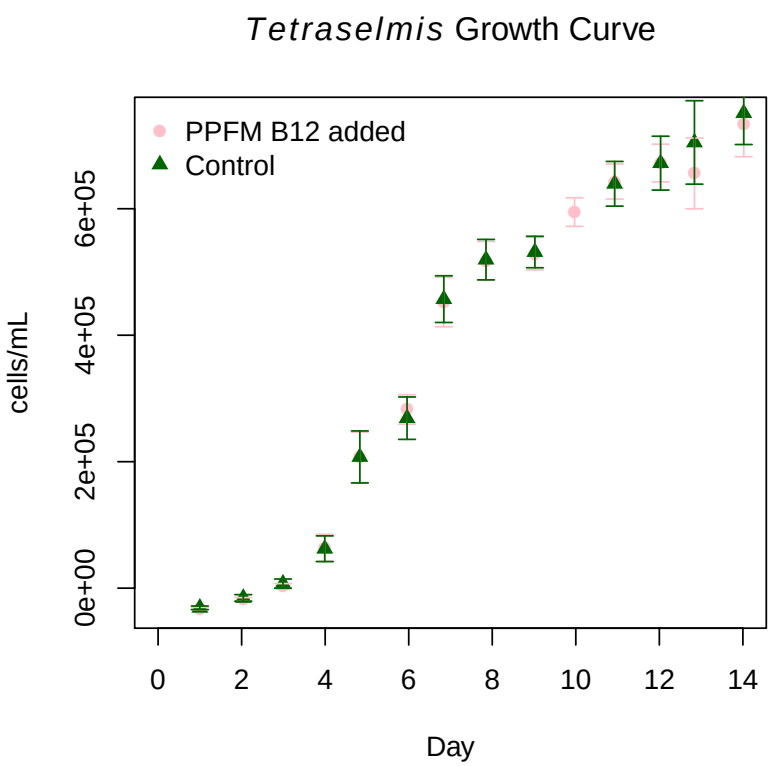
Figure: Research supported by Jon Stewart

