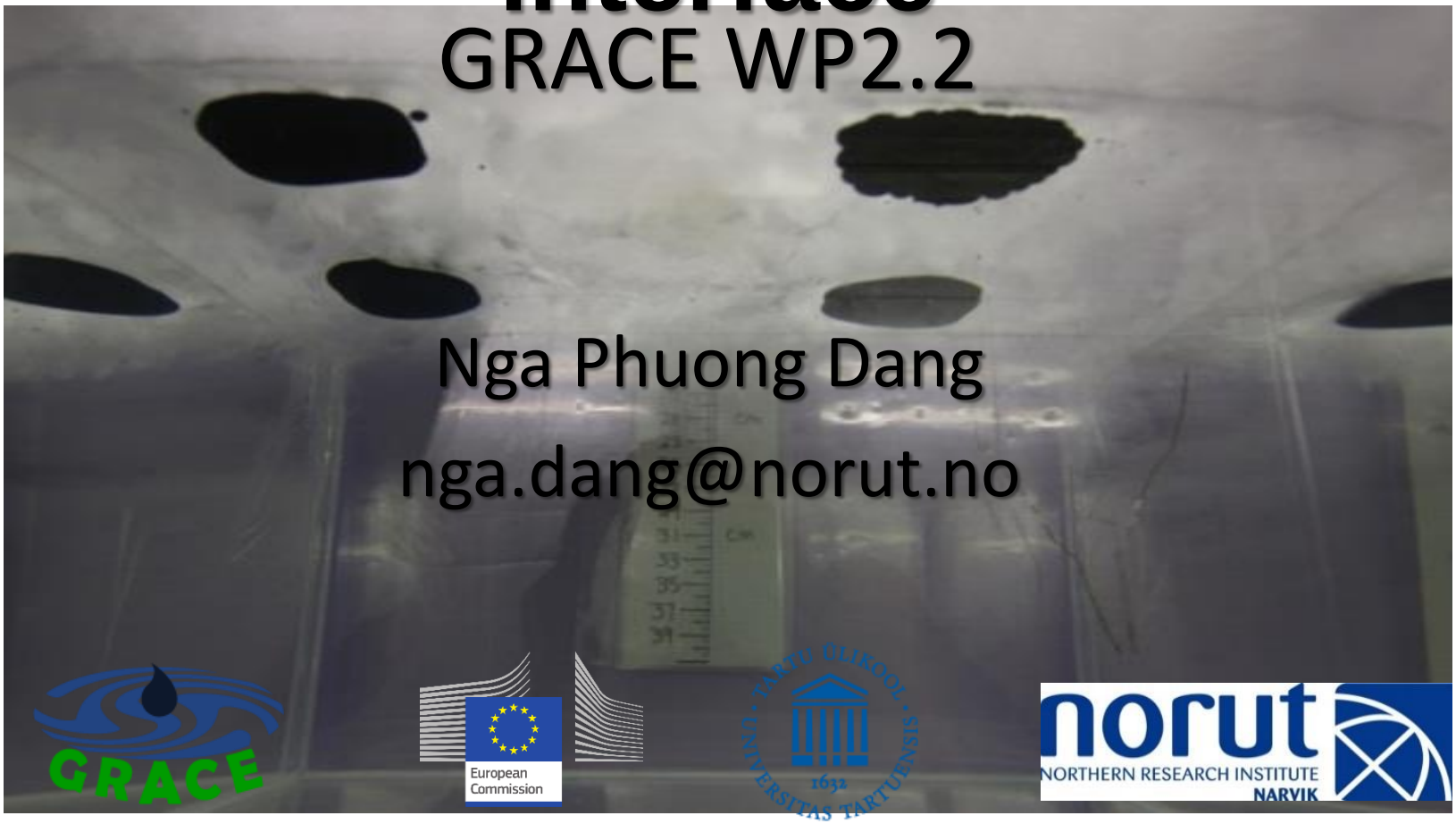
Biodegradation of oil seawater and ice-water interface

GRACE WP2.2



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Objectives & Approaches

- Degradation rate of crude oil in sea-ice covered water
- Key microbial species and metabolic pathways for degradation of oil

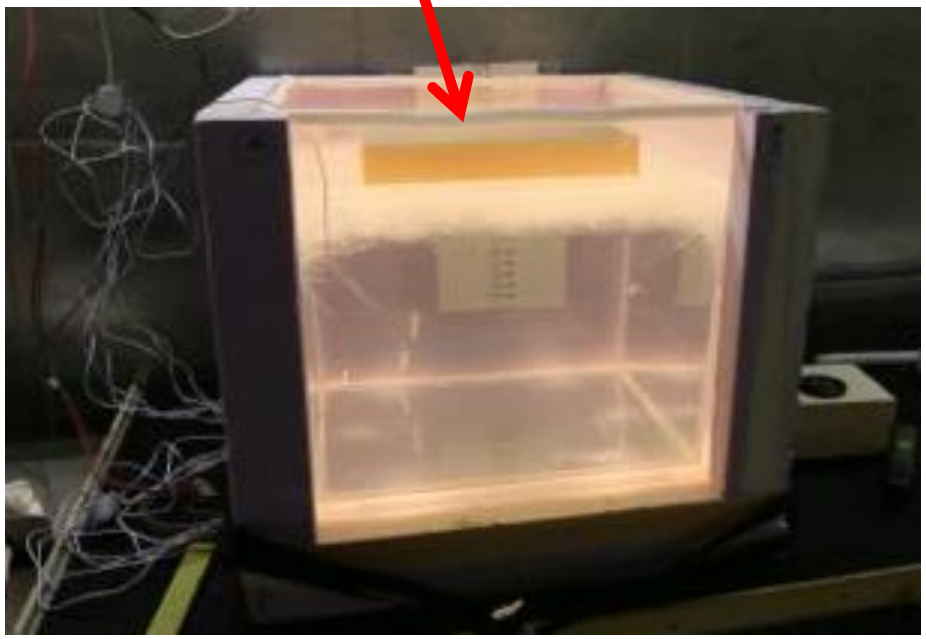
- Apply 16S rRNA amplicon & short-gun metagenomic sequencing for mesocosms & microcosms
- qPCR for 16S rRNA gene and functional genes
- Oil analyses: GC-FID, GC-MS

