MICROALGAL BIOFUEL:

Isochrysis sp. & *Phaeodactylum tricornutum* lipid characterization and physiology studies

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Overview

Introduction – Orientation & Motivations:

Petro fuel & primary energy; why algae, why biofuels?

Terminology

Research Study I:

Isochrysis, alkenones, & biodiesel cloud points

Research Study II:

Phaeodactylum physiology & lipid quality

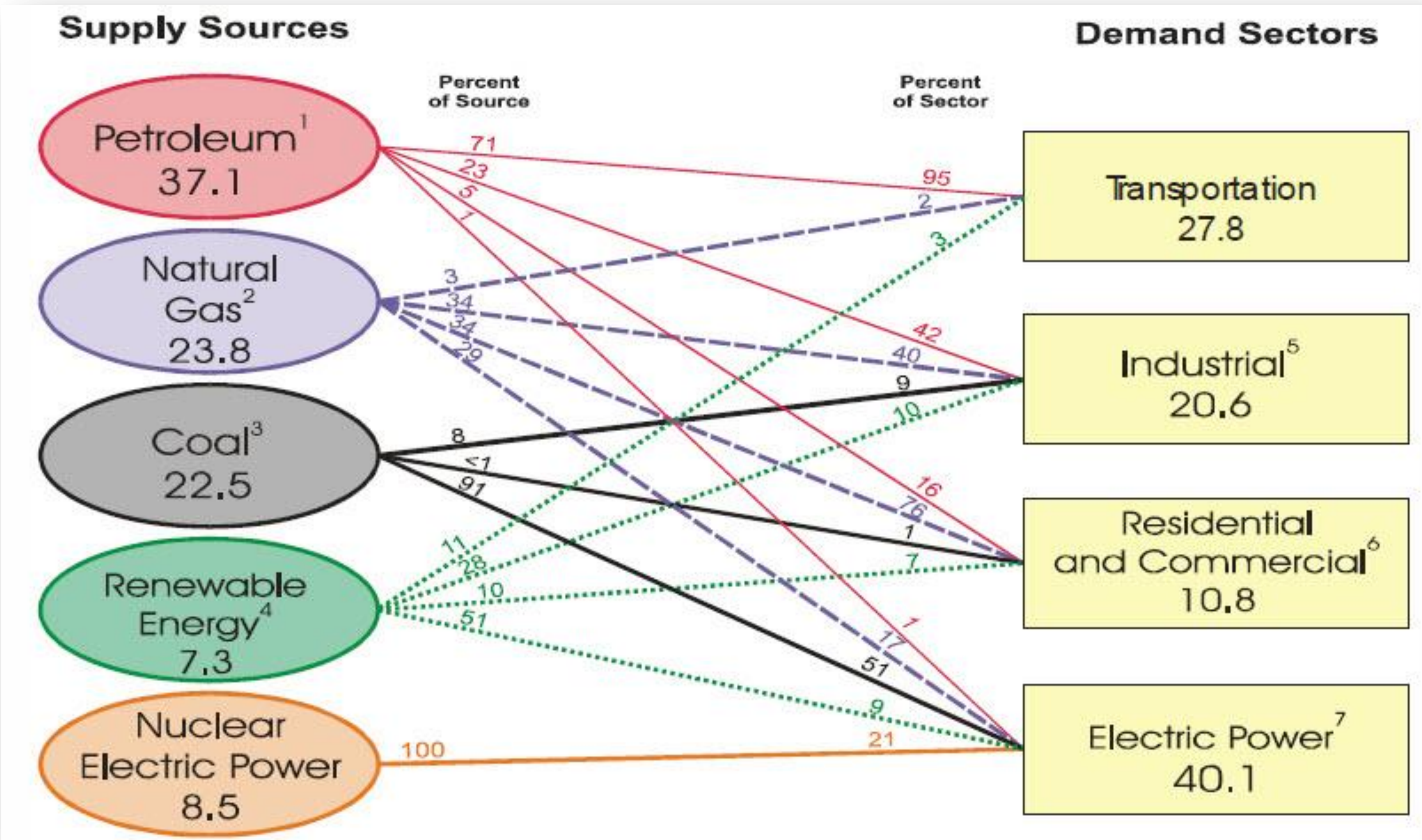
Discussion & Outlook:

Implications, and future challenges

The US consumes 25% of the worlds oil
50% of that oil goes to US autos

Mouawad, J., 2008

US primary energy consumption



Energy Information Administration, 2009

Biofuel defined…

Fuel made from living things or from the waste they produce.

Indispensable petro-commodities

Lubricants

Jet and auto fuels

Algae can provide these and more including:
cosmetics, pharmaceuticals, nutracueticals, bioplastics, more…

Comparison of algae & other feedstocks

Plant source	Seed oil a	Oil yield b	Land use c	Biodiesel productivity d
Corn/Maize (*Zea mays* L.)	44	172	66	152
Hemp (*Cannabis sativa* L.)	33	363	31	321
Soybean (*Glycine max* L.)	18	636	18	562
Jatropha (*Jatropha curcas* L.)	28	741	15	656
Camelina (*Camelina sativa* L.)	42	915	12	809
Canola/Rapeseed (*Brassica napus* L.)	41	974	12	862
Sunflower (*Helianthus annuus* L.)	40	1070	11	946
Castor (*Ricinus communis*)	48	1307	9	1156
Palm oil (*Elaeis guineensis*)	36	5366	2	4747
Microalgae (low oil content)	30	58,700	0.2	51,927
Microalgae (medium oil content)	50	97,800	0.1	86,515
Microalgae (high oil content)	70	136,900	0.1	121,104

Mata, T. M., 2010

a) % oil, dry weight
b) L oil / ha
c) m^2 / kg biodiesel
d) kg biodiesel / ha

(b–d) Annually

Oil content of some microalgae

Microalga	Oil content (% dry wt)
Botryococcus braunii	25–75
Chlorella sp.	28–32
Crypthecodinium cohnii	20
Cylindrotheca sp.	16–37
Dunaliella primolecta	23
Isochrysis sp.	25–33
Phaeodactylum tricornutum	20–30
Schizochytrium sp.	50–77
Tetraselmis sueica	15–23

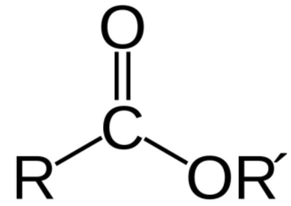
Chisti, Y., 2008

Terminology

Microalgae

"…all unicellular and simple multi-cellular photosynthetic microorganisms, including both prokaryotic microalgae, i.e., Cyanobacteria (Cyanophyceae) and eukaryotic microalgae, e.g., green algae (Chlorophyta) and diatoms (Bacillariophyta)." **Wang, B. 2008.**

GC **G**as **C**hromatography/chromatograph
FID **F**lame **I**onization **D**etection
FAME **F**atty **A**cid **M**ethyl **E**ster
FAEE **F**atty **A**cid **E**thyl **E**ster
TLE **T**otal **L**ipid **E**xtract
TAG **T**ri**a**cyl**g**lycerol



