SINTEF A19803

REPORT

Oil in Ice - JIP

Report no.: 34

The effects of use of dispersant and in situ burning on Arctic marine organisms - A laboratory study

Liv-Guri Faksness, Jan Fredrik Børseth, Thierry Baussant, Anne Helene S. Tandberg, Anna Ingvarsdottir, Dag Altin and Bjørn Henrik Hansen

SINTEF Materials and Chemistry

Marine Environmental Technology

Date: 24.06.2011

www.sintef.no

IRIS International Research Institute of Stavanger

SINTEF

Preface

SINTEF has in cooperation with SL Ross Environmental Research Ltd and DF Dickins Associates LLC on behalf of the oil companies AGIP KCO, Chevron, ConocoPhillips, Shell, Statoil and Total initiated an extensive R&D program; *Joint industry program on oil spill contingency for Arctic and ice covered waters.* This program was a 3-year program initiated in September 2006 and finalized in December 2009.

The objectives of the program were;

- To improve our ability to protect the Arctic environment against oil spills.
- To provide improved basis for oil spill related decision-making:
- To advance the state-of-the-art in Arctic oil spill response.

The program consisted of the following projects:

- P 1: Fate and Behaviour of Oil Spills in Ice
- P 2: In Situ Burning of Oil Spills in Ice
- P 3: Mechanical Recovery of Oil Spills in Ice
- P 4: Use of Dispersants on Oil Spills in Ice
- P 5: Remote Sensing of Oil Spills in Ice
- P 6: Oil Spill Response Guide
- P 7: Program Administration
- P 8: Field Experiments, Large-Scale Field Experiments in the Barents Sea
- P 9: Oil Distribution and Bioavailability

The program has received additional financial support from the Norwegian Research Council related to technology development (ending December 2010) and financial in kind support from a number of cooperating partners that are presented below. This report presents results from one of the activities under this program.

Stein Erik Sørstrøm
Program Coordinator
(stein.e.sorstrom@sintef.no)

Funding Partners

Eni Agip KCO ConocoPhillips Chevron Shell TOTAL Statoil

R&D Partners

IRIS International Research Institute of Stavanger SL ROSS DICKINS

Cooperating Partners

The Research Council of Norway NOFO acs alaska clean seas UNIS MMS Oil Spill Recovery Institute Cordova, Alaska KYSTVERKET Coastal Response Research Center at the University of New Hampshire

SINTEF Materialer og kjemi
SINTEF Materials and Chemistry

Address:
Postboks 4760 Sluppen
NO-7465 Trondheim
NORWAY

Telephone:+47 73593000
Telefax:+47 73595910

info.mk@sintef.no
www.sintef.no/mk
Enterprise /VAT No:
NO 948007029 MVA

Report

The effects of use of dispersant and in situ burning on Arctic marine organisms - A laboratory study

Oil in ice JIP – Report no. 34.

KEYWORDS:
Oil spill
Arctic
WSF
Amphipods
Copepods

VERSION
Final

DATE
2011-06-24

AUTHOR(S)
Liv-Guri Faksness, Jan Fredrik Børseth, Thierry Baussant, Anne Helene S. Tandberg, Anna Ingvarsdottir, Dag Altin, and Bjørn Henrik Hansen

CLIENT(S)
The Research Council of Norway, Chevron, ConocoPhillips, Shell, Statoil, and Total.

CLIENT'S REF.
Oil in ice JIP

PROJECT NO.
805341.04

NUMBER OF PAGES/APPENDICES:
27 /2

ABSTRACT
The effect and toxicity of a water soluble fraction (WSF) of oil versus oil added dispersant, and WSF versus the underlying water after in situ burning (ISB), have been studied. The exposure concentrations used were based on monitoring of WSF in the water column during an offshore field experiment.

A continuous flow-through system was established for the dispersant experiments. An Arctic amphipod, *Gammarus setosus*, was used as the test species. Seawater and gammarids were sampled for chemical and biological analyses. Chemical analyses of the water samples showed good correlation with data collected during the offshore field experiment, while the body burden measurements had higher level of PAHs in the gammarids exposed to oil+dispersants than in those exposed to oil alone. Among the effect markers monitored, general stress was indicated by a significant decrease in lysosomal stability in the dispersed oil treatment compared to WSF and control. The neutral lipid marker shows a relatively strong effect for gammarids exposed to both WSF and dispersed oil.

A system for allowing water sampling after ISB was developed. Seawater samples and oil were collected prior to and immediately after ISB, and chemical analysis was conducted. Acute toxicity tests with the marine copepod *Calanus finmarchicus* and Microtox® bioassay was performed to establish LC_{50}/EC_{50} values of the water. The results were compared to regular WAF systems, and indicated no increase in toxicity in the underlying water after ISB.

PREPARED BY
Liv-Guri Faksness

SIGNATURE
LG Faksness

CHECKED BY
Alf G. Melbye

SIGNATURE

APPROVED BY
Tore Aunaas

SIGNATURE

REPORT NO. SINTEF A19803
ISBN 978-82-14-05141-4
CLASSIFICATION Unrestricted
CLASSIFICATION THIS PAGE Unrestricted

SINTEF

Table of contents

1 Introduction

These studies have been an integrated part of a Joint Industry Program (JIP) to develop and advance the knowledge, methods and technology for an oil spill response in Arctic and ice-covered waters (Oil-in-Ice JIP). The research program was started in 2006 and the major parts of the activities were finalized in 2009, with the final scientific reports issued in 2010. The JIP summary report (Sørstrøm et al., 2010) gives an overview of the total program and technical reports.

In May 2009, a large-scale field experiment (FEX2009) took place in the marginal ice zone in the Barents Sea, northeast of Hopen Island (N77.6, E30.9). During the experiment, 7000 liters of crude oil were released uncontained between the ice floes to study oil weathering and spreading in ice. The processes for the drift, spreading and weathering of oil were monitored by multiple sampling throughout the six-day experiment (Faksness et al., 2011). Data on the potential bioaccumulation of oil components in the water column were collected by passive absorption devices (semi-permeable membrane devices (SPMDs)), while dissolved hydrocarbons in the water column were sampled by an in situ large-volume water sampler (Kiel In Situ Pump, KISP). The chemical monitoring showed low but detectable concentrations in the range between 0.1 (background) to 1.5 ppb dissolved hydrocarbons, 4 (background) to 32 ppb total hydrocarbons from the KISPs, as well as in the range between 0.6 (background) to 4 ppb dissolved hydrocarbons estimated from SPMDs (Faksness et al., 2011).

These results have been used as the basis for designing the laboratory exposure studies presented here. The objective was to investigate the toxicological effects of the water soluble fraction (WSF) of oil versus oil added dispersant, and versus the underlying water, after in situ burning (ISB). Different systems and organisms were used for the dispersant experiments and ISB. The water soluble oil fraction is of special interest since the components dissolved (e.g. naphthalenes, phenanthrenes, dibenzothiophenes and phenols) in an oil slick, or from the dispersed oil droplets beneath a slick, are considered to be the major contributor to any ecological effects from oil spills (Neff et al., 2006).

In this study, realistic exposure concentrations have been used to compare the biological effects of a WSF and oil added dispersant by measuring the body burden and biomarker responses (e.g. lysosomal stability in blood cells, MDA and catalase) on the Arctic amphipod *Gammarus setosus*. Acute toxicity tests with the marine copepod *Calanus finmarchicus* and Microtox® bioassay was performed to establish LC_{50}/EC_{50} values of the underlying water prior to and after an ISB, in addition to detailed chemical analyses of the oil and water.

The addition of a chemical dispersant to spilled oil increases the potential for the oil to become dispersed. Mixing energy is required to create small oil droplets to maintain the oil droplets within the water column, finally causing them to spread, dilute and naturally biodegrade. The smaller the oil droplets are, the more available they are for degradation by micro organisms in the water column. Dispersants effectiveness is influenced by many factors, and the most important is the oil characteristics, followed closely by sea energy (e.g. breaking waves).

There have been a large number of studies on toxicity of dispersed oil over the past decades. Most of these studies have been traditional lethal toxicity assay on various species, where usually a relatively high oil concentration has been required to establish LC_{50}-values. Baussant et al. (2009)

had chosen a biomarker-based approach their laboratory long-term experiments: Bivalve molluscs were exposed to nominal dispersed oil concentrations in the range of 15 to 250 µg/L for one month. Five biomarkers (enzymatic and cellular responses) and body burden of PAH were analyzed at the end of the exposure. They found that oil caused biomarker responses in the molluscs, but concluded that the relationship between exposure levels and enzymatic responses were complex. A viewpoint article by Chapman et al. (2007) describe the use of dispersants in past cases over a 10 year period (1995-2005) focusing on dispersant effectiveness and monitoring, toxicity and environmental effects, response planning and future research needs. Their review summarize that both naturally and chemically dispersed oil are unlikely to have acute effects on the marine environment provided that there is sufficient dilution to rapidly reduce hydrocarbon concentrations. However, in a review by Fingas and Banta (2009) of the literature in oil spill dispersants published from 2002 to 2008, it is summarized that more recent toxicity studies indicate that chemically-dispersed oil was more toxic than physically-dispersed oil, mostly caused by increased PAH content in the water column. Most tests showed that dispersant toxicity is less than the toxicity of dispersed oil.

Lately, a Joint Industry Program has been formed to investigate the toxicity and biodegradation of chemically and mechanically dispersed oil to valuable ecosystem components of the arctic marine environment of the Beaufort and Chukchi Seas. Phase I activities have been described in McFarlin et al. (2010), and results of toxicity are given in McFarlin et al. (2011). These studies concluded that physically dispersed oil (LC_{50} of 1.6 and 2.2 mg/L TPH) proved to be more toxic than chemically dispersed oil (LC_{50} of 3.3 and >3.7 mg/L) to both arctic cod (*Boreogadus saida*) and early season copepods (*Calanus glacialis*).

ISB is one of the response technique with the highest potential for the removal of oil spills, and has been proven and established as part of the oil spill contingency in many Arctic areas. The suitability of ISB depends on oil's initial characteristics (physical and chemical properties) and weathering state. Several factors, such as slick thickness, oil weathering, swell/waves and wind conditions are important factors for a successful burn.

The Newfoundland Oil Burn Experiment (NOBE, summarized in, e.g. Fingas et al., 1994) was performed in 1993 to look at the feasibility of burning oil offshore as a spill response measure. A toxicity component was built into NOBE to determine the potential toxic effects to aquatic organisms that could result from ISB and how they compare with the effects of unburned oil. Samples of the underlying water from laboratory experiments and the offshore field experiments NOBE, as well as weathered oil and burn residue, were collected to address the toxicity issues associated with burning (Blekinsopp et al., 1996; Daykin et al., 1994).

2 Materials and methods

A naphthenic Norwegian crude oil (Troll B) was used in all experiments, including the large-scale field experiment in the Barents Sea (Faksness et al., 2011). Corexit 9500A was selected as the chemical dispersant.

