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REPORT ON OIL SPILL EFFECTS

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WP4.2.3: Biology and potential effect of oil spill

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DELIVERABLE SUMMARY SHEET

Short Description
For the study of effects of oils spills on arctic sea ice biota, a field experiment was carried out in Van Mijenfjorden, Svalbard, during 22.2.-25.4.2004. During the 63 day long experiment, three different compounds (Statfjord crude oil, Inipol EAP 22 and fish food) were added in different combinations onto snow-free sea ice surface. In blank samples with no addition of chemicals, the abundance of photosynthesing algae (diatoms and euglenid flagellates) was ≤ 3400 cells 100 ml^{-1} melted sea ice, with highest abundance in the ice interior and bottom layers (50-120 cm). The treatment with oil only, oil and Inipol EAP 22, and oil and fish food lead to a general decrease in protist diversity and abundance. The most dramatic decrease in the abundance of all protists (microscopical invertebrate organisms, such as unicellular algae) was caused by the addition of oil only, and throughout the ice cover, while the addition of oil and Inipol EAP 22, and oil and fish food lead to the disappearance of all other protist groups than diatoms. The negative effects of Inipol EAP 22 and fish food were most severe in the ice surface, while the interior and the bottom parts of the ice cover were less impacted by the treatments. Heterotrophic flagellates seemed to be able to migrate downwards from the ice surface when only oil was added onto the ice. Also, the use of oil only, and oil and Inipol EAP 22 induced the formation of diatom resting spores. In control samples, the addition of fish food only lead to a notable increase of heterotrophic flagellates. Our experiment shows, that the addition of nutrients (either in the form of Inipol EAP 22 or fish food) accelerated the biodegradation of Statfjord crude oil in arctic sea ice notably.

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D4.2.3.3. Report on oil spill effects

An experimental study of the effects of crude oil, and application of Inipol EAP 22 and fish food on the sea ice biota and hydrocarbon content in Svalbard in February-April 2004

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Abstract
For the study of effects of oils spills on arctic sea ice biota, a field experiment was carried out in Van Mijenfjorden, Svalbard, during 22.2.-25.4.2004. During the 63 day long experiment, three different compounds (Statfjord crude oil, Inipol EAP 22 and fish food) were added in different combinations onto snow-free sea ice surface. In blank samples with no addition of chemicals, the abundance of photosynthesing algae (diatoms and euglenid flagellates) was $\leq$ 3400 cells 100 ml^{-1} melted sea ice, with highest abundance in the ice interior and bottom layers (50-120 cm). The treatment with oil only, oil and Inipol EAP 22, and oil and fish food lead to a general decrease in protist diversity and abundance. The most dramatic decrease in the abundance of all protists (microscopical invertebrate organisms, such as unicellular algae) was caused by the addition of oil only, and throughout the ice cover, while the addition of oil and Inipol EAP 22, and oil and fish food lead to the disappearance of all other protist groups than diatoms. The negative effects of Inipol EAP 22 and fish food were most severe in the ice surface, while the interior and the bottom parts of the ice cover were less impacted by the treatments. Heterotrophic flagellates seemed to be able to migrate downwards from the ice surface when only oil was added onto the ice. Also, the use of oil only, and oil and Inipol EAP 22 induced the formation of diatom resting spores. In control samples, the addition of fish food only lead to a notable increase of heterotrophic flagellates.

Introduction

Crude oil and oil product shipping is expected to increase in the Arctic, in particular in the Barents Sea, in the near future (UNEP 2004). In addition to increasing transportation rates, the risk of an oil spill or an accident is elevated by e.g. the lack of experience in large-tonnage tanker navigation under arctic conditions, and insufficient potential of emergency services (UNEP 2004). Environmental Risk Analysis (ERA) and Environmental Impact Assessments (EIA) are made for areas at risk (e.g. UNEP 2004, www.arctic-council.org). While birds and mammals suffer mostly from mechanical damages (oiling of the plumage or skin, thus problems with thermoregulation), most underwater nature is threatened also by chemical effects of hydrocarbons. Oil, in particular its photo-oxidation products can cause direct damage on marine organisms on several systematic levels (Sakshaug et al. 1994). The bioremediation by bacteria may be depressed due to the toxic effects of oil photo-oxidation in strong sun light. Eggs, as well as larval and juvenile stages of organisms are particularly sensitive to hydrocarbons. Sessile and filtrating organisms in the open water, littoral and benthos, such as macroalgae, crabs, sea stars, mussels and zooplankton,
may become covered by oil, their filtration apparatuses blocked by oil particles, or oil may be stored in lipid storages. This, in turn, will lead to biomagnification in the arctic foodweb, as a consumer may engulf large quantities of oil together with its prey organism. Indirect effects are for example skin irritation, causing increasing blood circulation and thus increased heat loss through the skin (Sakshaug et al. 1994).

