Investigating oil spill response products Corexit 9500 and Oil Spill Eater II: composition, fate, and effects on oil biodegradation in Alaskan waters

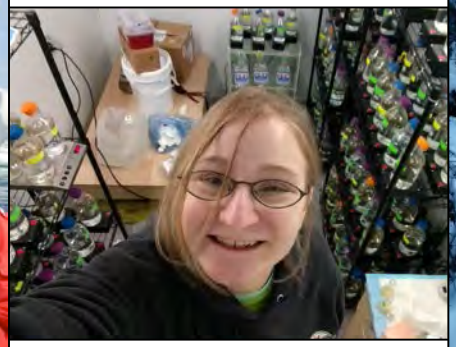
Mary Beth Leigh, Kelly McFarlin, Taylor R. Gofstein, & Ursel Schuette
(University of Alaska Fairbanks)

Matthew Perkins & Jennifer Field
(Oregon State University)

Research Team

Mary Beth Leigh (PI, Associate Professor)
with Vernon Brower (UIC Science boat captain)

Kelly McFarlin (former Ph.D. student)



Taylor Gofstein (Ph.D. student)

Ursel Schütte (Co-PI, Research Scientist)

Surfactant chemists
Oregon State University
- Jennifer Field (Prof.)
- Matthew Perkins (Ph.D. student)

Duke University
- Lee Ferguson (Prof.)
- Sarah Choyke (Ph.D. student)

Additional UAF personnel
- Eric Henderson (Lab technician)
- Alexis Walker (Ph.D. student)
- Kyle Dilliplaine (DNA Core Lab technician)
- Ben Hedges (B.S. student)
- Serena Fraraccio (postdoc)
- Kirsten Veech (B.S. student)

and **UIC Science personnel**, Utqiagvik

COASTAL MARINE INSTITUTE

PRINCE WILLIAM SOUND
OIL SPILL RECOVERY INSTITUTE

Alaska
INBRE
IDeA Network of
Biomedical Research Excellence

BOEM
Bureau of Ocean Energy Management

NORTH SLOPE SPILL RESPONSE
acs
alaska clean seas
A WORLD CLASS ORGANIZATION

Statoil

ConocoPhillips

ExxonMobil

Funding

Alaska
DEC

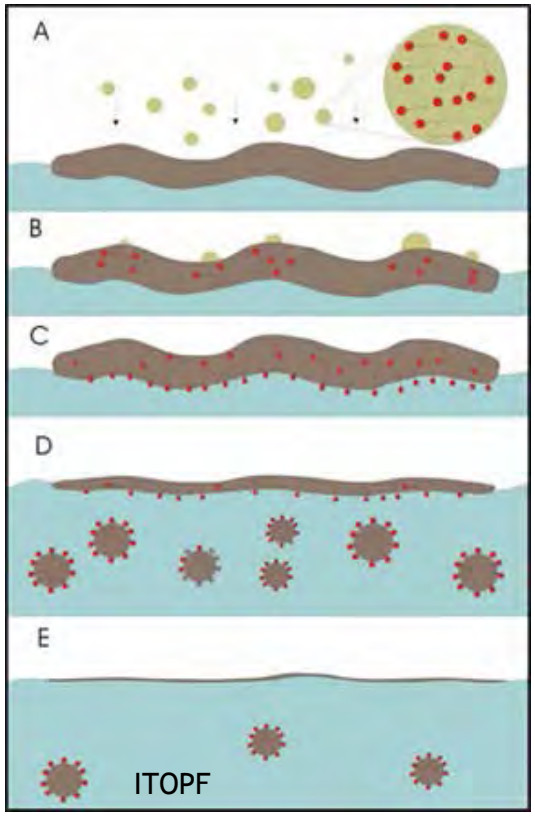
OSEI
OIL SPILL EATER INTERNATIONAL, CORP

Technical advice

Major goals: Oil and Corexit Studies in Arctic Seawater

- Quantify biodegradation in Arctic seawater of:
  - Crude oil
  - Chemical dispersant (Corexit 9500A's nonionic and anionic surfactants)
- Investigate if/how Corexit affects oil biodegradation
- Identify microbial taxa and genes implicated in oil and dispersant biodegradation

Chemical Dispersants (e.g., Corexit EC9500A)

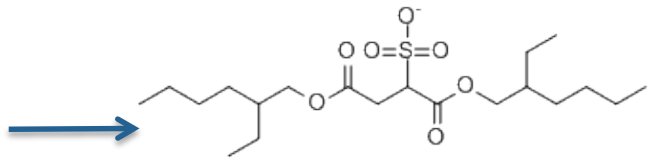


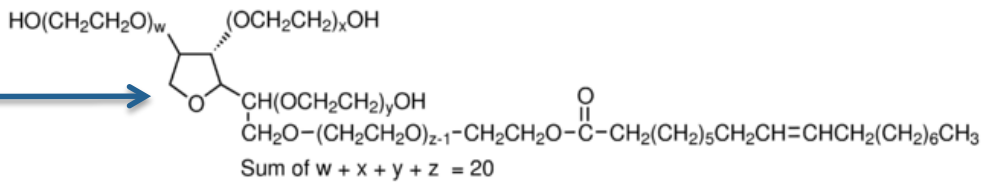
- Surfactant mixtures that send surface oil slicks into water column
- Increase bioavailability of oil by increasing surface area (Beal and Betts, 2000; Rosenberg, 1993, Prince & Butler, 2014)
- On NCP and eligible for use in Alaskan waters and elsewhere
- Little known about their environmental fate, especially in the Arctic (CRRC)

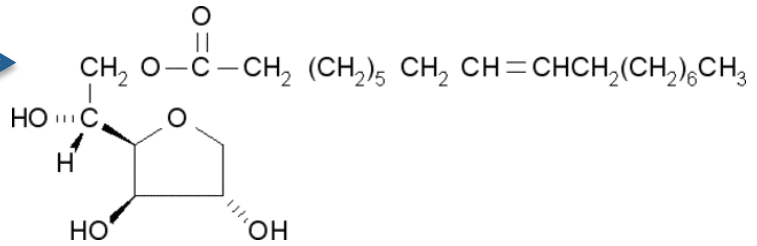
Composition of Corexit 9500A?

Surfactant components

- Anionic surfactant
  - 18% (w/w) dioctylsulfosuccinate (DOSS)
- Non-ionic surfactants
  - 18 % (w/w) Tween 80
  - 4.6% (w/w) Tween 85
  - 4.4% (w/w) Span 80







Other components

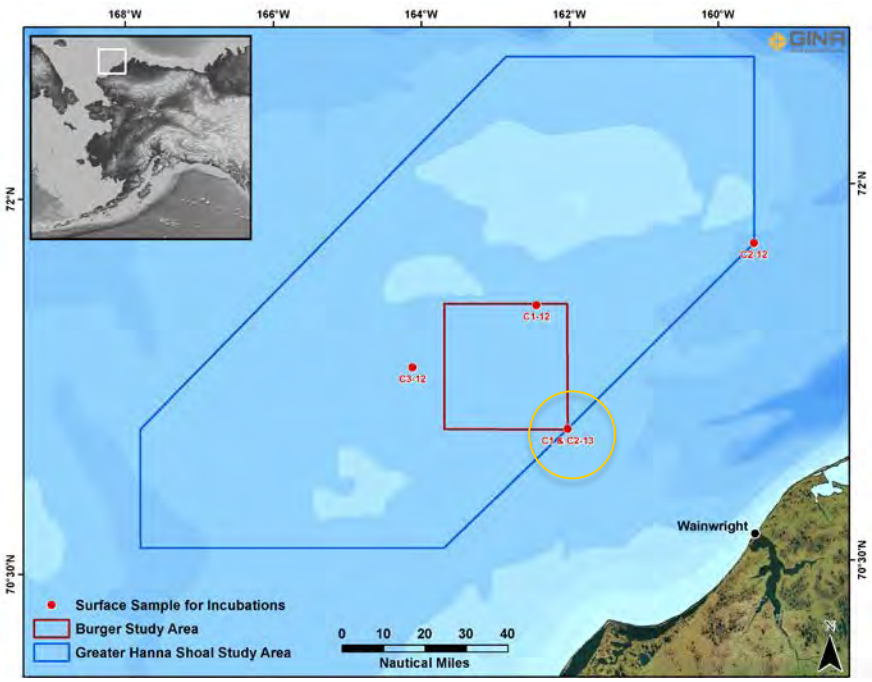
- Dipropylene glycol butyl ether
- 2-butoxy-ethanol
- Petroleum distillates
- and others

Place et al., 2016, Parker 2014, U.S. EPA

Surface Seawater Collection

Offshore

- Burger Study area, 90 km offshore of Wainwright
- 2013 – September & October



Offshore water collected by Chukchi Sea Environmental Studies Program

Ph.D. student Kelly McFarlin

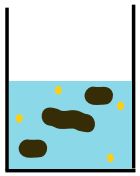
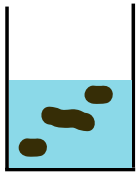
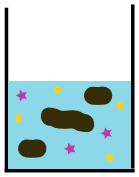
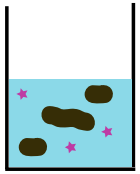
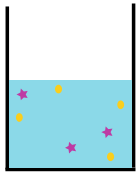
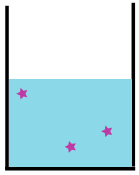
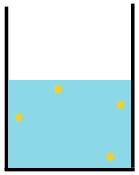
Near-shore

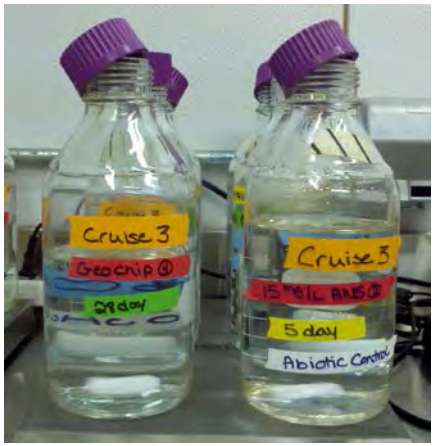
- 1 km offshore of Utqiagvik
- 2011 - February
- 2014 & 2016 – August

Ph.D. student Taylor Gofstein

Mary Beth Leigh
Vernon Brower (Boat Captain)

Biodegradation Incubation Experiments

	Live		Sterile (abiotic control)
I		Oil only	
II		Oil + Corexit (chemically dispersed oil plume)	
III		Corexit only	
V		Unamended (biotic control)	



800 mL or 6 L seawater stirred at *in situ* temperature

Oil concentration 2.5-50 ppm (depending on experiment)

Incubation time points (n = 3):
0, 5, 10, and 28 days

800-ml destructively harvested incubations

+

6-L repeatedly sampled incubations for surfactant analyses

Sarah Choyke, Ph.D. student, Duke University

Taylor Gofstein, Ph.D. student, UAF

Oil Loss over time (biodegradation + abiotic loss) Lower in Arctic than Temperate Seawater

Location		Citation	Oil mg/L	Temp	Day 10	Day 28
Arctic	near-shore	McFarlin et al., 2014	2.5	-1˚C	36%	45%
New Jersey	near-shore	Prince et al., 2013	2.5	8˚C	51%	69%

Corexit 9500A did not inhibit oil biodegradation

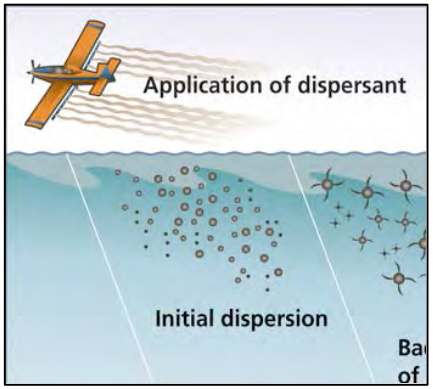
Location		Citation	Temp	Oil mg/L	Corexit mg/L	Day 10	Day 28
Arctic	near-shore	McFarlin et al., 2014	-1˚C	2.5	0	36%	45%
					0.167	47%*	54%*

DOR 1:15

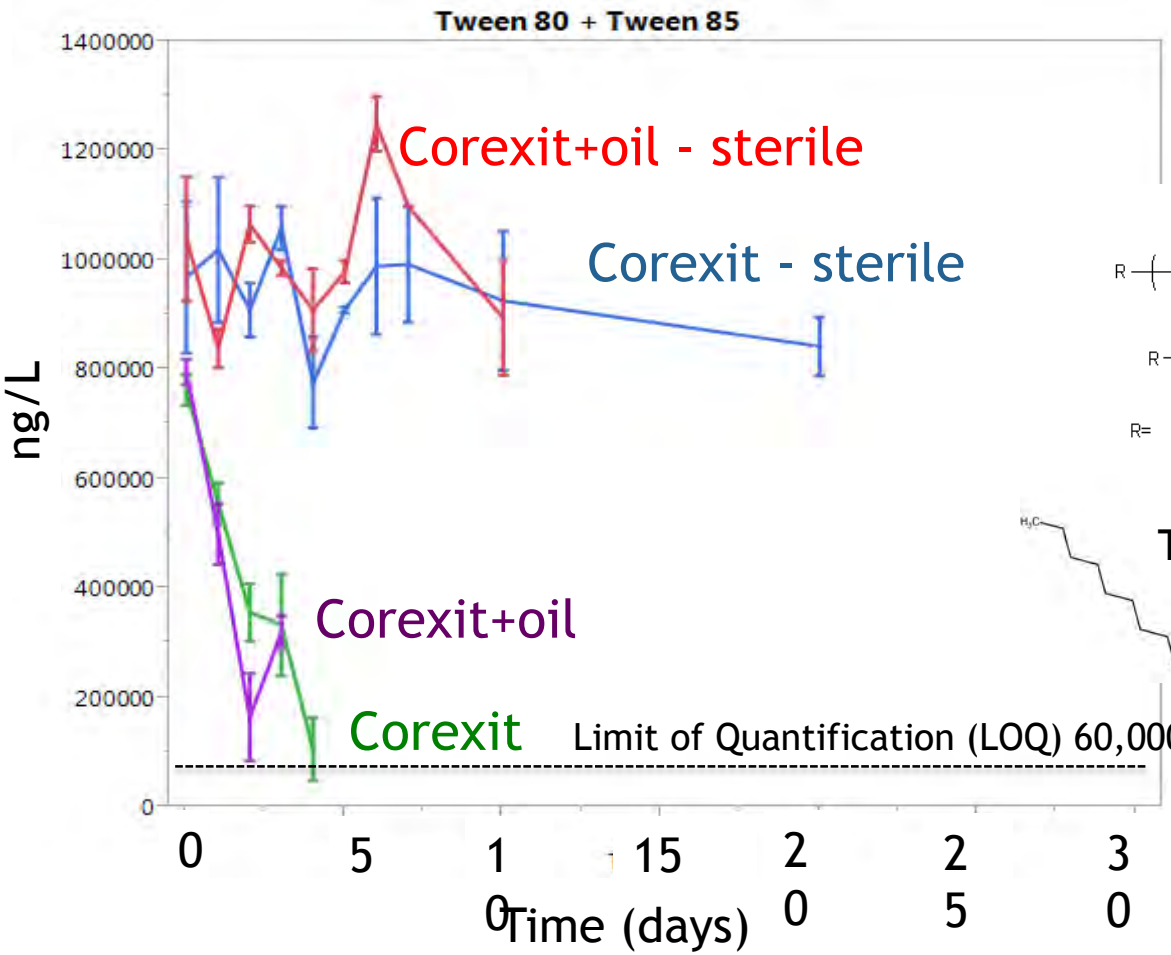
(McFarlin *et al.*, 2014)