Sampling seawater in Svalbard

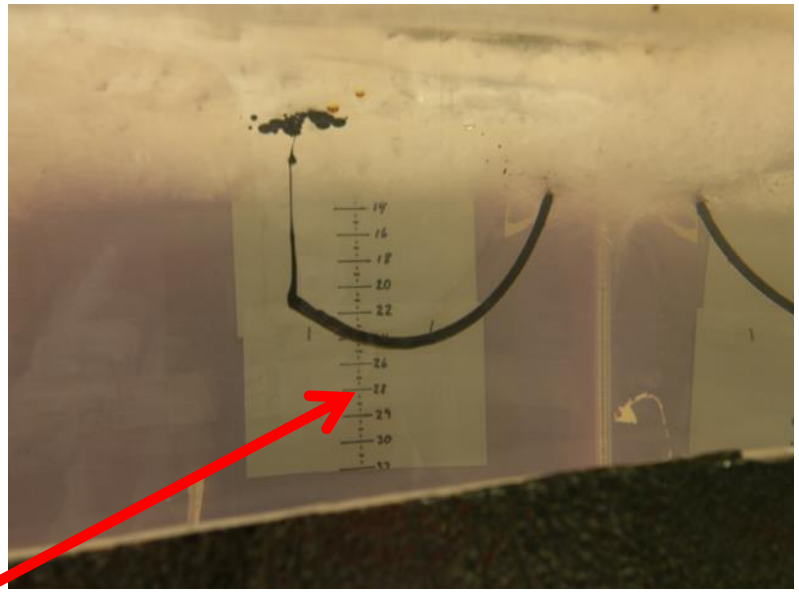
- 100 l of seawater

Oil encapsulated in ice

- Effect of oil on sea-ice microorganism communities



17 – 150 liter seawater
sea-ice -7 to -10°C



Injected crude oil

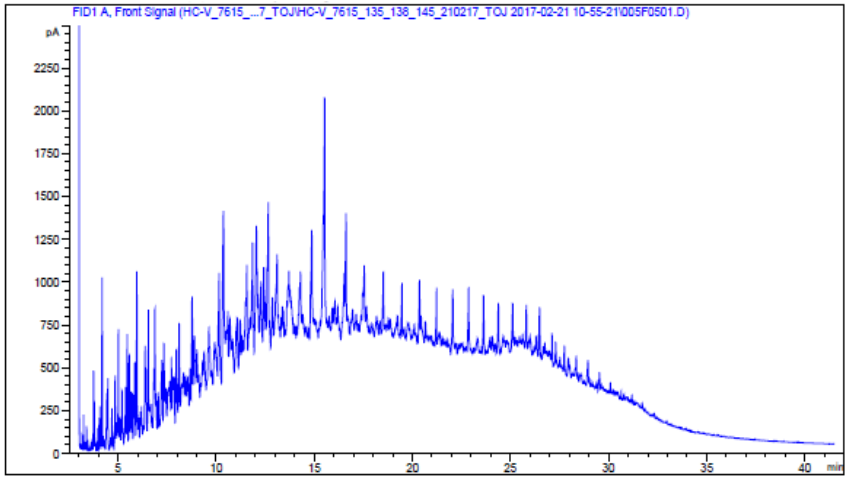
Oil encapsulated in ice

Oil encapsulated in ice at -10°C, 4-6 months in the dark

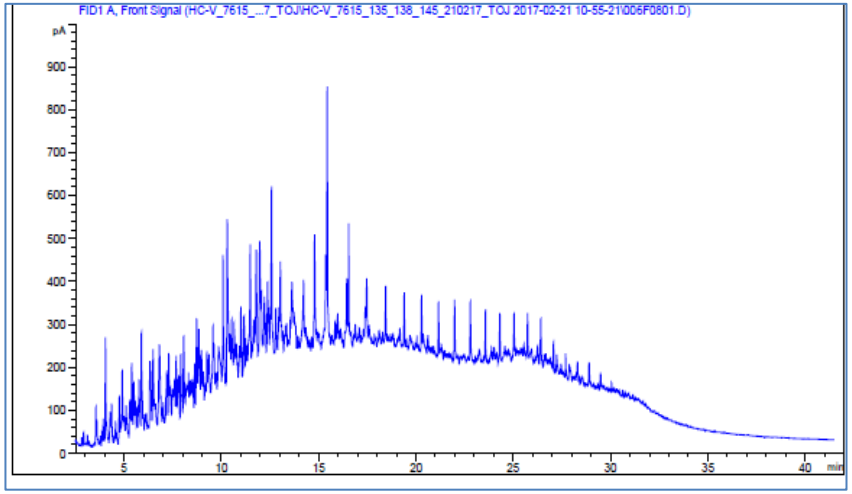
- Characteristics of ice: salinity, temperature and nutrients
- DNAs for qPCR of 16S rRNA gene and fuctional genes, amplicon sequencing and metagenomic sequencing

Oil encapsulated in ice

Starting oil



Oil after 6 months



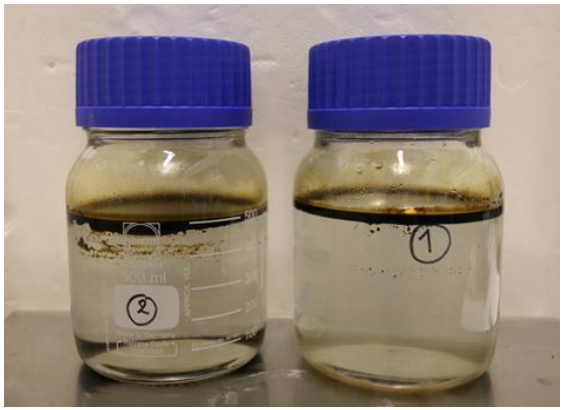
No change of C17/Pristane & C18/phytane ratio

No significant loss of oil

Increase of hydrocarbon-degraders and copy number of functional genes?