The knowledge of the consequences of oil contamination on unicellular organisms, in particular primary producers which are the foundation of sea ice covered arctic marine ecosystems is sparse and patchy. It is assumed, that algae remain unaffected or that the effects of oil on phytoplankton are limited to short term effects, such as temporary decrease of photosynthetic rates and species diversity, but that the recovery would be relatively fast (Cross 1987, Patin 2001). It is imperative that the matter be further investigated.

Our experiment was an effort to resolve some of the remaining questions regarding the effects of an oil spill on arctic sea ice biota. We exposed ice and its biota to Statfjord crude oil (provided by SINTEF, Norway), Inipol EAP 22, and nutrient rich fish food (also to improve oil bioremediation). Inipol EAP 22 is a commercial product with concentrations of phosphorous (tri(laureth-4) - phosphate, 0,7%) and nitrogen (urea, 7,4%) to accelerate bioremediation (e.g. www.atofinachemicals.com). Based on the results of our experiment, it seems that in case of an oil spill, the use of either Inipol EAP 22 or fish food would be recommended to a) minimize the negative effects of oil on unicellular organisms (in particular primary producers) in ice, and b) to accelerate the biodegradation of hydrocarbons. In experiments with only oil the ice biota was affected the most; both the species diversity and abundance decreased notably. Oil also caused the downward migration of heterotrophic flagellates within the ice cover. The addition of fish food alone lead to a dramatic increase of heterotrophic organisms.

Material and methods

The experiment was made during 22.2.-25.4.2004 (63 days). The total experimental area on ice was 10 m x 10 m (Fig. 1). Each experimental field (dimensions 64 x 45 cm) was surrounded by a hard plastic box to avoid contamination in the water column and the surrounding sea ice. Blank ice core samples were taken prior to any experimental treatments on 22.2.2004. Statfjord oil (150 ml), oil

and Inipol EAP 22 (150 ml + 15 ml), and oil and fish food (150 ml + 15 g) was distributed onto snow-free sea ice. Also control areas with no treatment, only Inipol EAP 22 (15 ml), or only fish food (15 g) were established.

All ice core samples were taken with a SIPRE type auger and cut into subsamples: blank samples into 0-16, 16-33, 33-56, 56-83 and 83-111 cm, experiment samples into top part 0-25 cm, interior part 50-75 cm, and bottom part (95-120, 98-123 or 103-128 cm). Control samples with no treatment, only Inipol EAP 22, or fish food were taken only from the uppermost 25 cm of the ice cover. Each subsample was melted in 200 ml sterile sea water with added sea salts corresponding the salinity of sea water (34). Melted samples were preserved either with formalin (22.2.2004) or Lugol's (25.4.2004) solution (5% final concentration).

Chlorophyll-*a* samples were made of melted ice by filtering various volumes of each sample onto a GF/F filter and stored in -20 ºC. The sample types and volumes are listed in Table 1. The top layer (0-16 cm) was destroyed during transportation from field to the laboratory, and could thus not be processed. Chlorophyll-*a* was determined later in the water analytical laboratory at the Tvärminne Zoological Station with a Cary Varian Eclipse, microwell plate reader, exc430nm/em671nm, slits 5 nm.

Samples for the analysis of the species composition of sea ice biota, and the abundance of protists in different experiment fields were concentrated according to the Utermöhl technique (Utermöhl 1958) and examined with an inverted Leitz DMIL microscope, 250-500x final magnification.

date	sample type	ice depth (cm)		sample volume [ml]
22.2.2004	blank	0-16	top	destroyed
	blank	16-33	interior	736,0
	blank	33-56	interior	1067,0
	blank	56-83	interior	302,9
	blank	83-111	bottom	472,5
25.4.2004	blank	0-25 cm	top	209,7
	blank	95-120 cm	bottom	440,7
	control, no treatment	0-25 cm	top	962,5
	oil only	50-75 cm	middle	1226,9
	oil only	103-128 cm	bottom	220,0
	oil + Inipol EAP 22	50-75 cm	middle	190,9
	oil + Inipol EAP 22	95-120 cm	bottom	374,8
	oil + fish food	50-75 cm	middle	66,8
	oil + fish food	98-123 cm	bottom	496,2

Table 1. Samples for chlorophyll-a analysis of sea ice during the experiment.