Oil loss higher with Corexit at 10 and 28 days (but similar at 60 d)

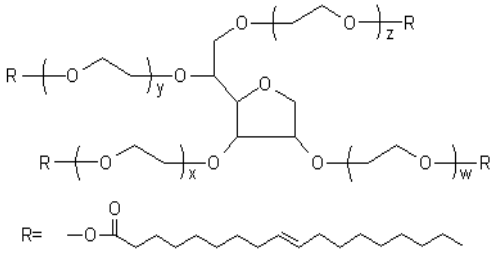
This experiment used a low oil concentration to simulate in situ levels after Corexit application: (**2-12 mg/L** within 60 minutes 0.75 m, 10 m downstream) (Li et al., 2009)



Biodegradation of Tween 80+85 (Corexit 9500A components) in Arctic seawater nearshore, August 2016 – 6-L incubations – 50 ppm oil 1:10 DOR



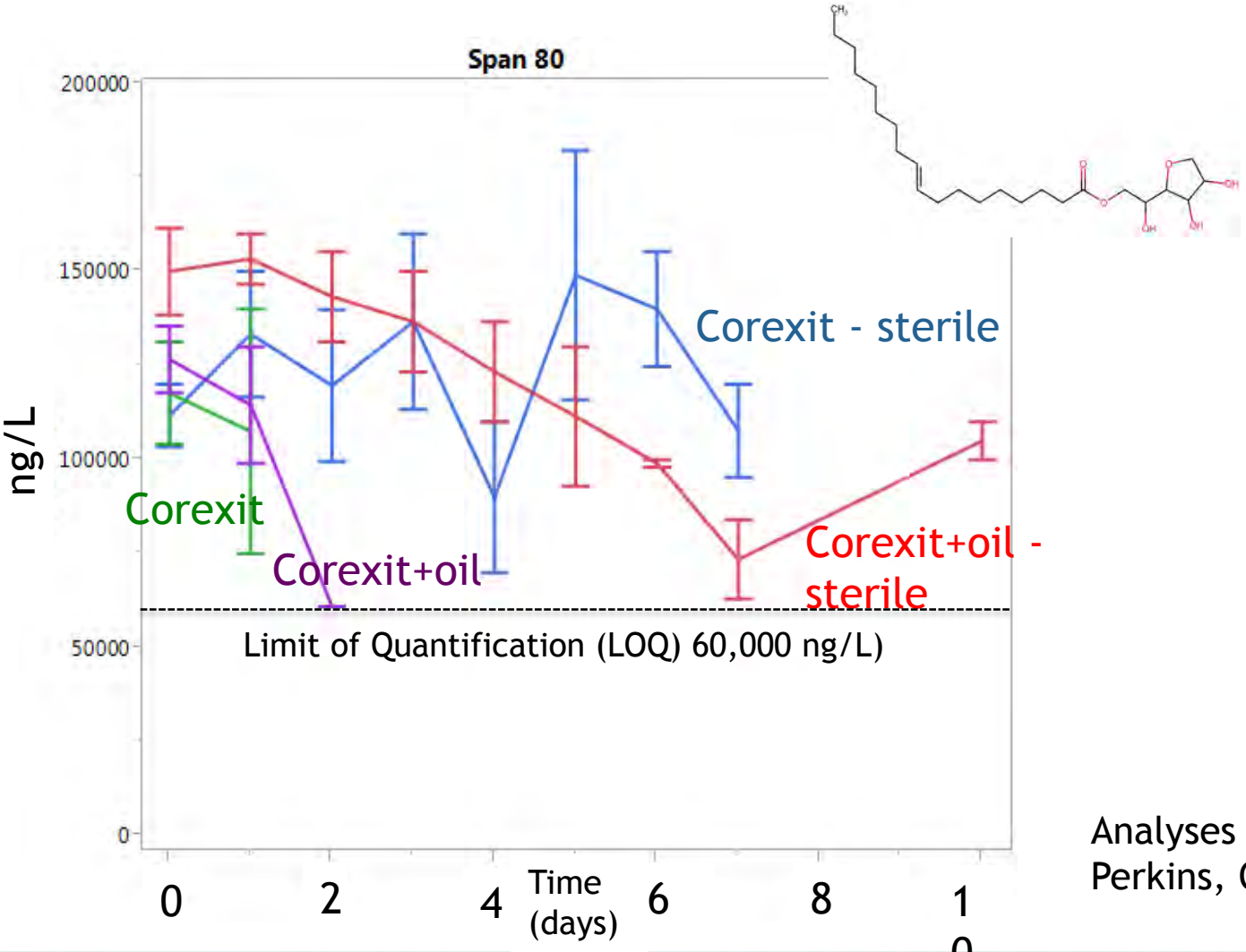
Tween 80



Tween 85

Analyses by Matt Perkins, OSU

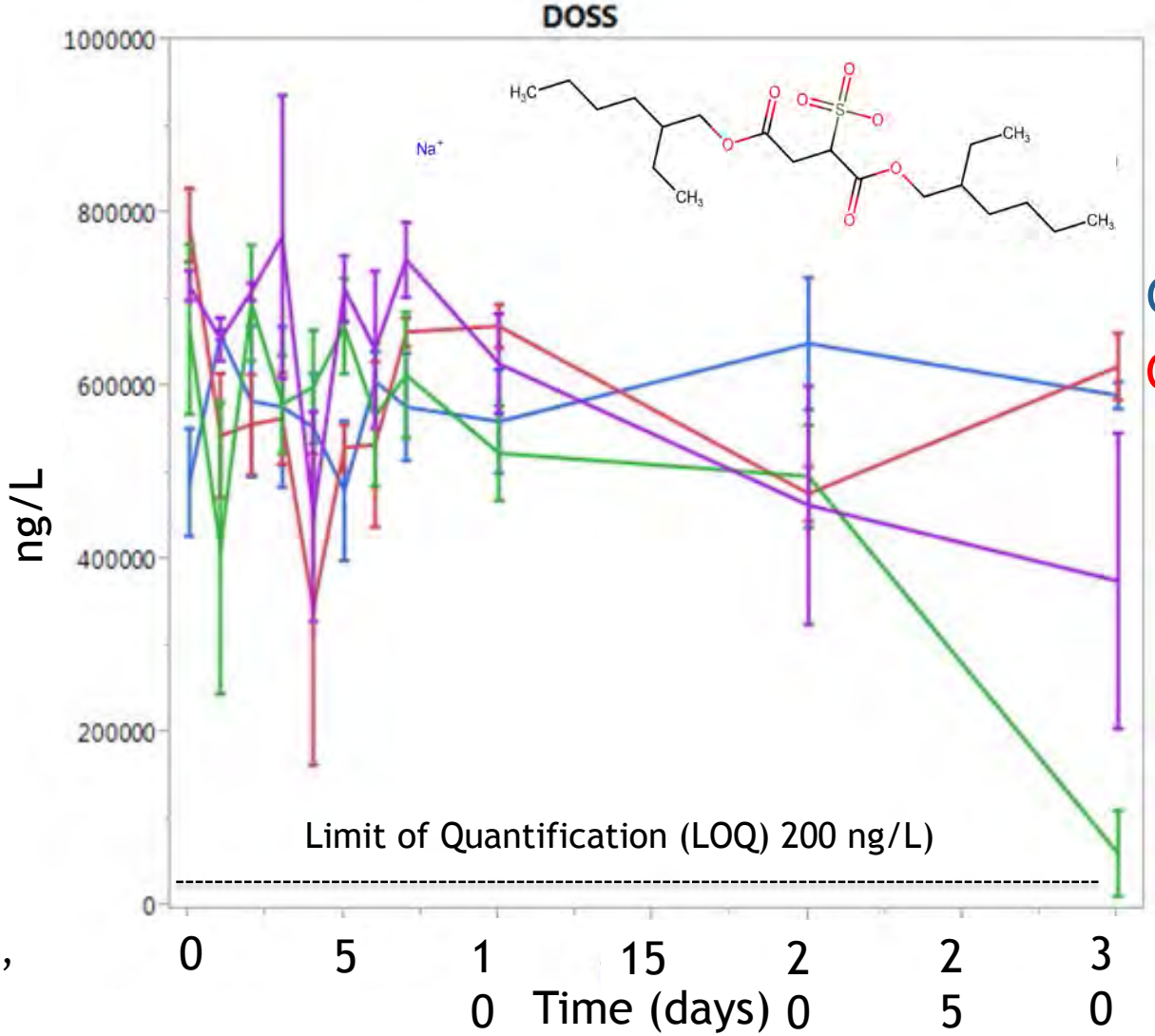
Biodegradation of Span 80 (Corexit 9500A component) in Arctic seawater
August 2016 – 6-L incubations – 50 ppm oil 1:10 DOR