2.1 Study organisms

In the dispersant experiments the test species was *Gammarus setosus* (Dementieva, 1931), a gammarid amphipod very common in the intertidal zone of Svalbard. The specimens were handpicked from the beach of Adventfjorden in Longyearbyen, and transported to the test facilities in Stavanger in thermos bottles filled with filtered and cooled seawater. A subsample of 25 specimens was taxonomically verified by a specialist, and all proved to be *G. setosus.*

Both *G. setosus* (Figure 2.1) and *G. oceanicus* are common in the intertidal of Svalbard (Spooner, 1951, Weslawski et al., 1993, Ronowicz, 2005), with *G. setosus* being considered the more cold-loving species of the two. It is often associated with ice (Hop et al., 2002), although it is not obligate sympagic as its sister species *Gammarus wilkitzkii*.

Amphipods are peracarid crustaceans; this implies that they, contrary to most other crustaceans, carry their young from egg to ready juvenile, and they do not easily spread planktonically as larvae. This results in a quite limited spreading in one generation, and it ensures that the juveniles will be kept at the same conditions as the adults. Littoral amphipods often group together under rocks and large pieces of gravel. *G. setosus* carries eggs in October-April, when the egg will hatch and juveniles appear. Each female carries approximately 70 eggs, and the females are ovigerous at an age of 3 years (Weslawski & Legezynska, 2002). They are grazers/detritus feeders, and are again food for fish and larger crustaceans.

Figure 2.1 Gammarus setosus *kept in an aquarium with rocks and gravel.*

A system for allowing water sampling after ISB has been developed. Seawater samples and oil were collected prior to and immediately after ISB, and chemical analyses conducted. An acute toxicity test, using the marine copepod *Calanus finmarchicus* (Gunnerus, 1765), in addition to Microtox® bioassay, was performed to establish the LC_{50}/EC_{50} values of the underlying water after ISB. *C.*

finmarchicus (Figure 2.2) was chosen as the test species because it is the most abundant copepod in Norwegian waters and an ecologically important prey for the early stages of several species of commercially important fish species. The test animals were from a continuous laboratory culture at SINTEF/NTNU Sealab, which was established with animals collected from the Trondheimsfjord in mid-Norway. The cultures are routinely kept at 10 ºC, and the details regarding the culturing conditions have been previously described by Hansen et al. (2007). The animals used in these experiments were from the 27th generation in culture.

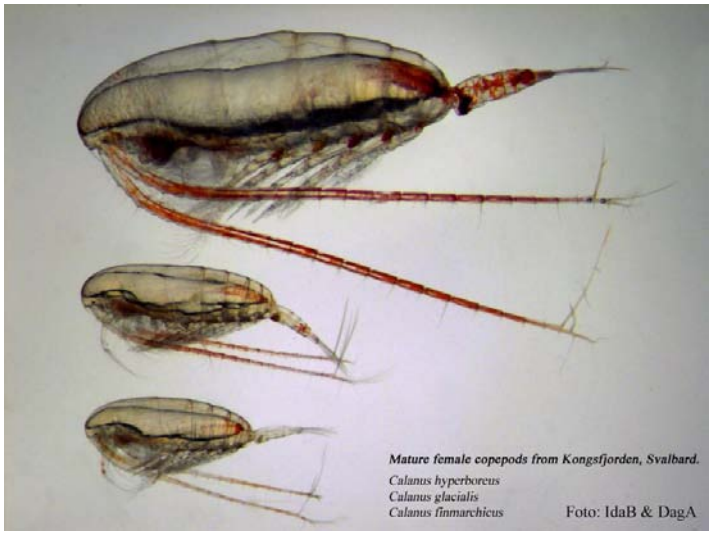

Figure 2.2 *Examples of Calanus species, including* C. finmarchicus, *collected in Kongsfjorden, Svalbard.*

2.2 Experimental systems

2.2.1 Dispersant experiment

A continuous flow-through system was designed to examine the biological effects from the water soluble components of oil in water, and oil and dispersant in water on *Gammarus setosus.* Three exposures (control, oil and oil + dispersant) were simultaneously tested, using animals from the same batch and collection and seawater from the same source and cooling. All animals were kept together until the exposure began.

Figure 2.3 illustrates the exposure system, which is a modified version of the system described by Bado-Nilles et al. (2009). The exposure scenario the modified system attempt to model is a low mechanical energy oil/seawater mixing system similar to the conditions documented in ice infested Arctic water. Each exposure system had a header-tank (110 cm x 110 cm), which was initially filled with fresh, natural seawater (2 °C). The header tank had a continuous exchange of seawater at a rate of 4 L/min, and the water level was maintained at 15 cm to produce a total water volume of 0.182 m^3 in the header tanks giving a theoretical mean residence time of renewed and clean seawater in the header-tanks containing exposure compounds of 45.5 minutes. This water fed three exposure aquaria with a water exchange of 50 mL/min. The aquaria were kept in cooled water baths to ensure a correct and stable water temperature for the exposure (2 ºC), and 33 amphipods were transferred to each aquarium at the start of the experiment. Each aquarium had a total biomass of

approximately 5.8 g. The sampling for the different parameters was evenly spread over the three aquaria from the same treatment/exposure.

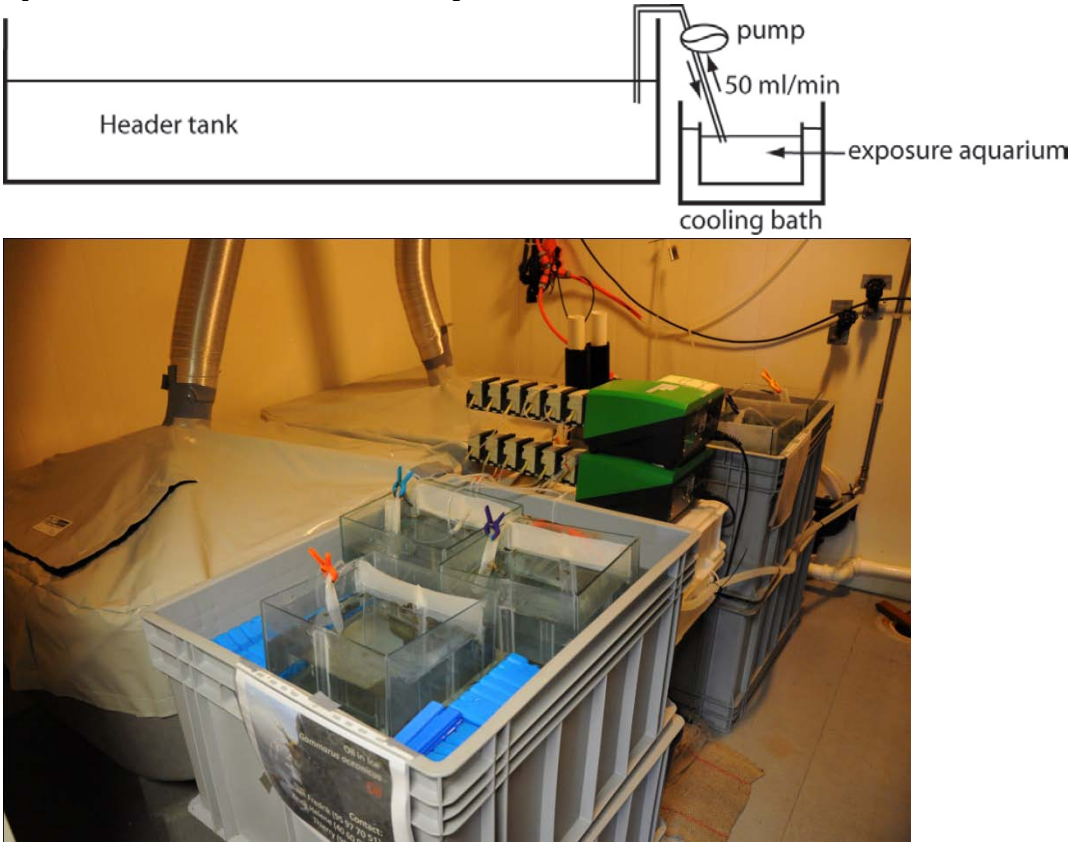

Figure 2.3 General view of experimental setup. Each header-tank fed three exposure aquaria.

All header-tanks were initially filled with cooled (2 °C) seawater taken from 80 m depth outside the lab in Stavanger, and after the initial fill-up the inflow was kept at a minimum. The different exposures were achieved by carefully adding oil (180 mL), oil (180 mL) and dispersant (2% per oil v/v) or nothing (control) to the header tank (Figure 2.3). The oil was added in a manner that formed a slick on the entire surface in the header tank. To minimize the risk for contamination, the control was kept in a separate climate-controlled room from the system the oil added. After 12 days of exposure the continuous supply of water in all aquaria was changed to clean seawater for recovery.

2.2.2 In situ burning experiment

The ISB experiments were performed in an indoor research facility (at a room temperature of 15 ºC) at SINTEF's NBL (Norwegian Fire Research Laboratory) in Trondheim. Two systems were established using barrels cut in two, with a bottom tap for water sampling. The barrels were filled to a level of 30 cm (around 80 L) with fresh seawater from the Trondheimsfjord. Fresh Troll B crude oil was applied on the water's surface to an approximate oil film thickness of 2.7 mm (800 mL). The oil was ignited after one hour in the first system and after two days in the second system. Photos illustrating the experiments are shown in Figure 2.4.

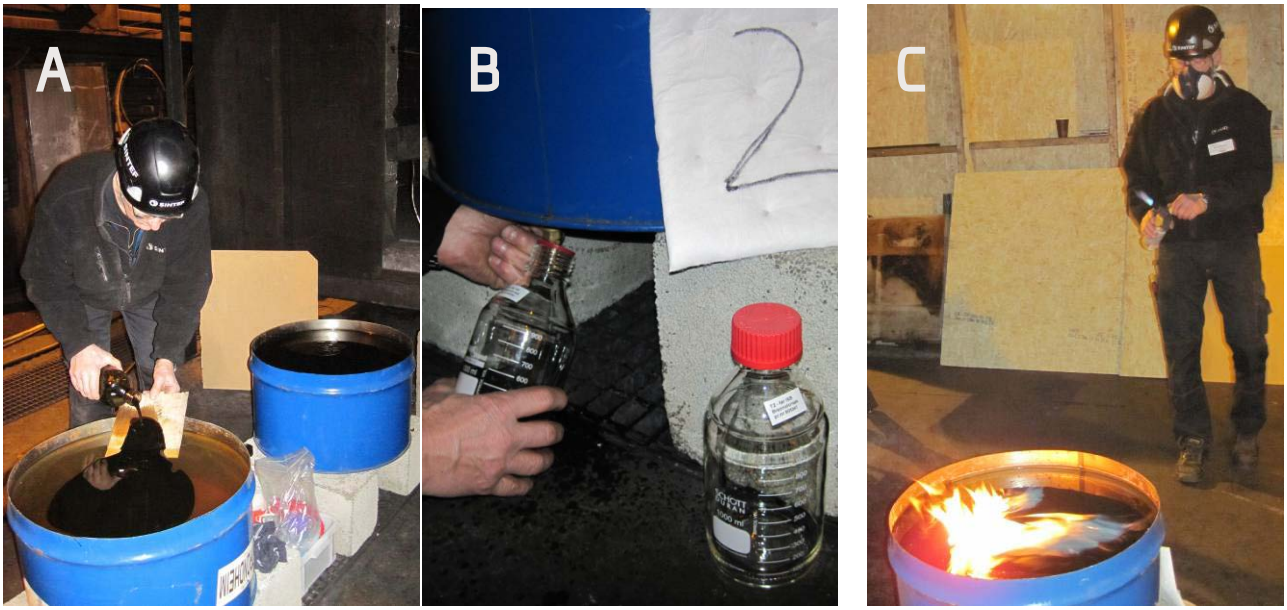

Figure 2.4 *Oil application (A), water sampling (B), and ignition (C).*

2.3 Sampling

2.3.1 Dispersant experiment

Animals were sampled at three different times: T0 at the start of the exposure (control, as none had been exposed so far), T12 after 12 days of exposure, which was the end of the exposure (all aquaria were then continuously supplied with clean seawater for recovery phase), and at the endpoint of the study after 13 days of recovery (T25). All specimens were wet weighed and measured for dorsal length (from the head at the base of the antennae to the base of the telson). The average weight for the entire study was 0.16 g (±0.06 g), with weight spread over the sampling dates of T0: 0.14 g (±0.09 g), T12: 0.18 g (±0.05 g) and T25: 0.18 g (±0.04 g) (see Figure 2.5, upper graph). The average length for the entire study was 20.6 mm (±2.8 mm), with length at T0: 17.7 mm (±2.4 mm), T12: 21.8 mm (±2.6 mm) and T25: 21.0 mm (±2.2 mm) (see Figure 2.5, lower graph). The analytical methods used were body burden (of PAH), lysosomal stability (NRRT), histochemistry and malondialdehyde (MDA). In addition, a sampling for gene expression was performed and stored for future analysis.