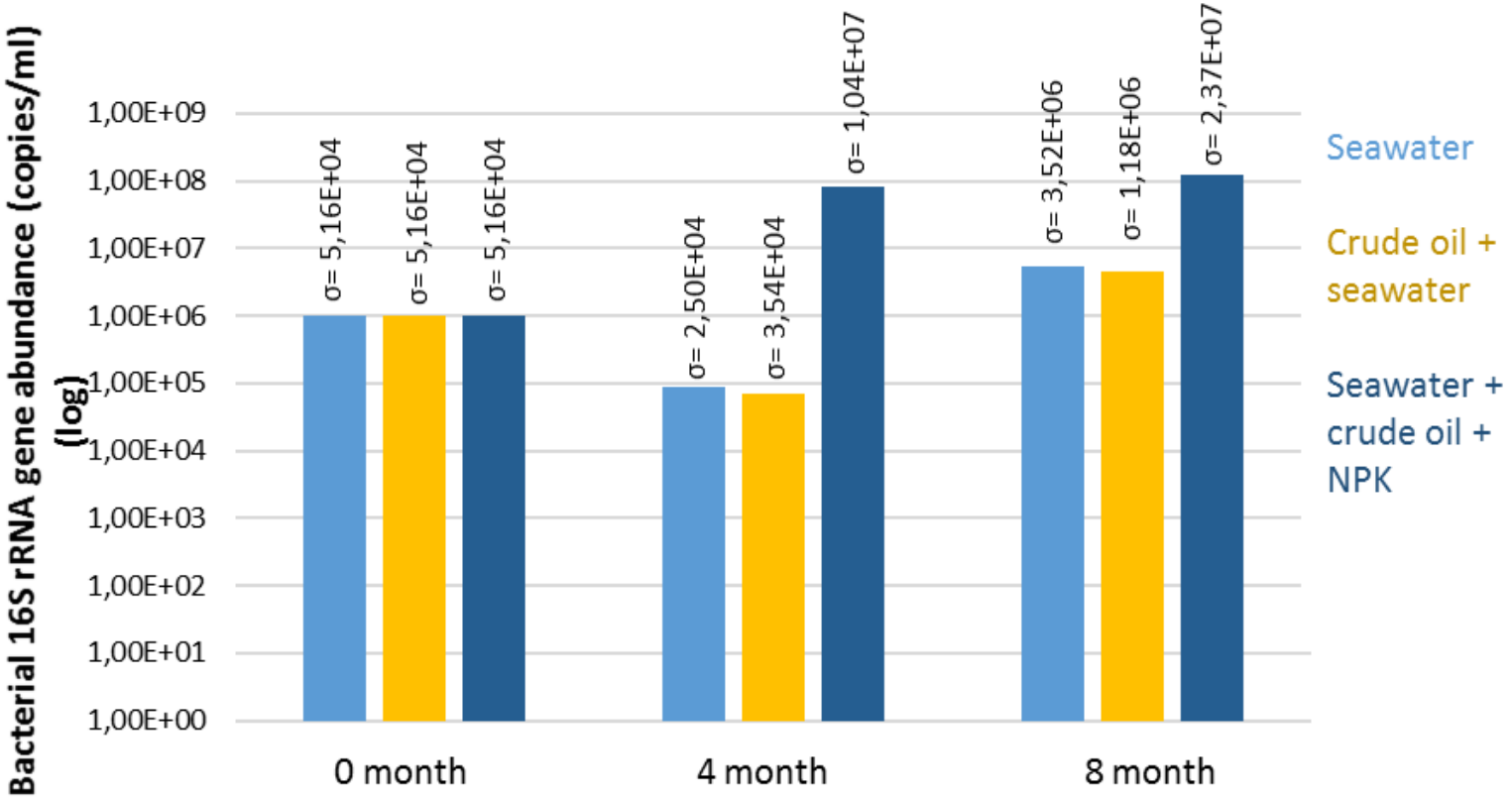
Biodegradation of oil film in seawater

- Biodegradation rate
- Effect of biostimulation
- Microorganisms



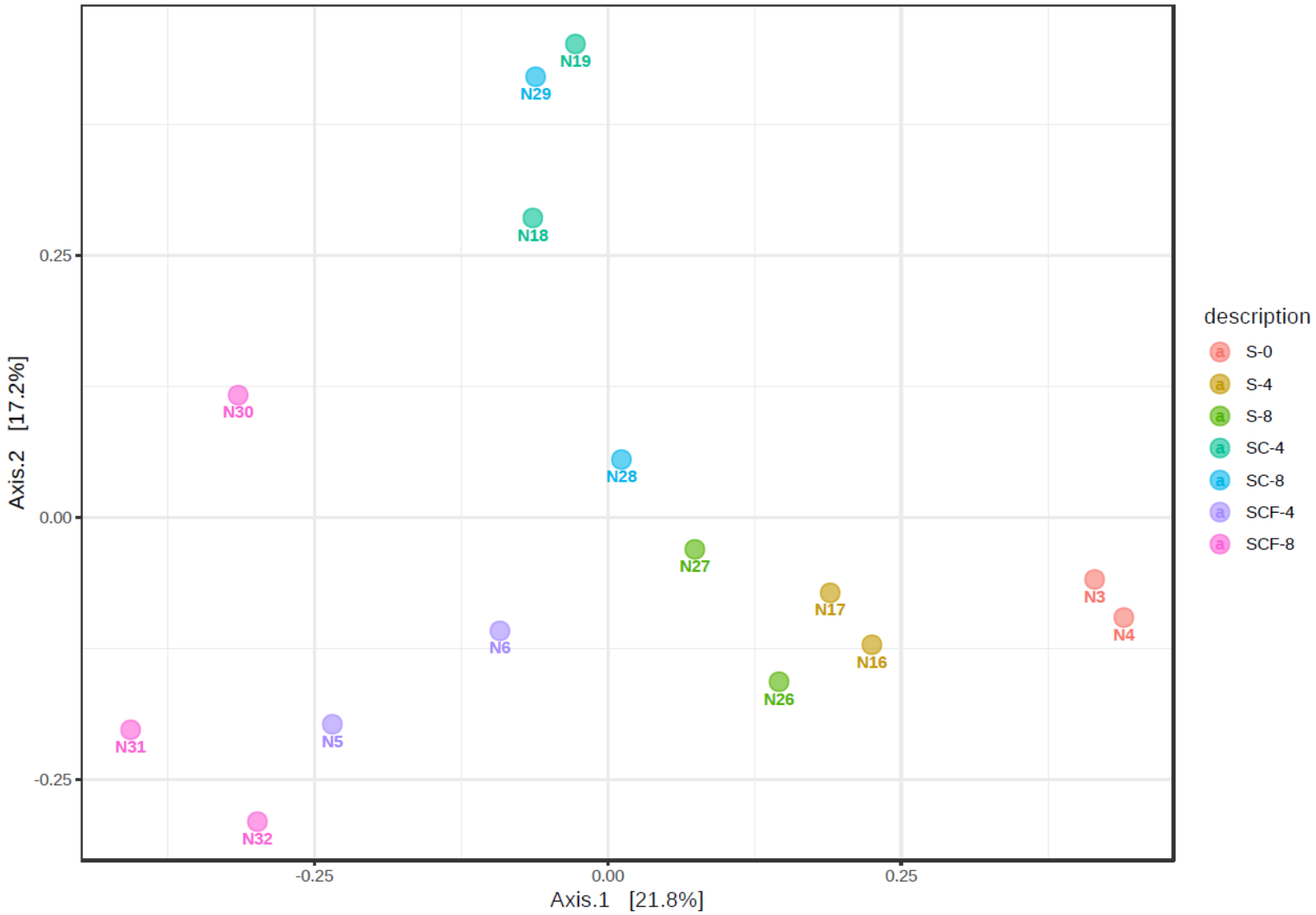
Seawater microcosms 4 °C, 8 months
1 %wt crude oil

