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Oil-in-Ice: Transport, Fate, and Potential Exposure

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ABSTRACT

This document provides a detailed work plan for the project, data collection and analysis methods, and division of responsibilities by task.

The project is split into three main activities:

1. Behavior (transport and physical fate) of oil hydrocarbons in ice;
2. Biodegradation of crude oil hydrocarbons in ice, with focus on the brine channels/inclusions; and
3. Modeling of behavior and biodegradation of crude oil hydrocarbons in ice (prototype model)

Procedures for all laboratory and data analysis methods are described.

KEYWORDS	ENGLISH	NORWEGIAN
GROUP 1	Oil spill	Oljeutslipp
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1 Project abstract

A transport/exposure laboratory study is suggested to determine how ice growth conditions affect the transport and fate of entrapped oil in ice. Quantitative data on the partitioning of oil (dissolved, particulate oil) components (bio-available fractions) into brine inclusions and channels, and rates of vertical transport, will be collected. Since biodegradation of petroleum hydrocarbons at subzero temperatures in marine ice has not yet been shown, it will be essential to determine *if* crude oil biodegradation takes place in marine sea ice within a defined span of time and to what extent. If so, the contribution of biodegradation to the depletion of hydrocarbons in comparison to other depletion processes will be quantified. Targeted analytes will include polycyclic aromatic hydrocarbons (PAHs) and BTEX compounds, as well as decalines and phenols.

The study directly addresses the need for exposure and injury assessment tools for oil spills in cold climates. The use of passive samplers is a fast and cost-effective method to detect PAHs, one of the most toxic groups of compounds present in oil. In this proposal, we suggest advancing the use of two different passive samplers as a tool to detect PAHs from oil spills in ice cores. The two types of passives samplers being considered are polyethylene (PE), and solid-phase micro-extraction (SPME) fibers. They will be used to detect the transport and fate of oil-derived PAHs in ice cores. The passive sampler testing methods will be established by the University of Rhode Island.

In a combination of laboratory and field studies, performance reference compounds will be included in the polyethylene matrix to enable their use as kinetic samplers and shorten deployment time in the field. In flow-through exposures using Narragansett Bay water, deployment will be undertaken to verify the use of the passives samplers to reflect dissolved concentrations as either equilibrium or kinetic samplers. Finally, in simulated oil spills in ice cores in the laboratory, dissolved concentrations of oil components will be detected using the passive samplers. The developed passive samplers will enable the oil spill community to deploy passive samplers to measure baseline conditions before a spill, as kinetic samplers during a spill and during the recovery phase of the natural ecosystem.

The findings from the laboratory experiments will be used in the development of an oil-in-ice sub-model. In contrast to most other recent and on-going work at the macro-scale, this project will start at the micro- (roughly mm to cm) and meso-scale (~ cm to m or greater).

The development of the numerical model associated with this project will integrate knowledge, understanding, and data derived from other tasks within this project and from earlier work by other investigators, into an internally consistent and relatively comprehensive numerical framework. The goal is to produce a dynamic module focused on micro- and macro- physical scales, built up as much as possible from first principles, to serve as a building block in an eventual large scale model of ice dynamics. The available level of funding is insufficient to complete the modeling work, in addition to all the necessary laboratory work. We therefore propose to produce a prototype model at the end of the first year, and seek additional funding to complete the model calibration, testing and documentation during the second year.

2 Project Organization and Responsibilities

Liv-Guri Faksness will be project manager and primary contact between SINTEF and CRRC. Alf mG. Melbye will have primary responsibility for quality assurance regarding all deliverables from the project.

Table 1. Responsibilities by task

Position	Participant	Responsibilities
Principal Investigator	Liv-Guri Faksness	Design, planning, coordination, data analyses, reporting, responsible for the behavior activity
Co- Principal Investigator	Odd Gunnar Brakstad	Planning, coordination, data analyses and reporting of biodegradation activity
Co- Principal Investigator	Mark Reed	Planning, coordination, model development, testing, reporting
Participant	Per Johan Brandvik	Laboratory equipment design on behavior activity
Participant	Øistein Johansen	Design, model development
QA/QC	Alf G. Melbye	Quality control

All three principal investigators have been involved in oil spill research for many years:

Dr. Faksness has been working for several years with weathering and behavior of oil in Arctic and ice-infested seawater, and with chemical characterization and toxicity testing of oil compounds in seawater. She defended her doctoral theses in 2008 with the title “Weathering of oil under Arctic conditions. Distribution and toxicity of water soluble oil components dissolving in seawater and migrating through sea ice. A combined laboratory and field study”.

Dr. Brakstad has been responsible for biodegradation and microbial community studies related to oil spills in cold seawater and marine ice.

Dr. Reed and Dr. Johansen have a long history in oil spill model development, including the use of laboratory and field data to develop numerical algorithms, including the well-known SINTEF oil weathering methodology and Oil Weathering Model.

Dr. Per Johan Brandvik received his PhD in 1997 with a thesis on multi-variate analysis for optimization of oil spill dispersants for weathered oils, and will be a key contributor in design of laboratory equipment used in this study.

Alf G. Melbye is Research Manager for the Fate and Effect Group within SINTEF Marine Environmental Technology. He has been working in the field for more than 15 years.

3 Project Methods and Approach

The project will be split into three main activities:

1. Behavior (transport and physical fate) of oil hydrocarbons in ice;
2. Biodegradation of crude oil hydrocarbons in ice, with focus on the brine channels/inclusions; and
3. Modeling of behavior and biodegradation of crude oil hydrocarbons in ice (prototype model)

Laboratory findings from the transport and biodegradation experiments will be used as input data to the model.