The seawater was sampled at the start (T0), after 6 days (T6), after 12 days (T12) and after 25 days (T25) for chemical analyses (semi-volatiles (SVOC) and volatiles (VOC)). A description of the exposure systems is given in Table 2.1.

Table 2.1 *Description of the flow-through exposure experiments with* Gammarus setosus.

System	Description
Control	Clean seawater, no oil added
Oil or WSF	Water soluble fraction (WSF) of oil in seawater
Oil + disp or WSF + disp	Oil and dispersant (2% Corexit)

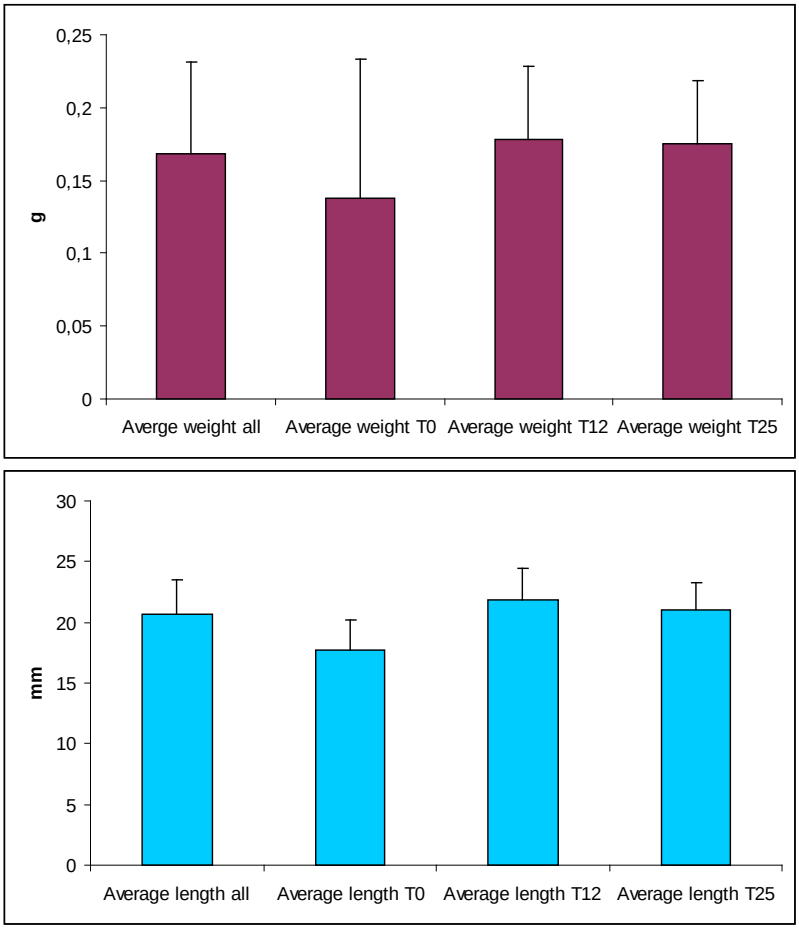

Figure 2.5 *Weight of amphipods (in g) shown in upper figure, and length of amphipods (in mm) shown in lower figure. Mean values and SD are given.*

2.3.2 In situ burning experiment

Sampling of oil and seawater for chemical analysis was performed prior to and after ISB. Water sampling for acute toxicity tests with *C. finmarchicus* (copepods) and Microtox® was performed after the ISB. An overview of the sampling and analyses is given in Table 2.2.

Table 2.2 *Sample description (oil and water) and analysis in the ISB experiments*

Sample ID	Description	Parameters
T0 prior to ISB	Sampled 1 hr after oil release prior to ISB	SVOC
T0 after ISB	Sampled after ISB	SVOC, VOC, Microtox, *Calanus*
T2 prior to ISB	Sampled 2 days after oil release prior to ISB	SVOC, VOC, Microtox
T2 after ISB	Sampled after ISB	SVOC, VOC, Microtox, *Calanus*

2.4 Sample preparation

Surrogate internal standards (SIS, *o*-terphenyl, naphthalene-d_8, phenanthrene-d_{10}, chrysene-d_{12}, phenol-d_6, 4-methylphenol-d_8) were added to the water samples and amphipods prior to processing and recovery internal standards (RIS, 5α-androstane, fluorene-d_{10}, and acenaphthene-d_{10}) prior to analysis on GC/FID (gas chromatography/flame ionization detection) and GC/MS (gas chromatography/mass spectrometry). No measurements for the body burden were performed for the copepods.

2.4.1 Extraction of water samples

For analyses of semi-volatile organic compounds (SVOC) and total petroleum hydrocarbons (TPH), the water samples were spiked with the appropriate surrogate internal standards and serially extracted with dichloromethane (DCM), thereby following a modification of EPA method 3510C (US EPA, 1996). The combined extracts were dried with sodium sulphate and concentrated to approximately 1 mL using a Zymark Turbovap® 500 Concentrator. The final extract was spiked with the appropriate recovery internal standards and analyzed on GC/FID and GC/MS.

2.4.2 Extraction of *Gammarus setosus*

The extraction of the animals was performed according to a modification of the method described in Baussant et al. (2001).

The gammarids were weighed in a round bottom flask and potassium hydroxide (7%) in methanol (50 mL) and internal standards (SIS) was added. This mixture was boiled under reflux for two hours to achieve saponification, and then followed by filtration and serial extraction with cyclohexane (3 x 30 mL). The combined extracts were dried with sodium sulphate and concentrated to approximately 0.5 mL using a Zymark Turbovap® 500 Concentrator. Cleanup of the extracts was performed by solid phase extraction using 3 mL columns containing 0.5 g normal phase silica packing (Superclean LC-Si, Supelco). The samples were eluted through the column with 3 x 2 mL of DCM:cyclohexane (1:3). The purified extracts were concentrated to 0.5 mL, spiked with RIS components and analyzed on GC/FID and GC/MS.

2.5 Chemical analyses

The samples were analyzed for SVOC (decalins, PAHs and phenols)) using GC/MS, for TPH using GC/FID, and volatile organic compounds (VOC, C_5-C_9), including BTEX (benzene, toluene, ethylbenzene, and xylenes), by use of P&T GC/MS (Purge and Trap Gas Chromatography Mass Spectrometry). The P&T GC/MS analysis was only performed on selected water samples. A list of all target analytes is shown in Appendix A. This list includes the recommended analytes given by Singer et al. (2000), and is a typical standard list for the target compounds used during post-oil spill damage assessments.

The GC/FID analyses were performed according to a modification of EPA Method 8100 (US EPA, 1986). TPH (resolved plus unresolved TPH) was quantified by the method of internal standards, using the baseline corrected total area of the chromatogram and the average response factor for the individual C_{10} to C_{36} n-alkanes. However, due to low concentrations of TPH in the flow-through

experiments and the resultant high uncertainty in the quantifications, the GC/FID analysis has only been used as a screening for the samples.

The semi-volatiles were quantified by modifications of EPA Method 8270D (US EPA, 2007). The mass spectrometer was operated in the selective ion monitoring mode to achieve optimum sensitivity and specificity. The quantification of target compounds was performed by the method of internal standards, using average response factors (RF) for the parent compounds. The PAH and phenol alkyl homologues were quantified using the straight baseline integration of each level of alkylation and the RF for the respective parent PAH compound. The response factors were generated for all targets and surrogates versus fluorene-d_{10}.

The volatiles were analyzed in selected water samples. A total of 30 target volatile analytes in the C_5 to C_{10} range were determined by P&T GC/MS using a modification of EPA method 8260C (US EPA, 2006). The samples were spiked with SIS (toluene-d_8 and ethylbenzene-d_8) and RIS (chlorobenzene-d_5). The quantification of individual compounds was performed by using the RFs of the individual compounds relative to the internal standards. All standards and samples were analyzed in a full scan mode.

2.6 Biomarker effects in amphipods

A suite of different parameters were analyzed for each of the groups at all sampling times. The lysosomal stability was analyzed immediately, body burden was sampled as a pooled sample (10 individuals in one tube) and stored at -20 °C, all other samples were individually snap-frozen in liquid nitrogen and stored at -80 °C.

Lysosymal stability of haemolymph cells by the NRRT test (Neutral Red Retention Time) uses the ability of the haemolymph cells to absorb and retain the cationic vital dye Neutral Red, a well-known measure of cytotoxicity (Dierickx and Vandevyer, 1991). This assay is demonstrated as a reliable general stress indicator of fish haemocytes (Lowe et al., 1992), invertebrate digestive cells (Lowe and Pipe, 1994) and invertebrate haemolymph cells (Lowe et al., 1995). In several studies by Lowe et al., the invertebrates have always been molluscs. In this study we have verified the technique for amphipod crustaceans. The NRRT gives a value of the stress level of the individual analyzed. Basically, the longer the cells stay alive, the better their condition is and the lower the stress affects them.

As an indication of increased peroxidation processes and xenobiotic-mediated lipidosis, the accumulation of neutral lipids was measured by cryo sectioning, followed by staining with the dye Oil Red O and image analysis by densitometry, according to the method described by Bancroft (1967). These lipids do not carry any charged groups (ionizable group) such as phosphate, amino groups or choline.

The reactive aldehyde malondialdehyde (MDA) was used as a biomarker to measure the level of oxidative stress in the organisms. Polyunsaturated lipids are degraded by reactive oxygen species forming MDA. This analytical method is an assay based on the reaction with thiobarbituric acid followed by the spectrophotometric determination (540 nm) of supernatant from homogenized muscle tissue (Wheatley, 2000). Moderate stress is indicated by activated antioxidants and a

decreased MDA level, while high stress is indicated by decreased antioxidant capacity and an increased MDA level.