16S rRNA copy number in seawater microcosm



Effect of nutrient addition

Principle component analysis of bacterial communities



Hydrocarbon reduction & microbial community change

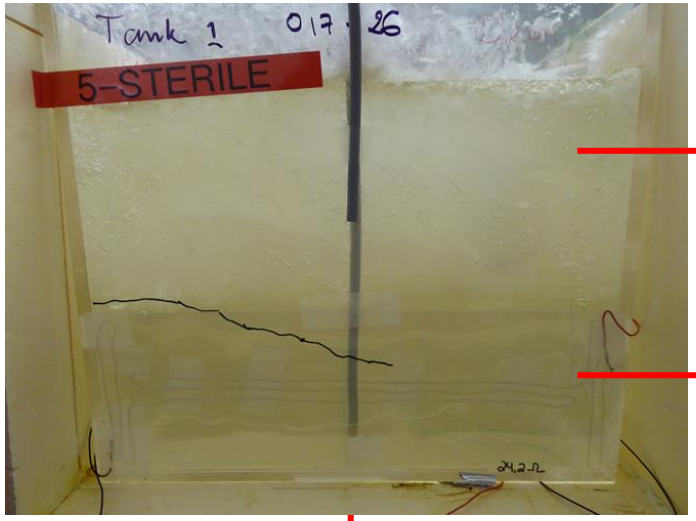
Hydrocarbon group		Oil addition		Oil and fertilizer		Control no oil	
		4 months	8 months	4 months	8 months	4 months	8 months
Reduction of C17/Pristane or C18/Phytane ratio		–	+	+	+		
2 ring PAHs		68.3	77.6	90.4	95		
3 ring PAHs		19.8	26	18.9	59		
4-5 ring PAHs		23	22.4	18.8	22.4		
Bacterial group							
Flavobacteriaceae unclassified	Bacteroidetes	52	32	14	10	10	7
Ulvibacter		3	<1	12	2	3	<1
Aequorivita		<1	<1	3	9	<1	<1
Jejudonia		<1	12	<1	<1	<1	1
Total of Bacteroidetes		**55**	**44**	**29**	**21**	**13**	**8**
Gammaproteobacter unclassified	Gammaproteobacteria	<1	3	<1	9	<1	2
Marinomonas		3	5	13	4	2	<1
Paraperlucidibaca		<1	<1	3	14	<1	<1
Cycloclasticus		<1	<1	<1	6	<1	2
Pseudomonas		1	<1	8	1	<1	<1
Colwellia		<1	8	<1	1	<1	3
Total of Gammaproteobacteria		**4**	**16**	**24**	**33**	**2**	**7**
Pacificibacter	Alphaproteobacteria,	4	<1	1	<1	2	<1
Sphingorhabdus		<1	<1	4	4	1	1
Sneathiella		1	<1	3	3	<1	<1
Hyphomonas		3	1	0	0	0	0
Total of Alphaproteobacteria		**8**	**1**	**8**	**7**	**3**	**1**