3.1 Oil

A North Sea crude oil (Statfjord) will be used in all laboratory the experiments

This oil has the following characteristics:

Categorization: Paraffinic

Pour point (°C): -6

Density (g/ml): 0.828

Viscosity (cP): 292

3.2 Behavior experiments

3.2.1 Establishment of laboratory methods for freezing columns

When working with ice in the laboratory it is important to have realistic properties with respect to the distribution of dissolved components from the oil in the ice. A setup with a series of up to 8 columns for ice core freezing has to be established for this purpose as a part of the project (illustrated in Figure 1). The columns will be operated in a temperature controlled room (at -20 °C) and will be insulated and with heating panels placed in the bottom. The columns will be constructed to simulate the freezing of first year sea-ice to quantify changes in dissolved oil component concentrations over time through freezing and thawing cycles. Column height will be designed such that salinity of under-ice water during growth will be limited and of no significant impact on ice growth and brine fluxes (as verified by salinity and ice micro-structural analysis). The columns will be filled with natural seawater, pre-filtered through a sand filter. If there is an effect of biodegrading the oil components in the ice, the findings from the experiments described in 3.3 will give us an indication of the extent and rate.

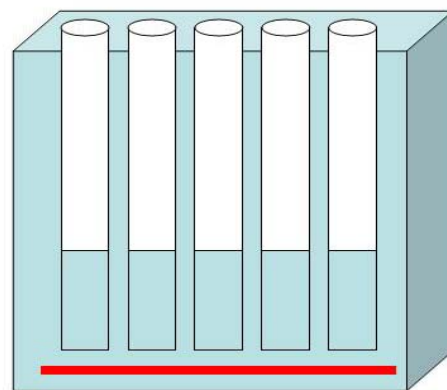


Figure 1. Illustration of the equipment for freezing ice cores in the laboratory

Column material and size

Transparent poly carbonate cylinders with an inner diameter of 144 mm and with a height of at least 80 cm will be used. The column size is chosen to minimize wall effects on brine channel development and to limit the increase of salinity in the water during freezing, while providing a suitable matrix volume for low-level organic chemistry analysis.

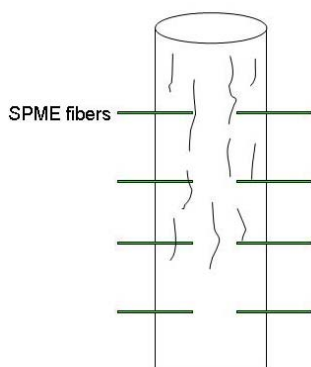


Figure 2. Possible experimental approach for SPMEs in ice core columns.

Introduction of oil into ice columns

An oil film thickness of 3 mm will be introduced into the ice. Oil application: A hole will be drilled from the top, halfway through the ice before the oil is injected into the ice. “Slush” from the drilling will be used to cover the oil. This method to apply the oil has been used with success in field experiments on Svalbard.

Testing of SPME fibers with ice columns

The analytical method for using SPMEs at low temperatures and at varying salinity developed by URI will be used in this work. SINTEF will test sampling with the SPME in the ice columns, for example as scheduled in Figure 2: Four SPMEs positioned at e.g. four levels in the ice and one in the water under the ice for on site sampling. Sample one SPME at all levels at each sampling time. The advantage will be that we do not have to sacrifice the column at sampling, but can

follow the behaviour of oil in the same column through the whole experimental period.

3.2.2 Laboratory experiments

Freezing of columns

The experiment will be conducted at -20 °C during which a layer of ice will be established (20-30 cm). It is assumed that it will take in the order of one week to generate 20-30 cm thick ice. Oil will be introduced into the columns, and then ice will be allowed to encapsulate the oil and grow slowly in order to replicate the brine channel networks. The ice growth will be monitored closely by thermistor strings positioned at different depths in the ice for temperature readings during the experiments. Salinity and density measurements will be performed after whole ice cores are sampled. To simulate spring thawing, the temperature is increased to enhance the size of the brine channels and increase the transport and migration of oil phases in the ice. The total length of the experiment (freezing and later simulation of spring thawing) is expected to be 8-10 weeks. However, establishing the freezing-thawing procedure is one of the main activities in phase-I (see below).

The experiments will be performed in three phases (Table 2 gives an overview of the experiments, and phase II and III are described in Table 3 and Table 4):

- Phase I (Table 2): Testing and establishment of freezing and thawing cycles/times with different temperature regimes. The application method for oil and the sampling techniques will also be implemented, including the initial testing of SPMEs (insertion and sampling).
- Phase II (described in Table 3): Different sampling techniques will be compared together with one reference ice core (column 1) with temperature logging and for characterization of ice properties. The established sampling technique with cutting the ice core into sections before melting followed by liquid-liquid extraction and SPME passive sampling will be compared. Three replicate experiments (columns) with SPMEs will be performed.
- Phase III (described in Table 4): Replicate experiments using different sampling techniques.
 - Column 1: Reference ice with temperature logging and for characterization of ice properties

- Columns 2 to 4: Three replicate columns will be cut and melted for liquid-liquid extraction (to estimate standard deviation). Sampling time depends on findings from phase II.
- Column 5: Reference ice core (no oil applied) for background level measurements using SPMEs
- Columns 6 to 8: Three replicate experiments using passive samplers (if successful in phase II) are planned.