Statistical analyses of biomarker data was carried out by using JMP version 5.1 by SAS Institute Inc. Data from the lysosomal stability test is of ordinal type and was investigated by Kruskal Wallis nonparametric test. A visualization of statistical analyses is shown at the right part of the diagrams for the biomarkers neutral lipids (Figure 3.5) and MDA (Figure 3.7). All red colored circles mean that there are no significant differences. When a circle appears in grey, it indicates a significant different group of data. The size of the circles indicates the power of the data with regard to number of individual samples, e.g. small circles means high power. The Dunnett's multiple comparison with control test was used with a level of significance set at 0.05.

2.7 Acute toxicity of water from in situ burning

2.7.1 Acute toxicity to *Calanus finmarchicus*

The acute toxicity testing of *C. finmarchicus* was performed according to ISO 14669:1999 (ISO, 1999), with modifications described in Hansen et al. (2011). Briefly, the WSFs from the ISB experiments were diluted in a series of seven concentrations, covering the range from undiluted to 4% WSF in seawater. Glass bottles (0.5 L) with Teflon lined screw caps were used as the exposure vessels. Each exposure concentration was done in triplicate, and six groups were used as controls containing seawater only. The exposure vessels were filled close to the rim to keep evaporative loss to a minimum during exposure, and each vessel was stocked with seven copepodites V of *C. finmarchicus*. Mortality was monitored at 24, 48, 72 and 96 hours. The temperature was monitored throughout the exposure period, and the saturation of oxygen was measured at the end of the exposure. The test animals were not fed during exposure.

2.7.2 Assessment of toxicity by Microtox®

A Microtox® M500 instrument was used to measure the acute toxicity of the water from the ISB, following a modified version of the Microtox® Acute Toxicity Test System (Azur Environmental Ltd., 1995). This method relies on the principle of measuring the inhibition of light emitted from the luminescent marine bacteria *Vibrio fisheri*. The Microtox® test was performed on the same day as the sampling, using the Microtox® bacteria reagent (Microtox® Acute Toxicity Testing Reagent), and prepared according to the test protocol (Azur Environment Ltd, 1995). Closed vials were used instead of the standard open Microtox® cuvettes to better control the volatile compounds during exposure/incubation of the sample. The toxicity was measured from the testing of eight dilutions from 100% to 0.78%. MTX7 software (Azur Environment Ltd.) was used to calculate the EC_{50}-values.

The Microtox® method is based on the measurement of a single response from a primitive organism. Thus, the method is only suitable as an indication of potential acute toxicity, and the results from the Microtox® analysis should only be used as an "early warning."

3 Results and discussions

3.1 Dispersant experiment

A colour change in the water phase of the oil + dispersant tank was clearly visible one day after adding the dispersant; this milky looking water remained for three days before gradually being reduced to a sheen, and at T12 the water looked the same in all exposures. Nevertheless, there was a distinct smell of hydrocarbons from the oil-water and the oil + dispersant-water.

3.1.1 PAH in seawater and body tissue

The chemical analyses demonstrated that the concentrations of oil components were relatively low compared to concentrations tested in similar experiments in which the mechanical mixing of oil and dispersant was conducted. Nevertheless, some lethality was observed during the experiments. However, the amphipods used in this study were taken directly from their natural cold water habitat in Svalbard, and were therefore probably well acclimated for managing a relatively standard metabolic rate. Despite low oil concentration and low temperature, the acclimation of the enzyme system means that they might be efficiently producing toxic metabolic intermediates of PAH, which could explain the mortality observed.

The total SVOC concentrations for the water and body burden (BB) in amphipods are given in Figure 3.1 and Figure 3.2. At the start of the exposure, the total SVOC concentration in the water was 6.3 ppb in the experiments with WSF and 4.3 ppb in the experiments with dispersed oil. The concentration level in both systems decreased during the exposure, and after 12 days of exposure the SVOC concentrations were 1.2 ppb in the system with WSF and 1.5 ppb in the system with dispersed oil. The naphthalenes dominated the SVOC in all systems, together with 2-3 ring PAH and decalins.

Measurements of BB in the amphipods indicated that the SVOC concentration in the animals exposed to dispersed oil for 12 days was more than three times higher (5.5 ppb) than in the animals exposed to a WSF (1.7 ppb). The concentration of naphtalenes and 2-3 ring PAH dominated the BB in the animals exposed to dispersed oil. After 13 days of recovery in clean seawater, the BB in the amphipods was reduced to 0.3 ppb (WSF) and 0.6 ppb (dispersed oil). Swimming activity and mortality were observed once each day. There were some mortality observed among the animals during the first days of exposure to dispersed oil as well as a decrease in mobility compared to the WSF and the non-exposed animals (control). Despite the low degree of the mechanical mixture of oil and dispersant, visual differences between the WSF and dispersed oil systems were observed during the first days. The first sampling of amphipods was performed after 12 days of exposure. The SVOC concentration in water was higher during the first days of exposure, so perhaps a more abundant biomarker effect could have been observed if the sampling of animals was performed earlier, e.g. after three and six days.

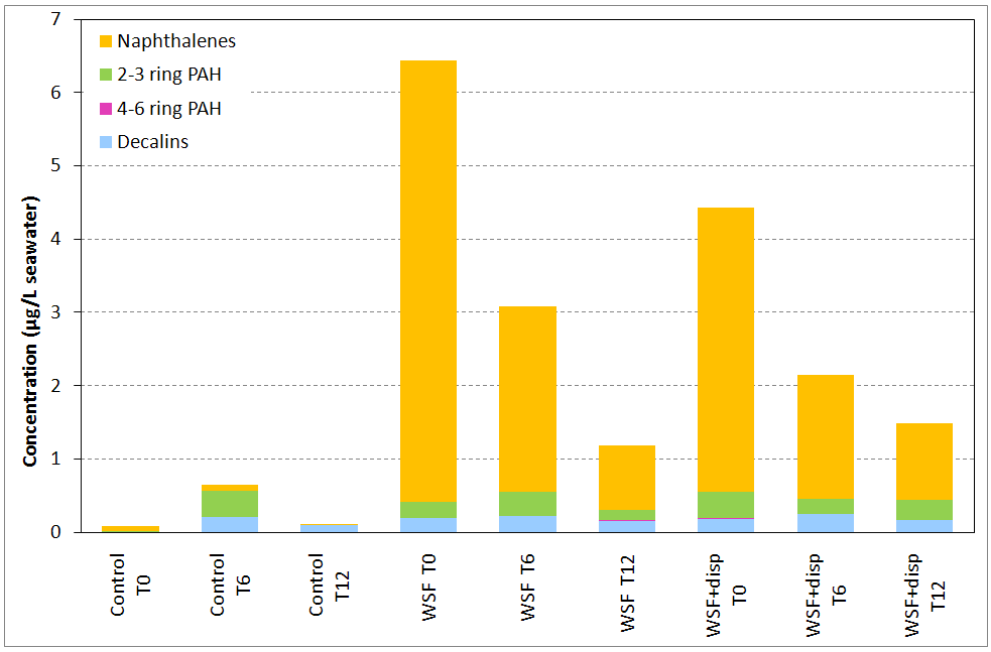

Figure 3.1 Concentration of SVOC in exposure water (sample description is given in Table 2.1).

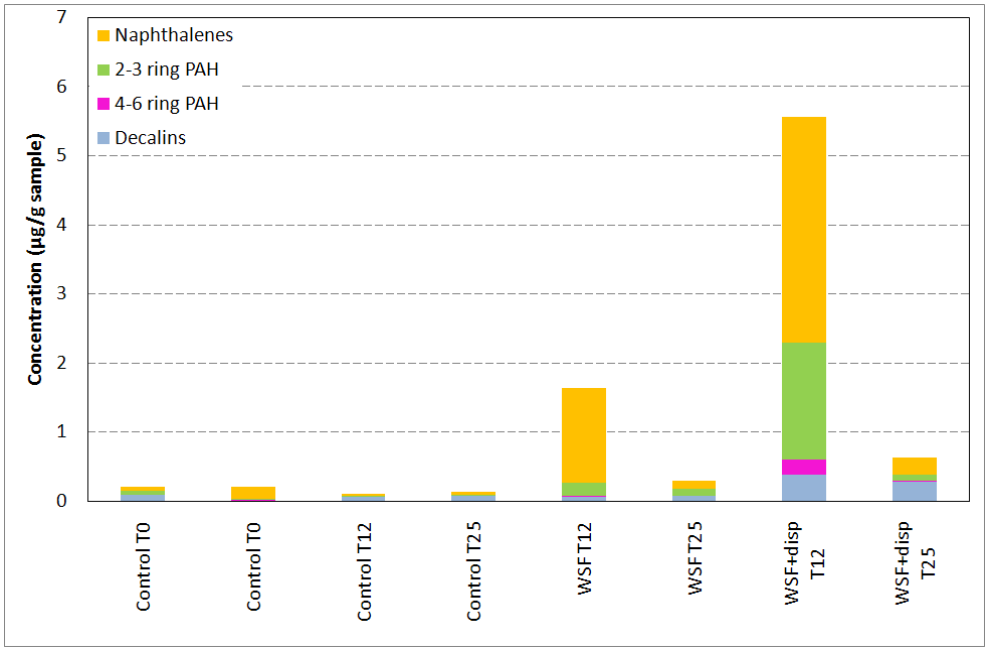

Figure 3.2 Body burden (SVOC) in Gammarus setosus (sample description is given in Table 2.1).

3.1.2 Biological effects

One of the more noticeable biological effect patterns is provided by the lysosomal stability Figure 3.3). The combination oil+dispersant seem to effect the animals more than oil alone and the clean seawater. Between oil and clean seawater there are no significant differences on this parameter, but there is a significant difference between the control group and the oil+dispersant. Figure 3.4 shows examples of live/healthy cells (A) and dying/unhealthy cells (B) from *G. setosus* haemolymph.