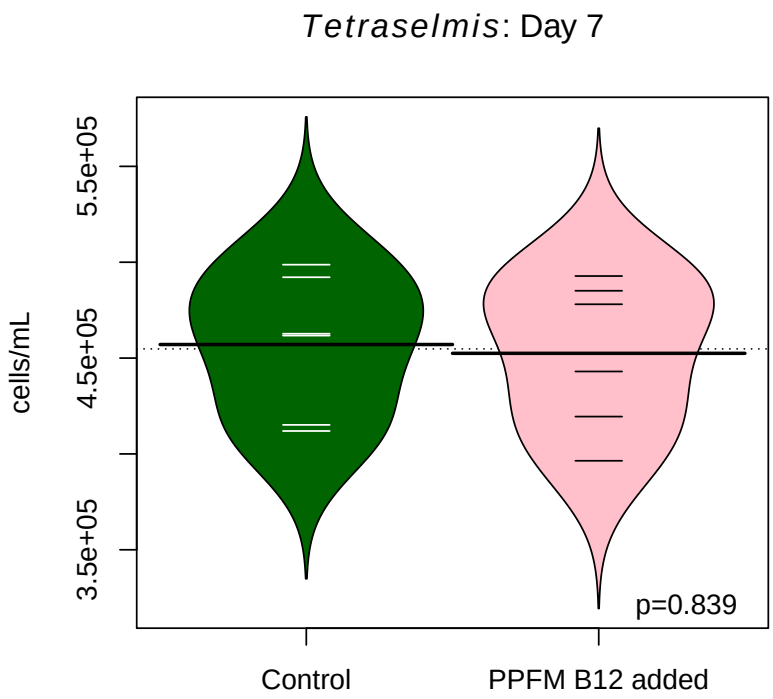
Results: *Thalassiosira*





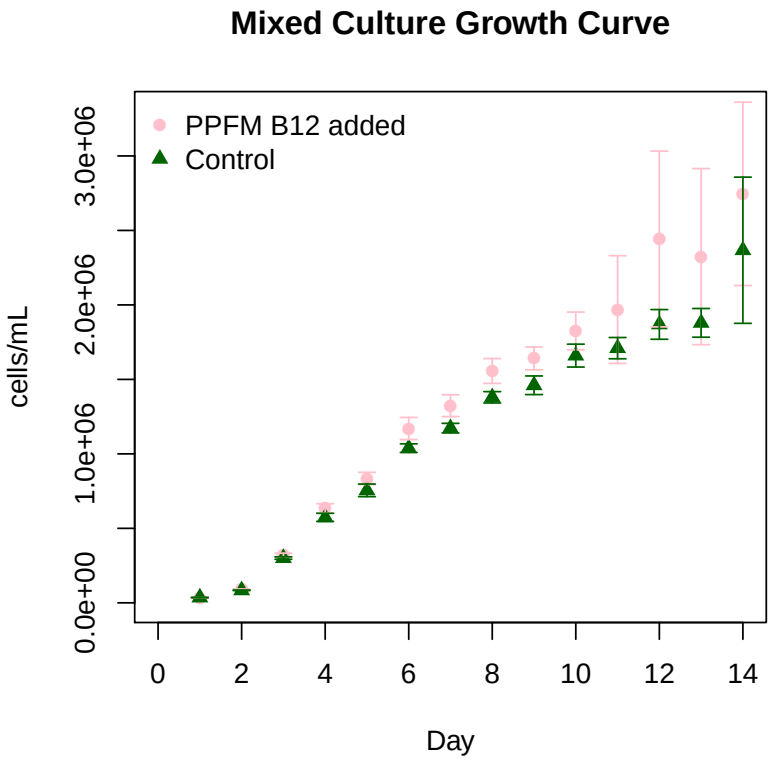
Results: *Tetraselmis*

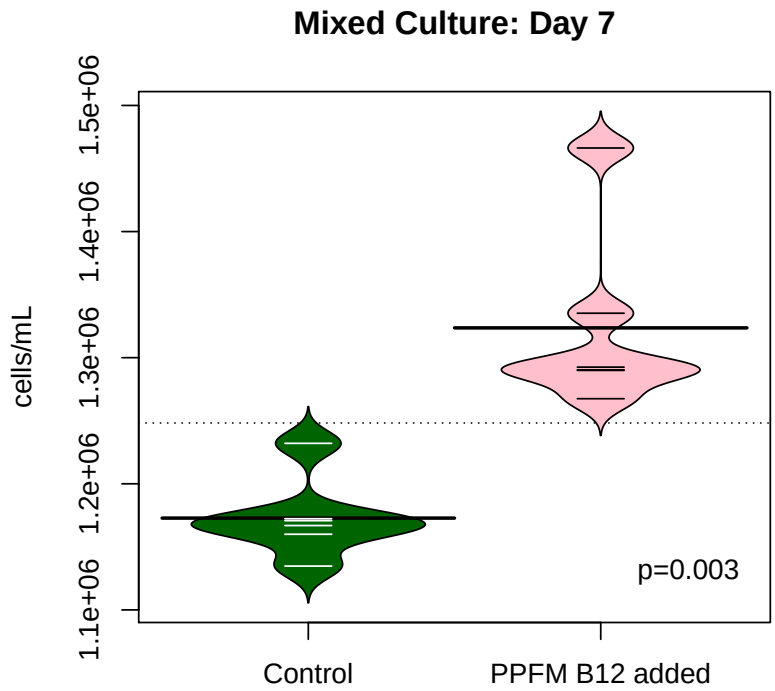