Ester

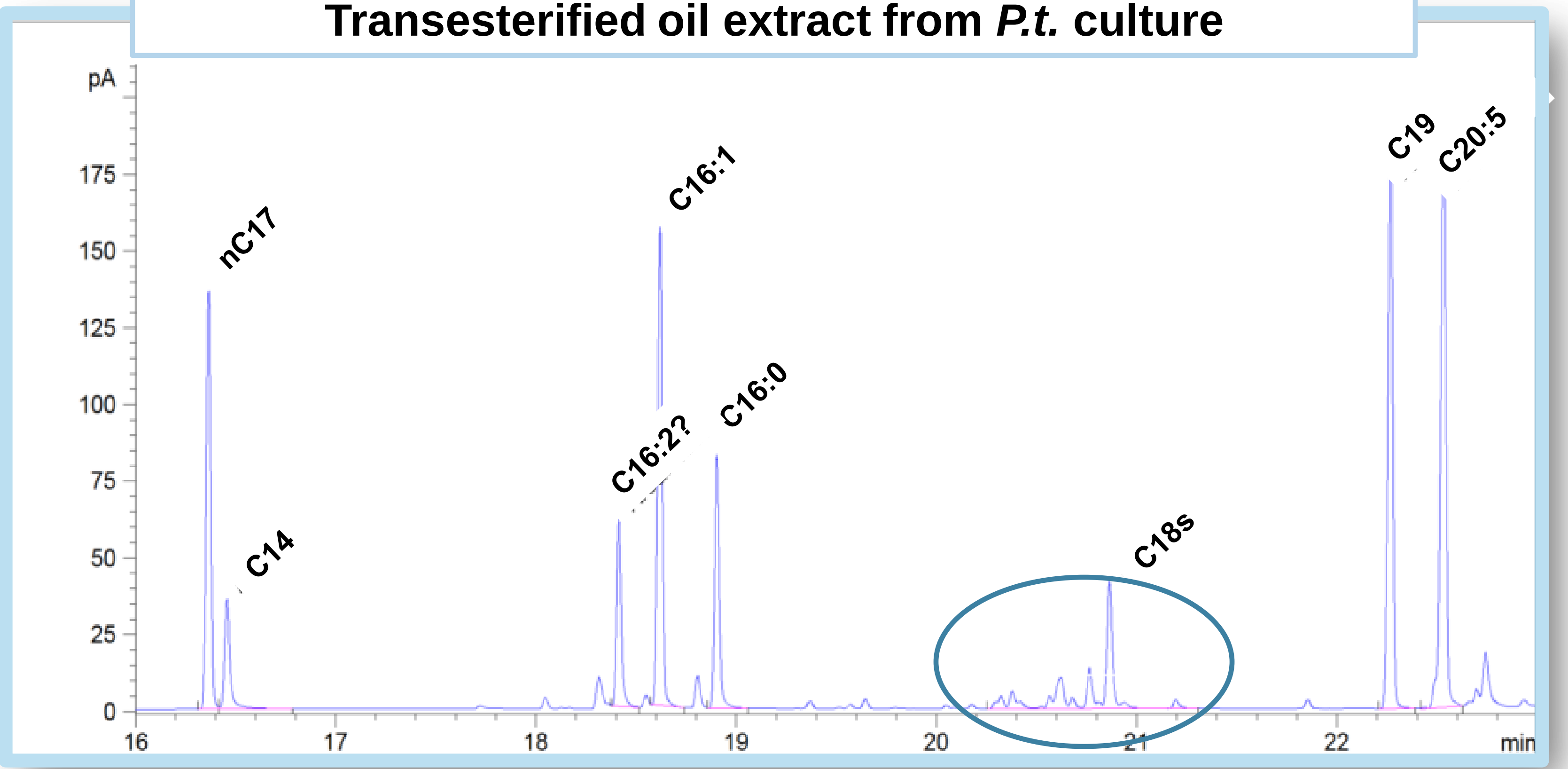
C#$_a$:#$_b$ Alkenone nomenclature (e.g., C20:5)
#$_a$ is **C**arbon-chain length, and #$_b$ is number of double bonds

Transesterification/Transmethylation

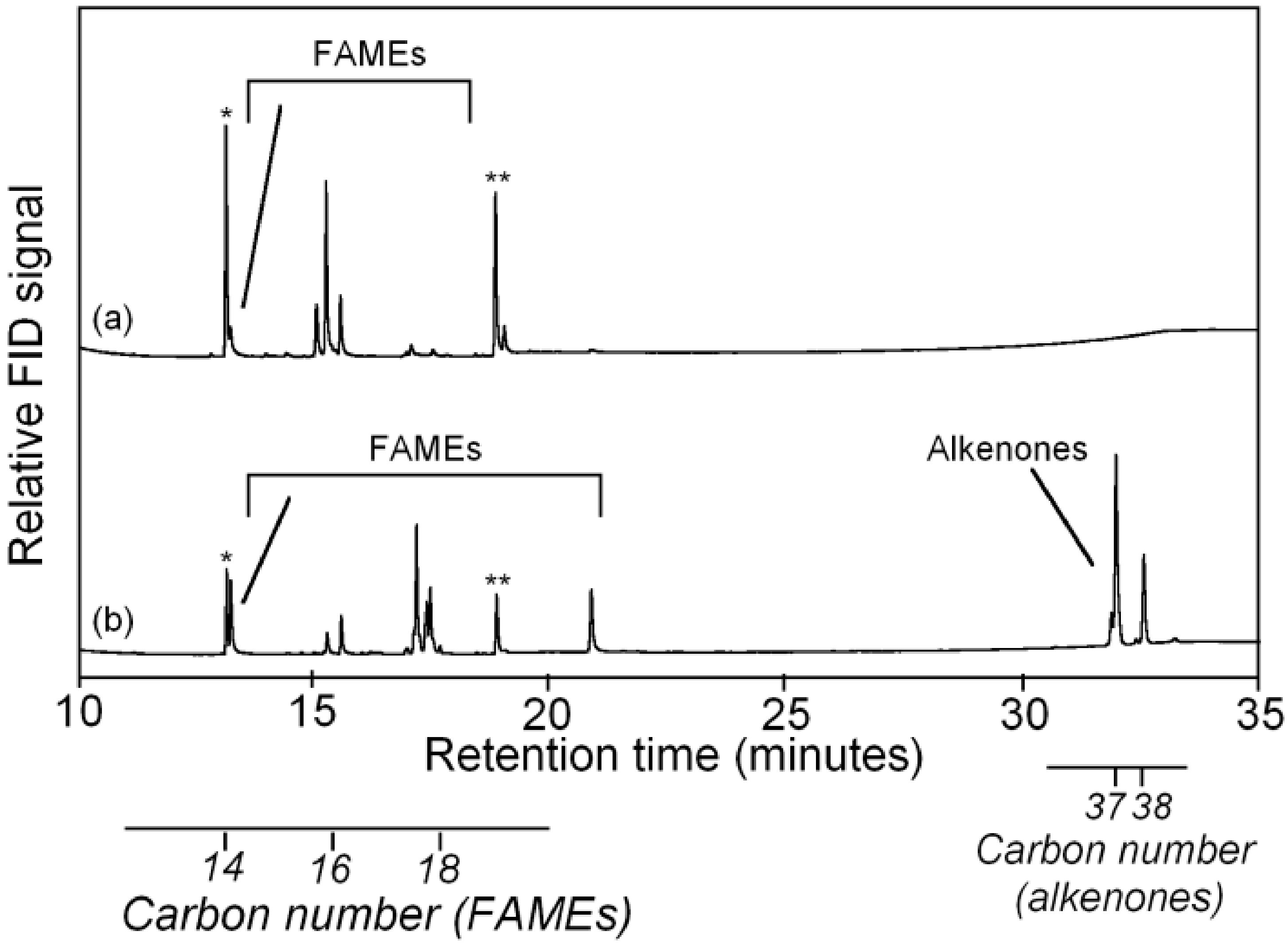
A conversion process for TAGs to make biodiesel