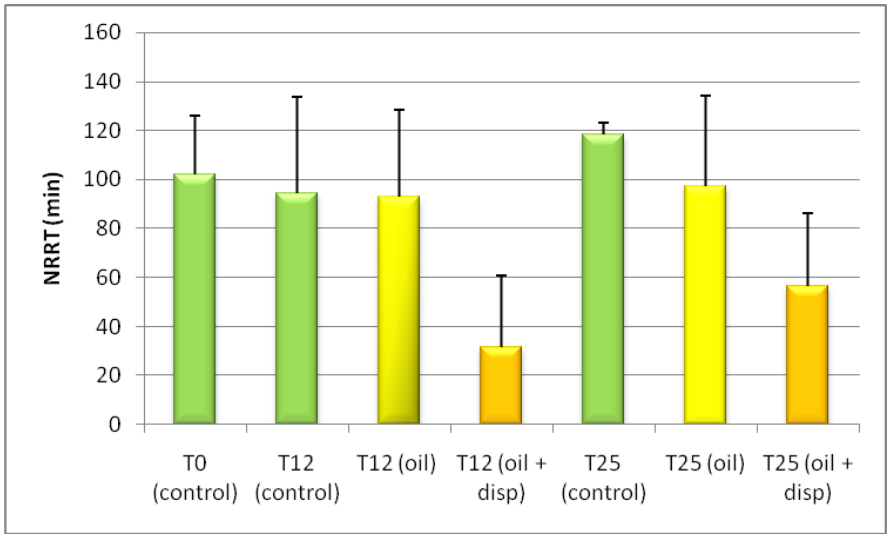

Figure 3.3 *NRRT for* G. setosus *in control (green), oil (yellow) and oil + dispersant (orange) from the start (T0), the end of the exposure (T12) and the end of the recovery in clean water (T 25). Error bars give standard deviation for the measurements – bars give average NRRT for the group, 10 individuals on all occasions except T0 (15 individuals). P-values are <0.0012 for T12 (oil+disp) and <0.0017 for T25 (oil+disp).*

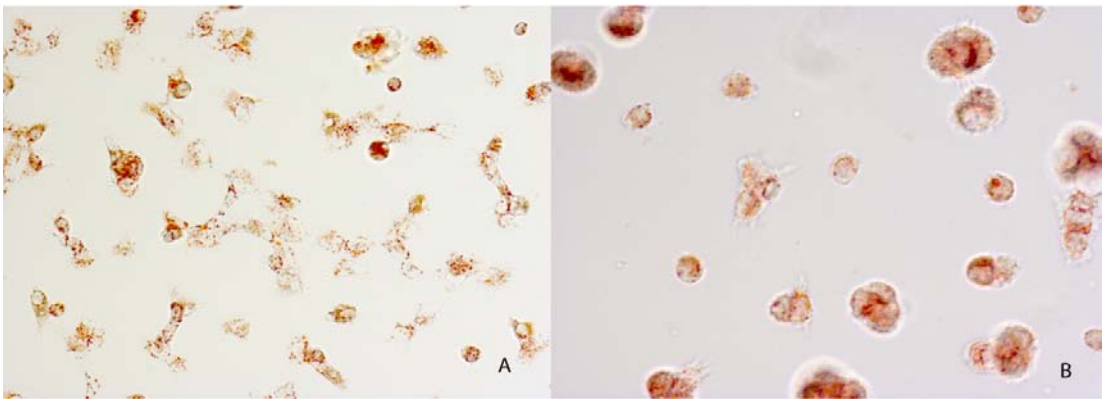

Figure 3.4 *Haemolymph cells of* G. setosus *during lysosome stability assay. A: healthy /live cells (10x magnification) B: unhealthy/dying cells (40x magnification).*

In Figure 3.5 the neutral lipids accumulation is shown for whole animal tissue sections from the thorax region. The results exhibit an increased level in both exposed groups at day 12. There was no significant difference between the amphipods exposed to a WSF in comparison to dispersed oil. The level of neutral lipids in the exposed groups maintained elevated after 13 days of recovery in clean seawater.

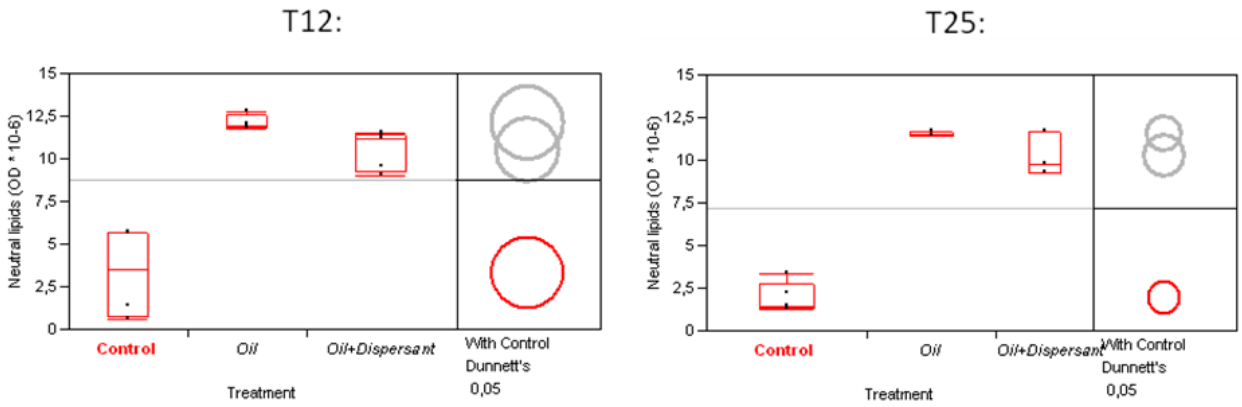

Figure 3.5 *Neutral lipids (NL) accumulation given as optical density (OD*10^{-6}) in amphipods after 12 days of exposure (T12) and 12 days exposure followed by 13 days of recovery (T25).*

The photos in Figure 3.6 illustrate the oxidative stress, with the upper right and lower photos (animals exposed to a WSF and dispersed oil for 12 days) indicating that the lipids in these animal groups were affected by peroxidation due to both WSF in the water and dispersed oil.

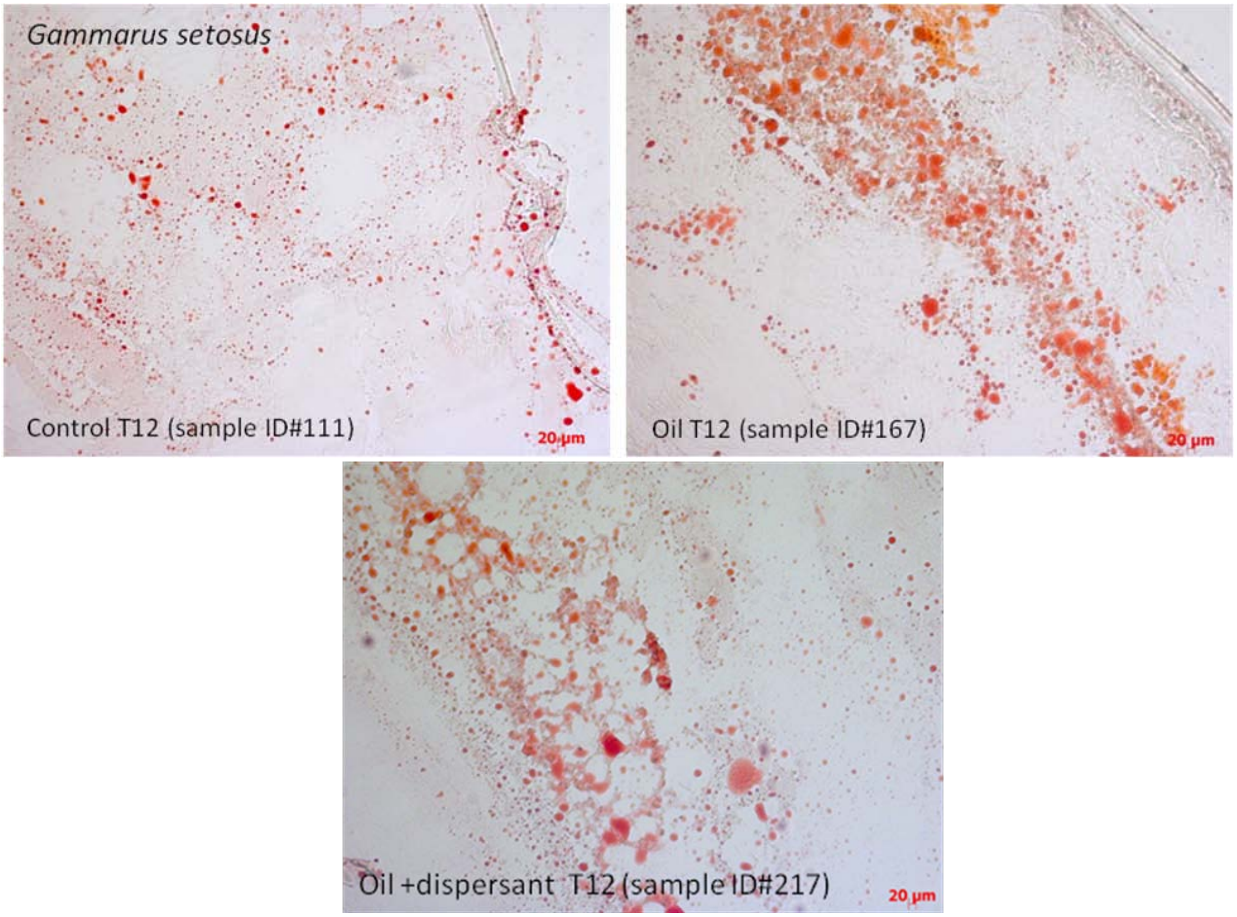

Figure 3.6 *Cryo sections (100x magnification) showing neutral lipid accumulation in amphipods after 12 days: Control group in upper left photo (Control T12) and animals exposed to water soluble oil components in upper right photo (Oil T12), and to dispersed oil in lower photo (oil+dispersant T12).*

Figure 3.7 shows the monitoring of MDA as a reactive product of lipid peroxidation in the animals. According to this, the exposed groups have significantly decreased levels, thereby possibly indicating that the antioxidant system is activated and still able to take care of the reactive product. After 13 days of recovery (T25), there are no significant differences between the groups, thus showing that the exposed animals have recovered. Altogether, the MDA marker indicates a moderate and reversible effect from the exposures.

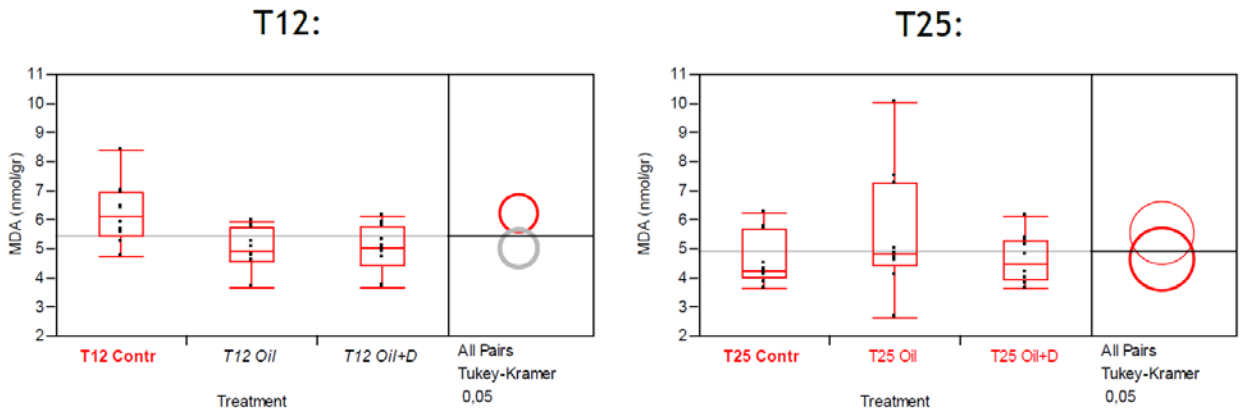

Figure 3.7 *Malondialdehyde (MDA, nmol/g) in amphipods after 12 days of exposure (T12) and 12 days exposure followed by 13 days of recovery (T25).*

3.2 In situ burning experiments

3.2.1 Chemical characterization of oil and water

The GC chromatograms from the oil slick, both prior to and after ISB, are given in Figure 3.8. The oil used, Troll B, is a biodegraded naphthenic oil. The T2 system was covered in the period from oil was released in the barrel until ignition, though the chromatograms show that some of the lighter components had evaporated. As the experiments were performed indoors, a very efficient ventilating system was in operation. In combination with the evaporation of lighter components in the oil in system T2, this resulted in the oil not igniting with the use of only a butane torch. The oil in the T0 system ignited immediately. The burning time was less than two minutes and the burning efficiency was approximately 65% in T2 and 70% in T0 (estimated from the amount of applied oil and collected oil residue). The GC chromatograms illustrate that the residue from the two experiments are similar.