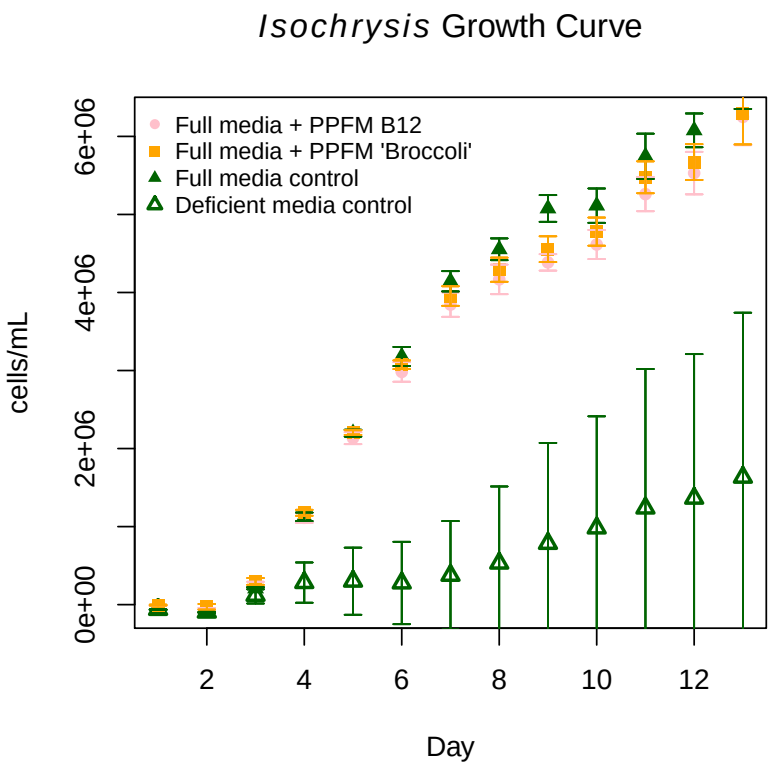
Results: Mixed Culture

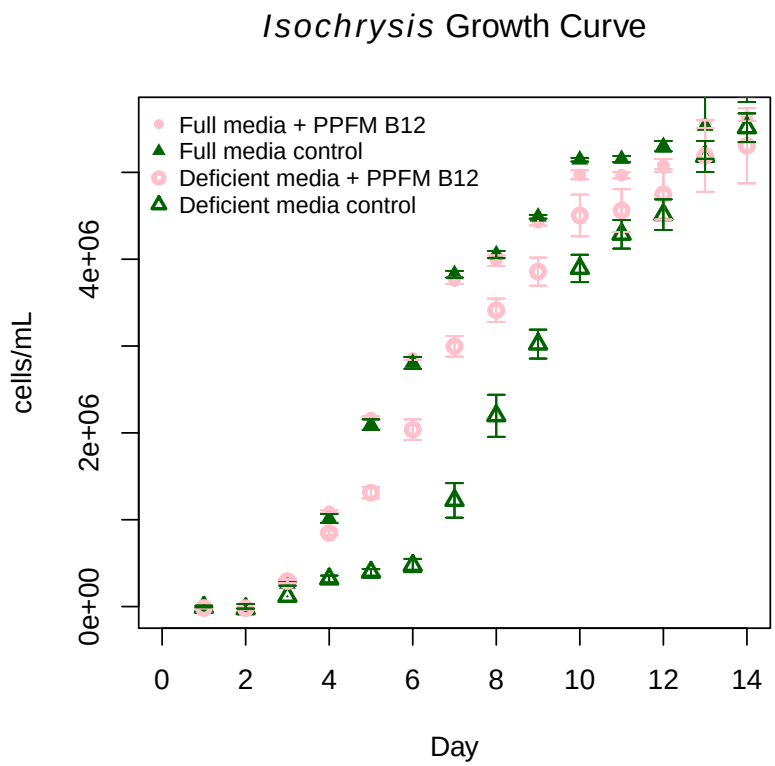
- Four aquaculture species grown together
  - *Tetraselmis*, *Thalassiosira*, *Isochrysis*, *Chaetoceros*