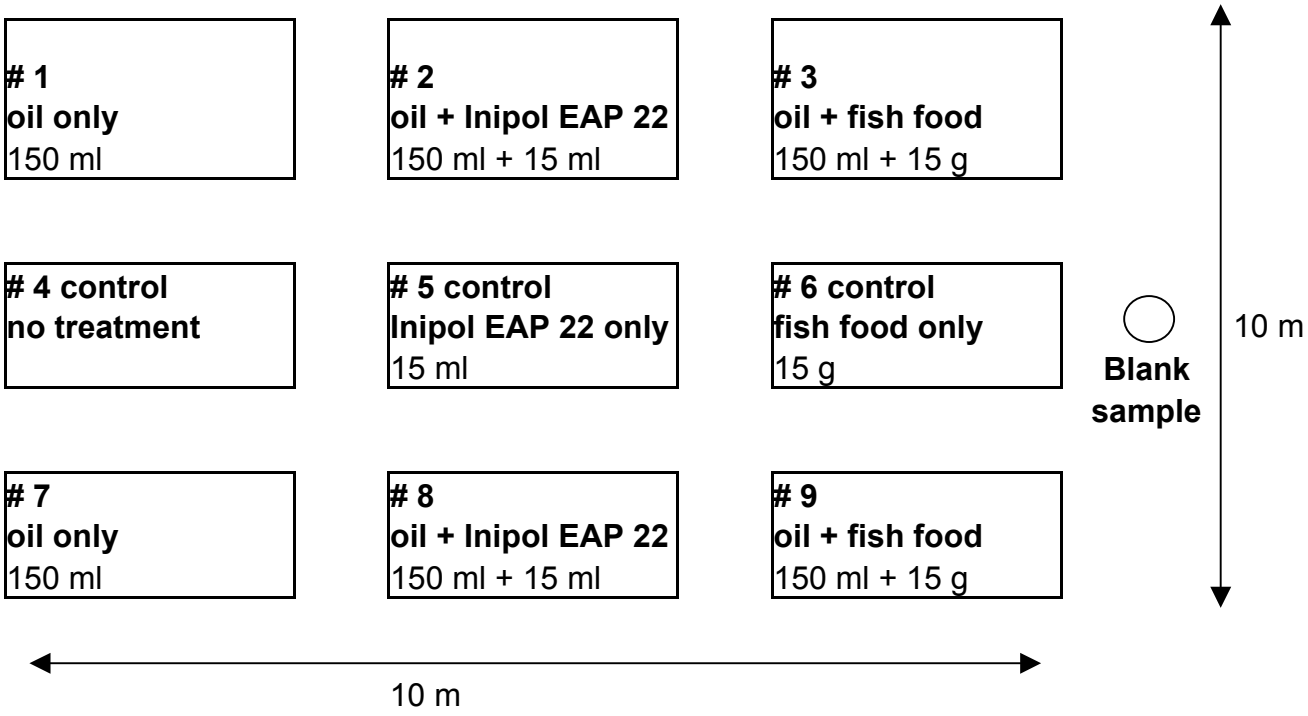


Figure 1. Experimental design for the study of effects of oil spills on the arctic sea ice biota.

Total petroleum hydrocarbons (TPH), aliphatic hydrocarbons and aromatic hydrocarbons of the oil samples were analyzed according to the Texas Natural Resource Conservation Commission methods 1005 and 1006 (www.tnrcc.state.tx.us). Hewlett Packard 5890 gas chromatograph with flame ionization (FID) detection, On-Column injection port, and a BPX-5 (15 m, 0.32 mm, 1.0 μm) column was used. Samples were extracted with hexane (Mallinckrodt for residual analysis). Aliquot of the extract was used to determine TPH content of the extract. Aliphatic and aromatic hydrocarbons were separated using a silica column (Merck 60 mesh). One millilitre of the extract was added to the top of the column. Aliphatic hydrocarbons were eluted from the column with hexane, and aromatic hydrocarbons with dichloromethane (Mallinckrodt for residual analysis). Finally, a 1:1 mixture of dichloromethane and acetone (Mallinckrodt for residual analysis) was used to elute heavier aromatic hydrocarbons from the column.

Results
Prior to the species identification of protists, we made remarks of the overall appearance of each sample with a 250x final magnification (Table 2).