The GC chromatograms from the water samples collected under the oil slick, both prior to and after the ISB, are given in Figure 3.9. The chromatograms show that the WSF (0.19 ppm TPH) from the oil that had been on the water for only one hour was lower than the WSF from the oil that had been on the water for two days (1.89 ppm). The chromatograms of the water samples collected after the ISB indicate that the removal of WSC during the ISB was insignificant. This is also revealed in the results from the VOC and SVOC analysis shown in Figure 3.10.

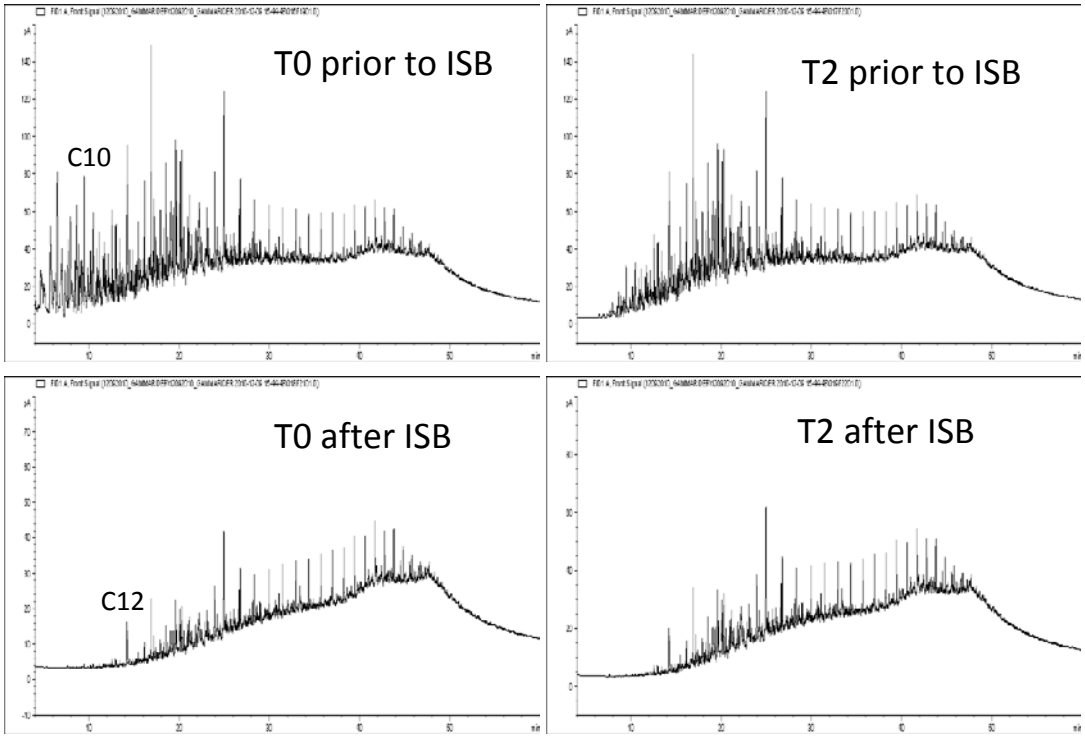

Figure 3.8 *GC chromatograms of the oil slick prior to and after ISB. T0 was ignited within one hr after the oil release and T2 after two days.*

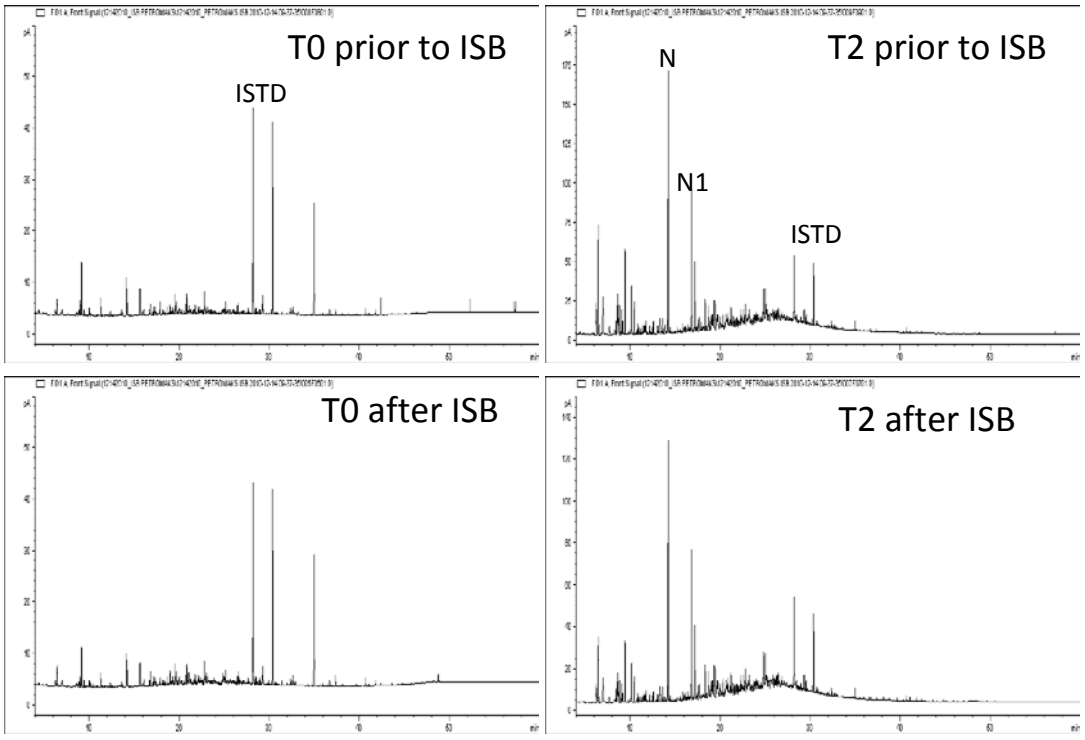

Figure 3.9 *GC chromatograms of water prior to and after ISB. The peaks for the added internal standards (ISTD, two peaks) and naphthalene (N) and C1-naphthalenes (N1) are labelled.*

Figure 3.10 illustrates that the water soluble oil components quantified in the water have not been affected by the burning. The water temperature was not measured prior to or after the ISB, but no significant increase was observed. The composition of the oil slicks prior to, in addition to the oil residue after ISB, is shown in Figure 3.11. No VOC analysis was performed, but a removal of the lighter semi-volatile components, primarily the decalines and naphthalenes, has occurred during the ISB. More detailed results are given in Appendix B. Nonetheless, the composition of the oil slick collected in the two experiments, both prior to and after ISB, is similar.

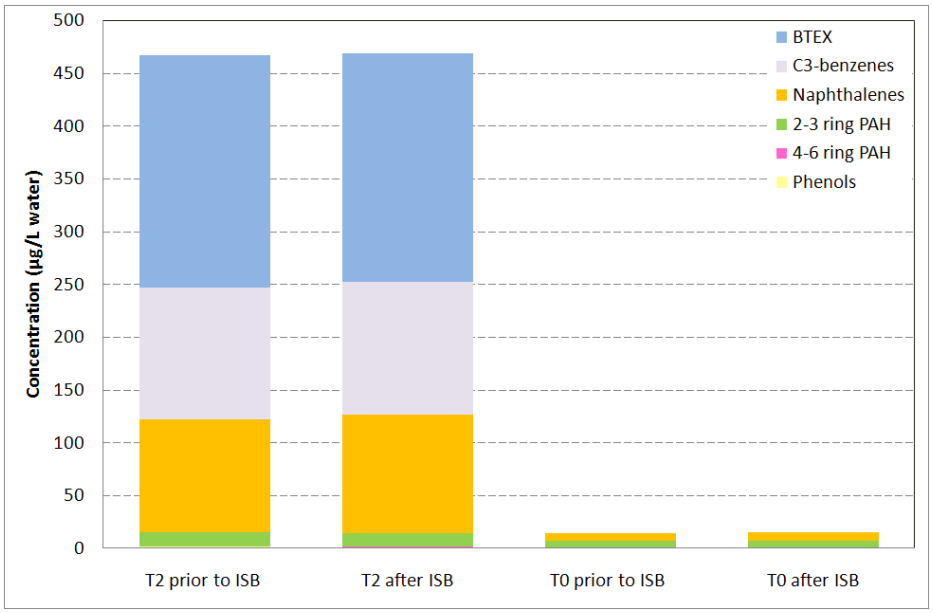

Figure 3.10 *Concentration of WSF in water (µg analyte/L water) sampled prior to and after ISB. T0 was ignited within one hr after oil release and T2 after two days.*

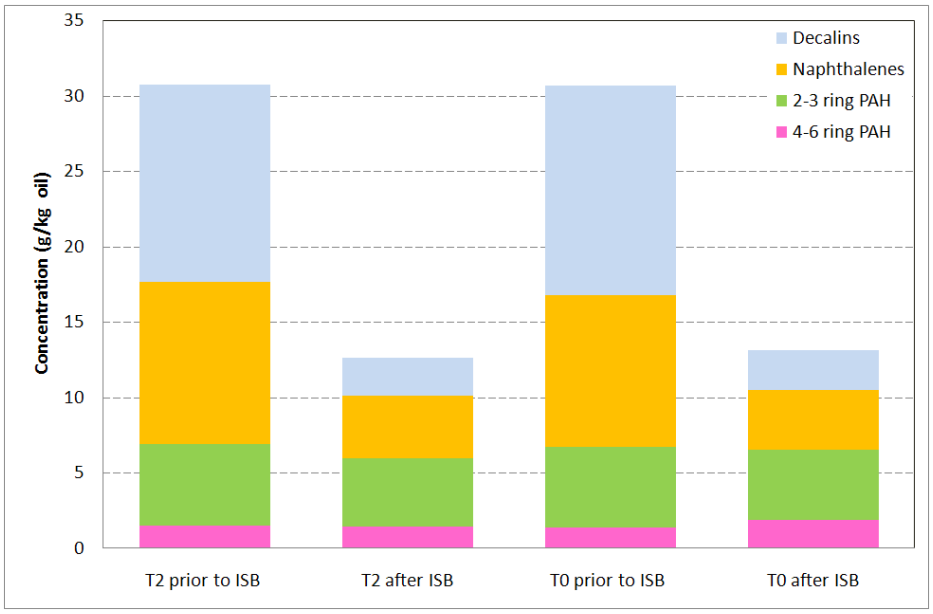

Figure 3.11 *Oil composition (in g analyte/kg oil) prior to and after ISB. T0 was ignited within one hr after oil release and T2 after two days. A VOC analysis was not performed.*

3.2.2 Acute toxicity of the water

The Microtox® test is very sensitive to water soluble oil components, and is only suitable as an indicator of potential acute toxicity, as it is based on the measurement of a single response from a primitive organism. There was no effect observed in the T0 experiment, probably due to the fact that the concentration of WSF was too low. In the T2 experiment, the relative acute toxicity (EC_{50} (%)) was 47% prior to the ISB and 39% after the ISB, thus corresponding to a specific toxicity of 1.1 and 0.8 ppm. These results indicate that the WSF after ISB did not become more toxic to the Microtox® bacteria than the WSF prior to ISB.