Table 2. Suggested experimental design for oil behavior experiments. The results from one phase will be evaluated before a detailed plan for next phase is made.

	freezing temp °C	time (to generate ice) days	expected ice thickness cm	oil film thickness mm	Description
Phase I	-20	7-14	20-30	no oil	Testing of freezing/thawing temperatures
Phase II	-20	7-14	20-30	3	Dependent of findings in phase I
Phase III	-20	7-14	20-30	3	Dependent of findings in phase I and II

Table 3. Suggested sampling intervals methods for phase II: Comparison of different sampling techniques. The freezing/thawing cycles are based on the finding from phase I.

	Oil application	sampling intervals (after oil application)				
		1	2	3	4	
Col 1	no oil				x	Reference and temperature logging (characterize ice properties)
Col 2	oil	x				The whole column, cut in sections and melted
Col 3	oil		x			The whole column, cut in sections and melted
Col 4	oil			x		The whole column, cut in sections and melted
Col 5	oil				x	The whole column, cut in sections and melted
Col 6	oil	x	x	x	x	Column with passive sampler
Col 7	oil	x	x	x	x	Column with passive sampler
Col 8	oil	x	x	x	x	Column with passive sampler

Table 4. Suggested sampling intervals and sampling methods for phase III: Replicate experiments.

	Oil application	sampling intervals (after oil application)				
		1	2	3	4	
Col 1	no oil				x	Reference and temperature logging (characterize ice properties)
Col 2	oil				x	The whole column, cut in sections and melted
Col 3	oil				x	The whole column, cut in sections and melted
Col 4	oil				x	The whole column, cut in sections and melted
Col 5	no oil	x	x	x	x	Reference ice core (blank), passive samplers
Col 6	oil	x	x	x	x	Column with passive samplers
Col 7	oil	x	x	x	x	Column with passive samplers
Col 8	oil	x	x	x	x	Column with passive samplers

3.2.3 Sampling and analysis

Both the established sampling technique with cutting the ice core into sections before melting followed by liquid-liquid extraction and in situ SPME passive sampling will be used. Usually the SPME is placed vertically into the matrix, but we want to try to insert it horizontally into the ice core and let the brine pass it. A so-called “fiber in needle” SPME device with PDMS (polydimethylsiloxane) fiber (100 μm film thickness) cut in appropriate length will be tested. As far as we know, the approach with in situ SPMEs in ice is not published by others. In the initial experiments both whole ice core column sampling and SPME sampling will be performed. Suggested experimental design, replicates, and sampling intervals are given in Table 2 to Table 4.

Analyses of ice cores:

- Characterization of ice properties: Temperature measurements in the ice through the experimental period, ice micro-structural characterization (on selected samples) of and salinity and density measurements of melted ice sections.
- Liquid-liquid extraction of the melted ice cores: Screening analysis by GC/FID and single component analysis by GC/MS of decalines, phenols, naphthalenes and PAH compounds (given in Appendix A)
- Purge-and-trap techniques for GC/MS analysis of BTEX in select samples (underlying water and melted ice, no extraction) might also be included
- Analysis of SPMEs: Extraction and analysis are described in the “Grant Funding Application” and will be developed by Dr. Lohmann and his project team at URI.
- Compare quantitative results from SPME samples and extracted ice core samples.
- Inter-laboratory comparison (URI-SINTEF) of quantitative results from SPMEs extracts: SPME’s extracts from ice cores sampled in phase II will be split in two and analyzed by both laboratories. The remaining samples will be analyzed by SINTEF.

The experiments will give detailed quantitative data of water soluble oil components in the ice and the rate of vertical transport through the ice. They will provide concentrations of oil components migrating through the ice as the ice is subject to different freezing-thawing cycle. How the thawing conditions affect the transport and fate of entrapped oil in ice will be observed. Knowledge of the exposure concentrations and composition to which ice associated organisms can be exposed can be used to estimate toxicity (e.g. as described in Faksness and Brandvik, 2008). All findings will be used as input to the oil-in-ice sub-model (described in section 3.4).

3.2.3.1 Sample preparation

The ice sections will be melted in closed containers at 10 °C. The volume of the melted ice is measured and the water sample will be spiked with surrogate internal standards (SIS, *o*-terphenyl, naphthalene- d_8 , phenanthrene- d_{10} , chrysene- d_{12} , phenol- d_6 , 4-methylphenol- d_8) and serially extracted with dichloromethane (Modified EPA Method 3510). The combined extracts are dried with sodium sulfate and concentrated to approximately 1 mL using a Zymark Turbovap® 500 Concentrator. The final extract is spiked with the appropriate recovery internal standards (RIS, 5 α -androstane, fluorene- d_{10} , and acenaphthene- d_{10}) and will be analyzed by gas chromatography/flame ionization detection (GC/FID) and gas chromatography/mass spectrometry (GC/MS). The SIS and RIS are added to the samples to monitor procedural efficiencies on a sample-by-sample basis and to allow for accurate determination by internal standards. The results are reported as μg component/L melted ice (ppb).

3.2.3.2 Chemical analyses

The samples are analyzed for SVOC (PAHs and phenols) using gas chromatography/mass spectrometry (GC/MS), for TPH using gas chromatography/flame ionization detection (GC/FID), and for volatile organic compounds (C₅-C₉), including BTEX (benzene, toluene, ethylbenzene, and xylenes), by Purge and Trap Gas Chromatography Mass Spectrometry (P&T GC/MS). A list of the target analytes is shown in Appendix A.