SAMPLE	REMARKS
All control samples (February 2004)	"clean" samples, intact algal cells and some sediment particles present
Control: no treatment	"clean" sample, intact cells and some sediment particles present
Control: treatment with Inipol EAP 22 only	only large (Ø ≤100 µm) aggregations of Inipol EAP 22 observed
Control: treatment with fish food only	high abundance of bacteria, some aggregations of fish food
Treatment: oil only (0-25 cm)	algal cells shrunk, partly broken, high abundance of rod-shaped bacteria. Oil as a non-transparent film on the innerside of the sample vial.
Treatment: oil only (50-75 cm)	very empty and "clean" sample, no oil, low abundance of bacteria. Oil as a non-transparent film on the innerside of the sample vial.
Treatment: oil only (103-128 cm)	very empty and "clean" sample, no oil, few bacteria. Oil as a non-transparent film on the innerside of the sample vial.
Treatment: oil + Inipol EAP 22 only (0-25 cm)	very "clean" sample, small (Ø ≤10 µm) oil droplets present, low abundance of bacteria. Oil as a semi-transparent film on the innerside of the sample vial.
Treatment: oil + Inipol EAP 22 only (50-75 cm)	very "clean" sample, no oil droplets observed, no or very few bacteria, intact algal cells. Oil as a semi-transparent film on the innerside of the sample vial.
Treatment: oil + Inipol EAP 22 only (95-120 cm)	very "clean" sample, no oil droplets observed, no or very few bacteria, intact algal cells. Oil as a semi-transparent film on the innerside of the sample vial.
Treatment: oil + fish food only (0-25 cm)	Very high abundance of rod-shaped bacteria, large (Ø ≤100 µm) aggregations of oil and fish food, no intact algal cells. Oil as a transparent film on the innerside of the sample vial.
Treatment: oil + fish food only (50-75 cm)	High abundance of bacteria, aggregations of fish food (Ø <100 µm), broken and intact algal cells present. Oil as a transparent film on the innerside of the sample vial.
Treatment: oil + fish food only (98-123 cm)	High abundance of rod-shaped bacteria, algal cells covered by bacteria. Oil as a transparent film on the innerside of the sample vial.

Table 2. Remarks of samples, made with naked eye and an inverted microscope (a 250x final magnification).

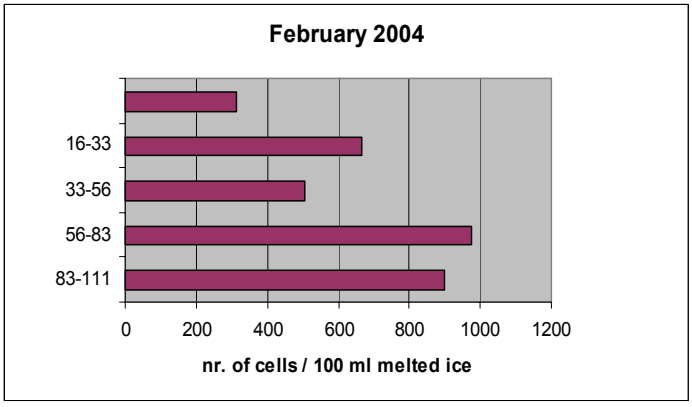
Tables 3 and 4 show protist taxa that we recorded in samples from February (Table 3: controls prior to the experiment) and in April (Table 4, Appendix I: controls, blanks and experimental treatments).

	0-16 cm	16-33 cm	33-56 cm	56-83 cm	83-111 cm
Bacillariophyceae					
Bacillaria paxillifera		x		x	
Banquisia belgicae	x	x	x	x	x
Coscinodiscus oculus-iridis		x			
Cylindrotheca closterium	x	x	x	x	x
cf. Haslea wawrikae		x	x	x	x
Navicula spp.	x	x			x
Nitzschia frigida	x	x	x	x	x
Pseudonitzschia sp.		x			x

Table 3. Species composition of sea ice biota on 22.2.2004

Table 4. Species composition of sea ice biota in different treatments on 25.4.2004 (Appendix I)

In samples from 22.2.2004, the ice biota consisted solely of diatoms (Table 3 , Fig. 2). The dominant species were *Banquisia belgicae*, *Cylindrotheca closterium*, cf. *Haslea wawrikae* and *Nitzschia frigida*. Cell abundance of diatoms was generally, however, low (<1000 cells 100 ml melted ice^{-1}). The ice surface was very dry (no brine seeping), thus most organisms were concentrated in the lower layers of the ice sheet (56-111 cm). After 63 days (25.4.2004), the ice biota was more diverse (Table 4, Fig. 3), comprising of some dinoflagellates (phototrophic *Gymnodinium* spp., heterotrophic *Katodinium* sp.), and some heterotrophic flagellates, in particular *Thaumatomastix* sp. (Protista Incertae Sedis) in the upper 0-25 cm. Diatoms were distributed evenly in the ice cover, and their abundace had increased 2-10x within 63 days (≤ 3400 cells 100 ml^{-1} melted sea ice). The diatom community comprised of e.g. *Bacillaria paxillifer*, *Banquisia belgicae*, *Thalassionema nitzschioides, Nitszchia frigida*, and *Cylindrotheca closterium* (Table 4).