Analyses by Matt Perkins, OSU

Biodegradation of DOSS (Corexit 9500A component) in Arctic seawater

August 2016 – 6-L incubations – 50 ppm oil 1:10 DOR



Corexit - sterile

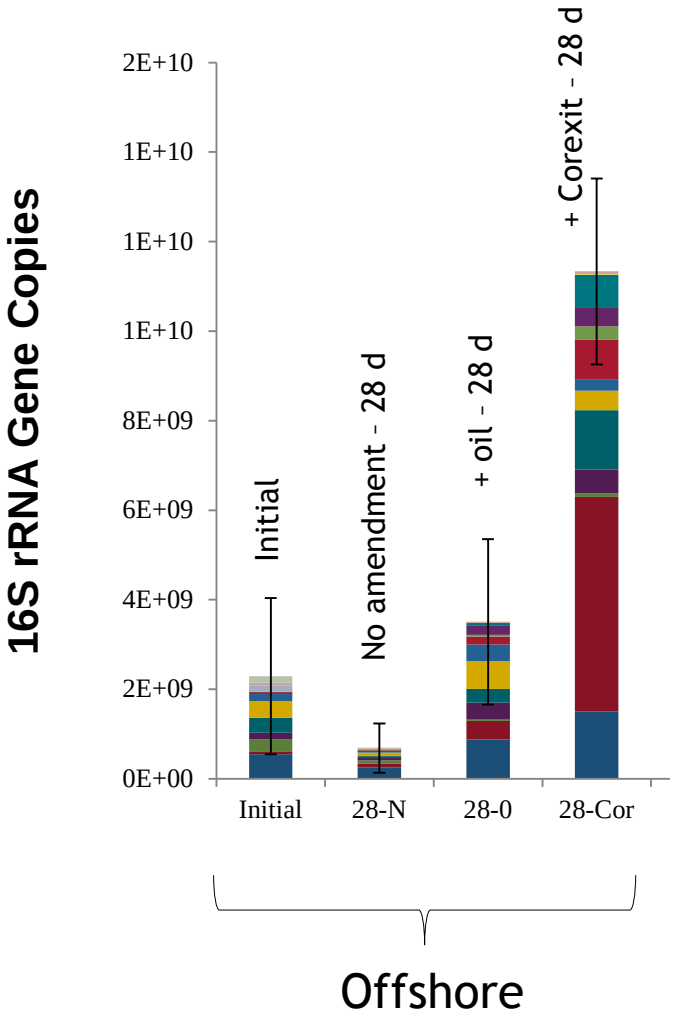
Corexit+oil -sterile

Corexit+oil

Corexit

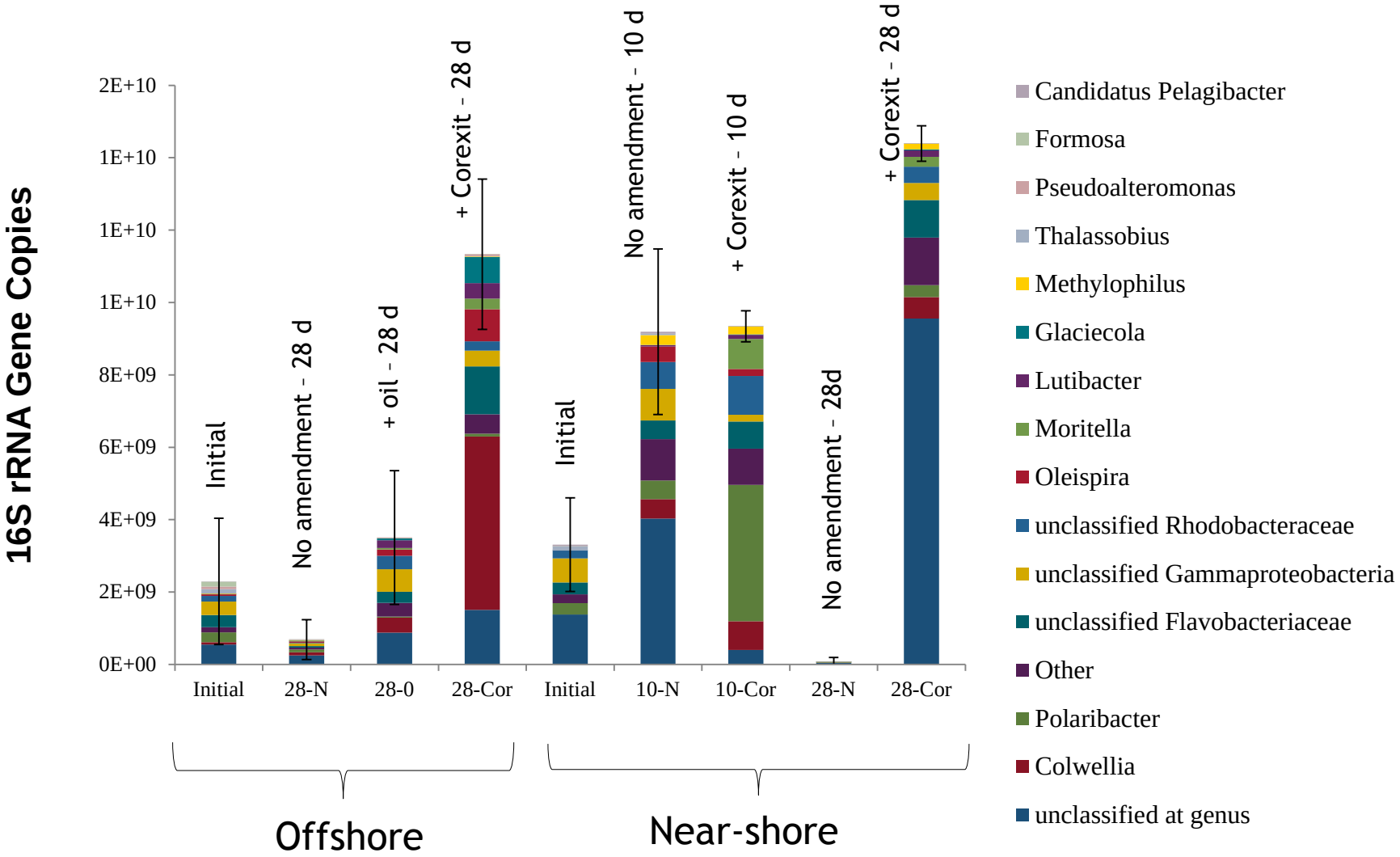
Analyses by Matt Perkins, OSU

Abundance of bacteria in incubations containing oil (15 ppm) or Corexit 9500 (15 ppm) at 2°C



- Candidatus Pelagibacter
- Formosa
- Pseudoalteromonas
- Thalassobius
- Methylophilus
- Glaciecola
- Lutibacter
- Moritella
- Oleispira
- unclassified Rhodobacteraceae
- unclassified Gammaproteobacteria
- unclassified Flavobacteriaceae
- Other
- Polaribacter
- Colwellia
- unclassified at genus

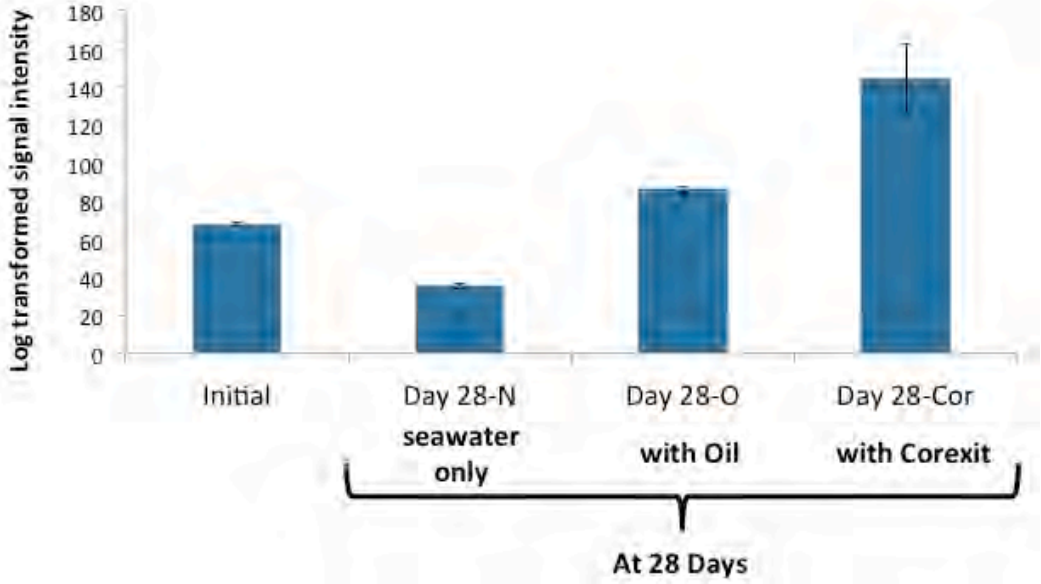
Abundance of bacteria in oil and Corexit 9500 incubations over time (2°C)



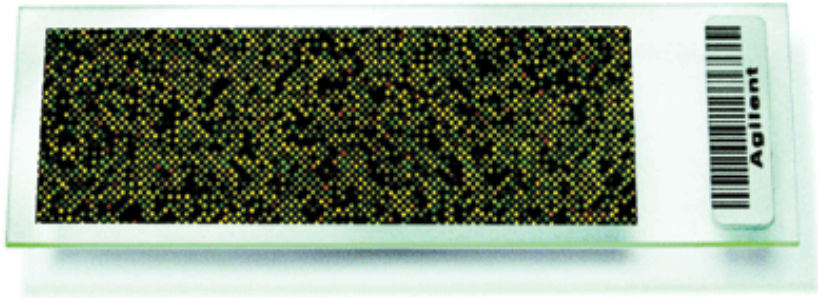
Biodegradation gene detection

- GeoChip Microarray
  - 167,044 probes covering 395,894 coding sequences targeting 1,500 functional gene families for microbial processing of **organic contaminants**, C, N, S, P, and many other functions
  - Semi-quantitative

Alkane biodegradation gene (*alkB*) relative abundance increased in response to oil and Corexit in offshore seawater

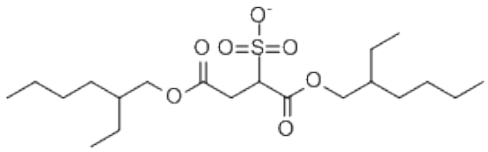


www.glomics.com



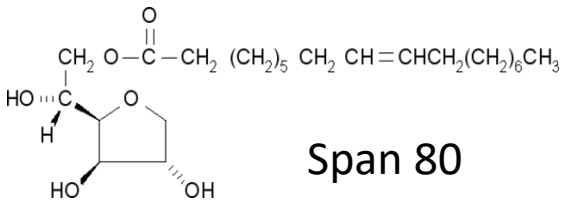
Alkanes (hydrocarbon chains) present in oil are also present in Corexit 9500A…

- Solvent fraction of Corexit
  - made of petroleum distillates
    - include alkanes (C_9 - C_{16}) and paraffins (MSDS)



DOSS

- Side chains of DOSS (Seidel et al., 2016)
- Nonionic surfactants
  - Span 80, Tween 80, and Tween 85



Span 80

- Bacteria may use similar genes/pathways when biodegrading some components of oil and Corexit
- Under a new BOEM-CMI project, we're examining gene expression (metatranscriptomics) during oil and Corexit 9500A biodegradation

Oil Spill Eater II (OSEII)

- Marketed by Oil Spill Eater International Corp. (Dallas, TX)
- Listed on NCP schedule (2017) as bioremediation agent (enzyme additive)
  - added to the NCP Schedule in 1996, removed in 2005, and then re-listed in 2009 (U.S. EPA, 2016)
  - Currently listed as **enzyme additive** (in past was enzyme and nutrient additive)
- Marketed for use on soil, pavement, and water

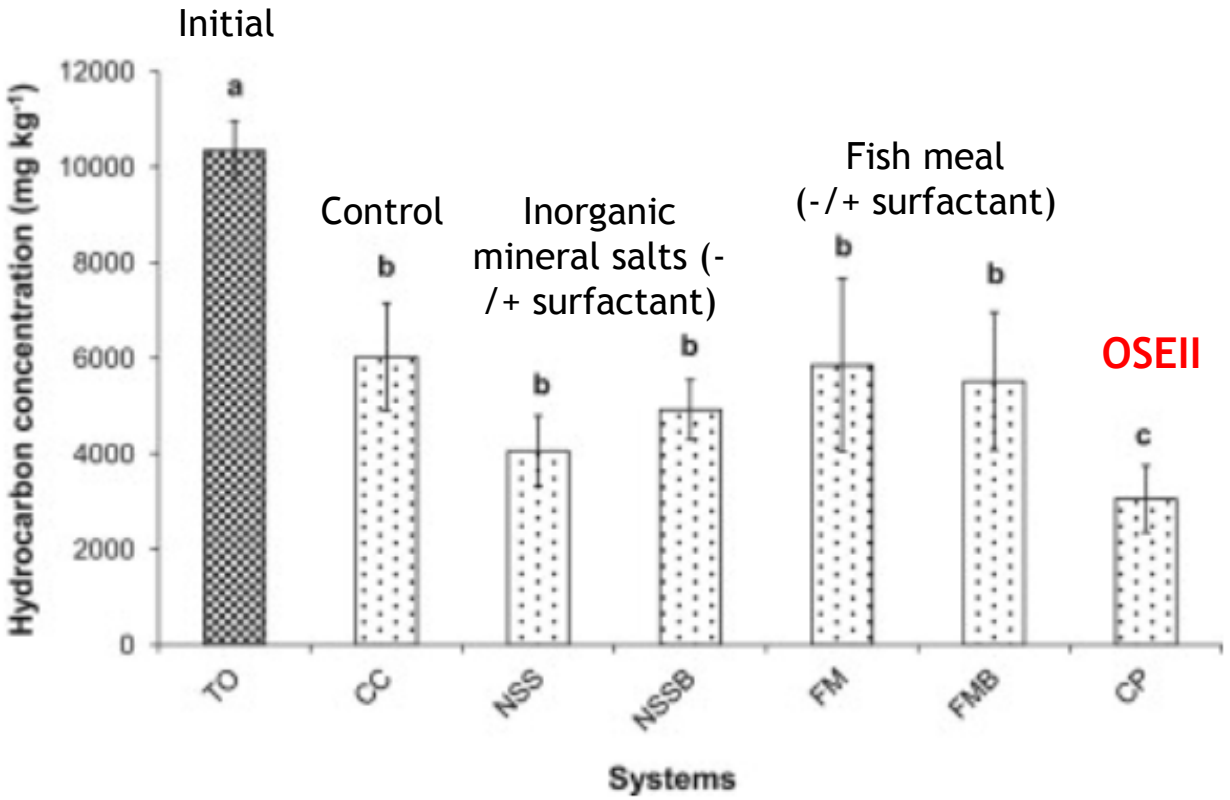
"OSE II is **not a bacteria** (bug), **fertilizer or dispersant** product. OSE II is a **biological enzyme** that converts the waste into a natural food source for the enhanced native bacteria found in the environment." (www.osei.us)

OSEII Composition

- Not publicly released, but
  - Literature and documents/reports released by OSEI suggest it may contain:
    - Enzymes - amylase and protease
    - Mineral nutrients
    - Molasses as a carbon source
    - Oleophilic surfactant

OSEI
OIL SPILL EATER INTERNATIONAL, CORP.

Effects of biostimulation methods on aged diesel loss in Antarctic soils (45 d)



Dias, et al.(2012). *International biodeterioration & biodegradation*, 75, 96-103.

Effects of OSEII and other commercial products on oil biodegradation in salt marsh mesocosms

- "Commercial products failed to enhance total oil or total petroleum hydrocarbon (TPH) degradation."
- "Products generally did not increase population sizes of heterotrophs or hydrocarbon-degrading microorganisms."

Wright, A. L., & Weaver, R. W. (2004). Fertilization and bioaugmentation for oil biodegradation in salt marsh mesocosms. *Water, air, and soil pollution*, 156(1), 229-240.