FAME analysis by GC-FID

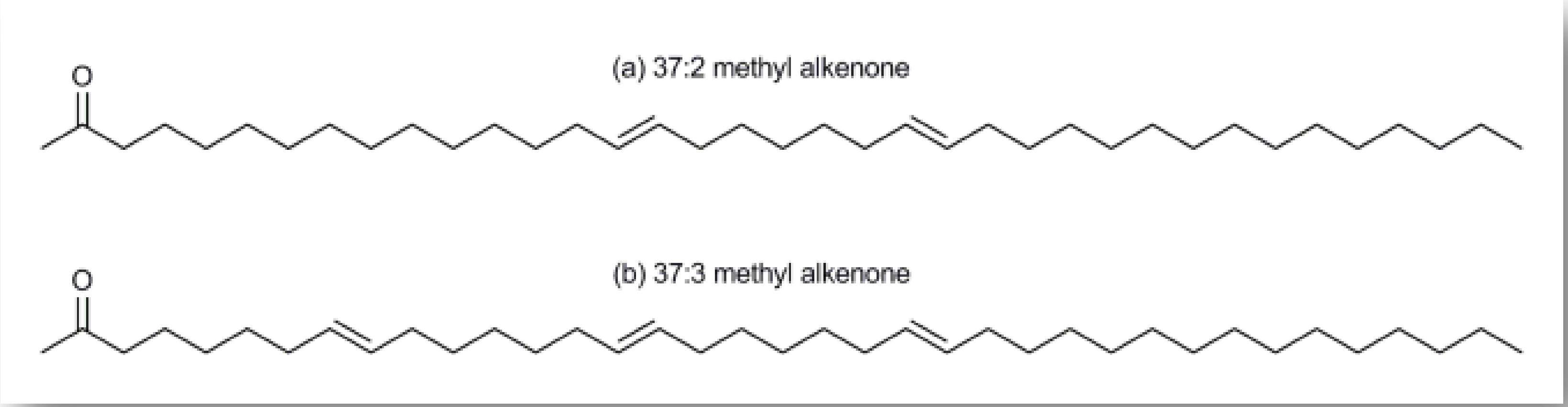
Transesterified oil extract from *P.t.* culture



Alkenones in *T.w.* (a), and *I.* sp. (b)



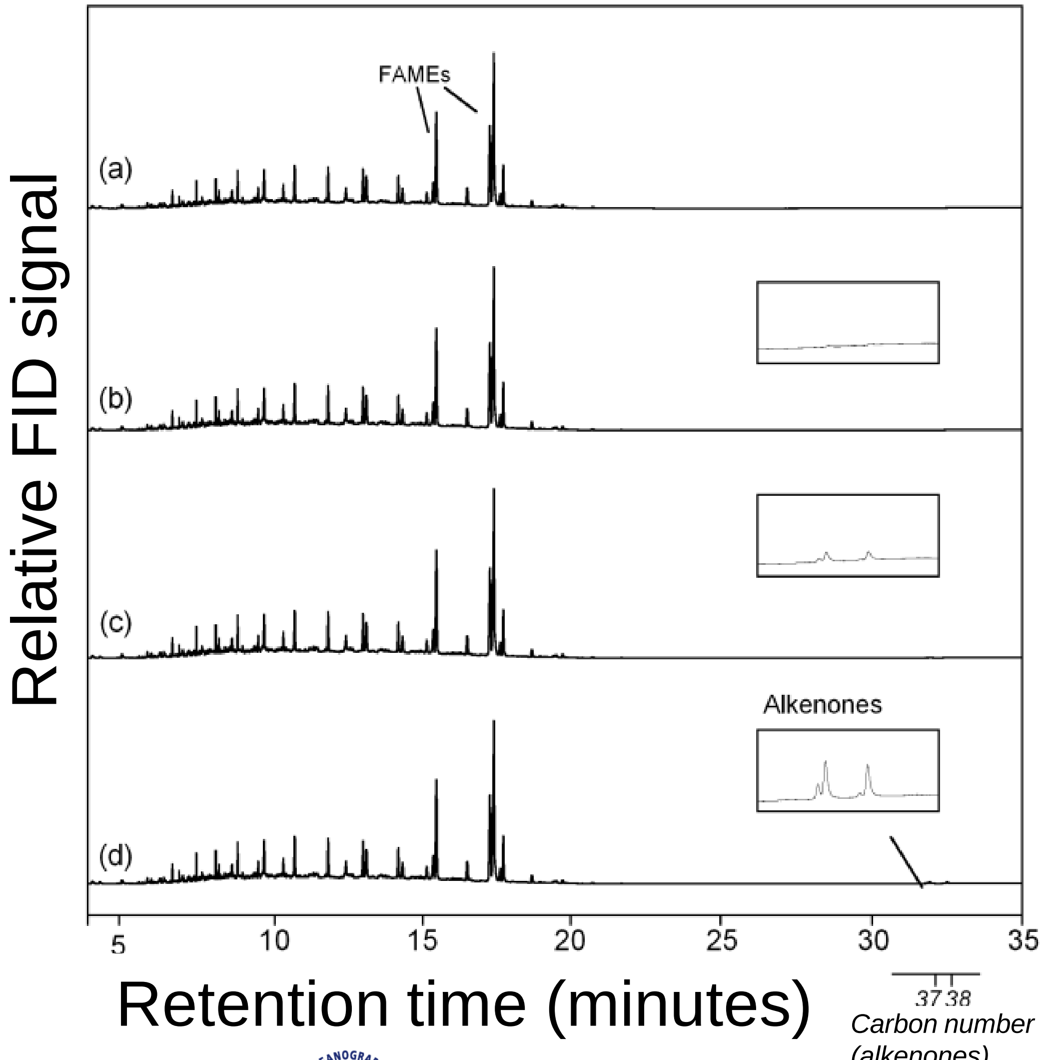
Alkenone structure; implications



- **Trans (E)-double bonds: increased CP.**
  (Vs. Cys (Z)-double bonds: more prone to oxidation, but lower melting points)

- **Long-chain length (C35-40): increased MP.**

B20 Perturbation study (1)



Alkenone content
(parts per thousand, PPT)