Analysis of total petroleum hydrocarbons (TPH)

GC/FID analysis will be performed according to a modification of EPA Method 8100. TPH (resolved plus unresolved TPH) is quantified by the method of internal standards using the baseline corrected total area of the chromatogram and the average response factor for the individual C₁₀ to C₄₀ n-alkanes. If the sample contains high amounts of dispersed oil, an external calibration curve using Statfjord crude oil will be established. A daily calibration of the instrument is performed.

Analysis of semi-volatile organic compounds (SVOC)

The semi-volatiles will be quantified by modifications of EPA Method 8270. The mass spectrometer is operated in the selective ion monitoring (SIM) mode for optimum sensitivity and specificity. Quantification of target compounds will be performed by the method of internal standards using average response factors (RF) for the parent compounds. The PAH and phenol alkyl homologues are quantified using the straight baseline integration of each level of alkylation and the RF for the respective parent PAH and/or phenol compound. The response factors are generated for all targets and surrogates versus fluorene-*d*₁₀. The GC/MS method recommended by OLF (The Norwegian Oil Industry Association) in the guidelines for analysis of produced water (OLF, 2003) will be used for quantifying the alkylated phenols (C₁ to C₄). A daily calibration of the instrument is performed.

Analysis of volatile organic hydrocarbons (VOC)

A total of 35 target volatile analytes in the C₅ to C₁₀ range (Appendix A) will be determined by P&T GC/MS, using a modification of EPA method 8260. The samples are spiked with SIS (toluene-*d*₈ and ethylbenzene-*d*₈), and RIS (chlorobenzene-*d*₅). Quantification of individual compounds is performed by using the response factors of the individual compounds relative to the internal standards. All standards and samples are analyzed in full scan mode. A daily calibration of the instrument is performed.

3.3 Biodegradation experiments

3.3.1 Experimental parameters

Artificial brines

The biodegradation experiments will be conducted as simple laboratory experiments. The conditions of the brines in the first-year marine ice will be simulated at different temperatures with respect to salinity. Artificial marine salts used for salt fish aquaria will be used for salting up normal seawater, resulting in the following temperature-related salinities:

- - 5°C: 100 practical salinity units (PSU)
- -10°C: 145 practical salinity units (PSU)

For other sub-zero temperatures included salinities will be used according to Mock and Thomas, 2005.

Bacterial inocula

Bacterial will be collected from permanently cold seawater at SINTEF's laboratory. This water is collected from a depth of 90 m, with constantly low temperature ($< 10^{\circ}\text{C}$). The seawater will be mixed with synthetic salt mixtures to generate the artificial brines before the experiments (see above). Sterile controls will be included in all experiments to compare biotic and abiotic depletion processes.

Water-soluble compounds

Water-soluble fractions (WSFs) of the crude oil will be generated in artificial brines with natural seawater bacterial communities, or in sterile artificial brine controls. WSFs will be achieved by the well established CROSERF method (Aurand and Coelho, 1996) in large glass flasks (Figure 3) with the following ratios between oil and water-medium:

- 1 part oil to 1000 parts of water
- 1 part oil to 10000 parts of water

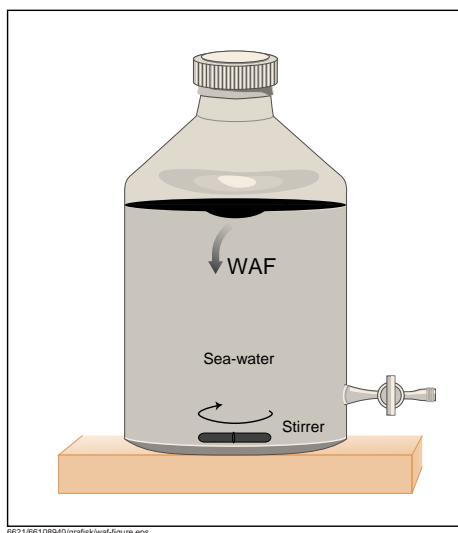


Figure 3. Generation of WSF by the CROSERF method.

WSFs will be generated at a fixed temperature ($0-4^{\circ}\text{C}$) for a period of 96 hours for equilibration. The equilibrated WSFs will be distributed in closed bottles without headspace.

Adsorbed oil

Thin oil films ($10-20\ \mu\text{m}$ thick) will be adsorbed on hydrophobic Fluortex fabric adsorbents ($1 \times 1\ \text{cm}^2$) by generating a thin oil film on the surface of sterile water (Figure 4), the excess oil removed by careful rinsing with sterile water, and the oil-coated fabrics submerged in closed bottles with artificial brines with natural seawater bacterial communities, or in sterile artificial brine controls.

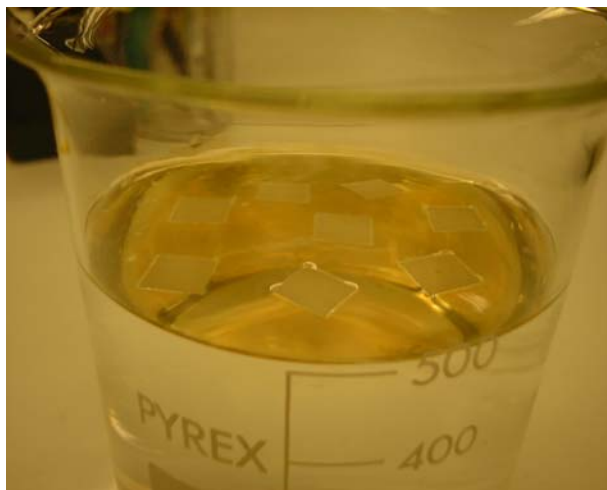


Figure 4. Thin oil film adsorbing to Fluortex fabrics on a bed of sterile water.