Reduction of hydrocarbons

Bacterial groups abundance ≥ 10%

Bacterial groups abundance ≥ 3% and increased vs control no oil

Mesocosm WAF+Finasol 51
Narvik seawater

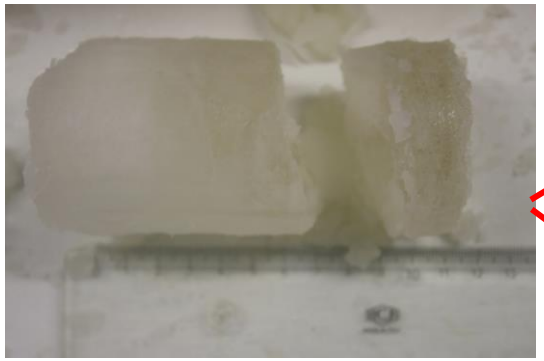


-2 to -10°C

-1 to -2°C

Aquaeous phase

Ice growth



DNA

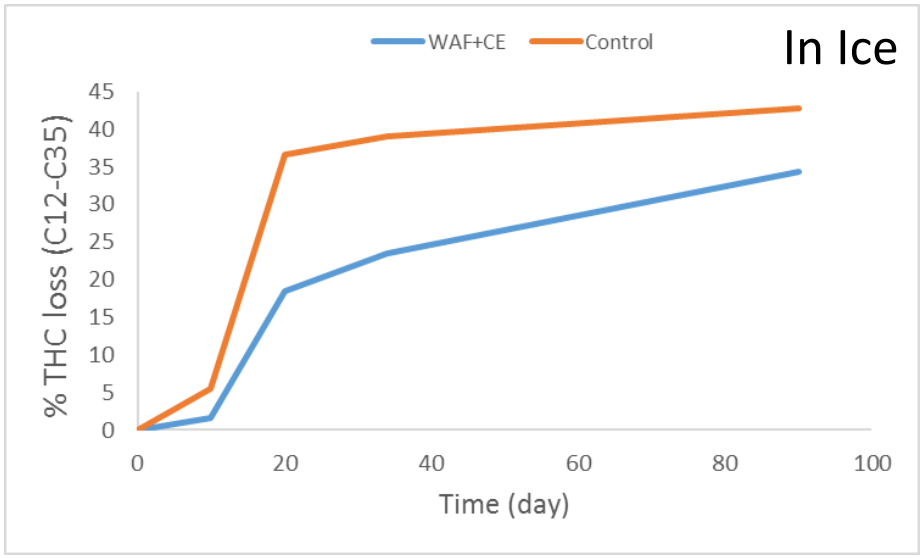
Oil analysis

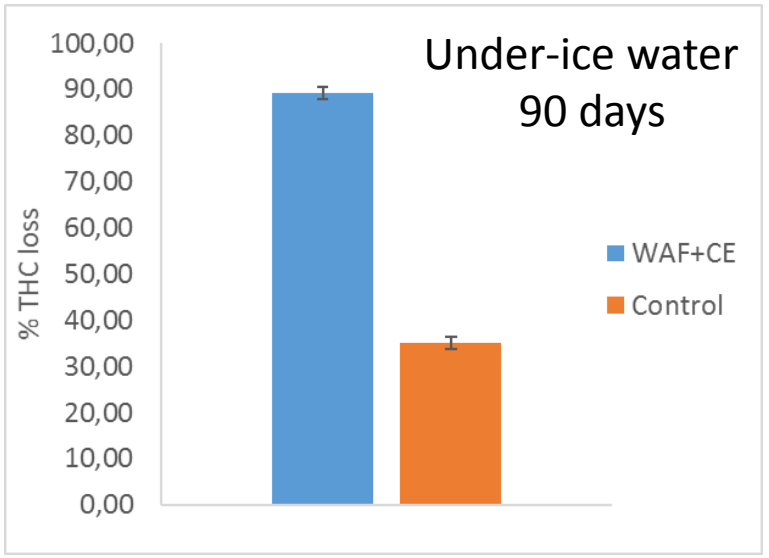
Preparation WAF + Finasol 51

10: 1 (Oil : dispersant)
1g oil/1l seawater

THC reduction in ice & under-ice water

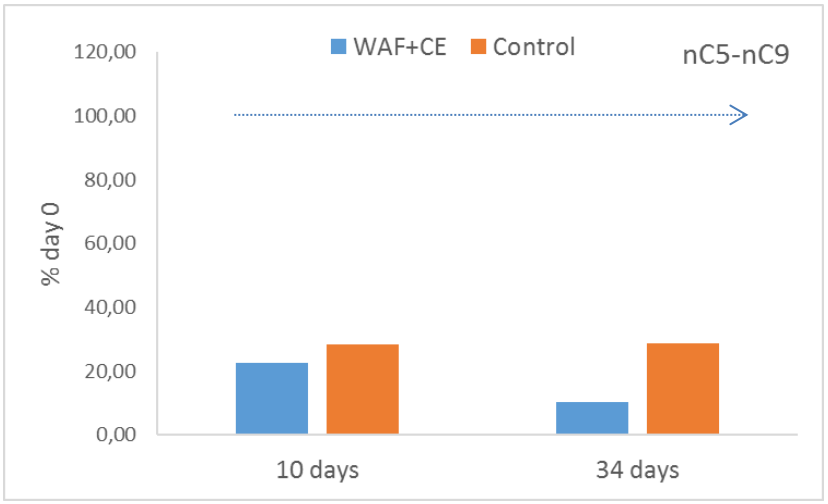
THC 25 mg/l

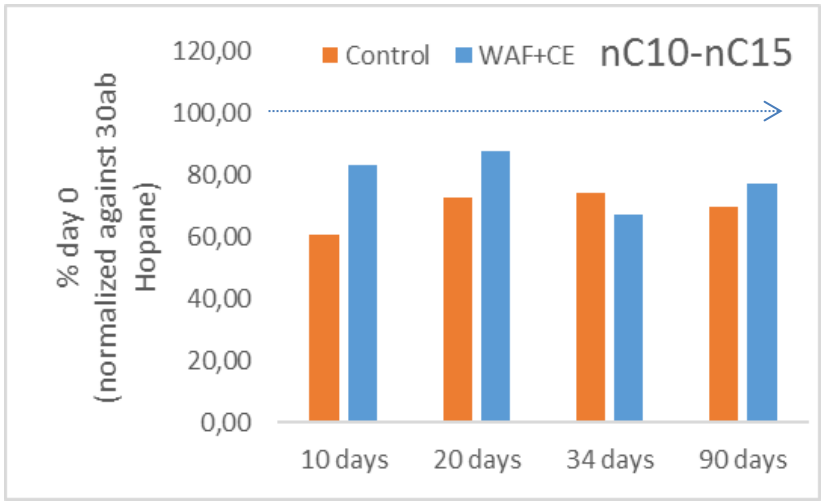


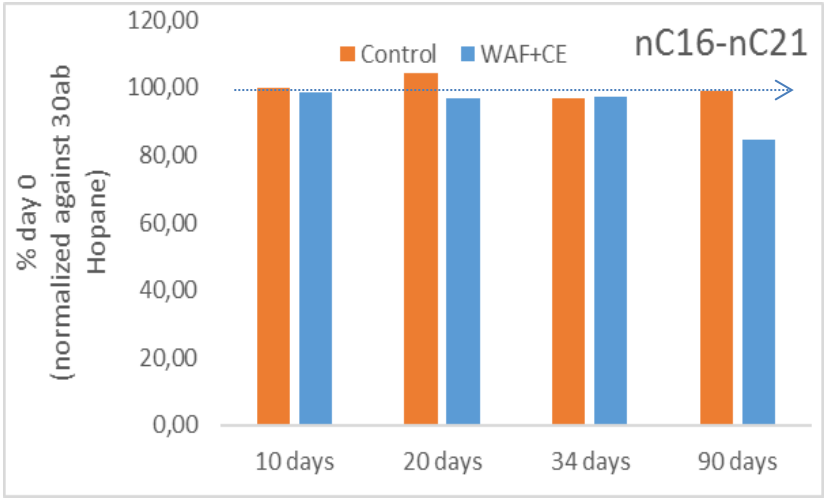


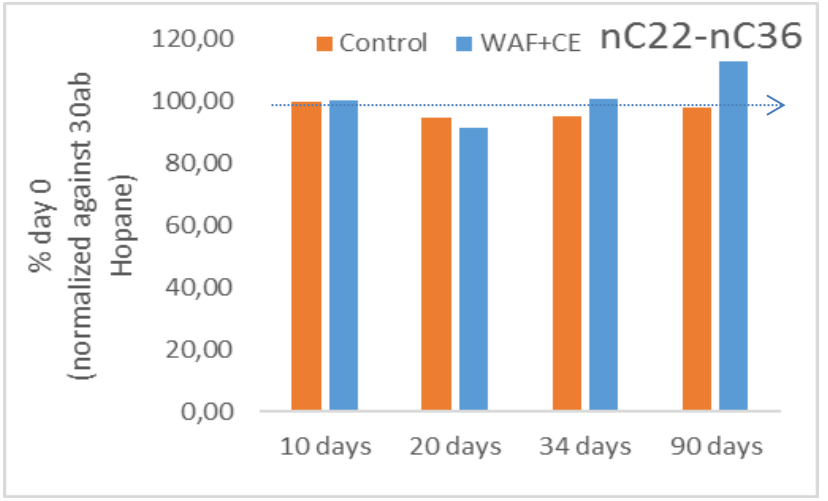
90% loss in under ice water
35-45% loss in the ice