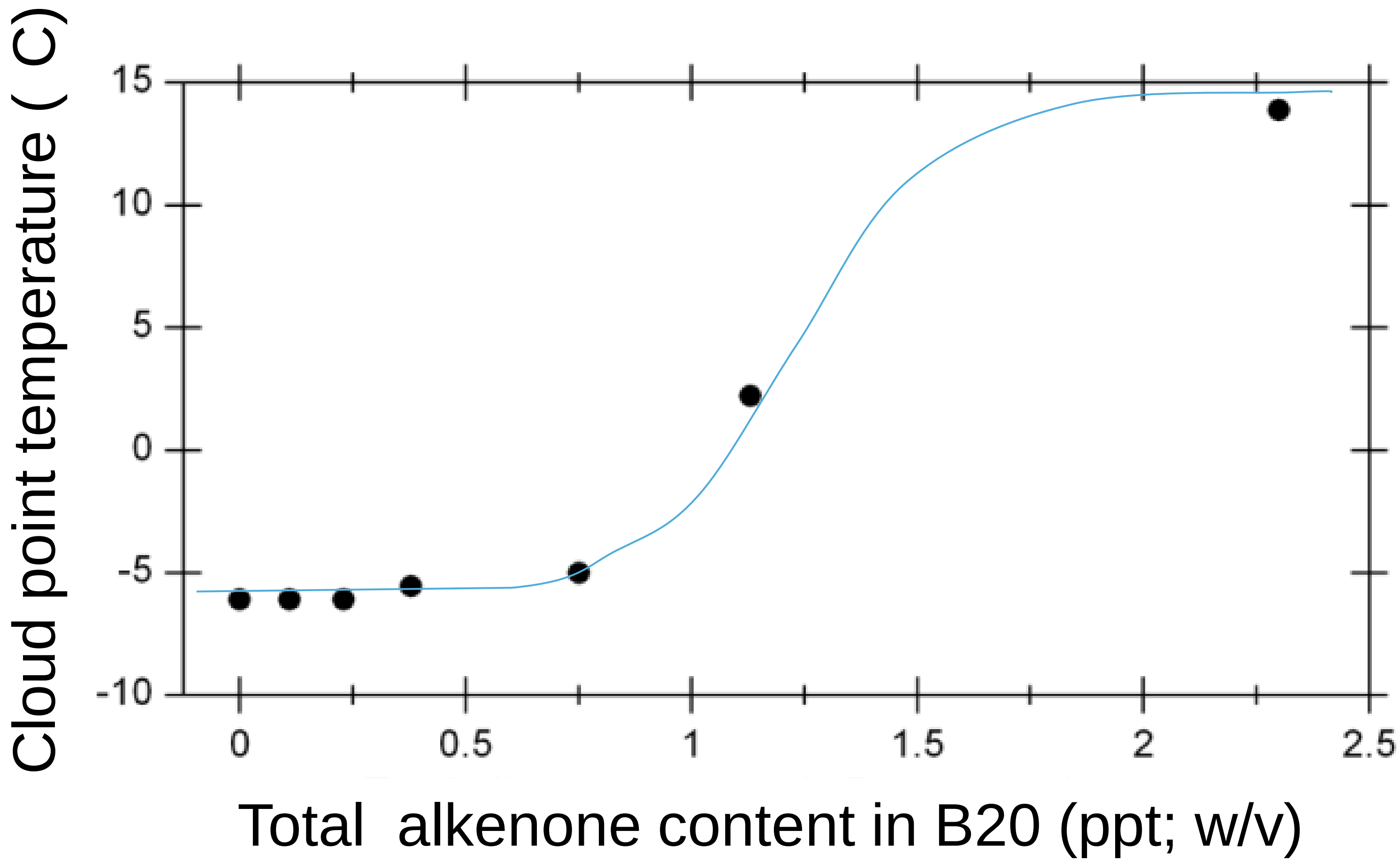
(a) None

(b) 0.11

(c) 0.75

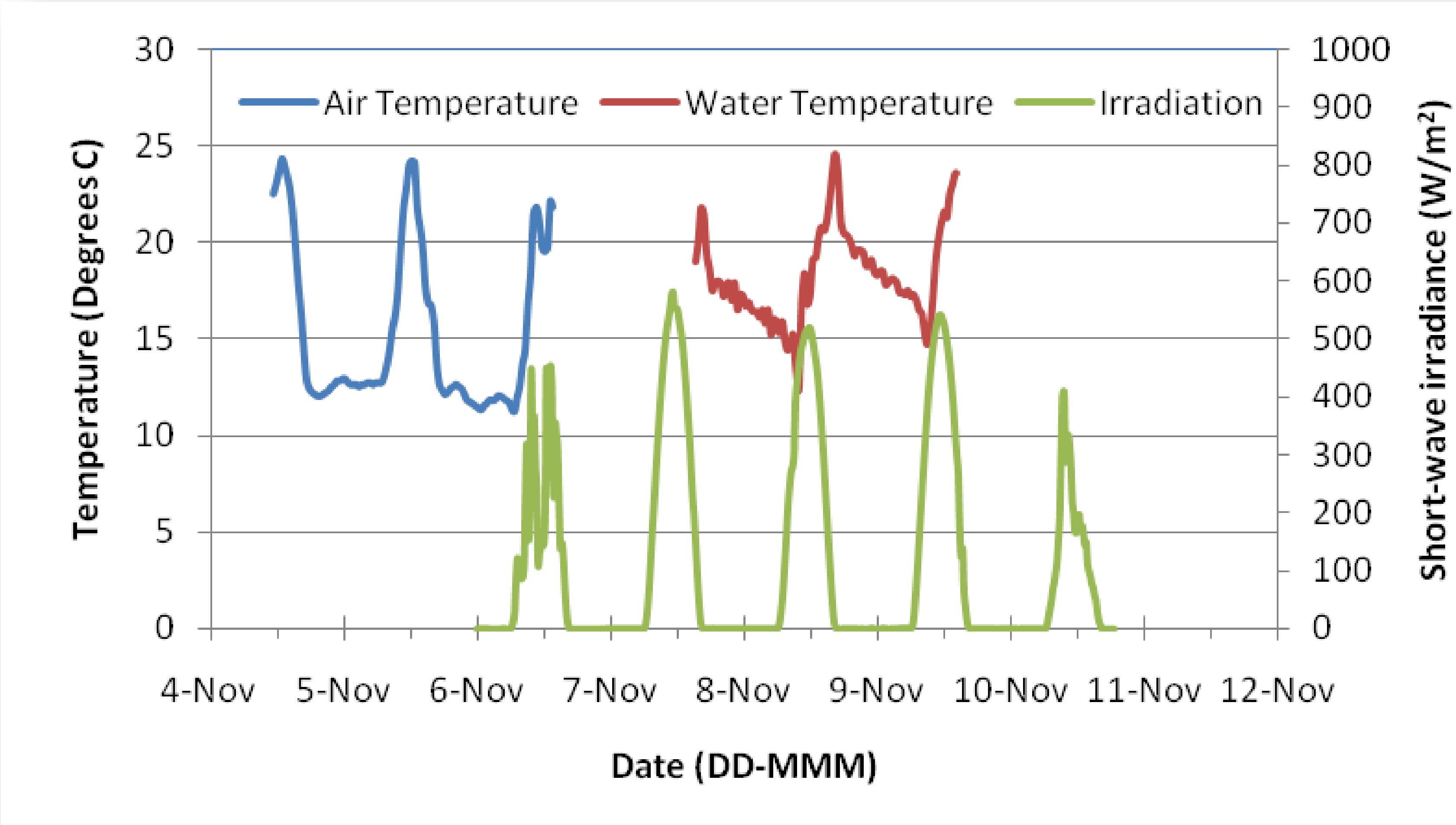
(d) 2.25

B20 Perturbation study (2)