UAF Research Objectives

- Evaluate the effects of **OSEII** on **crude oil** and **marine diesel** biodegradation in **Alaskan Arctic** and **sub-Arctic seawater**
- Investigate the **mode(s) of action** through which OSEII affects crude oil and diesel biodegradation
- Compare the effects of OSEII on crude oil biodegradation in Arctic seawater to those of the chemical dispersant, Corexit 9500A
- Evaluate the response of indigenous marine microorganisms to oil and OSEII in order to:
  - Identify microbes and genes important to the biodegradation of oil
  - Determine if/how OSEII application affects microbial ecology and biogeochemical cycling potential

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OSE II Composition - Nutrients

	OSE II, neat (µM)
PO_4	40,712
SiO_4	1,279
NO_3	161
NO_2	23
NH_4	679,878
Fe	121

OSE II Composition - Nutrients

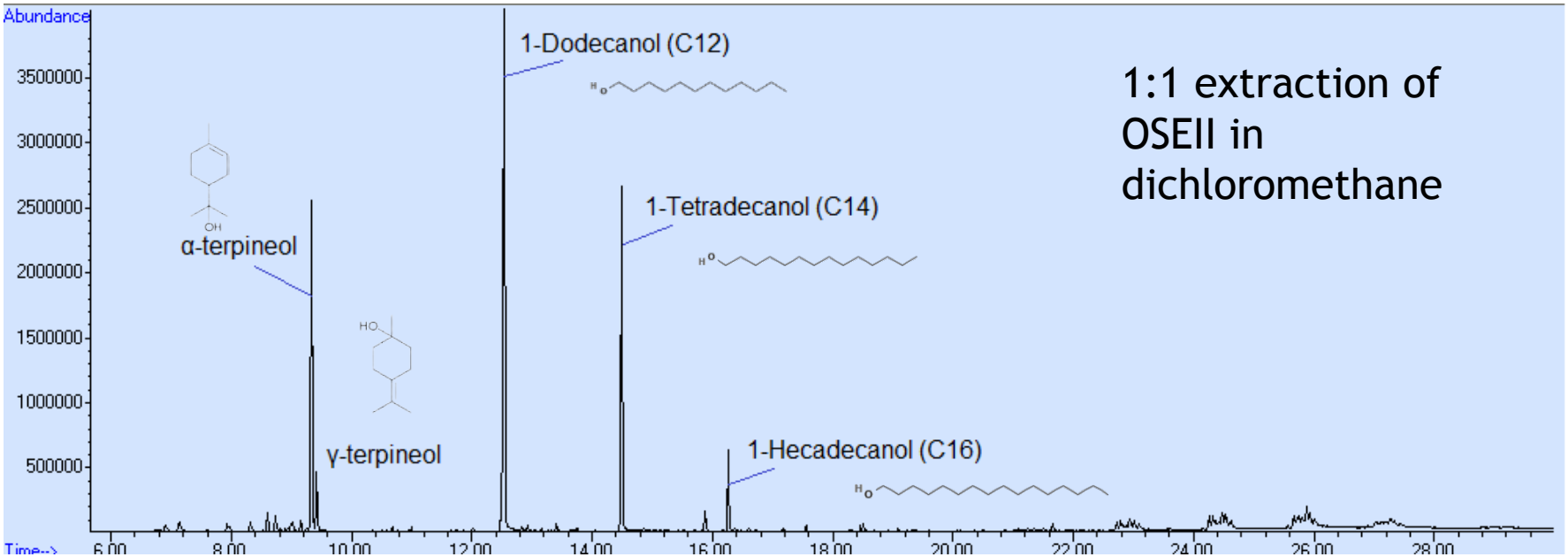
	OSE II, neat (µM)	OSE II, 1:25 dilution (µM)	OSE II in mesocosms (µM)	Arctic seawater (µM)	Subarctic (PWS) seawater (µM)
PO_4	40,712	1,628	1×10^{-1}	0.66	1.28
SiO_4	1,279	51	3×10^{-3}	12.53	19.22
NO_3	161	6	4×10^{-4}	0.19	14.07
NO_2	23	1	5×10^{-5}	0.03	0.08
NH_4	679,878	27,195	1.6	0.05	0
Fe	121	5	3×10^{-4}	$<1 \times 10^{-9}$	$<1 \times 10^{-9}$

- Increases the NH_4 in Arctic seawater by 30X (5X total N)
- Increases Fe 100,000X in Arctic and subarctic seawater

OSE II Composition

Assay	Analyte	Result
Benedict's test	Reducing sugars	+
Bradford protein assay	Protein	+
Hiai et al. saponins test	Saponins	+

OSE II Composition – GC/MS analysis



Terpineols – plant-derived alcohols (e.g. in pine oil) - lilac/pine scent, used as fragrance in disinfectants, polishes, household products

Long-chain alkanols –emollients, precursors for surfactants, derived from palm oil or petroleum

Future OSE II characterization

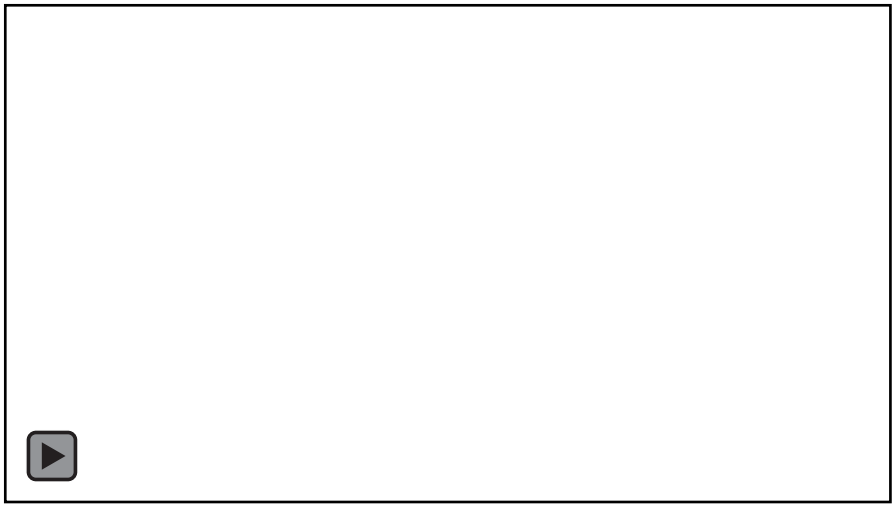
Ongoing and future analyses:

- Iron - confirmation by Microwave Plasma Atomic Emission Spectroscopy (UAF)
- Enzyme assays
  - Protease
  - Amylase
- Liquid chromatography-mass spectrometry (LC/MS) - Non-volatiles such as surfactants
- Baffled Flask Dispersion test

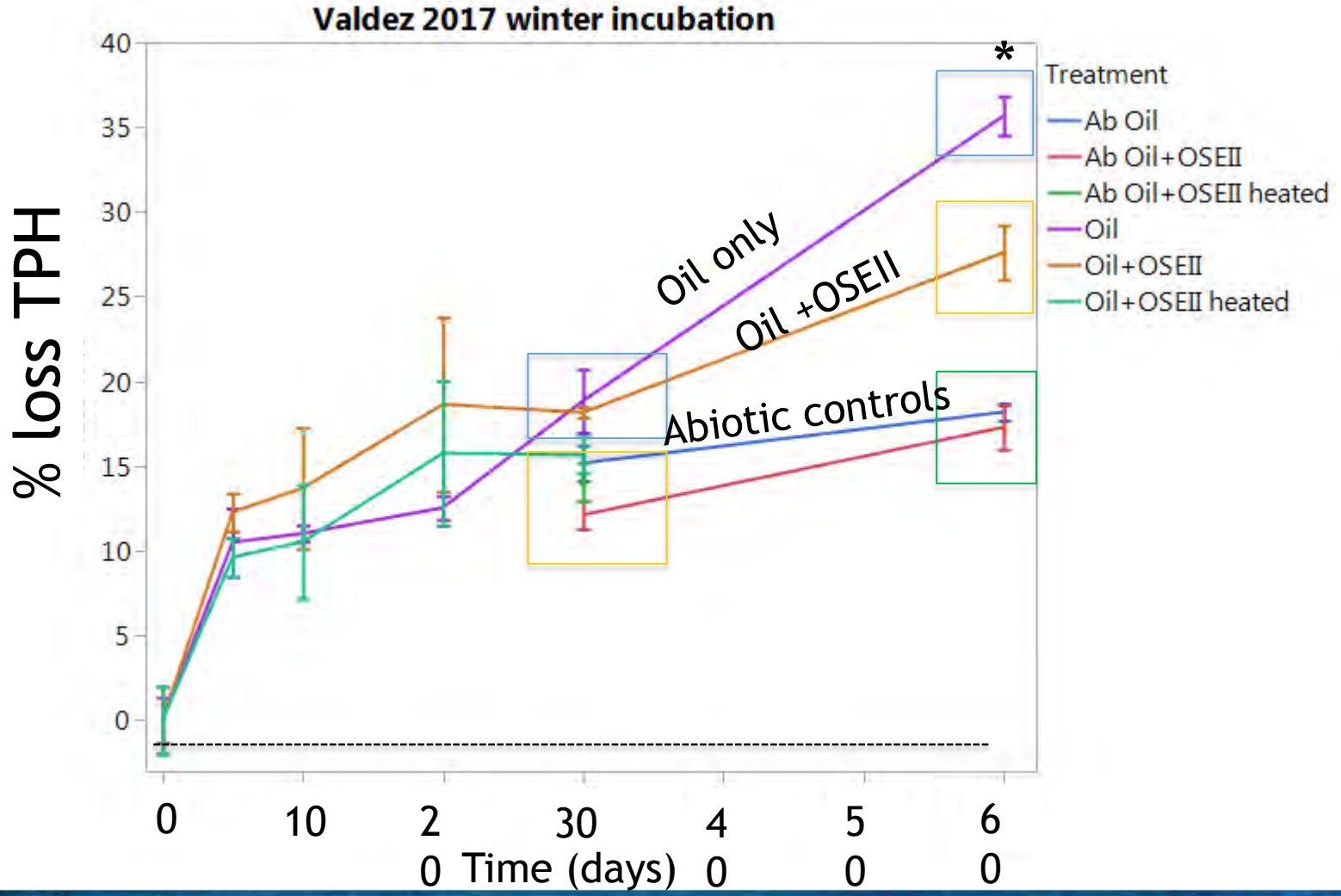
Methods: Effects of OSEII on petroleum biodegradation in seawater

- Collect fresh surface seawater
  - Sub-Arctic: Prince William Sound (Valdez area)
  - Arctic: 1 km offshore of Utqiagvik (formerly Barrow)
- Mesocosm incubation studies in UAF cold room
  - Oil (50 ppm) +/- OSEII
  - Oil + pre-heated OSEII (enzymes deactivated)
  - Sterile controls

- Analyze over time-course:
  - Total petroleum hydrocarbons (TPH)
  - Nutrients
  - Microbial community shifts

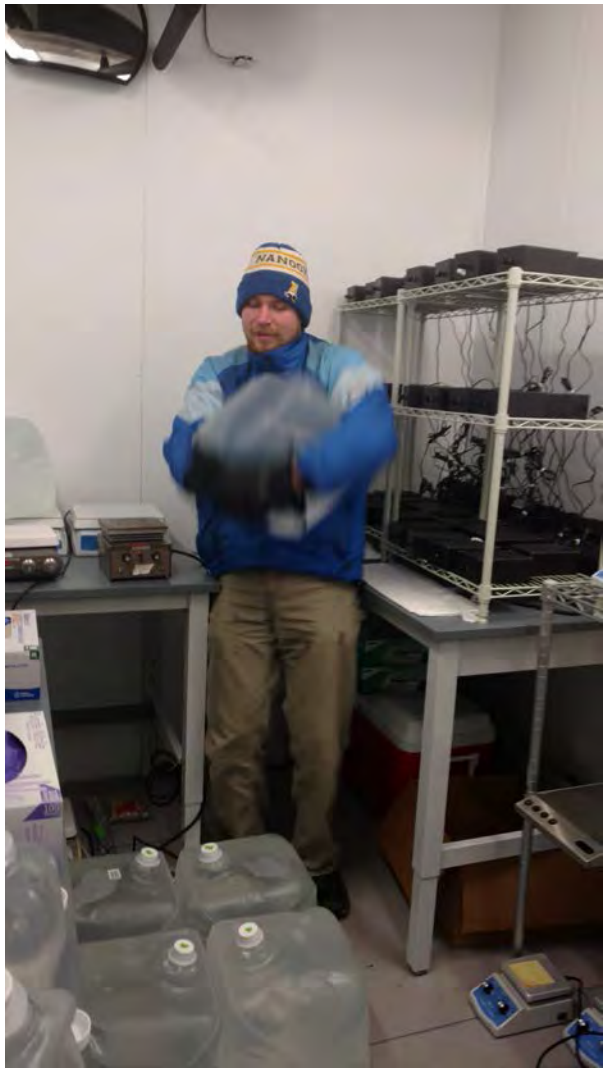


OSE II did not significantly enhance oil loss in PWS seawater (with supplemental nutrients…)



*=p<0.05

Adding nutrients to seawater (since discontinued)



Nutrient additions standard for our previous Arctic studies to prevent depletion in microcosms

At the start of this study, we didn't know about OSEII's high N and P levels

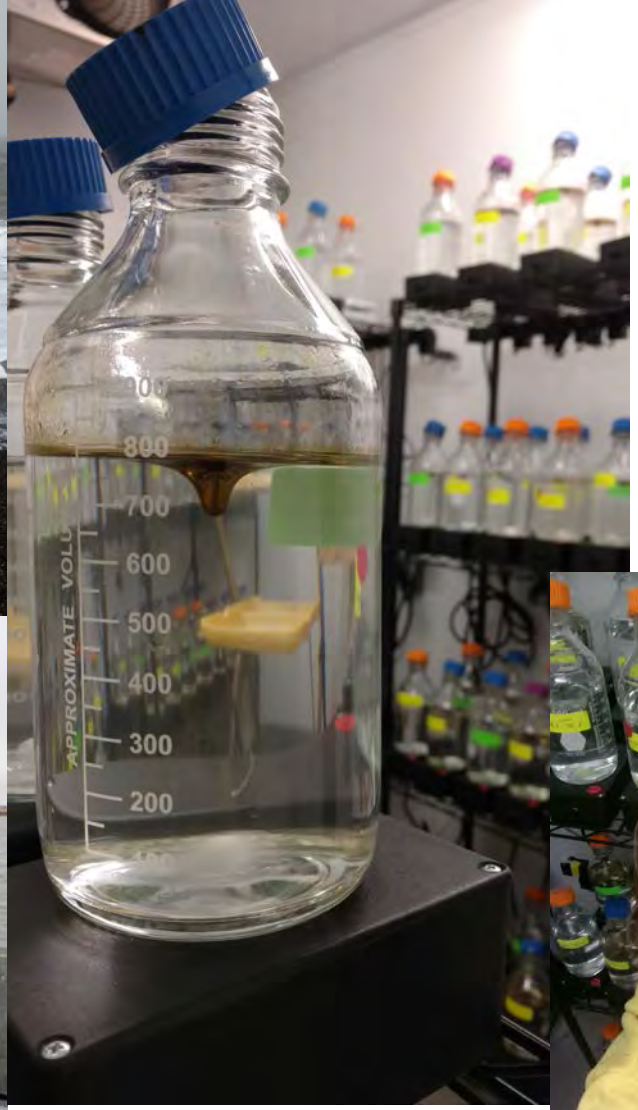
Our nutrient supplements exceeded those in OSEII by about 60-fold (NH4, PO4)