n-alkane reduction in ice

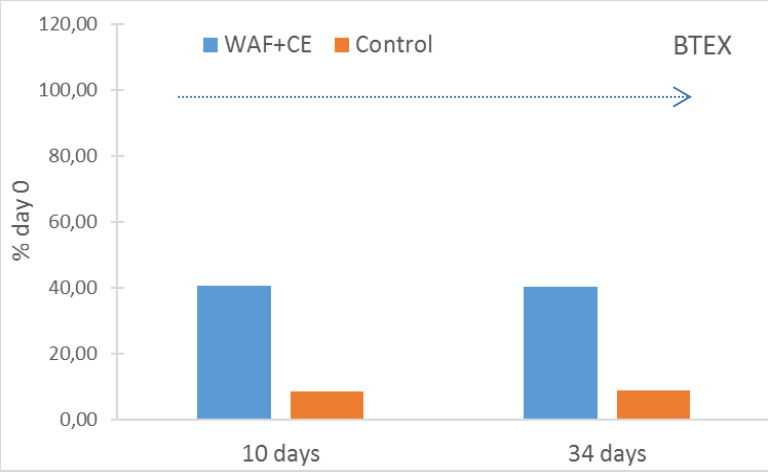


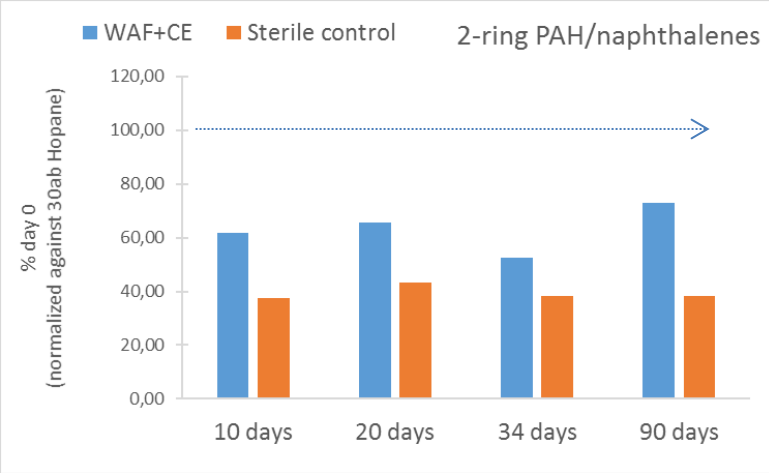


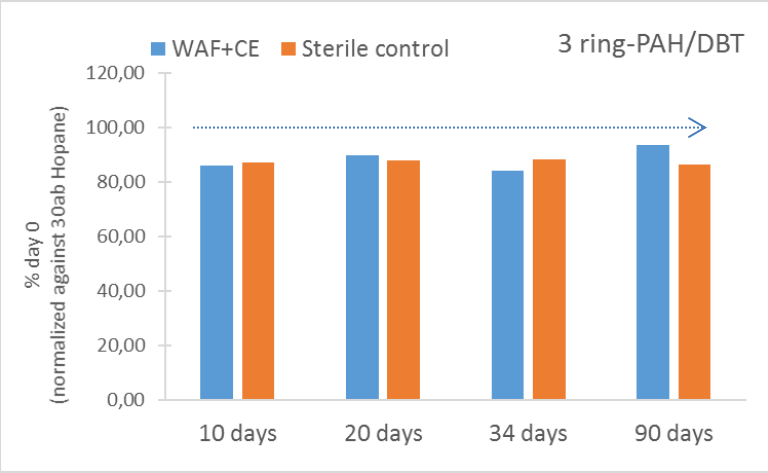


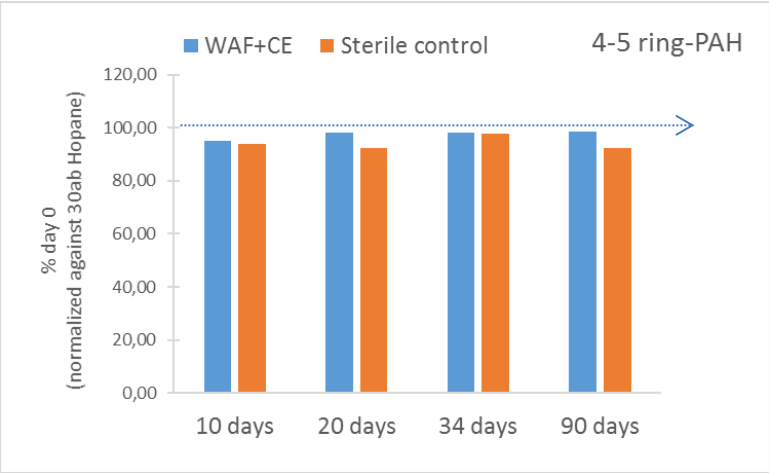


PAH reduction in ice



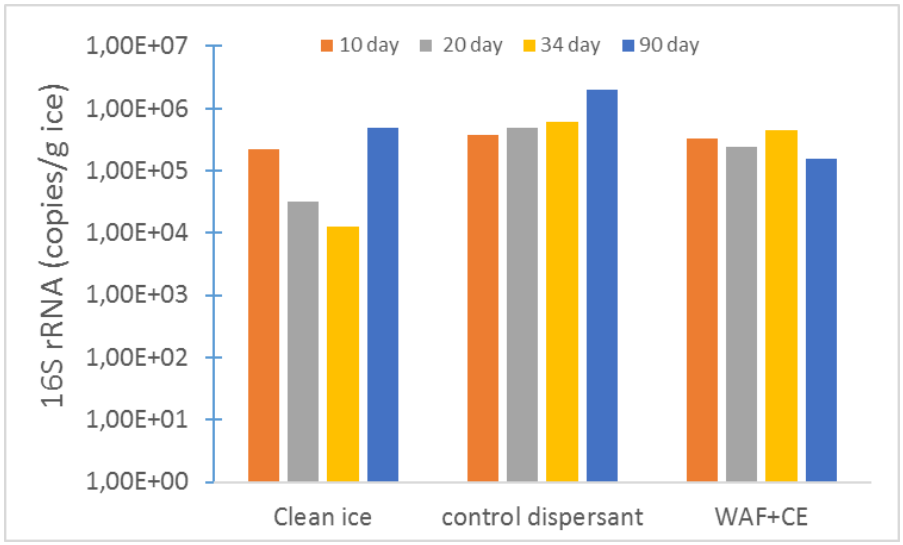




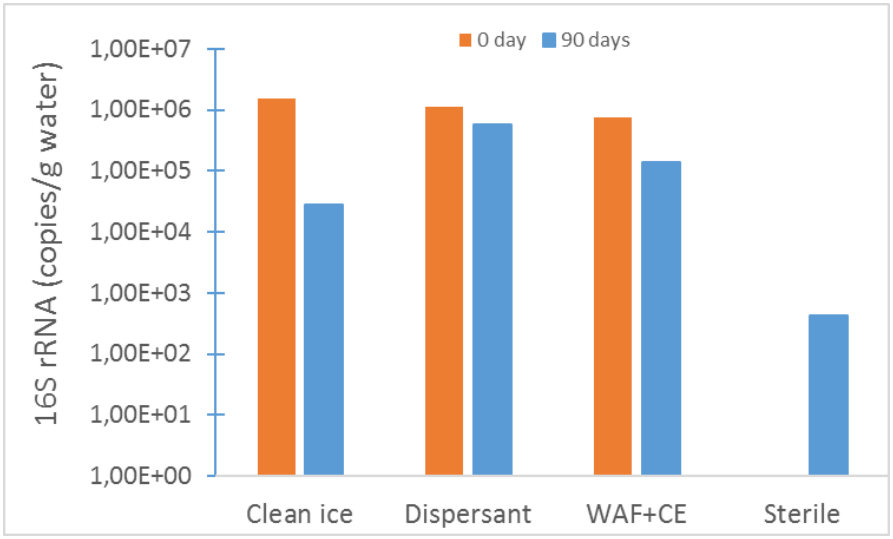


Bacterial 16S rRNA copy

In the ice



Under ice water



- ✓ Increase bacterial abundance in presence of oil
- ✓ Stimulation effect of dispersant

Biodegradation of oil film in sawater

- Biodegradation of oil occured in seawater even at low temperature (4 to -2°C).
- Inorganic fertilizer stimulated the bacterial growth & biotransformation of low-molecular weight PAHs, n-alkanes.
- Bioavailability is important limiting factor (dispersed vs non-dispersed)
- *Bacteroidetes* (*Flavobacteriaceae)* were abundant in oil film microcosms
- *Bacteroidetes* (*Flavobacteriaceae* and *Ulvibacter*) and *Gammaprotebacteria* (*Marinomonas* and *Pseudomonas*) were abundant in fertilizer amended microcosms