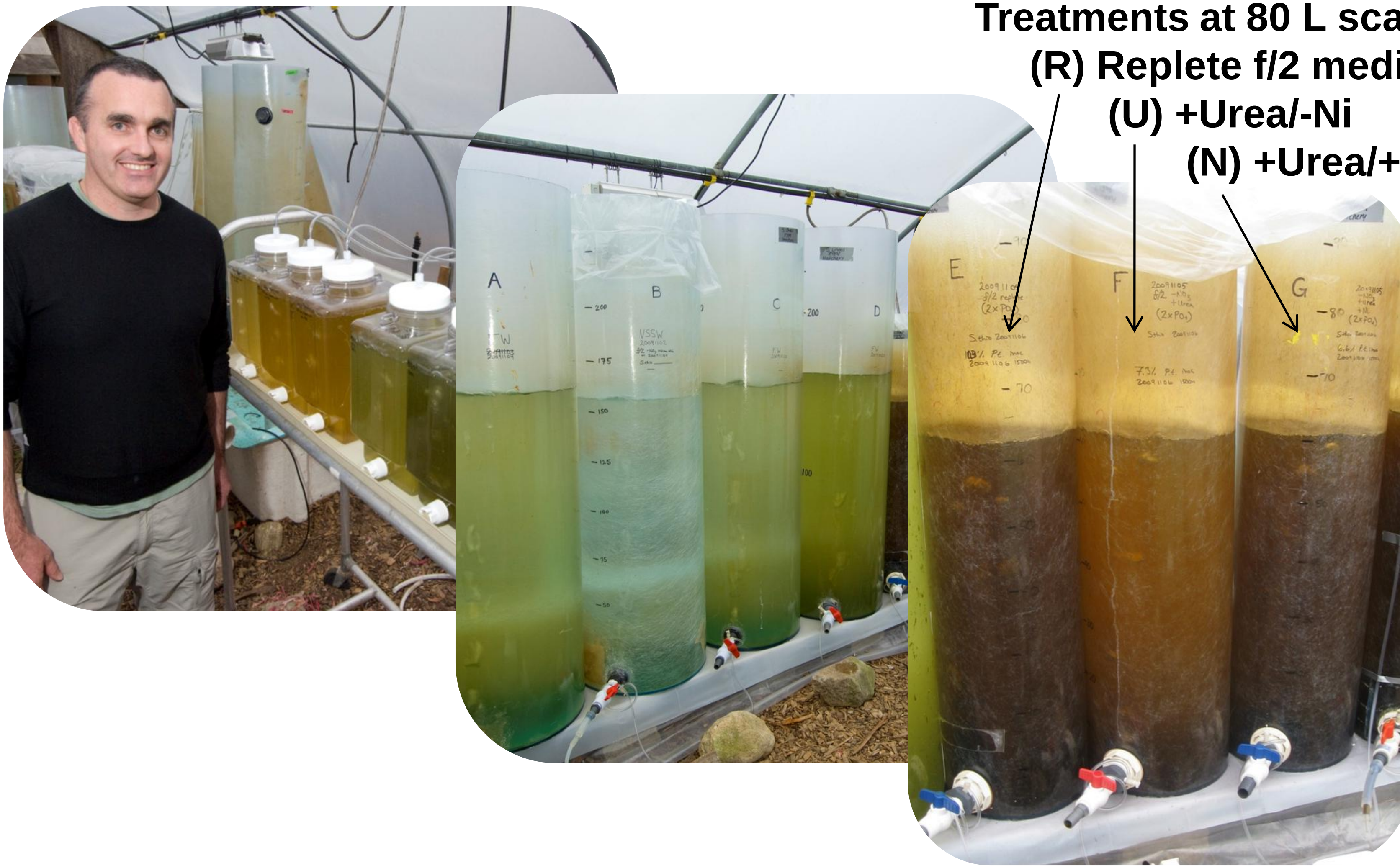
Greenhouse in Woods Hole, MA, USA

Greenhouse climate control

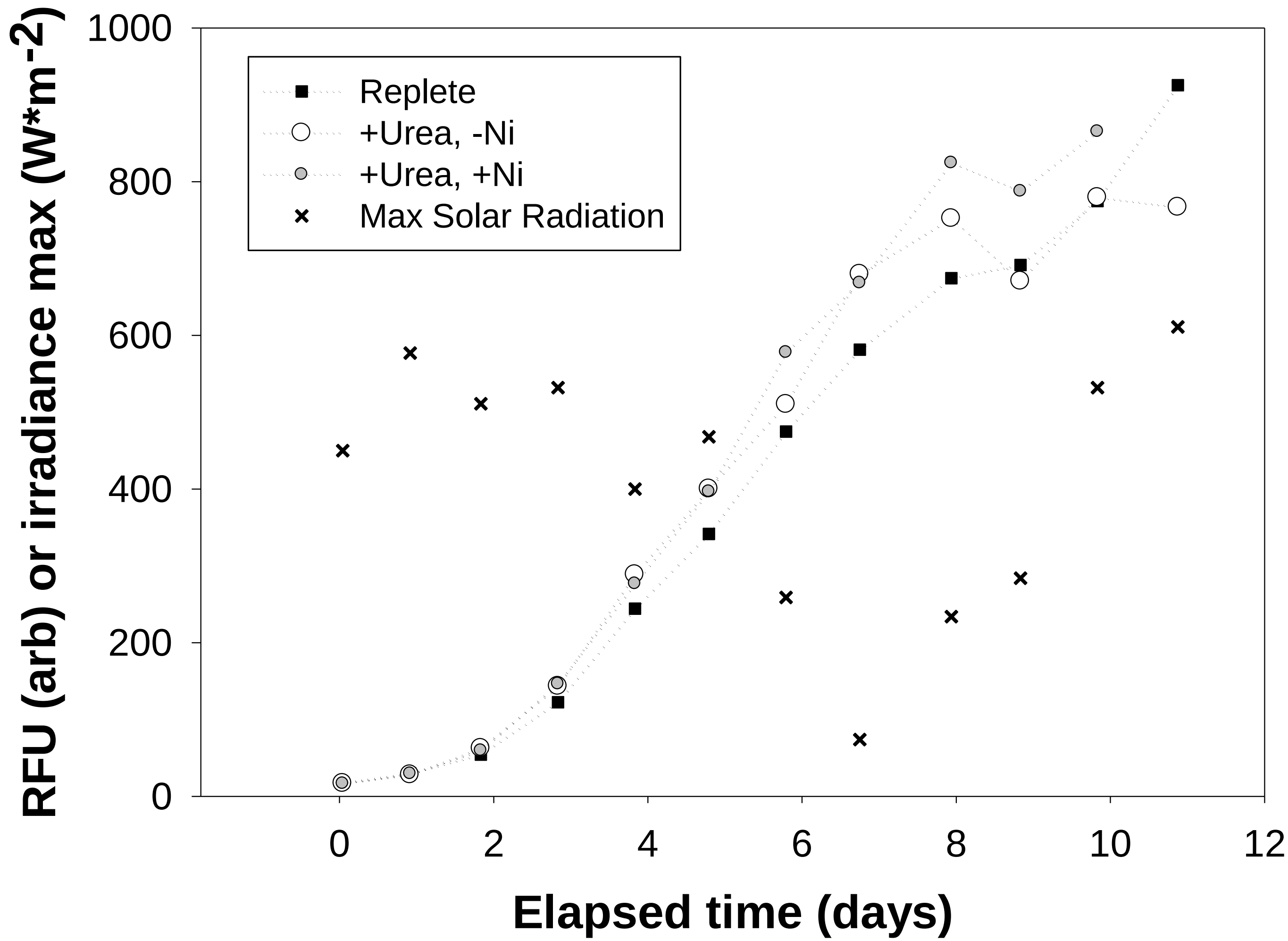


Batch cultures

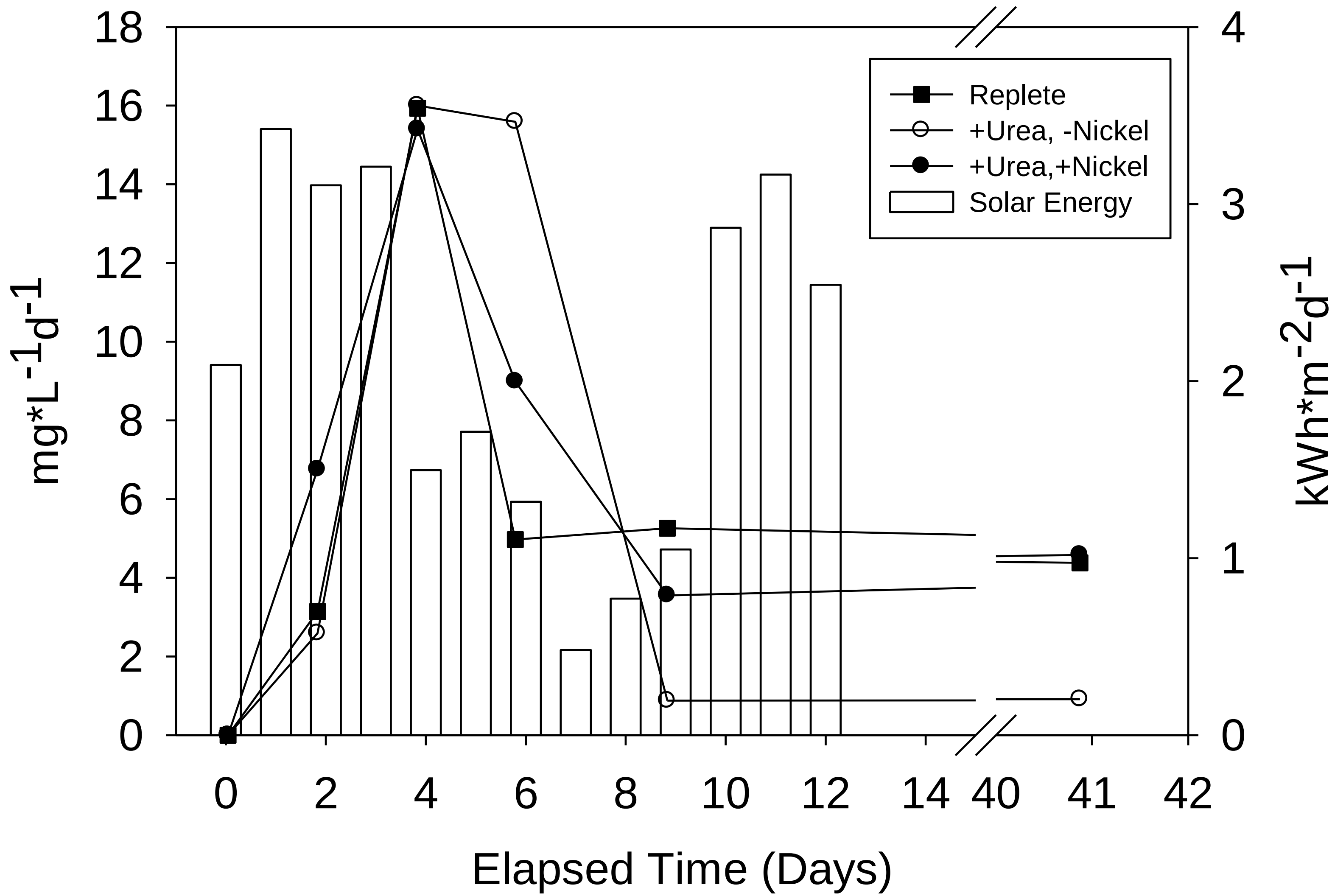
Treatments at 80 L scale:
(R) Replete f/2 media
(U) +Urea/-Ni
(N) +Urea/+Ni