Valdez
March 2017

Valdez incubation – preliminary conclusions

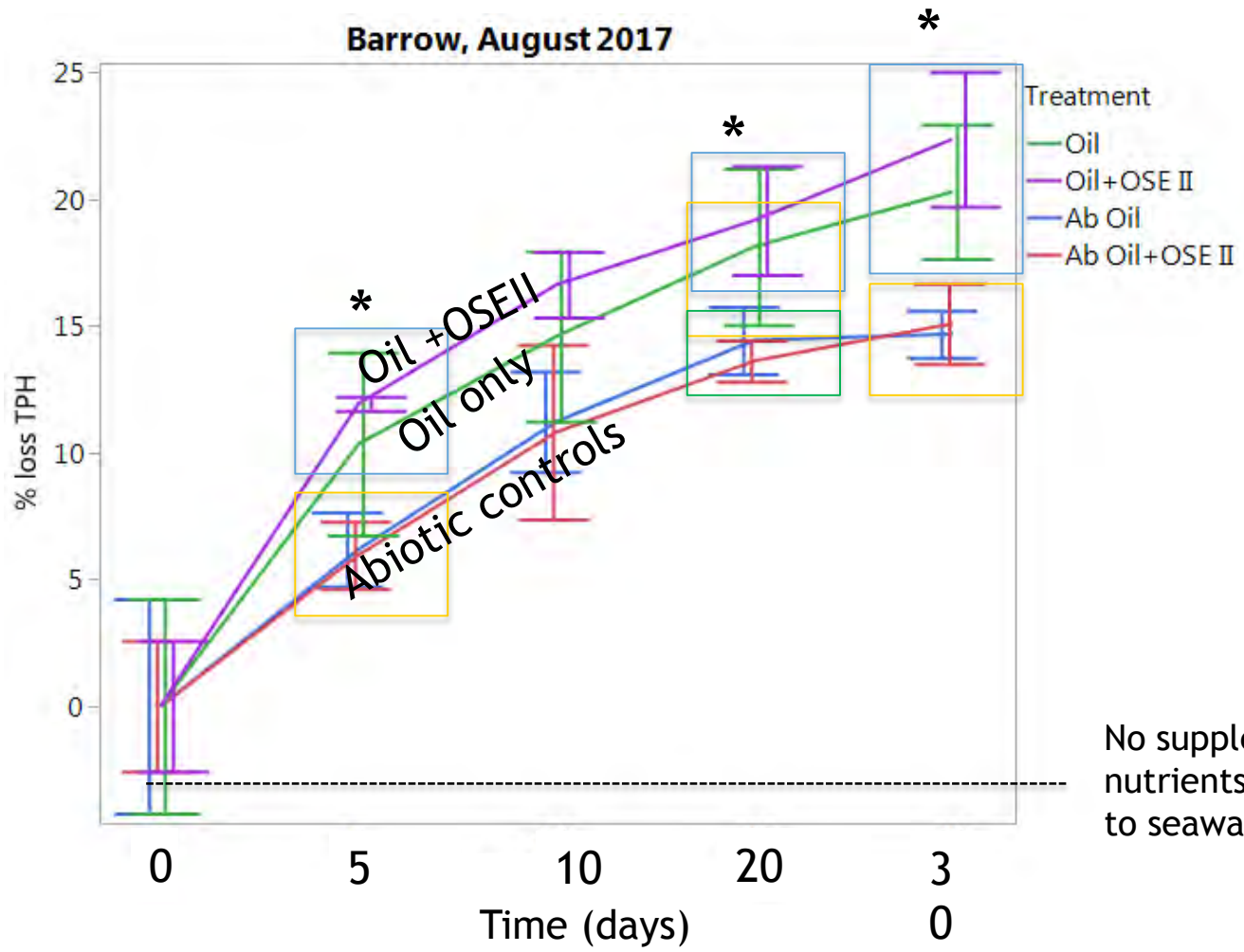
- OSEII did not enhance oil biodegradation, BUT…
  - our nutrient supplements overshadowed those present in OSEII
- If OSEII acts due to nutrient addition we would not have detected it due to our nutrient supplementation
- OSEI Corp. advised against adding nutrients to our experiments
- **Future studies: eliminate nutrient additions**

Utqiagvik (July, 2017)



Ph.D. students
Alexis Walker (L) and
Taylor Gofstein (R)

OSE II slightly increased oil loss in Arctic seawater (5, 10, and 20 d; not at 30 d)

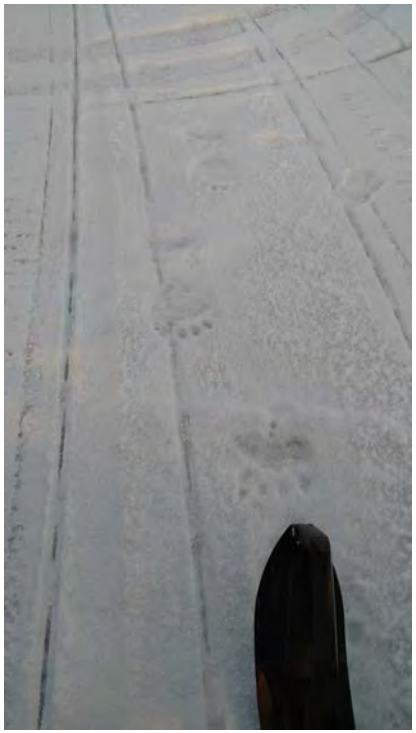


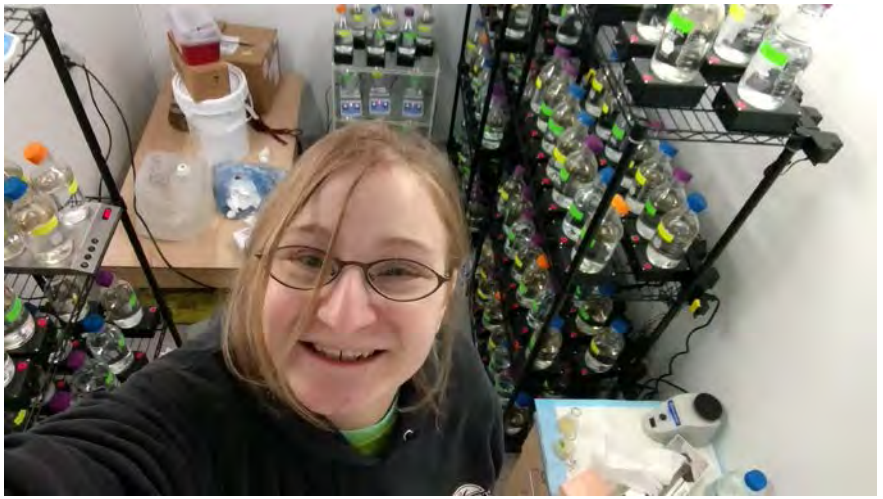
No supplemental nutrients added to seawater

*=p<0.05

Barrow Winter 2018 incubation

- Seawater collected from under sea ice
- Comparing effects of Corexit 9500 and OSE II (without addition of nutrients)





Future OSEII work

- Valdez seawater incubations (no nutrients added) – Summer 2018
  - Crude oil
  - Marine diesel
- Dispersion testing
- Comparison to Corexit 9500

- Microbial analyses
  - Identify putative oil degraders
  - Determine how OSEII affects oil-degraders and other microbes
- Toxicological assays
  - Microtox - assess detoxification of petroleum
  - Predicted toxicity based on petroleum remaining

Conclusions

Oil and Corexit studies

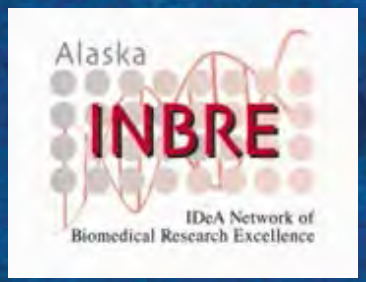
- Oil losses in Arctic waters are substantial (abiotic + biotic) but slower than in temperate waters
  - See McFarlin 2014 and Taylor Gofstein's poster for more details
- No evidence that Corexit inhibits oil biodegradation
- Nonionics degrade quickly (2-5 days)
- DOSS more persistent, especially in presence of oil
- Some of the same organisms involved in oil and dispersant biodegradation

Oil Spill Eater II

- Contains high levels of NH_4 and Fe (as well as complex organics)
  - List on NCP as nutrient supplement?
  - Consider potential ecological effects
- Enhanced oil biodegradation in Arctic water, but only slightly
- No effect in PWS (subarctic water) when nutrients added (stay tune for future tests)

Thank you

Acknowledgements

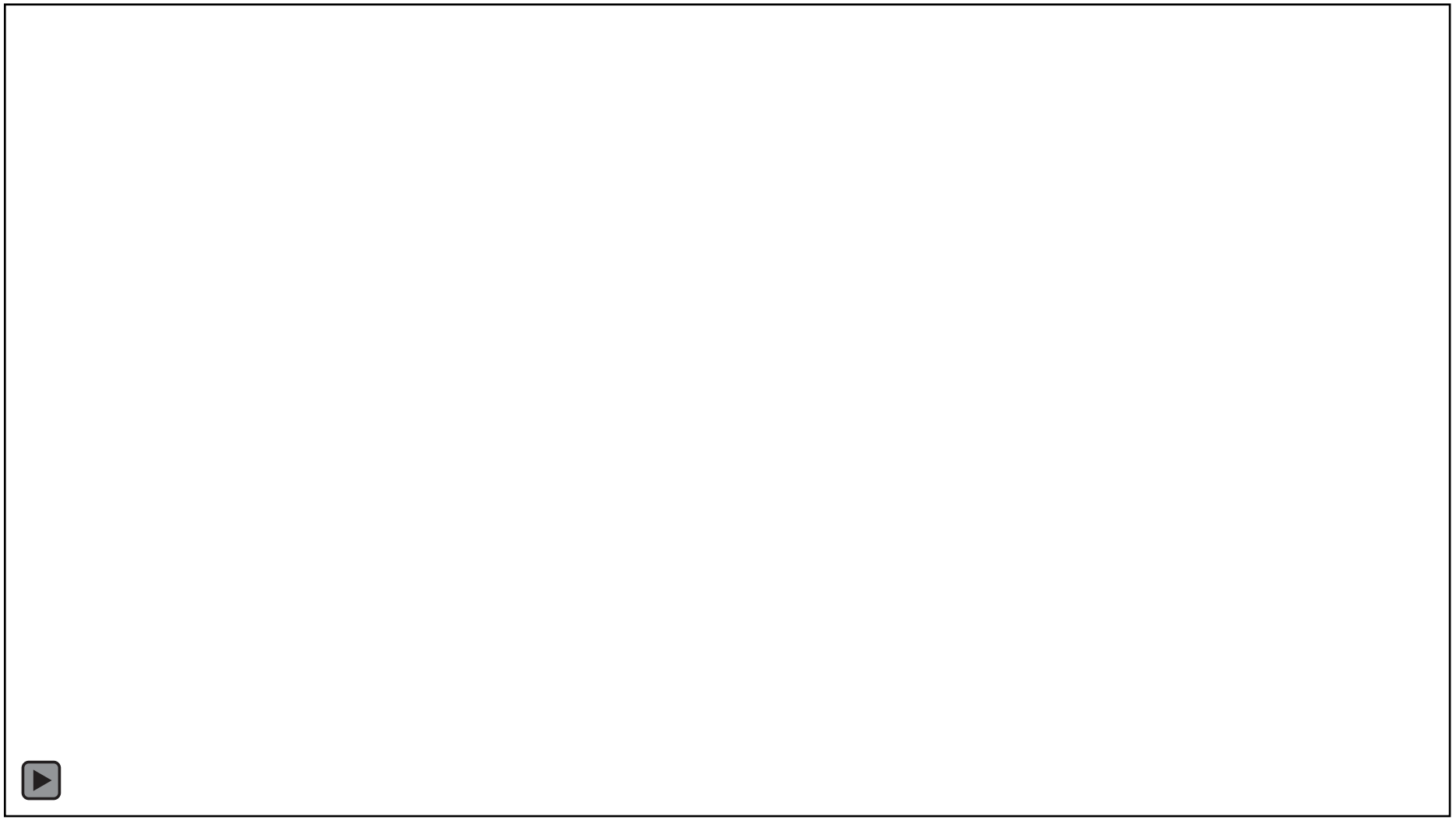


Baffled Flask Dispersion Test

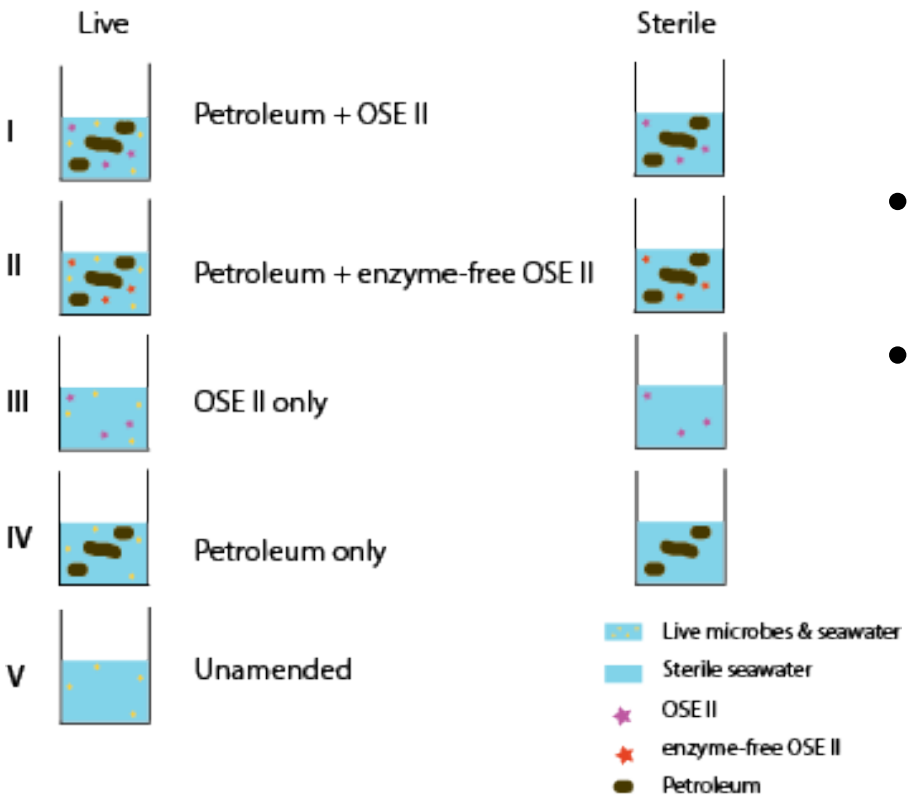
- Capacity to disperse oil into water
- Method: Venosa et al. (2011). US EPA, 600-699.
- Will analyze petroleum by UV and GC/MS spectroscopy
- Flasks currently under construction