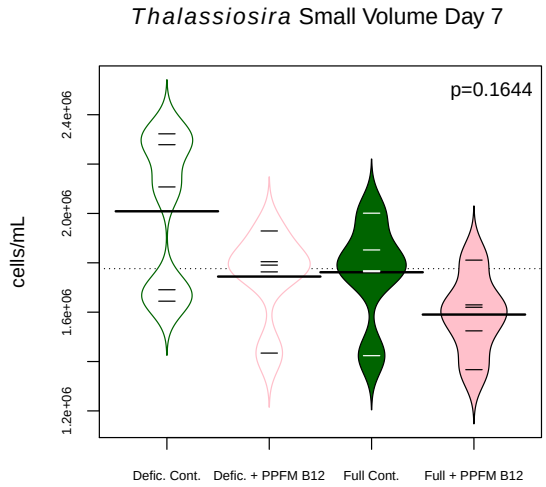


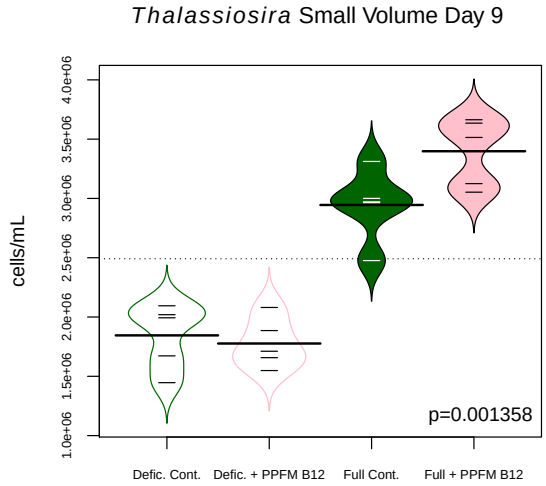
Results: B12 Deficient Media

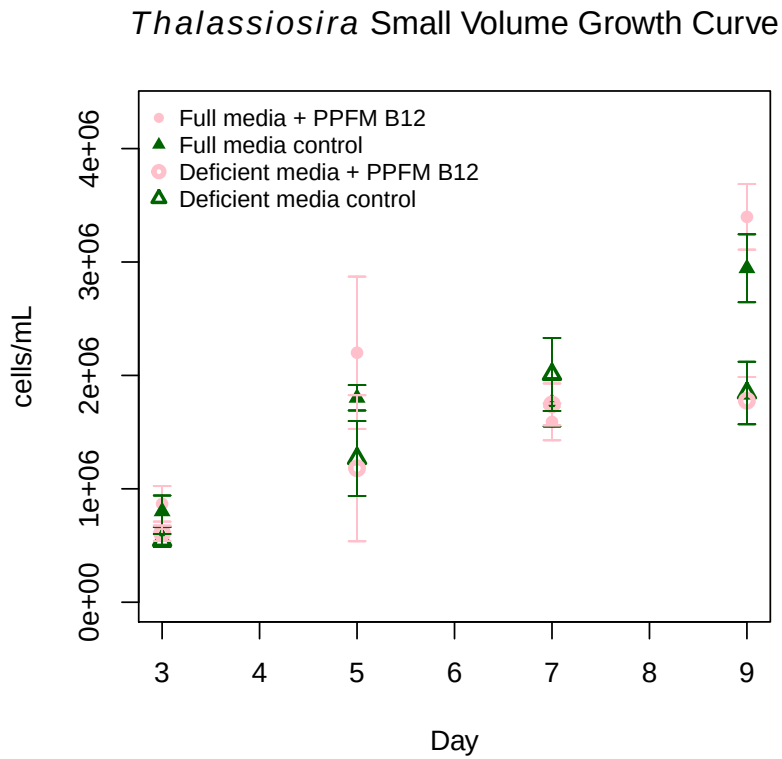




Results: B12 Deficient Media cont.