- **Hydrocarbon biodegradation pathway?**

Oil encapsulated in ice & Dispersed oil in ice

Oil encapsulated

- No significant loss of oil which is encapsulated in ice
- Reduce abundance of some bacteria
- Increase of hydrocarbon-degrading bacteria
- **Increase copy number of functional genes?**

Dispersed oil

- Dispersed oil is biodegraded in the under-ice water at -2°C
- Significant loss of n-alkanes (C5-C15), BTEX, 2 ring-PAHs in the ice, but not of n-alkanes (C16-C36), or HMW-PAHs
- **Contribution of biodegradation?** (quantify genes related to hydrocarbon-degradation, analysis of microorganisms community involved in biodegradation of oil in ice)

Contributors

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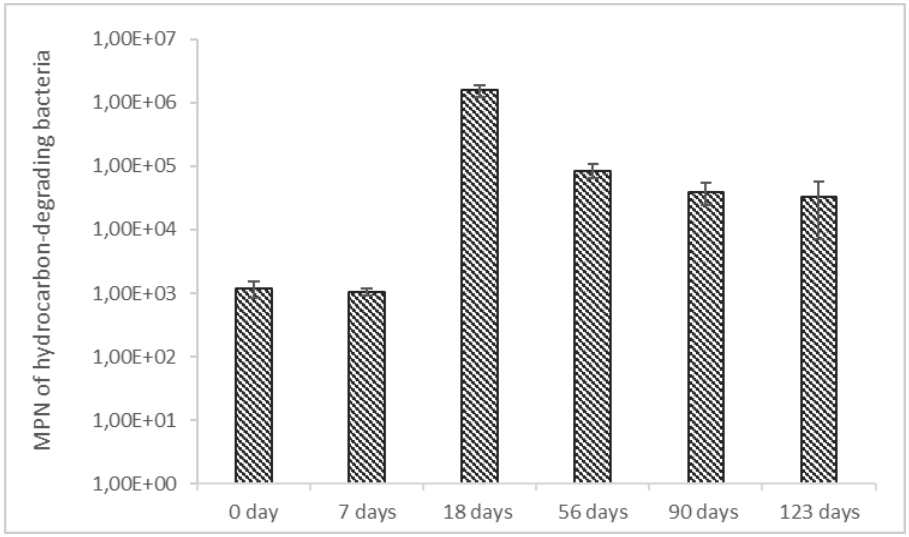
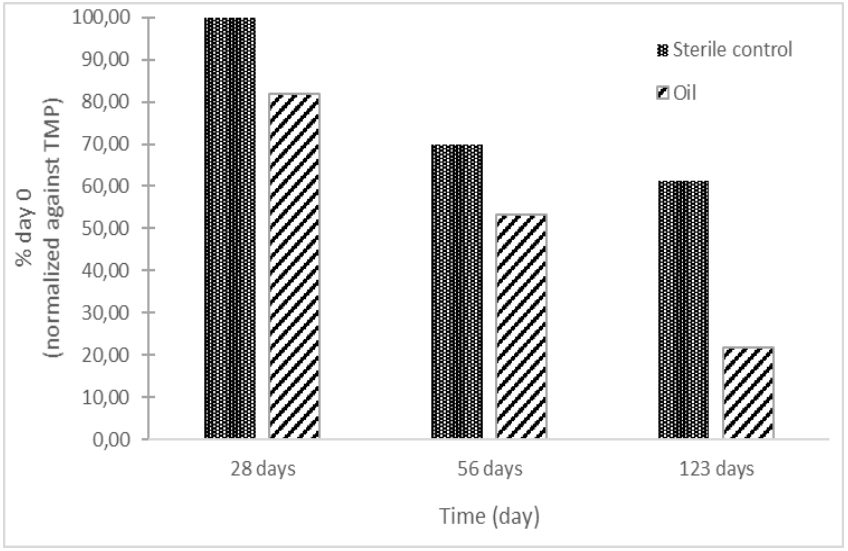
Biodegradation of dispersed diesel at -2 °C