Extra slides

Prince William Sound seawater incubations (March 2017)



Petroleum incubation design



crude oil: 0, 5, 10, 20, 30 d; sterile controls 30 d (diesel 20 d)

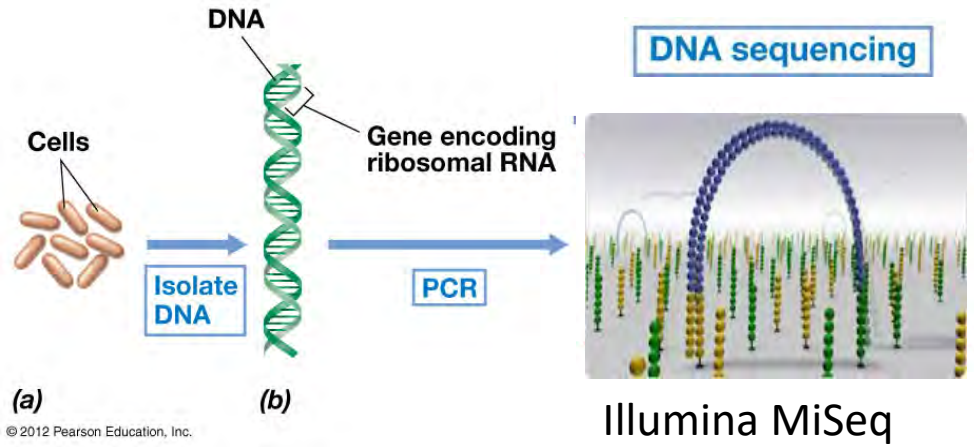
3 replicates for each sampling time point

Duplication of treatment I, II, IV for crude oil analysis

Total number of microcosms 123

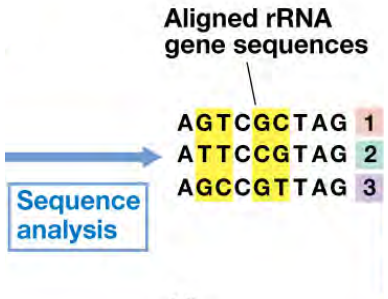
- 50 ppm oil
- OSEII diluted 1:25 then added to oil in 1:1 ratio

Bacterial Community Analysis Using 16S rRNA Gene Sequencing



DNA sequencing

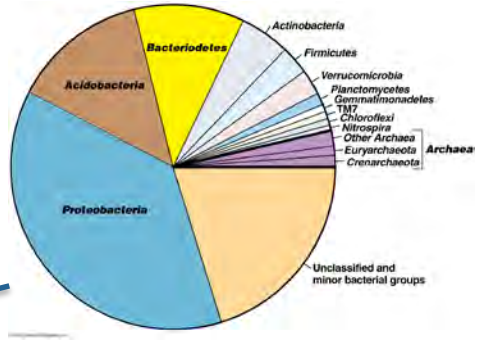
Illumina MiSeq
(hundreds of thousands of 16S rRNA sequences per sample)



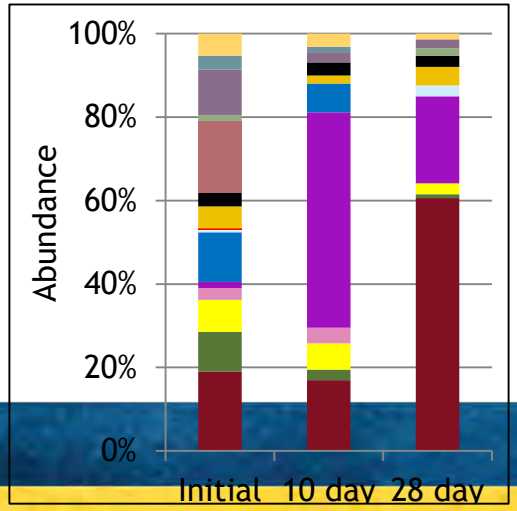
Compare to databases for phylogenetic identification

Group sequences by taxonomic affiliation

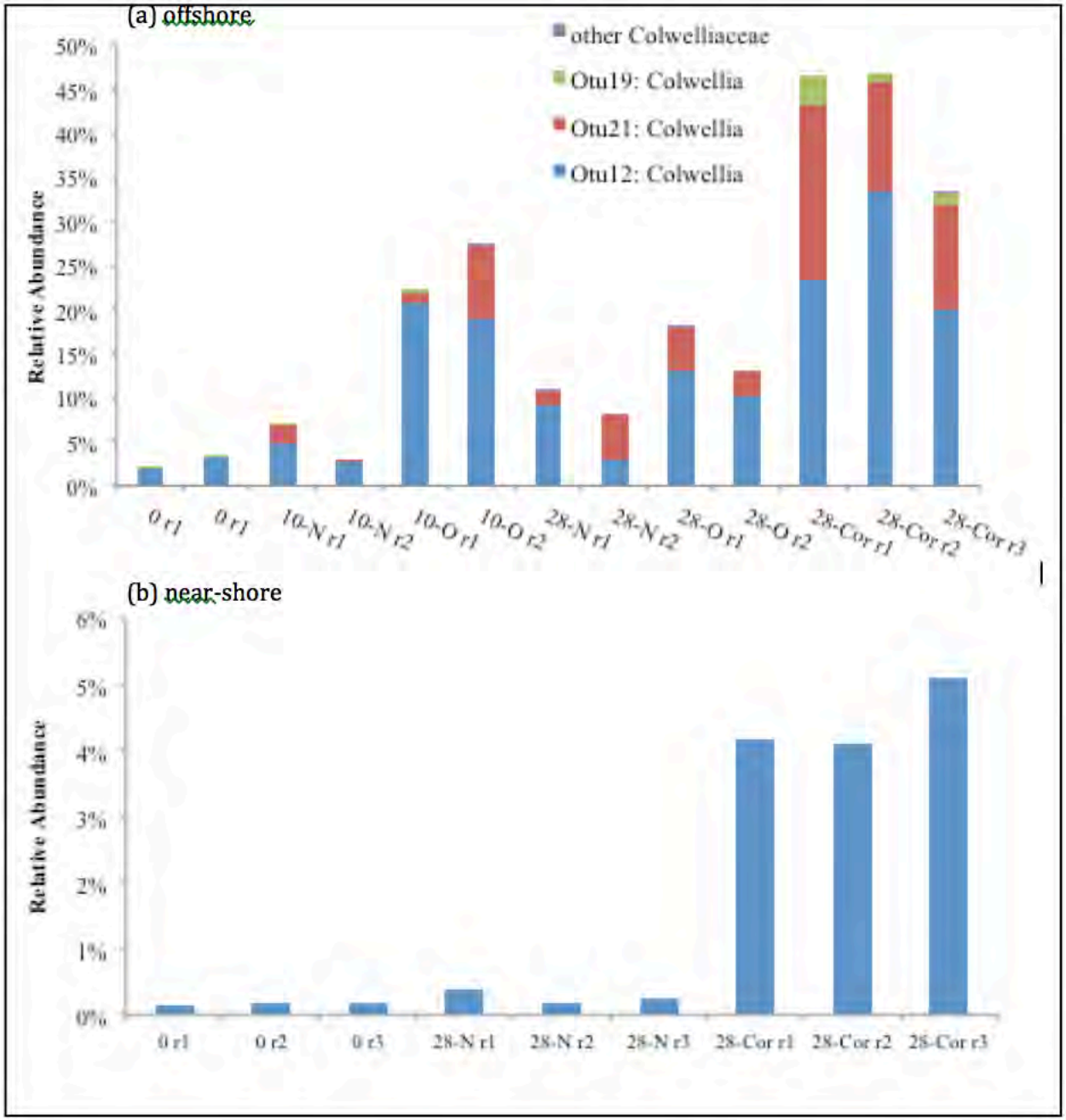
Count # reads per taxonomic group



Community composition and structure

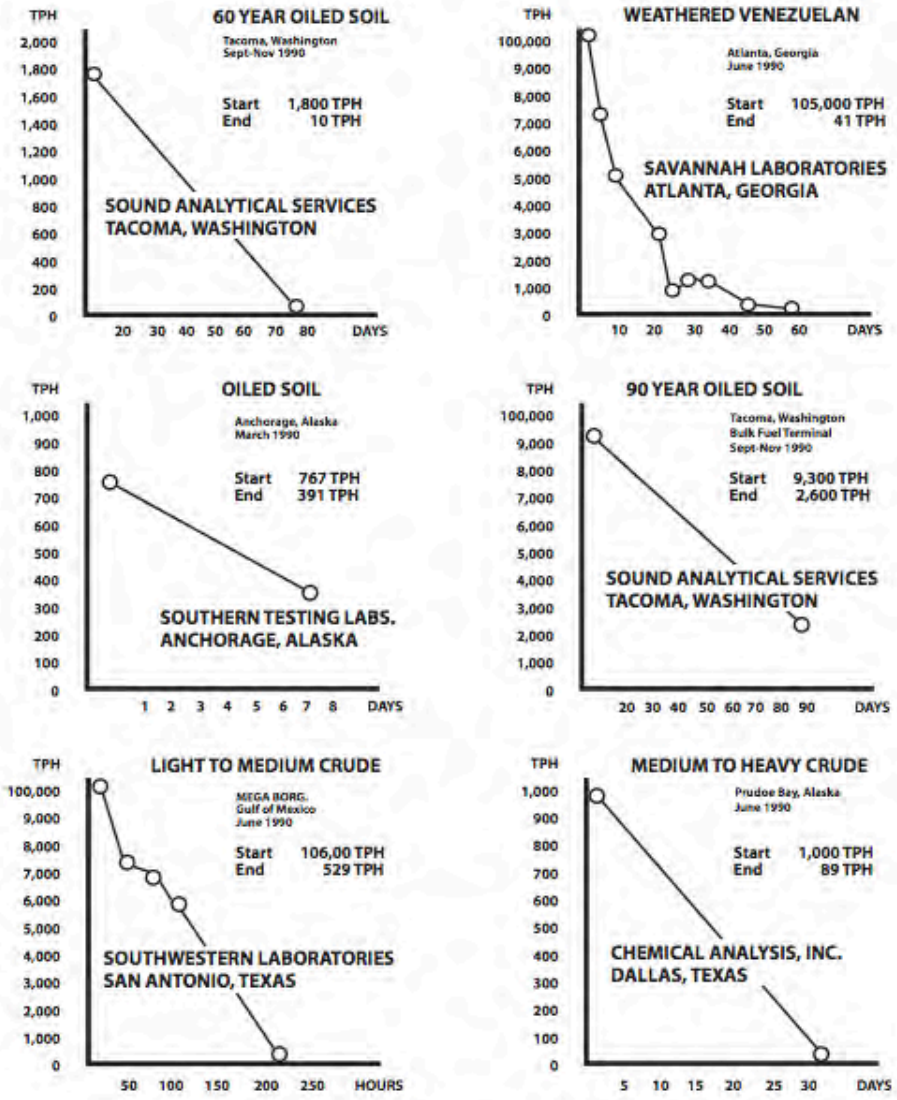


Compare community structure of different samples


(a) offshore
other Colwelliaceae
Otu19: Colwellia
Otu21: Colwellia
Otu12: Colwellia
Relative Abundance
50%
45%
40%
35%
30%
25%
20%
15%
10%
5%
0%
0 r1
0 r1
10-N r1
10-N r2
10-O r1
10-O r2
28-N r1
28-N r2
28-O r1
28-O r2
28-Cor r1
28-Cor r2
28-Cor r3
(b) near-shore
Relative Abundance
6%
5%
4%
3%
2%
1%
0%
0 r1
0 r2
0 r3
28-N r1
28-N r2
28-N r3
28-Cor r1
28-Cor r2
28-Cor r3

Sample of technical report data (www.osei.us)