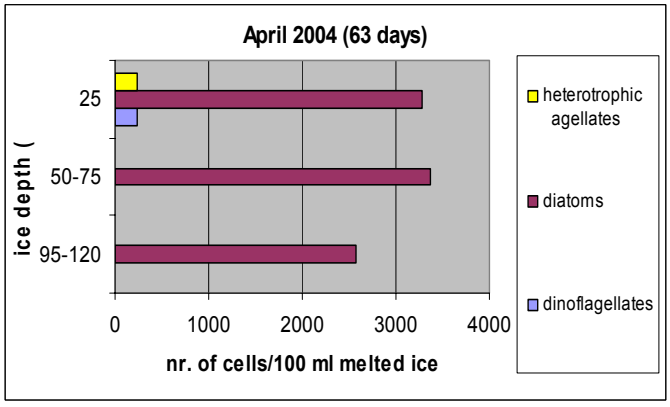


Figure 2. Organisms in sea ice on 22.2.2004

Figure 3. Organisms in sea ice on 25.4.2004

After the addition of Inipol EAP 22 only (Fig. 4) both the diversity and the abundance of algae broke down. The abundance of diatoms (mainly *Nitzschia frigida)* was <1000 cells 100 ml^{-1} melted sea ice. The addition of fish food only (Fig. 4) caused a remarkable increase of heterotrophic

flagellates, in particular *Protaspis* sp. and *Telonema subtile* (Protista Incertae Sedis; abundance ≤ 25 000 cells 100 ml^{-1} melted ice), and the colourless *Polytoma papillata* (Chlorophyceae; abundance 6000 cells 100 ml^{-1} melted ice). Also with this treatment, the diatom abundance decreased to < 1500 cells 100 ml melted ice^{-1}.

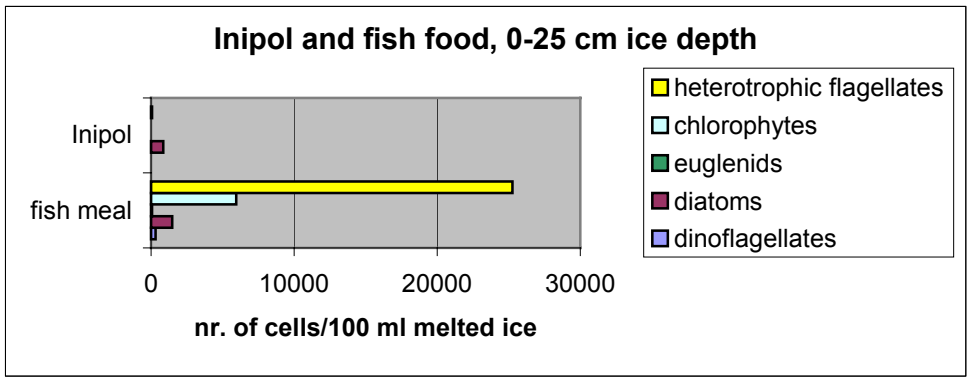


Figure 4. Organisms in the upper 25 cm of sea ice after treatments with Inipol EAP 22 and fish food.

The addition of only oil (Fig. 5) caused a dramatic decrease of diatoms (<500 cells 100 ml^{-1} melted sea ice) throughout the ice cover (total thickness 128 cm), the formation of diatom resting spores (<100 cells/spores 100 ml^{-1} melted sea ice), and the migration of heterotrophic flagellates from the ice surface into the ice interior. The measured chlorophyll-*a* concentrations were low (0,363 µg 100 melted sea ice^{-1} in 50-75 cm (the ice interior), and 0,032 µg chl-*a* 100 melted sea ice^{-1} in 103-128 cm (the bottom layer).