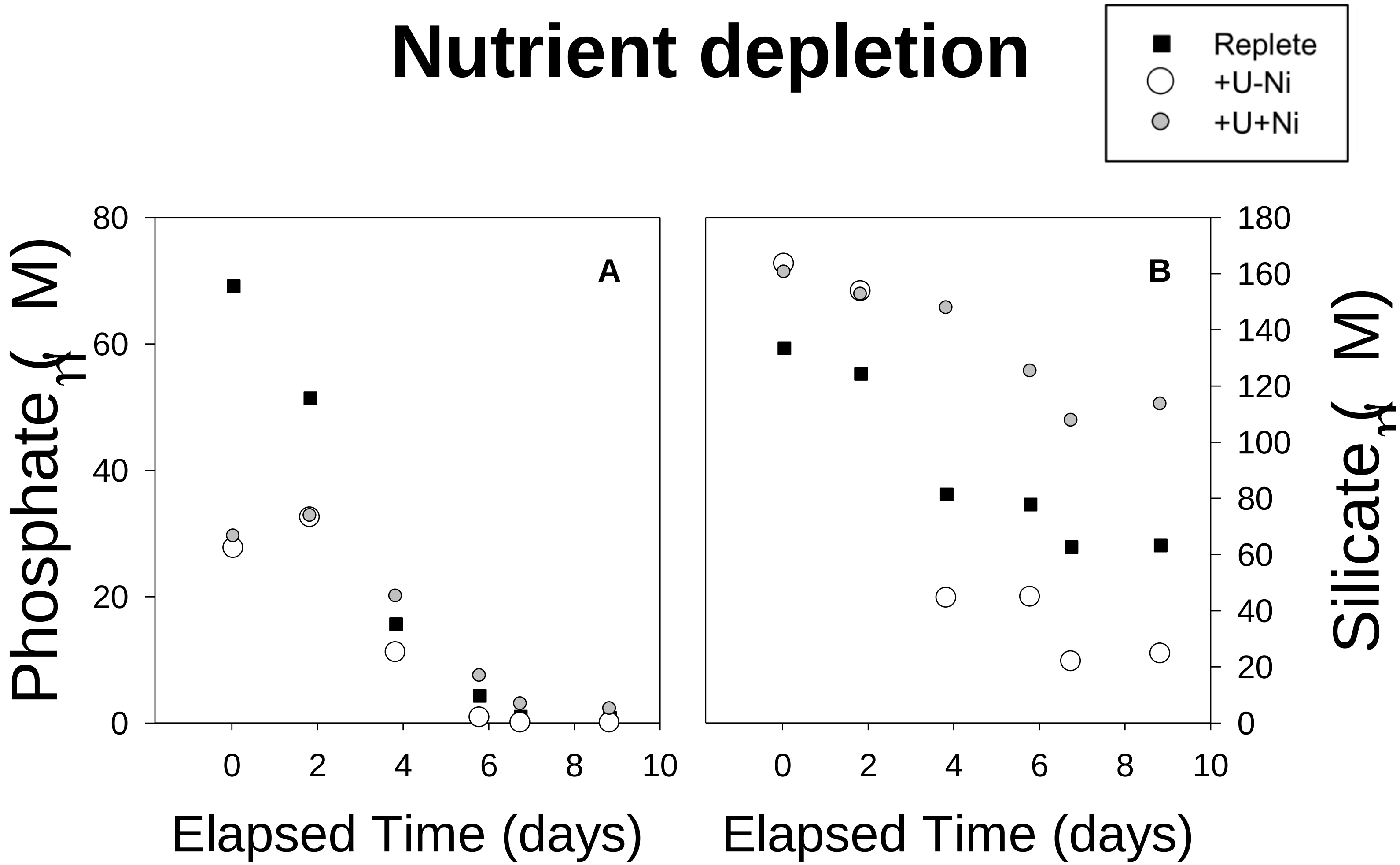
Growth Curves + Solar Data



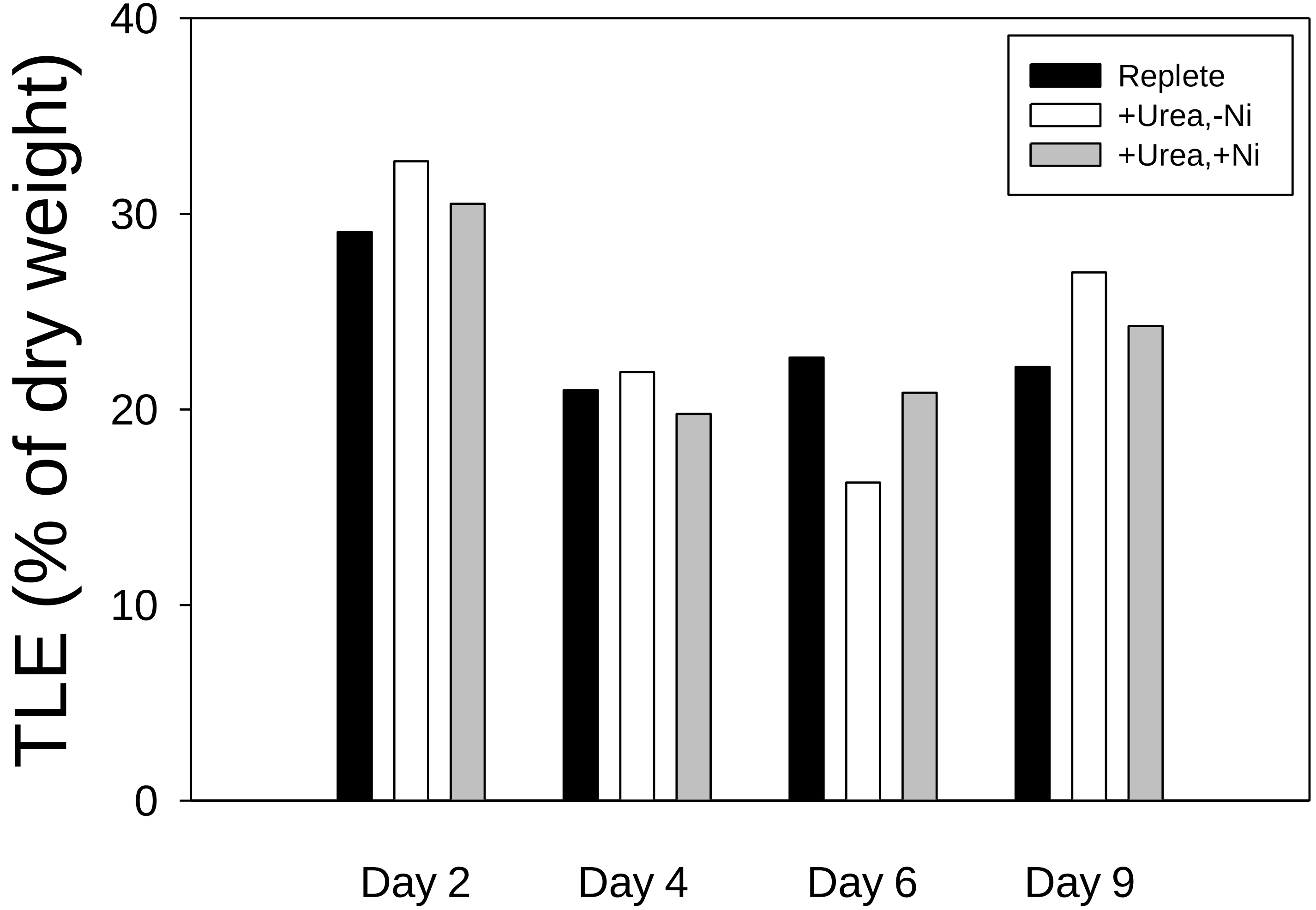
Biomass productivity & solar energy



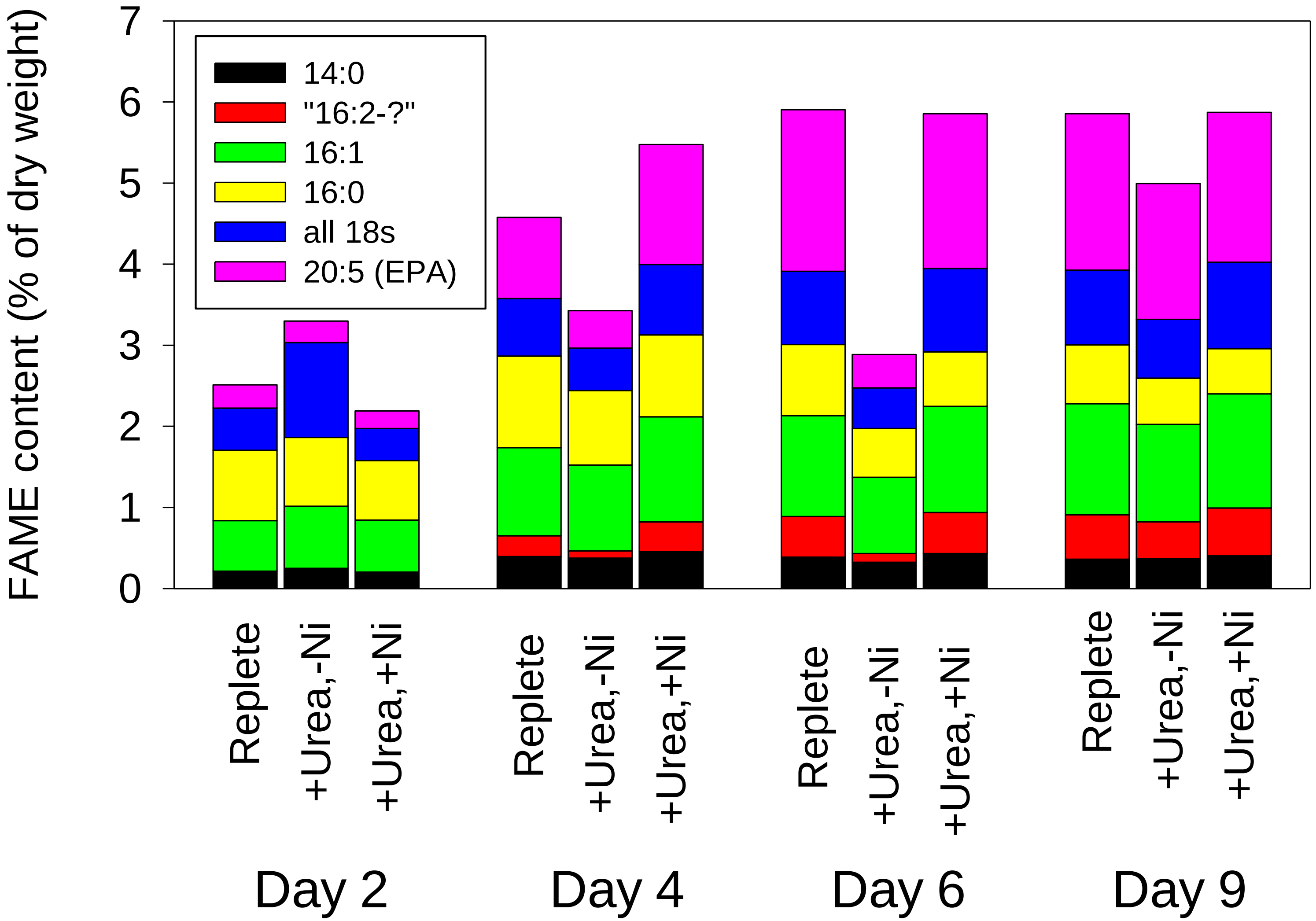
Nutrient depletion



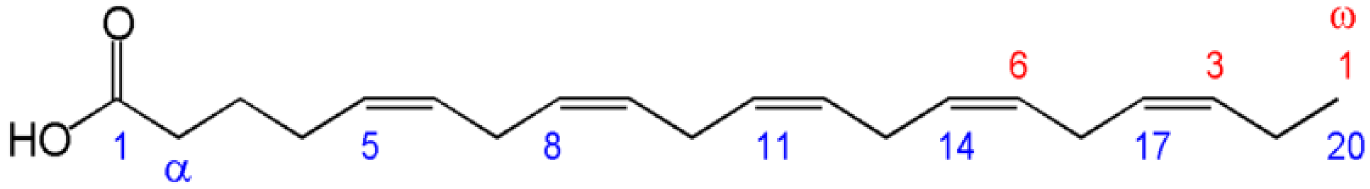
Total lipid extract



FAME distribution



Eicosapentaenoic acid (EPA)



Cis-double bonds (increased susceptibility to oxidation)

So what does this mean for biofuel potential?

Maybe not so good for "biodiesel"…

Could be a "renewable diesel" superstar!
(perhaps hydrocracking?)

Pharma/nutracceuticals, EPA & DHA TGs better than EEs

Points of interest from study II

Transition to neutral lipid domination was not observed.

Perhaps due to light limitation or incomplete nitrogen depletion.
Urea may function as a good N reservoir with *P.t.*'s urease
…future proteomic assessments may answer this more conclusively.

Trends in TLE are absent, however for FAMEs, the urea treatment (sans nickel) appears suppressed.

A feature also noted in biomass productivity (post stationary)

EPA is most prevalent compound following day 2.