Nutrients

For initial investigations of bioremediation potential of oil in ice nutrient mixtures will be amended in some of the experiments as a fertilizer mixture of carbon, nitrogen and phosphorous compounds. The formula of the fertilizer will be generated with a molar ratio between these elements of 100 parts carbon, 5 parts nitrogen, and 0.1 parts of phosphorous.

3.3.2 Experimental design

The experiments will be conducted as simple batch tests in flasks resistant to the low temperatures used and with volumes of 500 ml. Some experiments will also be performed with agitation to include a situation of brine movement. Experimental periods will be for a maximum of 6 months. Series of flasks will be incubated at three different subzero temperatures: -5, -10 and -20°C with seawater as inoculum and dissolved fractions of petroleum or oil immobilized to Fluortex fabrics as hydrocarbon sources. During the experimental periods flasks will be sacrificed for chemical and microbiological analysis.

We propose to perform the project in three separate steps:

- Initial experiment
- Biodegradation experiment 1
- Biodegradation experiment 2

In addition an experiment 3 will be conducted at CRRC

Initial experiments

Initial experiments will be performed to finally decide on the practical experimental design and conductance for biodegradation experiments 1, 2, and 3. During these experiments we will establish a number of procedures, e.g. for brine generation at the three temperatures and for sampling strategies for specific chemical and microbiological analysis. A number of brine characteristics will be analyzed here, as well as the impact on the seawater microbial population.

Biodegradation experiment 1 (BE1)

One major objective of this experiment is to gain experience. BE1 will be conducted at two temperatures, -5° and -10°, with two different oil/water ratios for the generation of WSFs with oil/brine ratios of 1/10000 and 1/1000, and without added fertilizers. Experiments run at -10° serve to verify measurable activity at lower temperatures and inform BE2. Limited reference experiments will also be run at 0, 5°C, and experimental period will last for a maximum of 6 months. All experiments will be run in the dark (no light exposure). Depletion of hydrocarbons will be determined primarily by GC-MS analysis (see below). A suggested setup of the experiment is shown in Table 5. Sterile controls will be included to correct for depletion caused by abiotic depletion. All samples will be analyzed by GC-MS, and selected samples will be collected for microbiological analysis.

Table 5. Suggested experimental setup for biodegradation experiments BE1, with oil/water (O/W) ratios of 1/1000 and 1/10000.

Temp. (°C)	O/W (ratio)	Sampling (weeks)								
		0	1	2	4	8	12	16	20	24
+5	1/10000	X			X	X				
0	1/10000	X			X	X		(X)		(X)
-5	1/1000	X		X	X	X	X	X	X	X
	1/10000	X	X	X	X	X	X	X	X	X
	Adsorbents	X	X	X	X	X	X	X	X	X
-10	1/1000	X					X			X
	1/10000	X					X			X
	Adsorbents	X					X			X

Biodegradation experiment 2 (BE2)

Based on the outcome of BE1, the final design of this experiment will be established. We propose testing a number of parameters, with a limited number of samples for each of these parameters, including, different temperatures (-5, -10 and -20°C) and the influence of nutrients. Only one oil-water ratio will be used for generation of WSFs, based on the results of BE1. The suggested setup of the experiment is described in Table 6. As in BE1, sterile controls will be included to correct for depletion caused by abiotic depletion. The need to run experiments at -20° will be determined based on BE1 results.

Table 6. Suggested experimental setup for biodegradation experiments BE2.

Temperature (°C)	Nutrients	Sampling time						
		0	1	2	3	4	5	6
-5 (static)	+	X	X	X	X	X	X	X
	-			X		X		X
-5 (agitation)	+	X	X	X	X	X	X	X
	-			X		X		X
-10 (static)	+	X	X	X	X	X	X	X
	-			X		X		X
-20 (optional)	+	X		X		X		X
	-					X		X

3.3.3 Analytical methods

Hydrocarbon analysis

Hydrocarbon analysis will be conducted by GC-FID of total petroleum hydrocarbons (TPH) and GC-MS of decalines, phenols, naphthalenes and PAH compounds. We may also include purge-and-trap techniques for GC-MS analysis of BTEX compounds in selected samples. The analytical methods are described above (section 3.2.3.2) and specified in Table 8.

Biodegradation determination

In order to compare approaches for field analyses we will determine various ratios of biodegradable vs. recalcitrant compounds present in the saturated solutions (e.g., comparison of phenanthrene to dimethylphenanthrenes, 2-methylnaphthalene to 1- methylnaphthalene, naphthalene vs. phenanthrene and alkylnaphthalene ratio – MN/(MN+DMN+TMN), as described by Siron et al., 1995. The assumption for the ratios is that biodegradation appear if depletion of a biodegradable compound is higher than for a more recalcitrant are analogue. In that respect it is assumed that phenanthrene is more degradable then dimethylphenanthrenes, 2-methylnaphthalene more degradable than 1- methylnaphthalene, and naphthalene more degradable than phenanthrene.

For GC-FID analyses n-C17/Pristane and n-C18/Phytane will be assessed. These form close peaks in the GC-FID chromatogram. During biodegradation of oil in seawater the n-alkane is depleted before the isoalkane, which also eventually is depleted (see Figure 5).