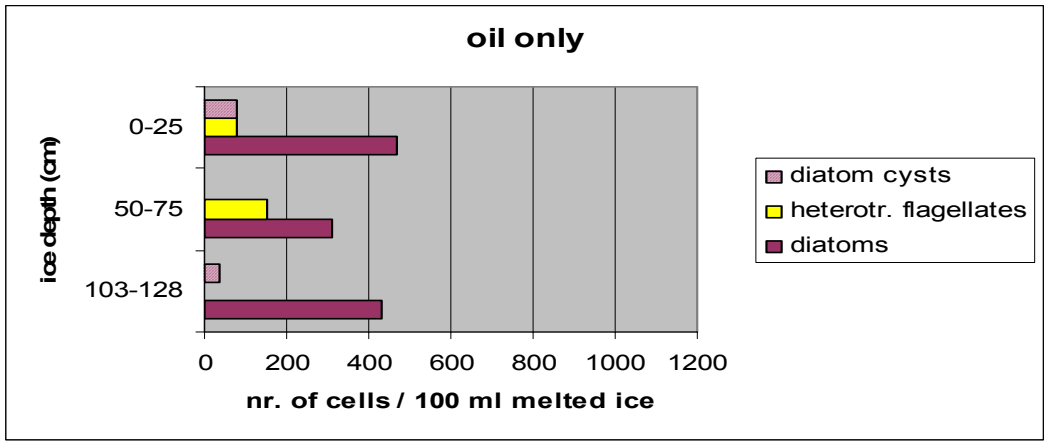


Figure 5. Organisms in sea ice when only oil was added.

The negative effects of oil and Inipol EAP 22 (Fig. 6) were more restricted to the ice surface (0-25 cm), where diatoms decreased dramatically (abundance <200 cells 100 ml^{-1} melted sea ice). Live, healthy looking diatom cells and diatom resting spores were present only in the lower ice layers (95-120 cm). In the ice interior (50-75 cm), the use of oil and Inipol EAP 22 caused a notable decrease in diatom abundance (≤ 3400 cells 100 ml^{-1} melted sea ice in 50-75 cm in the blank samples (Fig. 3) to ≤ 1000 cells 100 ml^{-1} melted sea ice; Fig. 6). Here, the chlorophyll-*a*

concentrations varied from 1,242 in 50-75 cm (the ice interior) to 0,14 in 95-120 cm (the bottom layer) µg chl-*a* 100 melted sea ice^{-1}.

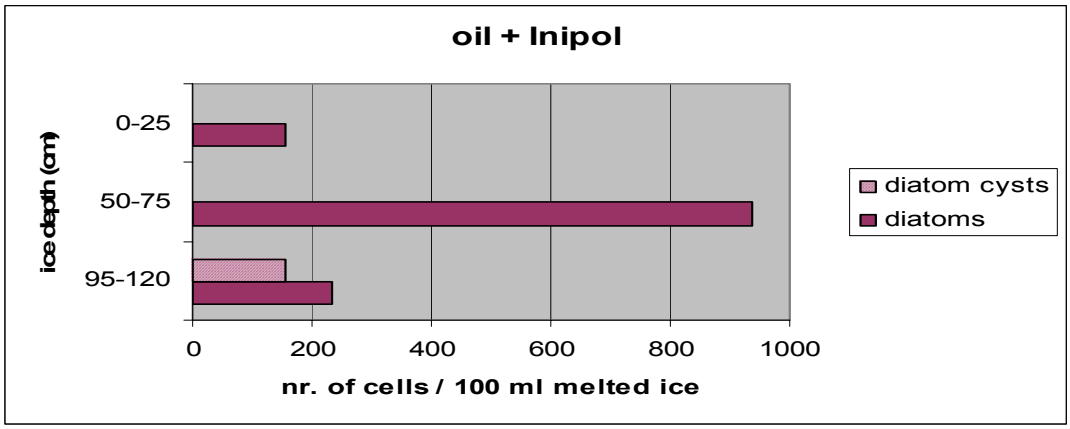


Figure 6. Organisms and chlorophyll-a concentration in sea ice when oil and Inipol EAP 22 were added.

When oil and fish food was added (Fig. 7), no live organisms were present in the surface ice (0-25 cm). In the ice interior and bottom layers, only diatoms were present but in low abundance (≤ 1100 cells 100 ml^{-1} melted sea ice). With oil and fish food, the chlorophyll-*a* concentration of diatoms was higher (0,524 in 50-75 cm to 0,44 in 98-123 cm µg chl-*a* 100 melted sea ice^{-1}) in the ice interior than with other treatments.