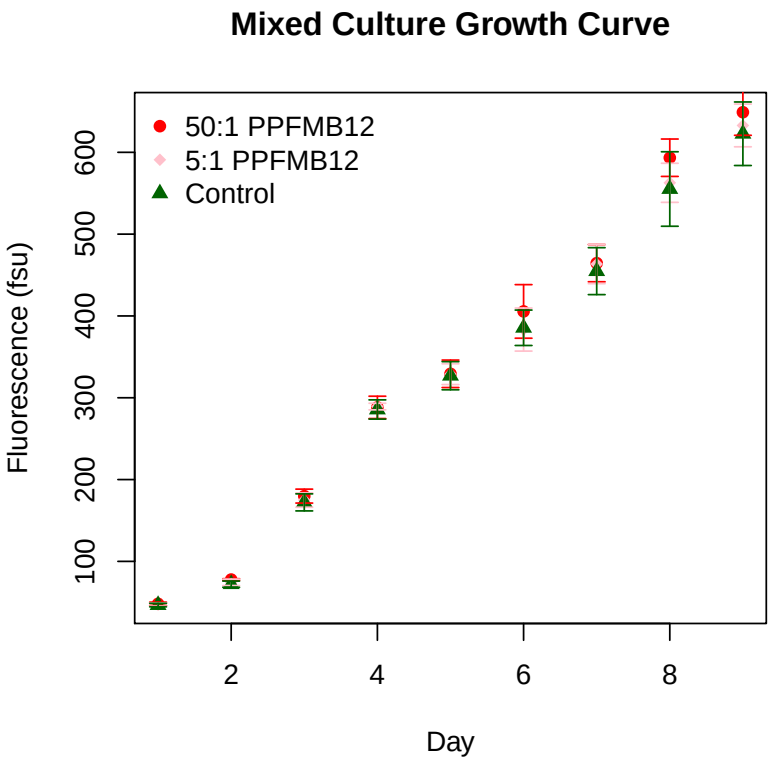


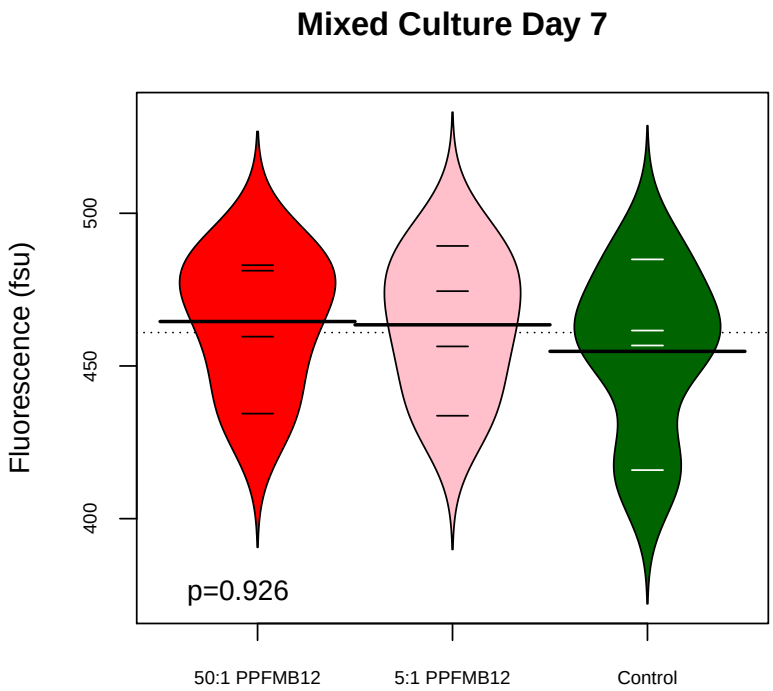




PPFM Ratio

- Test PPFM supplementation at higher level





Vitamin B12 Assay

- Competitive ELISA assay test

	Species	Type	Processing	Avg. Conc. (ng/mL)	St.Dev. (ng/mL)
1	Mixed Culture	Control	Homogenate	0.29	0.25
2	Mixed Culture	Control	Supernatant	0.00	0.00
3	Mixed Culture	Experimental	Homogenate	0.06	0.11
4	Mixed Culture	Experimental	Supernatant	0.92	1.59
5	Tetraselmis	Control	Homogenate	0.14	0.12
6	Tetraselmis	Control	Supernatant	0.00	0.00
7	Tetraselmis	Experimental	Homogenate	0.28	0.47
8	Tetraselmis	Experimental	Supernatant	0.00	0.00

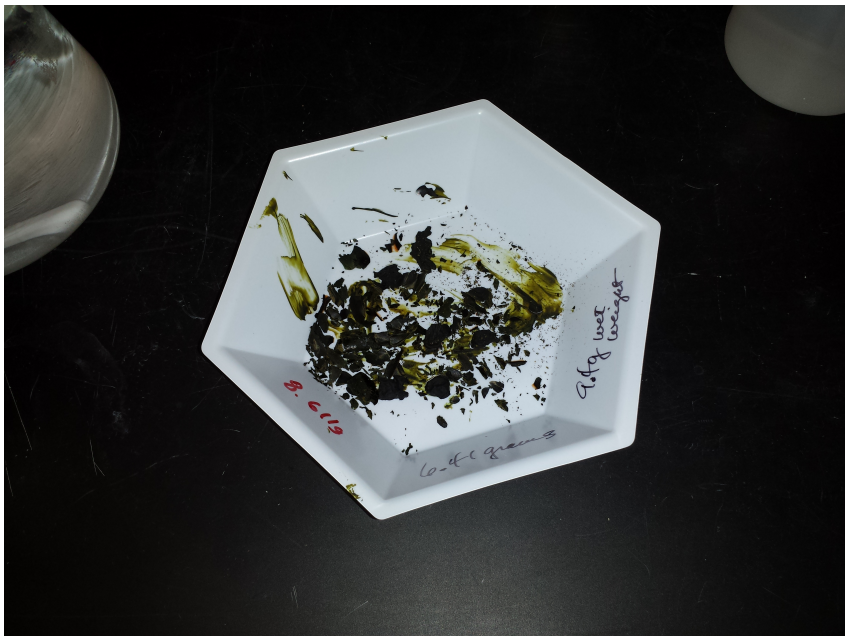
Table: Adjusted algal data. Concentration in ng/mL, n=3.

	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

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



Nutritional Analysis

	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

	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

	Compound	Algae Control	Algae + PPFM B12	% Change
1	DHA (22:6n3)	3.19	5.04	**57.99**
2	Linolenic (18:3n3)	5.14	7.61	**48.05**
3	Myristoleic (9c-14:1)	0.23	0.30	**30.43**
4	Myristic (14:0)	7.67	9.47	**23.47**
5	Linoleic (18:2n6)	3.37	4.14	**22.85**
6	Behenoic (22:0)	0.54	0.63	**16.67**
7	10c-17:1	1.53	1.60	**4.58**
8	Oleic (9c-18:1)	19.34	19.15	**-0.98**
9	Lignoceric (24:0)	0.16	0.14	**-12.50**
10	EPA (20:5n3)	4.58	3.93	**-14.19**
11	Stearic (18:0)	0.46	0.39	**-15.22**
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 species identified
- Potential increase in nutritional qualities independent of growth rate

Figure: Algal bioreactors, Austin, TX

Acknowledgements

- 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