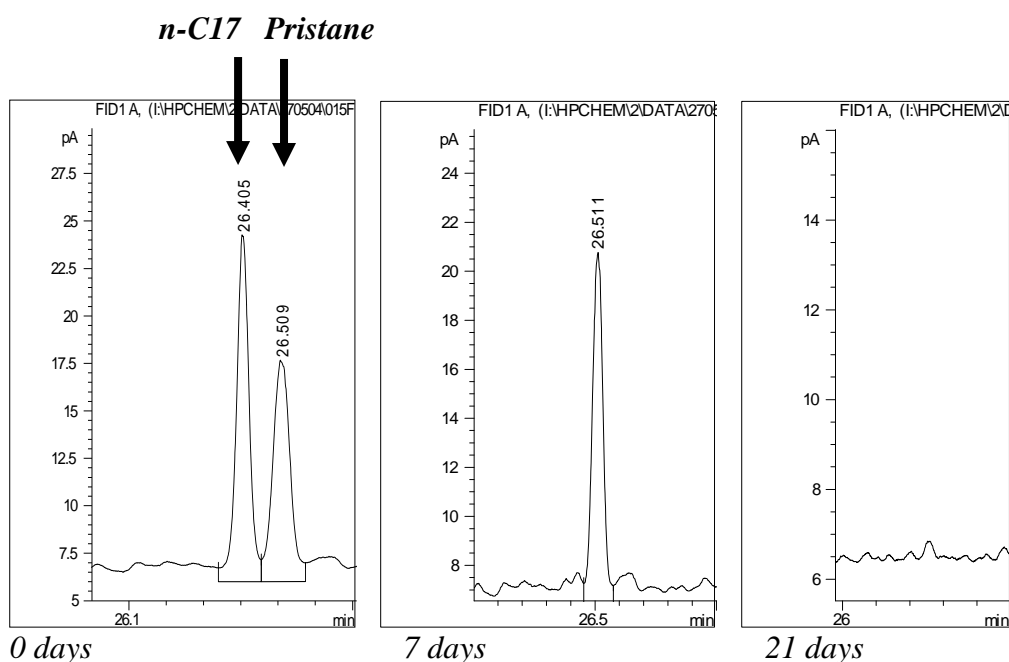


Figure 5 GC-chromatograms for C17/pristan peaks after 0, 7 and 21 days in an immobilised oil in seawater at a temperature of 5°C.

Epifluorescence microscopy

Picoplankton abundance in seawater before and after brine generation, and during biodegradation experiments, will be determined by epifluorescence microscopy. The method is routinely in our laboratory. Briefly, brine samples will be stained by the DNA-staining fluorochrome 4,6-diamino-2-phenylindole by standard procedures in our lab and filtered through black 0.2 µm polycarbonate

filters (Millipore). The filters will be applied to glass slides and analysed by fluorescence microscopy (Leitz Dialux with Ploemopak fluorescence unit and UV-filter A) at 1250x magnification and with oil immersion. Fluorescent cells were counted in 10x10 lattice system and concentration in original samples calculated.

Viable counts of heterotrophic and oil-degrading prokaryotes

Viable counts of bacteria will be performed on 10-fold serial dilutions of samples cultured in Marine Broth 2216 (Difco Labs.), while oil-degrading prokaryotes will be cultured in marine Bushnell-Haas broth (Difco) with 0.1 % crude oil (Statfjord) as carbon source. Methods are used routinely in our laboratory. The broth media will be distributed in sterile 24 well tissue culture plates (Costar) with lids (1.8 ml broth per well). Serial dilutions of samples will applied in triplicate in tissue culture plate wells with media and the plates incubated at low temperatures (5-10°C) for 7-14 days. Growth in each well will be judged visually, while metabolic activity in Bushnell-Haas will be measured after 0.1 mg/ml fluorescein diacetate (Sheen-Green method). MPN-results will be recorded according to tabulated MPN indices for 3 parallels (Standard Methods for the Examination of Water and Wastewater, 1995).

PCR and DGGE

Brine or seawater samples will be filtered through 0.22 µm filters (Sterivex GV filters, Millipore Corp., Bedford, MA, USA), which will be filled with a lysis buffer (50 mM Tris-HCl, pH 8.0; 40 mM EDTA; 750 mM sucrose) and sealed in both ends until nucleic acid extraction. Filters will be stored at -20 °C until further processing. Nucleic acids will then be extracted by a standard hot (60°C) phenol-chloroform-isoamylalcohol method, mainly as described by Sambrook et al., (2001).

Polymerase chain reaction (PCR) will be performed with the universal 16S rDNA bacterial primers 341fBac (sequence 5'-CCT-ACG-GGA-GGC-AGC-AG-3') and 907rBac (sequence 5'-CCC-CGT-CAA-TTC-CTT-TGA-GTT-3'), as originally described by Teske et al., 1996. For DGGE analyses the primer 341f Bac will include a 40 mer GC-clamp added to the 5'-end of the 341fBac primer (sequence 5'-CGC-CCG-CCG-CGC-GCG-GCG-GGC-GGG-GCG-GGG-GCA-CGG-GGG-G-3'). PCR will be conducted in a BioRad thermocycler (iCycler, BioRad Labs.) by standard method of 30 cycles of DNA denaturation (92°C), primer annealing (55°C) and DNA synthesis (72°C), resulting in an expected product of 567 bp. PCR products will be analysed by horizontal agarose gel electrophoresis.