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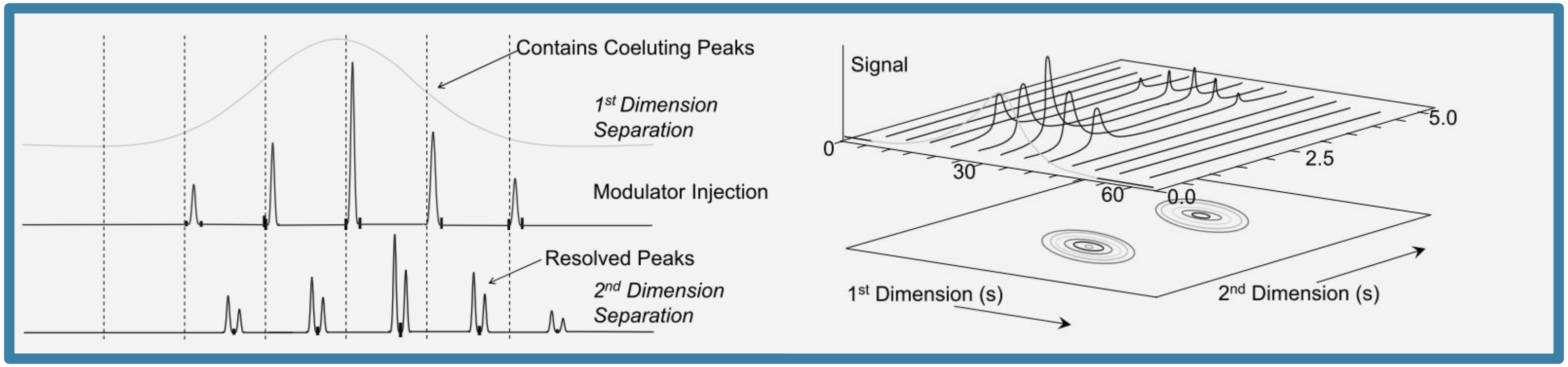
PPRE

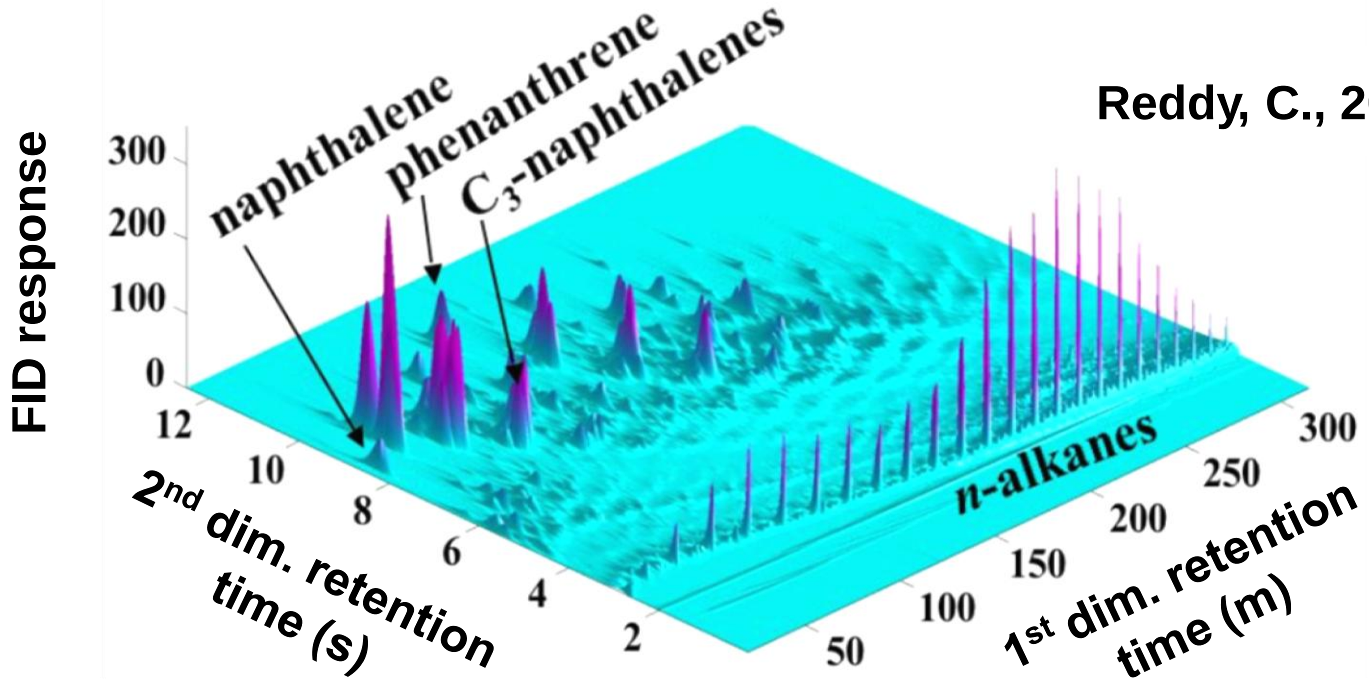
WOODS HOLE OCEANOGRAPHIC INSTITUTION 1930

Massachusetts Institute of Technology

Harvest and Extraction

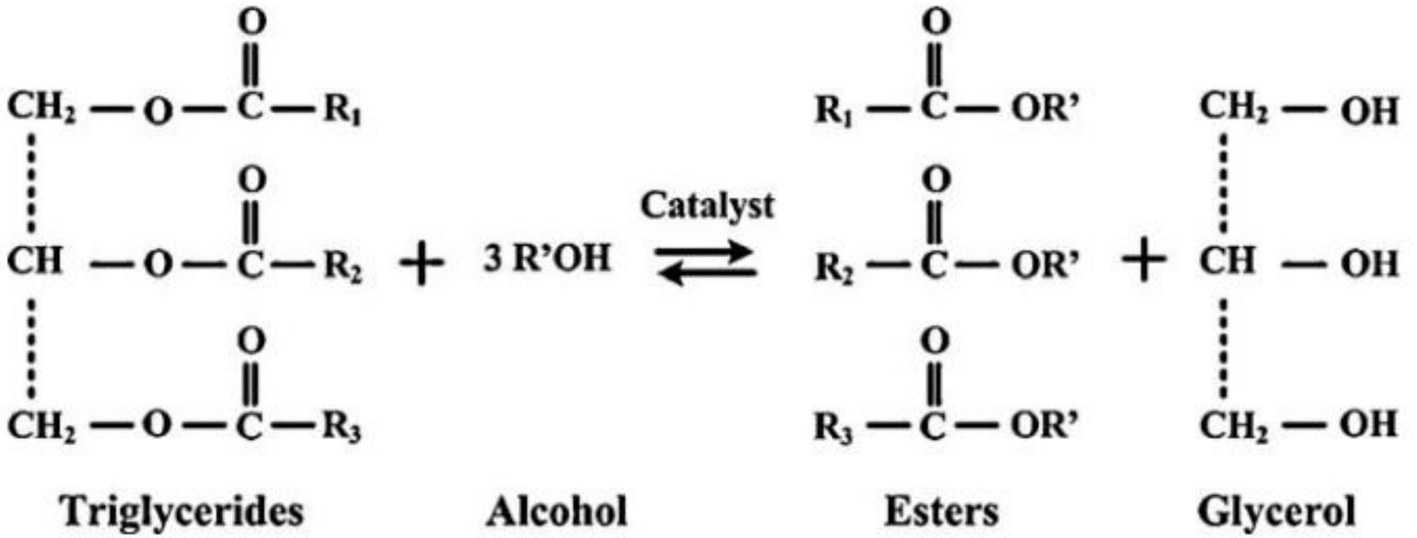
Comprehensive 2-D GC (GC x GC)





Reddy, C., 2009

Transesterification



Transesterification of triglycerides (overall reaction).

Mata, T. M., 2010

Looking ahead…

Interests for future research include:

Need to identify the putative "18:2" FAME.

Either a simple catalyzed hydrogenation or 2D-GC investigation

Chemostat culturing
Nutrient cycling (esp. nitrogen)
Hydrocracking and GCxGC characterization
Other lipid assays, intact lipids, TAGs, nile red staining
Identifying still unknown compounds and potential uses
Colonial mat-forming cultures (novel antarctic cyanobacteria)
Alternative extraction methods especially pulsed electric field (PEF)

Analyze nickel, cytometry, and proteomic samples

(develop heat maps for Urease and Acyl-CoA)

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*** Note:** A more extensive bibliography and literature review to the subject is available in the written thesis, for copies, please email your request to tyler.goepfert@uni-oldenburg.de.