The copepods *C. finmarchicus* were exposed to a serial dilution of the water from the ISB experiments in order to determine the LC_{50}. In Figure 3.12, copepod survival (as % of controls) is plotted as a function of Log_{10} of the total WSF concentration in the water. However, the level of toxic stress was not sufficient for calculation of a conclusive value for LC_{50} after an exposure of 96 hours, as the survival in both treatments was well above 50%. The highest immobilization was observed in the T0 treatment with a 33% immobility rate among the animals after 96 hours, while the immobilization in the T2 experiment was 19% after the same amount of time. No immobilization was observed in the control.

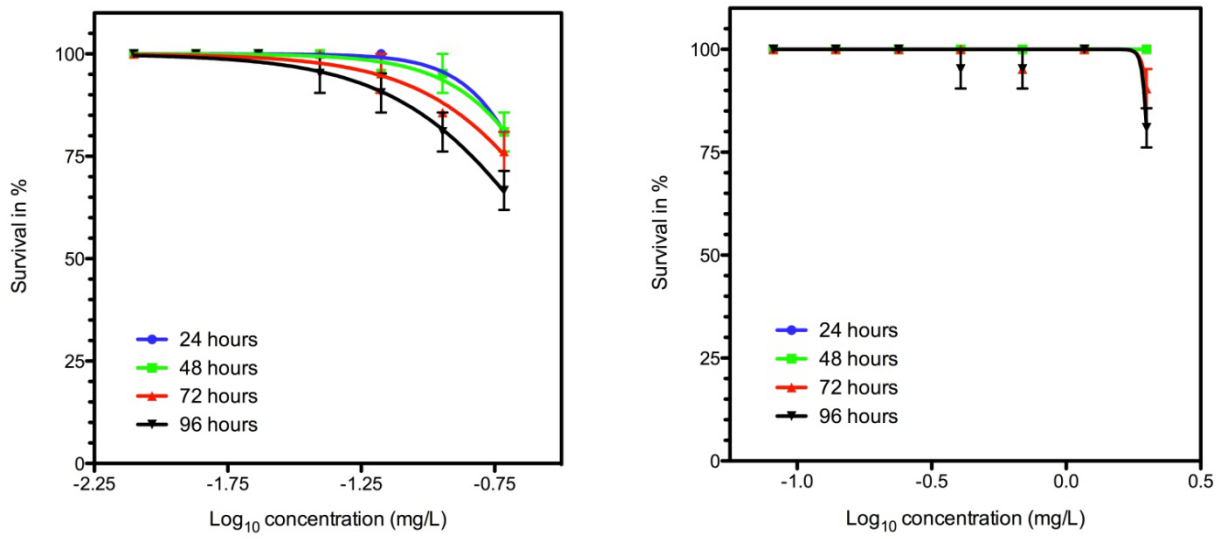

Figure 3.12 *Survival by percentage for Calanus finmarchicus exposed for 24, 48, 72 and 96 hours to different concentrations of water after ISB, T0 to the left (33% immobilized) and T2 to the right (19% immobilized). The dots represent the mean (±SE) of observed data, and solid line represents the data fitted to the non-linear Sigmoidal model.*

Several experiments have been performed in the past using the CROSERF methodology (Singer et al., 2000) to prepare standard water accommodated fractions (WAF). In a previous experiment that followed the same protocol as this study, *C. finmarchicus* was exposed to WAF prepared with an oil-to-water ratio of 1 to 10 000 from Troll crude oil artificially weathered to 200 ºC+ as described in Hansen et al. (2011). The total WAF concentration was 0.8 ppm, and this experiment yielded an immobilization rate of 24% for the copepods after 96 hours. These results show that there may not have been any increase in acute toxicity in the underlying water after an ISB when tested by a toxicity assay that measured acute toxicity.

One of the objectives of NOBE (summarized in e.g. Fingas et al., 1994) was to determine the potential toxic effects to aquatic organisms resulting from ISB and how these effects compared with the effects of unburned oil. One of the activities was designed to assess the effects of ISB on aquatic toxicity to the water column and the subsequent effects to aquatic organisms (Daykin et al., 1994). Several toxicity tests were conducted on the laboratory generated water samples and on the field burn samples. The chemical analyses and toxicity testing performed on the water samples indicated that an ISB did not adversely affect the underlying water column beyond those effects already associated with the unburned weathered oil. These findings are in accordance with the results presented here, and indicate that severe effects in the underlying water column may be neglectable under an ISB when traditional lethal toxicity assays are applied. The toxicity seems to be low compared to for instance mechanically and/or chemically dispersed oil in the water column. However, future effect studies of ISB should include other potential environmental compartments (e.g. air and residue).

4 Conclusions

Realistic exposure concentrations have been used to compare the biological effects of WSF of oil versus chemically dispersed oil by measuring body burden and biomarker responses (e.g. lysosomal stability in blood cells, MDA and catalase) on the Arctic amphipod *Gammarus setosus*. Chemical results from the analyses of water samples in the laboratory exposure experiments exhibited a good correlation with comparable data monitored during the offshore field experiment with oil in ice-infested water. Due to low input of energy during the exposure period in the laboratory study, there were weak indications of oil droplet formation in the water fraction caused by the dispersant. Among the effect markers monitored, general stress was indicated by a significant decrease in the lysosomal stability of amphipod haemocytes in the dispersed oil treatment in comparison to the WSF and control. The neutral lipid marker shows a relatively strong effect for gammarids exposed to both a WSF and dispersed oil, even after recovery in clean seawater. The MDA marker shows a weak effect after exposure, but the animals recovered after they were transferred to clean seawater.

Seawater samples and oil were collected prior to and immediately after ISB, and chemical analyses were conducted. Acute toxicity tests with the marine copepod *C. finmarchicus* and Microtox® bioassay was performed to establish LC_{50}/EC_{50} values of the water. The chemical characterization of the underlying water prior to and after ISB indicated that the disappearance of water soluble oil components during ISB was insignificant, but an evaporation of the lighter semi-volatile components, mainly the decalines and naphthalenes, had occurred from the oil slick. It was not possible to measure a level of toxic stress sufficient for calculating a conclusive value for LC_{50} for *C. finmarchicus* after an exposure of 96 hours, as the immobilization did not exceed a 33% undiluted WSF at its highest. There seems to be no increase in acute toxicity in the underlying water after ISB compared to corresponding experiments with regular WAF systems. The acute toxicity of the water to the Microtox® bacteria demonstrated the same trend as for the copepods. These results show that there is no increase in acute toxicity in the water column beneath the slick after ISB compared to before the ISB was initiated when investigated by traditional assays measuring acute toxicity.

These results can be used as a basis for environmental risk modelling, including decision support in oil spill response planning, as one of the critical issues for the authorities and the oil industry are to be able to choose the mitigation method that will give the largest net environmental benefit when a

spill has occurred. There are several elements that must be considered, such as impacts from chemically versus mechanically dispersed oil, impacts of dispersed oil in the water column as basis for further spreading, dilution and biodegradation versus impacts of spreading on surface to potential sensitive habitats (e.g. ice edge and shoreline), and the efficiency of other combat strategies such as ISB and mechanical recovery.

5 Acknowledgments

This work has been an integrated part of the Oil-in-Ice Joint Industry Program and the authors wish to thank the sponsors of this program: The Research Council of Norway and the five oil and gas companies ConocoPhillips, Chevron, Shell, Statoil, and Total for their valuable support. We gratefully acknowledge Trond Nordtug, Per Johan Brandvik, Bror Johansen, Kjersti Almås and Marianne U. Rønsberg at SINTEF, and Nadia Aarab, Claudia Lucas, and Solveig Apeland at IRIS for their valued discussions and/or assistance in the laboratory.

6 References

Azur Environmental Ltd. *Microtox® Acute Toxicity Basic Test Procedure*, Microbics Corporation, Carlsbad, CA, 1995.

Bado-Nilles, A., C. Quentel, M. Auffret, S. LeFloch, B. Gagnaire, T. Renault, and H. Thomas-Guyon, "Immune Effects of HFO on European Sea Bass, *Dicentrarchus labrax*, and Pacific Oyster, *Crassostrea gigas"*, *Ecotoxicology and Environmental Safety*, 72: 1446–1454, 2009.

Bancroft , J.D., *An Introduction to Histochemical Technique.* Butterworths, London, 268 p., 1967.

Baussant, T., S. Sanni, G. Jonsson, A. Skadsheim, and J.F. Børseth,"Bioaccumulation of Polycyclic Aromatic Compounds: 1. Bioconcentration in Two Marine Species and in Semipermeable Membrane Devices during Chronic Exposure to Dispersed Crude Oil", *Environmental Toxicology and Chemistry*, 20(6): 1175-1184, 2001.

Baussant, T., R.K. Bechmann, I.C. Taban, B.K. Larsen, A.H. Tandberg, A. Bjørnstad, S. Torgrimsen, A. Nævdal, K.B. Øysæd, G. Jonnson, and S. Sanni, "Enzymatic and Cellular Response in Relation to Body Burden of PAHs in Bivalve Mulloscs: A Case Study with Chronic Levels of North Sea and Barents Sea Dispersed Oil", *Mar. Poll. Bull.*, 58: 1796-1807, 2009.

Blekinsopp, S.A., G. Sergy, K. Doe, G. Wohlgeschaffen, K. Li, and M. Fingas. "Toxicity of the Weathered Crude Oil used at the Newfoundland Offshore Burn Experiment (NOBE) and the Resultant Burn Residue", *Spill Science & Technology Bulletin*, 3(4): 277-280, 1996.

Chapman, H., K. Purnell, R.J. Law and M.F. Kirby. "The Use of Chemical Dispersants to Combat Oil Spills at Sea: A Review of Practice and Research needs in Europe", *Mar. Poll. Bull.*, 54: 287-838, 2007.

Daykin, M., G. Sergy, D. Aurand, G. Shigenaka, Z. Wang, and A. Tang. "Aquatic Toxicity Resulting from In Situ Burning of Oil-on-Water", in *Proceedings of the 17th Arctic and Marine Oil Spill Program (AMOP) Technical Seminar*, Environment Canada, Ottawa, ON, pp. 1165-1193, 1994.

Dierickx, P. J, and I. E. Van de Vyver. "Correlation of the Neutral Red Uptake Inhibition Assay of Cultured Fathead Minnow Fish Cells with Fish Lethality Tests", *Bulletin of Environmental Contamination and Toxicology*, 46(5): 649-653, 1991.

Faksness, L.G. P.J. Brandvik, R.L. Daae, F. Leirvik and J.F. Børseth. "Large-scale Oil-in-Ice Experiment in the Barents Sea: Monitoring of Oil in Water and MetOcean Interactions", *Mar. Poll. Bull.*, 62: 976-984, 2011.