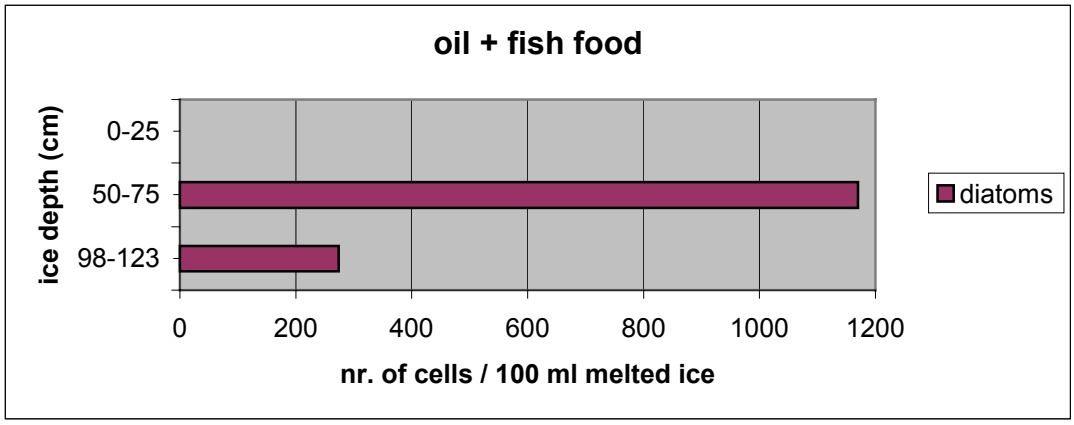


Figure 7. Organisms and chlorophyll-a concentration in sea ice when oil and fish food were added.

The analysis of hydrocarbons in Statfjord crude oil, and in ice with different treatments (only oil, oil and Inipol EAP 22, and oil and fish food; figure 8) shows clearly, that the addition of Inipol EAP 22 and fish food accelerated the degradation of hydrocarbons notably (from 803 mg/g total concentration of aliphatic and aromatic hydrocarbons in Statfjord oil to 136 mg/g in the treatment with Inipol EAP 22, and 174 mg/g with fish food) . When no nutrient source was added, the total hydrocarbon concentration decreased only to 643 mg/g. The concentrations of aliphatic hydrocarbons decreased the most in treatments with Inipol EAP 22 and fish food, from 575 mg/g in Statfjord oil to 98 mg/g (Inipol EAP 22), and 121 mg/g (fish food). Aromatic hydrocarbons

decreased more when Inipol EAP 22 was used (from 195 mg/g to 16 mg/g) than with the addition of fish food (45 mg/g). When no nutrient was added, the change in the concentrations of both the aliphatic and aromatic hydrocarbons was minor (aliphatic 426 mg/g, aromatic 182 mg/g).

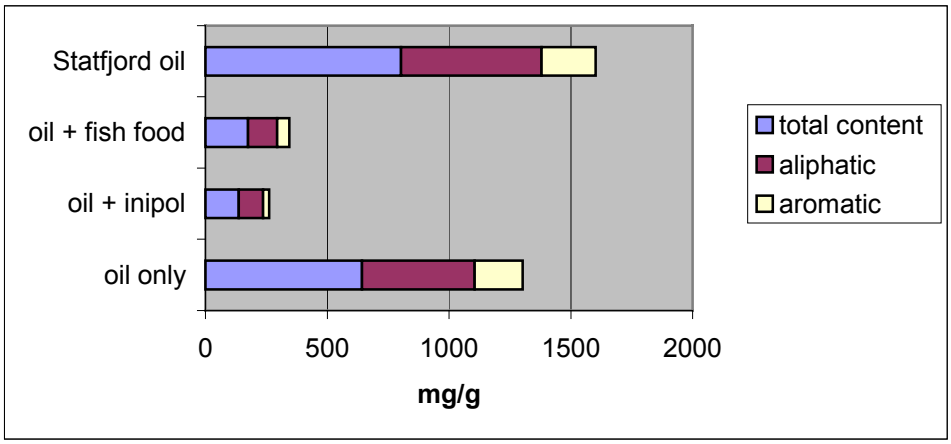


Figure 8. Total concentration of hydrocarbons, and concentrations of aliphatic and aromatic fractions in Statfjord oil and in ice with different treatments (only oil, oil and Inipol EAP 22, and oil and fish food).