TPH BIODEGRADATION TESTS OF OSE II PERFORMED BY INDEPENDENT LABORATORIES

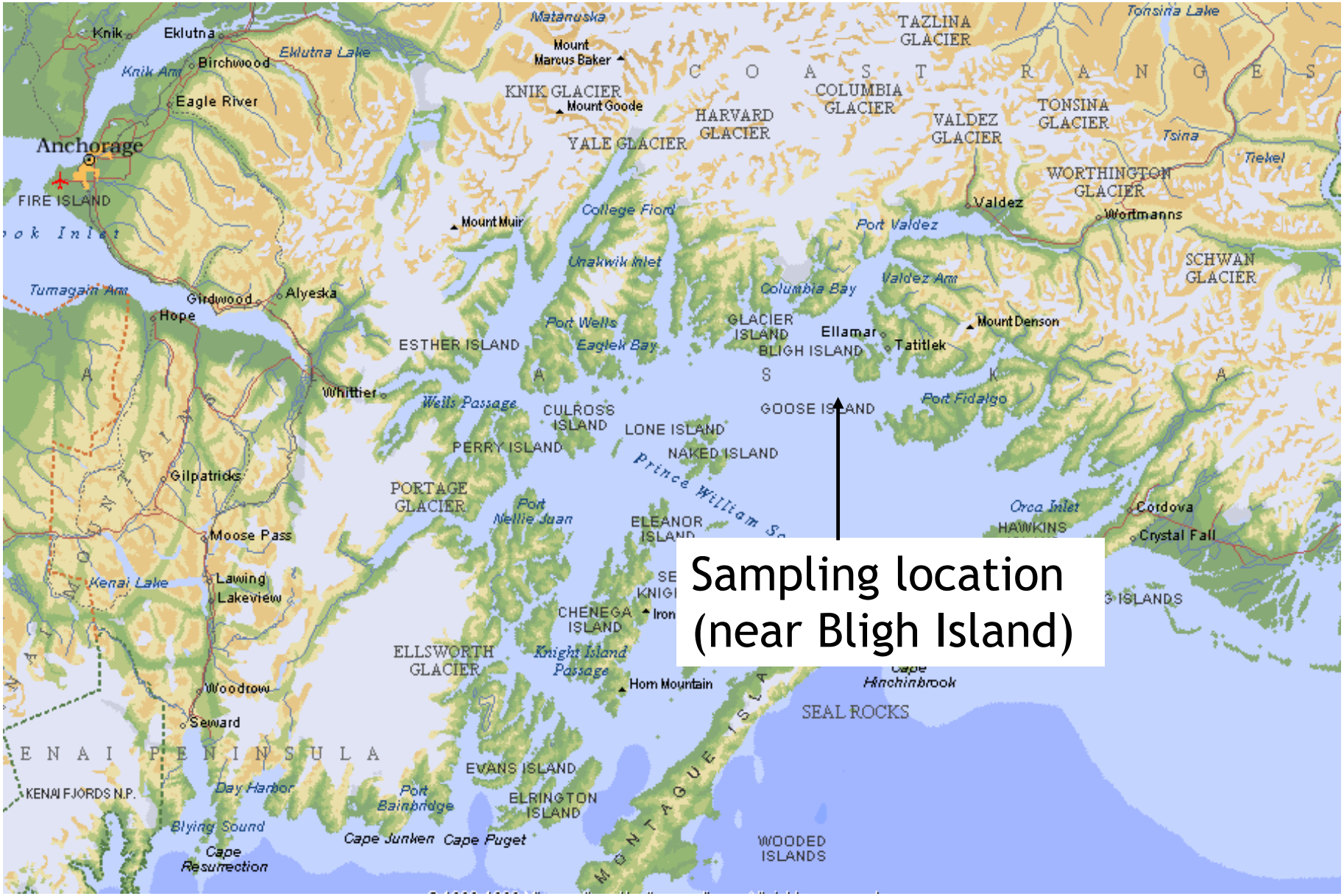


This page represents a sampling of laboratory test reports available upon request.

OSE II Composition

Analyses performed to date:

- Nutrients (N, P, Si)
- Gas chromatography-mass spectrometry (GC/MS)
- Colorimetric tests for
  - Iron
  - Reducing sugars
  - Protein
  - Saponins (natural surfactants, usually plant-derived)

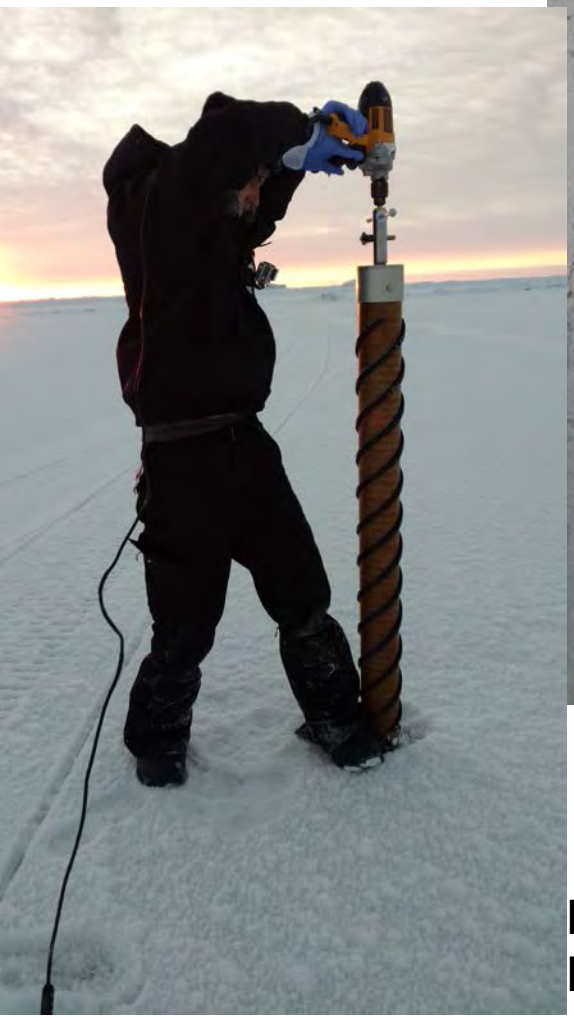

Sampling location
(near Bligh Island)
Knik
Eklutna
Eklutna Lake
Knik Arm
Birchwood
Eagle River
Anchorage
FIRE ISLAND
Matanuska
Mount Marcus Baker
KNIK GLACIER
Mount Goode
YALE GLACIER
HARVARD GLACIER
COLUMBIA GLACIER
TAZLINA GLACIER
Tonsina Lake
VALDEZ GLACIER
TONSINA GLACIER
Tsina
Tiekel
WORTHINGTON GLACIER
Valdez
Wortmanns
SCHWAN GLACIER
Mount Muir
College Fiord
Port Valdez
Unakwik Inlet
Valdez Arm
Columbia Bay
Tumagain Arm
Girdwood
Alyeska
Hope
Port Wells
Eaglek Bay
ESTHER ISLAND
GLACIER ISLAND
BLIGH ISLAND
Ellamar
Tatitlek
Mount Denson
Whittier
Wells Passage
CULROSS ISLAND
LONE ISLAND
PERRY ISLAND
NAKED ISLAND
GOOSE ISLAND
Port Fidalgo
Prince William Sound
PORTAGE GLACIER
Port Nellie Juan
ELEANOR ISLAND
Orca Inlet
HAWKINS
Cordova
Crystal Fall
Gilpatricks
Moose Pass
Kenai Lake
Lawing
Lakeview
CHENEGA ISLAND
Iron
ISLANDS
ELLSWORTH GLACIER
Knight Island Passage
Horn Mountain
Hinchinbrook
SEAL ROCKS
Woodrow
Seward
KENAI PENINSULA
EVANS ISLAND
KENAI FJORDS N.P.
Day Harbor
Port Bainbridge
ELRINGTON ISLAND
Blying Sound
Cape Junken
Cape Puget
Cape Resurrection
MONTAGUE ISLAND
WOODED ISLANDS

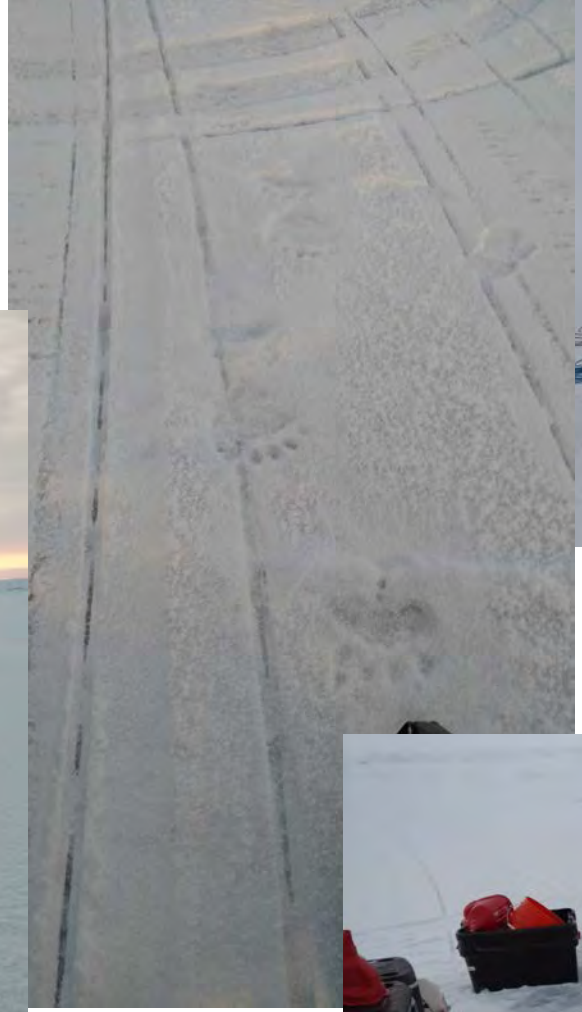
VALDEZ WINTER CRUDE OIL INCUBATION

Biodegradation of Corexit 9500 components in Arctic seawater (2013)

	DOSS		Span 80		Tweens	
Offshore (October)	µg/L	% Loss	µg/L	% Loss	µg/L	% Loss
Day 0	2880 ± 697^{a}		244 ± 30^{e}		2010 ± 20^{q}	
Day 28A	2270 ± 400^{a}	21	132 ± 91^{efg}	46	1230 ± 570^{q}	39
Day 28	71 ± 15^{d}	98	<LOD	>98	<LOD	>99
Near-shore (August)						
Day 0	2260 ± 210^{a}		179 ± 23^{efg}		2240 ± 110^{r}	
Day 10A	2190 ± 120^{a}	3	150 ± 10^{f}	16	409 ± 33^{s}	82
Day 10	1660 ± 200^{b}	27	44 ± 2^{h}	75	<LOD	>99
Day 28A	2210 ± 220^{a}	2	177 ± 8^{g}	1	298 ± 41^{t}	87
Day 28	1460 ± 170^{c}	35	<LOD	>97	<LOD	>99

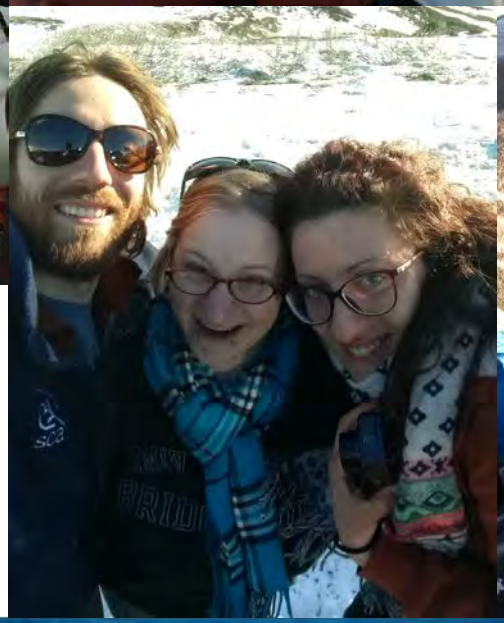
Analyses by Matthew Perkins (Ph.D. student Oregon State University).
Due to rapid degradation of non-ionics, we conducted more frequent sampling in subsequent studies…





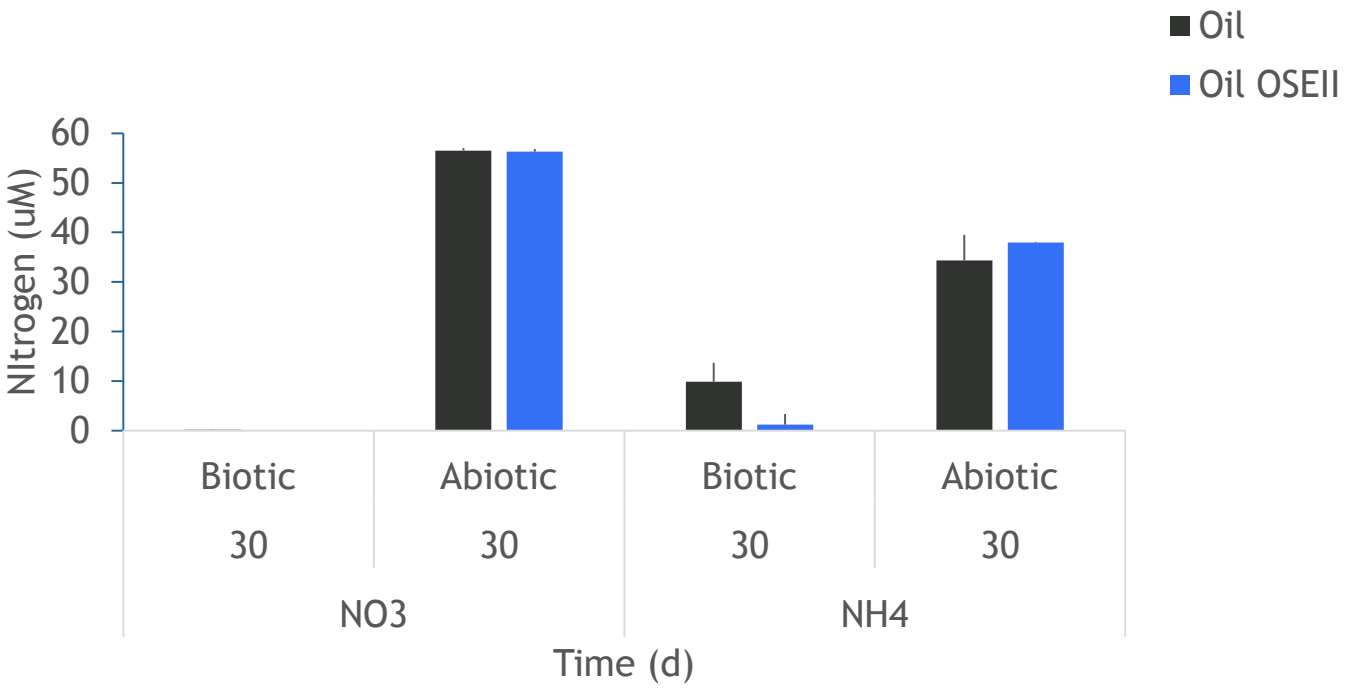
Barrow
February 2018

Barrow
February 2018



Valdez
March 2017

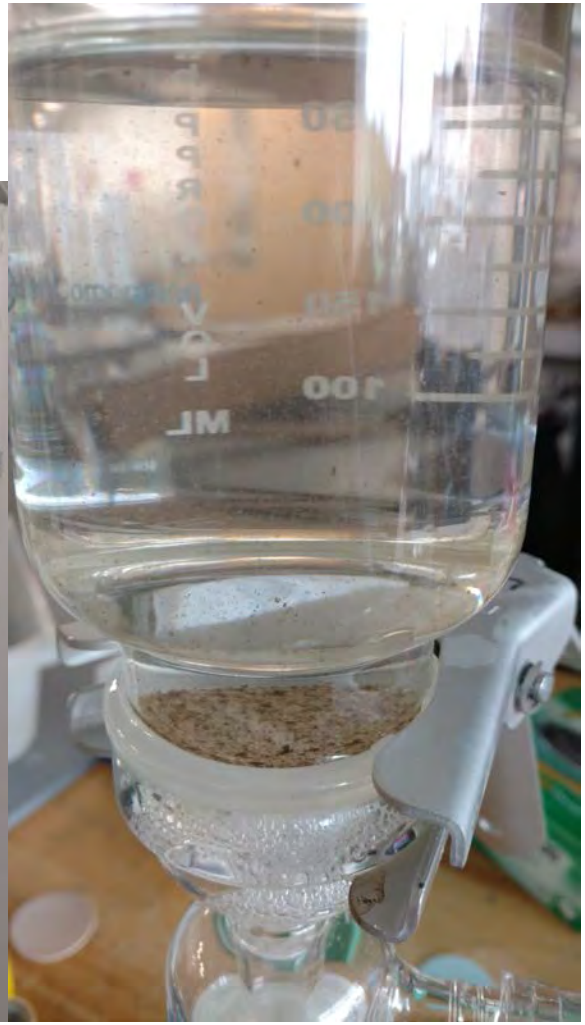
Biological uptake/processing of nitrogen in incubations
(all treatments combined)