Fingas, M.F., G. Halley, F. Ackerman, N. Vanderkooy, R. Nelson, M.C. Bissonnette, N. Laroche, P. Lambert, P. Jokuty, K. Li, W. Halley, G. Warbanski, P.R. Campagna, R. Turpin, M.J. Trespalacios, D. Dickins, E.J. Tennyson, D. Aurand, and R. Hiltabrand, "The Newfoundland Offshore Burn Experiment – NOBE. Experimental Design and Overview", in *Proceedings of the 17th Arctic and Marine Oil Spill Program (AMOP) Technical Seminar*, Environment Canada, Ottawa, ON, pp. 1053-1063, 1994.

Fingas, M., and J. Banta, "Review of Literature Related to Oil Spill Dispersants", in *Proceedings of the 32nd Arctic and Marine Oil Spill Program (AMOP) Technical Seminar*, Environment Canada, Ottawa, ON, pp. 869-920, 2009.

Hansen, B.H., D. Altin, T. Nordtug, and A.J. Olsen, "Suppression Subtractive Hybridization Library Prepared from the Copepod *Calanus finmarchicus* (Gunn.) Exposed to a Sublethal Mixture of Environmental Stressors", *Comp. Biochem. Physiol*., 2D:250-256, 2007.

Hansen, B.H., D. Altin, S.F. Rørvik, I.B. Øverjordet, A.J. Olsen, and T. Nordtug. "Comparative Study on Acute Effects of Water Accommodated Fractions of an Artificially Weathered Crude Oil on *Calanus finmarchicus* and *Calanus glacialis* (Crustacea: Copepoda)", *Science of the Total Environment*, 409: 704-709, 2011.

Hop, H., T. Pearson, et al. "The Marine ecosystem of Kongsfjorden, Svalbard", *Polar Research*, 21(1); 167-208, 2002.

ISO (International Organization for Standardization), "Water Quality - Determination of Acute Lethal Toxicity to Marine Copepods (Copepoda, Crustacea)", ISO 14669:1999.

Lowe, D.M., M.N. Moore, and B.M. Evans, "Contaminant Impact on Interactions of Molecular Probes with Lysosomes in Living Hepatocytes from Dab Limanda-Limanda", *Marine Ecology-Progress Series*, 91(1-3): 135-140, 1992.

Lowe, D.M., and R.K. Pipe, "Contaminant-induced Lysosomal Membrane Damage in Marine Mussels Digestive Cells: An *In Vitro* Study", *Aquat. Toxicol*. 30: 357-365, 1994.

Lowe, D.M., V.U. Fossato, and M.H. Depledge, "Contaminant-induced Lysosomal Membrane Damage in Blood Cells of Mussels *Mytilus galloprovincialis* from the Venice Lagoon: An In Vitro Study", *Marine Ecology-Progress Series*,129(1-3): 189-196, 1995.

McFarlin, K.M., R.A. Perkins, W.W. Gardiner, and J.D. Word, "Evaluating the Biodegradability and Effects of Dispersed Oil Using Arctic Test Species and Conditions: Phase 1 Activities", in *Proceedings of the 33rd AMOP Seminar on Environmental Contamination and Response*, Environment Canada, Ottawa, ON, pp. 1243-1251, 2010.

McFarlin, K.M., R.A. Perkins, W.W. Gardiner, J.D. Word, and J.Q. Word, *Toxicity of Physically and Chemically Dispersed Oil to Selected Arctic Species*, paper no. 2011-149, The International Oil Spill Conference, May 23-26, 2011, Portland, Oregon.

Neff, J.M., S. Johnsen, T.K. Frost, T.I.R. Utvik and G.S. Durell, "Oil Well Produced Water Discharges to the North Sea. Part II: Comparison of Deployed Mussels (*Mytilus edulis*) and the DREAM Model to Predict Ecological Risk", *Marine Environmental Research* 62, 224-246, 2006.

Ronowicz, M. , " Species diversity of Arctic gravel beach: case study for species poor habitats", *Polish Polar Research*, 26(4): 287-297, 2005.

Singer, M.M., D. Aurand, G.E. Bragin, J.R. Clark, G.M. Coelho, M.L. Sowby, and R.S. Tjeerdema," Standardization of the Preparation and Quantification of Water-Accommodated Fractions of Petroleum for Toxicity Testing", *Marine Pollution Bulletin*, 40 (11): 1007-1016, 2000.

Spooner, G.M., "On *Gammarus zaddachi oceanicus* Segerstråle" , Journal *of the Marine Biology Association of the United Kingdom*, 30(1): 129-146, 1951.

Sørstrøm, S.E., P.J. Brandvik, I. Buist, P. Daling, D. Dickins, L.G. Faksness, S. Potter, J. Fritt-Rasmussen, and I. Singsaas," Joint Industry Program on Oil Contingency for Arctic and Ice-covered Waters, Summary Report", SINTEF A14181, ISBN-no. 978-82-14-04759-2, 40 p., 2010.

US EPA, Method 3510C. Separatory Funnel Liquid-Liquid Extraction, 1996. http://www.epa.gov/wastes/hazard/testmethods/sw846/pdfs/3510c.pdf

US EPA, Method 8100. Polynuclear Aromatic Hydrocarbons, 1986. www.epa.gov/osw/hazard/testmethods/sw846/pdfs/8100.pdf

US EPA, Method 8260C. Volatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS), 2006. http://www.epa.gov/osw/hazard/testmethods/sw846/new_meth.htm#8260C

US EPA, Method 8270D. Semivolatile Organic Compounds by GC/MS, 2007. http://www.epa.gov/wastes/hazard/testmethods/sw846/pdfs/8270d.pdf

Weslawski, J.M., J. Wiktor, M. Zajaczkowski, and S. Swerpel, "Intertidal Zone of Svalbard. 1. Macroorganism Distribution and Biomass", *Polar Biology*, 13: 73-79, 1993.

Weslawski, J.M. and J. Legezynska, "Life cycles of some Arctic amphipods". *Polish Polar Biology*, 23 (3-4) 253-264, 2002.

Wheatley, R.A. "Some Recent Trends in the Analytical Chemistry of Lipid Peroxidation", *Trac-Trends in Analytical Chemistry*, 19(10): 617-628, 2000.

A Overview of analytes and component groups used in tables and figures.

Group	Compound	Group	Compound
SVOC			
	Decalin	C0-C4 phenols	Phenol
	C1-decalins		C1-phenols
	C2-decalins		C2-phenols
	C3-decalins		C3-phenols
	C4-decalins		C4-phenols
Naphthalenes	Naphthalene		
	C1-naphthalenes	VOC	
	C2-naphthalenes	(incl. BTEX	Isopentane
	C3-naphthalenes	C3-benzenes)	n-C5 (Pentane)
	C4-naphthalenes		Cyclopentane
2-3 ring PAHs	Biphenyl		2-methylpentane
	Acenaphthylene		3-methylpentane
	Acenaphthene		n-C6 (Hexane)
	Dibenzofuran		Methylcyclopentane
	Fluorene		Cyclohexane
	C1-fluorenes		2,3-dimethylpentane
	C2-fluorenes		3-methylhexane
	C3-fluorenes		n-C7 (Heptane)
	Phenanthrene		Methylcyclohexane
	Anthracene		2,4-dimethylhexane
	C1-phenanthrenes/anthracenes		2-methylheptane
	C2-phenanthrenes/anthracenes		n-C8 (Octane)
	C3-phenanthrenes/anthracenes		n-C9 (Nonane)
	C4-phenanthrenes/anthracenes		n-C10 (Decane)
	Dibenzothiophene		n-Butylbenzene
	C1-dibenzothiophenes		1,2,4,5-tetramethylbenzene
	C2-dibenzothiophenes		n-pentylbenzene
	C3-dibenzothiophenes		C4-benzenes
	C4-dibenzothiophenes		C5-benzenes
4-6 ring PAHs	Fluoranthene	BTEX	Benzene
	Pyrene		Toluene
	C1-fluoranthrenes/pyrenes		Ethylbenzene
	C2-fluoranthenes/pyrenes		*m*-xylene
	C3-fluoranthenes/pyrenes		*p*-xylene
	Benz[*a*]anthracene		*o*-xylene
	Chrysene	C3-benzenes	Propylbenzene
	C1-chrysenes		1-methyl-3-ethylbenzene
	C2-chrysenes		1-methyl-4-ethylbenzene
	C3-chrysenes		1,3,5-Trimethylbenzene
	C4-chrysenes		1-methyl-2-ethylbenzene
	Benzo[*b*]fluoranthene		1,2,4-trimethylbenzene
	Benzo[*k*]fluoranthene		1,2,3-trimethylbenzene
	Benzo[*e*]pyrene		
	Benzo[*a*]pyrene		
	Perylene	TPH	C10-C36
	Indeno[*1,2,3-c,d]*pyrene	WAF	Sum of VOC and TPH
	Dibenz[*a,h]*anthracene		
	Benzo[*g,h,i*]perylene		

B Chemical and toxicological characterization

Table B 1 *Chemical composition of the oil prior to ISB and the oil residue after ISB. T2 was ignited 2 days after oil release, and T0 within an hour.*

	Sum SVOC µg/mg oil	Naphthalenes µg/mg oil	2-3 ring PAH µg/mg oil	4-6 ring PAH µg/mg oil	Decalins µg/mg oil	Phenols µg/mg oil
Troll crude	28.2	9.33	4.85	1.19	12.8	0.02
T2 prior to ISB	30.8	10.7	5.43	1.51	13.1	0.01
T2 after ISB	12.6	4.13	4.53	1.46	2.51	ND
T0 prior to ISB	30.7	10.0	5.38	1.38	13.9	0.01
T0 after ISB	13.2	3.98	4.67	1.88	2.64	0.02

ND: Not detected or < 0.01 µg/mg oil

Table B 2 *Chemical composition of the underlying water prior to and after ISB. T0 was ignited within one hour after oil release and T2 after 2 days.*

	Sum SVOC µg/L water	Naphthalenes µg/L	2-3 ring PAH µg/L	4-6 ring PAH µg/L	Decalins µg/L	Phenols µg/L
T2 prior to ISB	123	107	14.0	0.11	0.26	1.50
T2 after ISB	127	113	12.9	0.22	0.18	1.21
T0 prior to ISB	14.6	7.54	6.47	0.18	0.03	0.40
T0 after ISB	15.2	7.81	6.76	0.19	0.06	0.33

Table B 3 *Chemical composition and measured acute toxicity by Microtox® in the underlying water prior to and after ISB. WSF is the sum of TPH and VOC.*

	WSF µg/L	TPH µg/L	VOC µg/L	BTEX µg/L	C3-benzenes µg/L	EC_{50} % WSF	EC_{50} µg/L WSF
T2 prior to ISB	2268	1888	380	220	125	47	1064
T2 after ISB	1989	1614	375	217	125	39	776
T0 prior to ISB	194	194	NA	NA	NA	NA	NA
T0 after ISB	193	177	15.6	8.37	1.65	>100	

NA: Not analyzed

Technology for a better society

www.sintef.no