Denaturing gradient gel electrophoresis (DGGE) will be performed with 6 % (w/v) polyacrylamide (PAA) gels in [0.5x] TAE buffer (20 mM Tris-acetat, pH 7.4; 10 mM acetat; 0.5 mM EDTA) with a 20-70 % gradient of the denaturing agents urea and formamide (100 % denaturing agents corresponds to 7 M urea and 40 % (v/v) deionised formamide) in a DCode Universal Mutation Detection system (BioRad). The procedure for preparing gels and running samples are described in the Manual of the DCode system. Vertical electrophoresis is performed with continuous temperature (60°C) and voltage (150 V) until the both markers migrates to the bottom of the gel (approximately 4.5 hours). After electrophoresis the gels will be stained in SYBR Gold (Molecular Probes) and the processed with the Quantity One option of the GelDoc software program (BioRad). For comparison of DGGE patterns between samples dendrograms will be generated according to the Dice Coefficient method of the Quantity One software.

All PCR and DGGE methods are performed as routine methods in our laboratory.

3.4 Numerical Modeling

The goal of this task is to develop and test a software module that can eventually link oil spill models to large-scale coupled sea ice-hydrodynamic models. The module will describe on a very local (mm to m) scale the physical-chemical properties of oil, following the water-soluble components and bulk oil from freezing-in to thawing-out. Key output will be potential exposure of organisms associated with the ice.

The development will occur in parallel with the experimental tasks, which will be coordinated to provide the parameters and datasets necessary for model calibration and testing. The SINTEF and University of Alaska teams will collaborate on the modeling efforts by further development of an existing fluid dynamics model, to be expanded for simulations of two-phase flow, into a more comprehensive model to be developed at SINTEF. The work will progress in stages: Literature review, model design, model development, calibration and testing, reporting, and publication.

The modeling activities are intended to help constrain the experiments and establish their applicability in different environmental settings, and to quantitatively examine transport of dissolved hydrocarbons with the ultimate goal to derive operationally relevant conclusions from these simulations. Thus, the model to be developed will help place the experimental results in a broader context, i.e. show what the implications of the studied processes may be under various scenarios. Implicitly, the model should show that the experiments are particularly suited to shed light on key unknowns. At the same time, the interpretation of SPME data (which are likely a function of the concentration history at that particular horizon in the ice) is not the primary goal, although it would nevertheless be desirable to simulate such data.

A preliminary assessment of the current CFD model (as described in Petrich et al., 2006, with further improvements and modifications) indicates that the fate and transport of dissolved hydrocarbons can be simulated and described with only minor modifications (since the oil phase is largely stationary). This lays the groundwork for a significant part of the modeling activities which can then focus on the operational implications and sensitivity studies.

The latter would be carried out by the Ph.D. student Whitney Blanchard who will visit UAF for a period of several months during the early project stages to familiarize herself with the model code and become proficient in its use and further development. Petrich will oversee this part of the work. He is fully funded as a UAF IPY Postdoctoral Fellow for the duration of this work. Since the present work ties in with his research interests (including simulations of multiphase flow) and since he is not bound by other research contracts he can devote significant time (1-2 months) to this project. Blanchard will then also be able to ensure integration of these simulations into the broader goals of the SINTEF work on oil transport simulations and biodegradation and toxicity.

This work will lay the foundation for the treatment of the next relevant process, the movement of oil through sea ice. At the simplest level, this would be an assessment of percolation of oil into the ice interior during the entrainment phase and later mobilization during increases in porosity and permeability. This semi-empirical approach would be based on past studies such as the NORCOR experiments and improved understanding of fluid flow through sea ice as constrained by permeability (Golden et al., 2007). It would also tie in with Petrich's efforts to simulate generic multiphase flow in sea ice using CFD approaches.

A summary modeling work plan is given in Table 7.

Table 7. *Proposed work plan: oil-ice modeling*

Task	Responsible	Timing
1. 2-D single-phase flow		
a. Describe processes to be included	Whitney/Mark	Fall 08
i. Dissolution		
ii. Evaporation		
iii. Advection-diffusion		
iv. Density and viscosity effects		
v. Adsorption to ice and/or particles		
vi. Biodegradation		
vii. Insolation, thermodynamics generally		
viii. Others (e.g. particle, droplet transport)		
b. Evaluate significance of processes/forces (dimensional analysis)	Whitney/Mark/Chris	Fall 08
c. Describe mathematical representation	Whitney/Mark/Chris	Fall 08
d. Plan and carry out pilot experiments on bulk oil movement in ice	Chris	2008
e. Implementation		Fall 08 – Summer 09
i. Design	Whitney/Mark/Chris	
ii. Increase ease of maintenance and portability of existing code	Chris	
iii. Make existing code available to CRRC project	Chris	
iv. Modify to include additional processes	Whitney/Mark/Chris	
v. Document code	Whitney/Chris	
vi. Testing (e.g. stability), calibration, verification (experimental data)	Whitney/Chris/Mark	
2. Publications		Summer 09-December 2009
a. Comparison of model and experimental output	(Chris et al, 2009 or 10)	
b. Model description, testing, benchmarks	(Whitney et al, 2009)	
c. Other systematic findings, implications		
3. Potential future work: implementation in other model systems	SINTEF	2010-2011

Progress and quality will be evaluated through the reports generated as a result of tasks 1a – 1e, and reflected finally in the publications planned in Tasks 2a and b.