Valdez
March 2017

Flocs in Valdez seawater – possibly due to high nutrient levels
Never seen in our previous Arctic studies

BARROW SUMMER CRUDE OIL INCUBATION



Barrow
July 2017

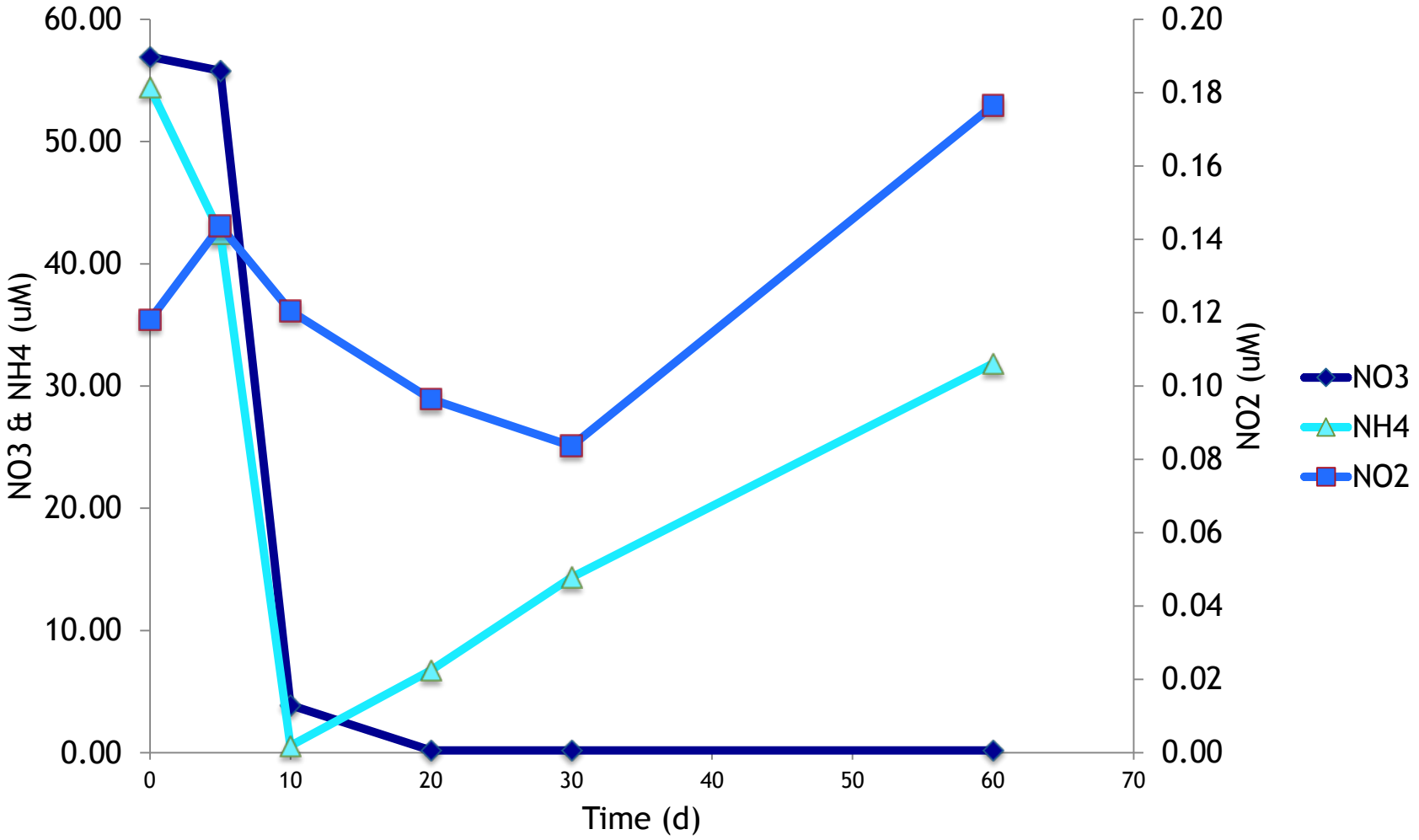
BARROW WINTER CRUDE OIL INCUBATION – COREXIT 9500 COMPARISON

Nutrients added

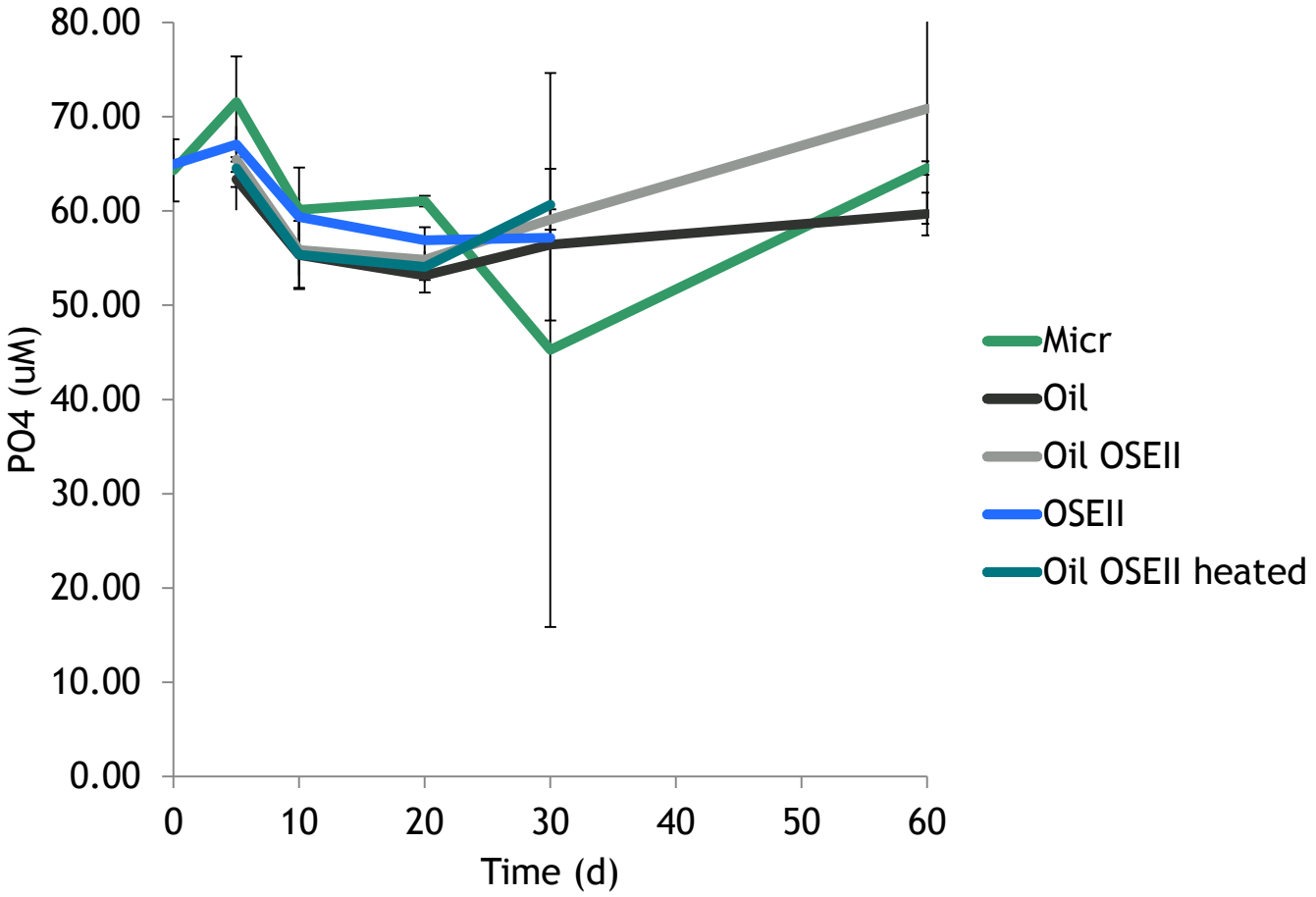
	From OSE II in mesocosms	Bushnell-Haas, in mesocosms 16 ppm	Valdez 3/17, before B-H	Valdez 3/17, after B-H
PO4	1×10^{-1}	64	1.28	64.32
SiO4	3×10^{-3}	0	19.22	
NO3	4×10^{-4}	61	14.07	57.13
NO2	5×10^{-5}	0	0.08	0.126
NH4	1.6	61	0	58.07
Fe	3×10^{-4}	1.5	$<1 \times 10^{-9}$	$<1 \times 10^{-9}$

Bushnell-Haas medium is our standard nutrient supplementaion

Nitrogen trends over time – all biotic treatments combined

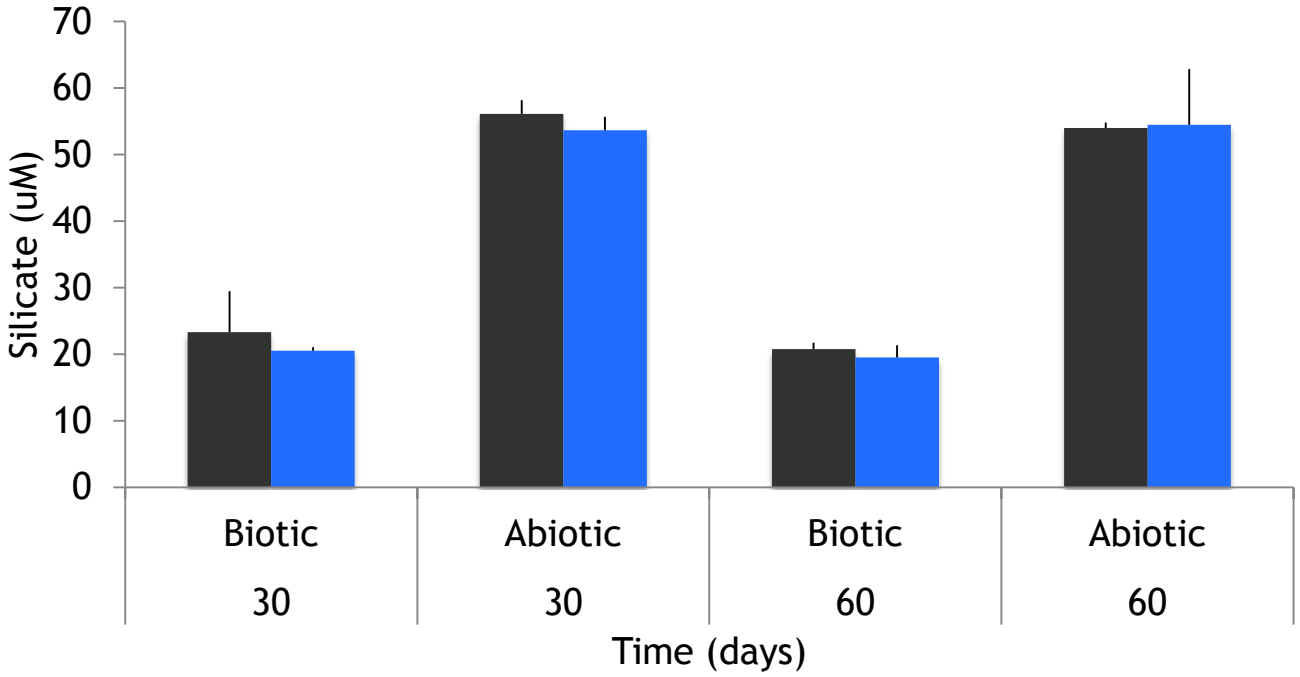


Phosphate trends over time



- No statistical differences in nutrient concentrations across treatment

Biological uptake/processing of silicate in incubations



Important nutrient for diatoms (phytoplankton)

Outline (30-min slot, ~20 min talk)

- Team
- Project themes and funders
- Corexit and oil
  - Background/res need: Dispersant state of sci doc, maybe others
  - Kelly's oil biodeg and effects of Corexit
  - Corexit composition and evidence of biodeg
    - Respirometry
    - LC-MS data – OSU (Taylor's comp pres) and Duke (AMSS)
  - Microbes involved in oil and Corexit biodeg – Kelly's data (AMSS)
  - Ongoing work – more studies, transcriptomics, sediments
- OSEII
  - Composition
  - Effects on oil biodeg – in progress
  - Other research plans in progress – direct Corexit/OSEII comparison, 2 more Valdez – marine diesel+crude