Abundance of oil-degrading bacteria

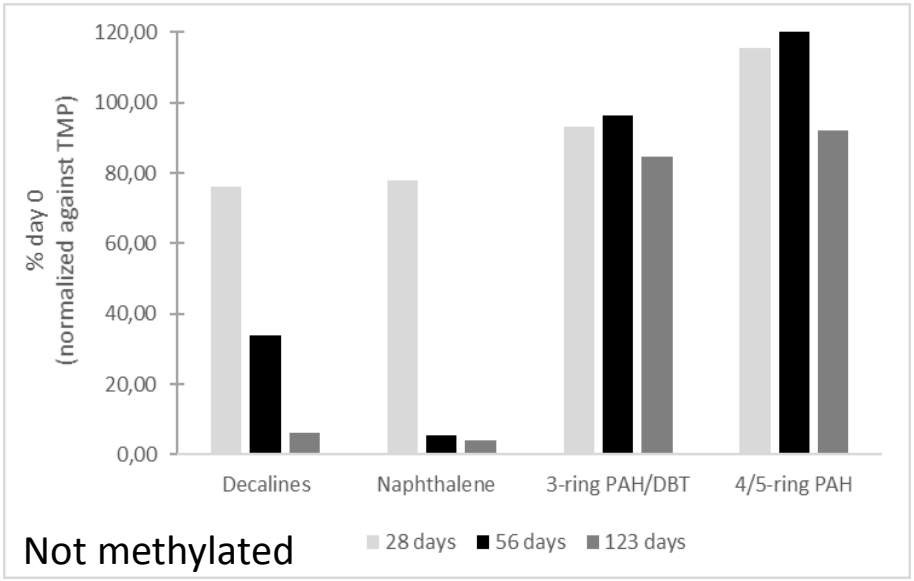


(2 ppm)

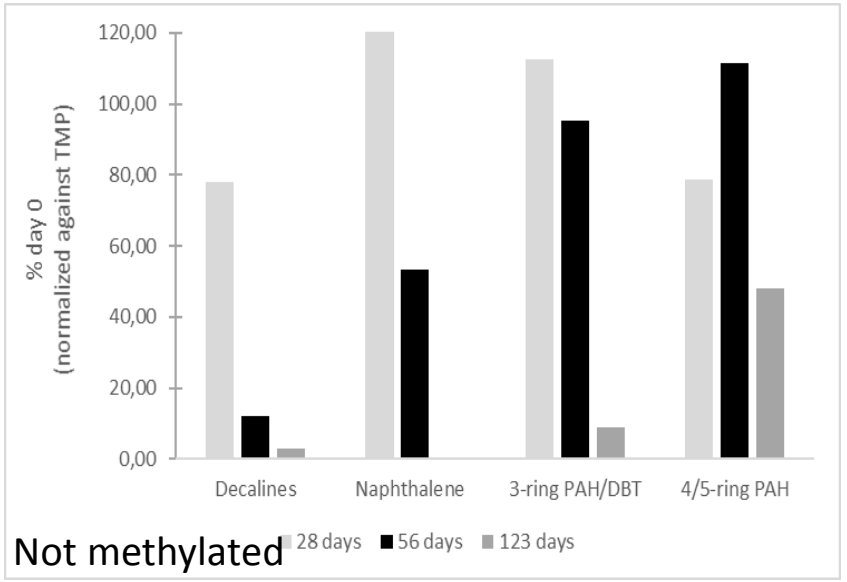
n- alkanes reduction



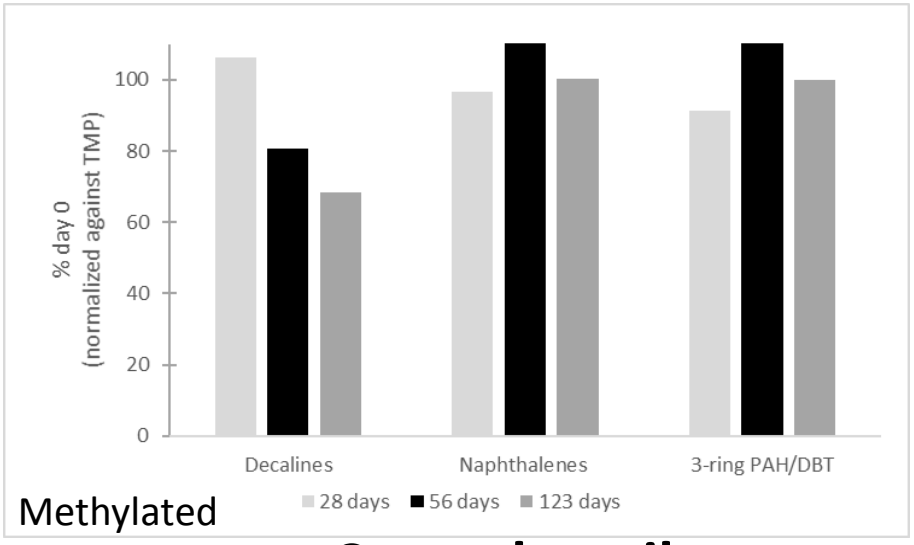
Biodegradation of dispersed diesel at -2 °C



Not methylated

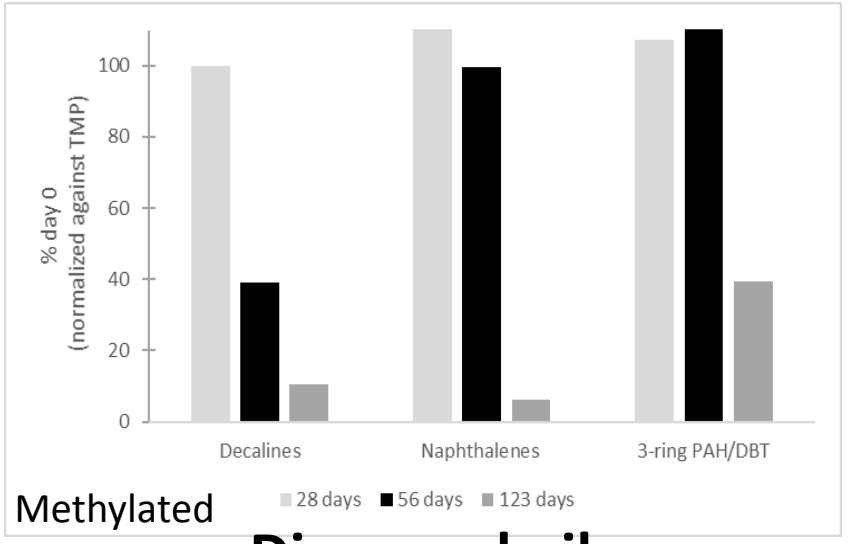


Not methylated



Methylated

Control sterile



Methylated

Dispersed oil