4 Quality assurance

A summary and specifications of the project methods are given in Table 8. The methods are described in more detail in section 3 (Project Methods and Approach).

Table 8. *Specifications of relevant laboratory procedures/analysis for the measurements and documentation of the experiments.*

Analysis	Method/Instrumentation
Characterization of ice properties:	
Temperature	Online logger
Salinity	Conductivity/salinity probe
Density	Volume and weight measurements
Microstructural characterization	Vertical and horizontal thin sections, microscope view under polarized light
Sample processing ice cores	Liquid-liquid extraction, EPA method 3510
Sample processing SPME	Method developed by Dr.Lohmann, URI, through this project
Chemical analysis:	
Total petroleum hydrocarbons	GC/FID (Agilent 7890A GC/FID), EPA Method 8100
SVOC (PAHs and phenols)	GC/MS (Agilent 5975 MSD), EPA Method 8270
VOC (C5-C9, including BTEX)	P&T GC/MS (Hewlett Packard P&T, HP7695), EPA Method 8260
Biodegradation methods:	
Epifluorescence microscopy	Leitz Dialux with Ploemopak fluorescence unit and UV-filter A, method according to internal SINTEF standard KS 66-21-L-303
Most Probable Number (MPN) counts	Method according to internal SINTEF standard KS 66-21-L-302
PCR	iCycler thermocycler, methods according to draft standard internal procedures
DGGE	DCode Universal Mutation Detection system (BioRad) Quantity One option of the GelDoc software program (BioRad). Methods according to the Manual of the DCode system

The instruments and methods are in constant use and are regularly calibrated in the SINTEF laboratories.

The assignment shall be executed according to SINTEFs normal QA procedures, which in this case in short implies the following:

- *Governing documents* are mainly the Project Contract between Client and SINTEF, and the Project Plan.
- Possible *major non-conformances* from agreed plans are to be discussed with the Contractor as soon as possible. In this assignment, *major non-conformances* are defined to be deviations which must be assumed to have influence on the completion date of the assignment, the total cost or the quality of the final result.
- Possible *minor non-conformance* should be dealt with immediately by SINTEFs appointed Project Manager.
- Client shall be kept informed of all non-conformances and corrective actions by means of *Status Reports*

- *Independent Quality Control* shall be carried out for all Draft Reports etc which are forwarded to Client, as well as the Final Report. The QA team assigned to this Contract shall be consulted in the planning phase and later when needed.
- *Final control and internal approval* of SINTEF's Final Report shall be carried out by the assigned responsible person(s), before dispatch to Client.

5 References

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Appendix A Overview of target analytes and component groups

Group	Compound	Group	Compound	
SVOC	Decalin	C0-C4 phenols	Phenol	
	C1-decalins		C1-phenols	
	C2-decalins		C2-phenols	
	C3-decalins		C3-phenols	
Naphthalenes	C4-decalins		C4-phenols	
	Naphthelene	VOC (incl BTEX and C3-benzenes)	Isopentane	
	C1-naphthalenes		n-C5 (Pentane)	
	C2-naphthalenes		Cyclopentane	
C3-naphthalenes	2-methylpentane			
2-3 ring PAHs	C4-naphthalenes		3-methylpentane	
	Biphenyl		n-C6 (Hexane)	
	Acenaphthylene		Methylcyclopentane	
	Acenaphthene		Cyclohexane	
	Dibenzofuran		2,3-dimethylpentane	
	Fluorene		3-methylhexane	
	C1-fluorenes		n-C7 (Heptane)	
	C2-fluorenes		Methylcyclohexane	
	C3-fluorenes		2,4-dimethylhexane	
	Phenanthrene		2-methylheptane	
	Anthracene		n-C8 (Octane)	
	C1-phenanthrenes/anthracenes		n-C9 (Nonane)	
	C2-phenanthrenes/anthracenes		n-C10 (Decane)	
	C3-phenanthrenes/anthracenes		n-Butylbenzene	
	C4-phenanthrenes/anthracenes		1,2,4,5-tetramethylbenzene	
	Dibenzothiophene		n-pentylbenzene	
	C1-dibenzothiophenes		C4-benzenes	
	C2-dibenzothiophenes		C5-benzenes	
	C3-dibenzothiophenes		BTEX	Benzene
	C4-dibenzothiophenes			Toluene
	Fluoranthene			Ethylbenzene
	Pyrene			<i>m</i> -xylene
	4-6 ring PAHs	C1-fluoranthenes/pyrenes		<i>p</i> -xylene
		C2-fluoranthenes/pyrenes		<i>o</i> -xylene
C3-fluoranthenes/pyrenes			C3-benzenes	Propylbenzene
Benz[<i>a</i>]anthracene				1-methyl-3-ethylbenzene
Chrysene				1-methyl-4-ethylbenzene
C1-chrysenes				1,3,5-Trimethylbenzene
C2-chrysenes			1-methyl-2-ethylbenzene	
C3-chrysenes			1,2,4-trimethylbenzene	
C4-chrysenes			1,2,3-trimethylbenzene	
Benzo[<i>b</i>]fluoranthene			TPH	C10-C40
Benzo[<i>k</i>]fluoranthene				
Benzo[<i>e</i>]pyrene				
Benzo[<i>a</i>]pyrene				
Perylene				
Indeno[1,2,3- <i>c,d</i>]pyrene				
Dibenz[<i>a,h</i>]anthracene				
Benzo[<i>g,h,i</i>]perylene				