Discussion

Once oil is released into the arctic marine environment in the presence of ice cover, several processes may take place, depending e.g. on the season, the site of the oil spill (below or on the ice cover), and the state of ice growth or melt. During the pack ice season, an under-ice spill will lead to the formation of oil lenses (uneven ice underside) or large sheets (even ice underside) beneath the ice sheet. The arctic pack ice underside has an irregular profile, and oil will fill ice cracks and other depressions. During the ice growth (new ice formation and rafting of ice floes), oil may become sealed in. At this stage, no evaporation occurs. It may take even 7 years for an oil lense within the ice to migrate to the top of the arctic multi-year ice (Nelson-Smith 1982, Payne et al. 1991).

Oil weathering is possible only when oil is in contact with moving water (i.e. fresh melt water on sea ice surface in summer, or water currents in the underlying water column). Therefore, hydrocarbon concentrations increase notably during ice-break up (Nelson-Smith 1982, Payne et al. 1991). When oil is spilled onto sea ice, as in our experiment, oil forms a hydrophilic sheet onto ice and/or becomes trapped in brine channels within ice. As long as the ice is not melting, oil weathering processes are very low or non existent. During the ice surface melting in summer, fresh meltwater migrates downwards, and finally through the multi-year arctic ice. Meltwater migration facilitates the release of ice associated organisms into the underlying water column, where they may act as a “seed” for vernal phytoplankton bloom. Thus, meltwater migration would also promote 1) oil weathering as the oil becomes in contact with moving water, and 2) the penetration of oil into the ice and the water column. Acute toxic effects of an oil spill on the arctic ice biota and the planktonic communities in the water column would thus not appear sooner than during the surface melting or break-up of the ice. Spring is probably the most critical period of the growth season in the Arctic, as that is when organisms’ metabolism - and thus hydrocarbon intake - is accelerated due to improved light climate and increased water temperature. These processes are concentrated in the marginal ice zone (MIZ) which makes this region particularly vulnerable to oil spills.

Petroleum and its products may also have a mechanical effect on marine organisms (Nelson-Smith 1982) and within ice. It can penetrate into brine channels and cling to its surfaces. As a substantial part of ice, associated organisms live attached to brine channel surfaces (either attached or e.g. gliding), oil films in brine channels have a direct mechanical effect on unicellular organisms. Furthermore, the presence of oil decreases albedo, thus causing heating, accelerating the ice melt and ice break-up, and preventing the gas exchange between the sea ice surface and the atmosphere.

The effect of PAH compounds on unicellular, in particular ice-associated organisms is to a large extent unstudied. Cross (1987) has conducted studies of the effects of oil spills on under-ice algae. No adverse effects could be detected for neither algal density, biomass, nor productivity after a moderate predisposition of crude oil (Cross 1987). Also, systematically higher organisms, such as marine invertebates remained unaffected by crude oil application (Cross & Martin 1987, Killie & Gulliksen 1994). However, the application of chemical dispersants lead to strong reduction in densities of these animals (Cross & Martin 1987). Generally, the effect of PAHs (without the addition of dispersants or other chemicals) is dependent on the organisms's ability to metabolise these compounds. It is suggested, that multicellular invertebrate organisms with more developed anatomy and physiology (e.g. crustaceans) have better PAH metabolism than the less developed ones, such as unicellular protozoans (Robertson 1998). PAH compounds affect cell functions in two ways (Robertson 1998). Firstly, they can interfere with several cellular processes by binding reversibly to lipophilic sites in the cell. Secondly, PAHs' hydrophilic metabolic products can interact with DNA, lead to the formation of so called adducts which, in turn are thought to play a role in the tumour induction caused by the carcinogenic PAH compounds.

Low concentrations (1-20 µg/l) of PAH compounds can inhibit the growth of multicellular algae (Anderson & Gossett 1986). Patin (2001) identified potential effects of oil spills on marine organisms in the Barents Sea ecosystem. In phytoplankton these were changes in photosynthesis, species composition and other impacts which disappeared after the elimination of oil (within hours or days), with no further explanation what these definitions actually mean (Patin 2001). The sea ice biota, however, live trapped within ice, and thus the elimination of hydrocarbon compounds is notably slower than in the open water ecosystems. Our experiment gave an indication, that the silica frustule of unicellular diatoms would, at least in moderate predisposition, protect the cells from acute lethal effects of oil. While all other protists, mainly with uncovered cells, suffered from the presence of oil in our experiments, the survival of diatoms was better. Diatoms are also capable of producing resting stages (i.e. spores or cysts) asexually when environmental conditions deteriorate. Later, after the improvement of environmental conditions, such as the disapperance of PAHs through biodegradation, migration or other pathways, a new, vegetative diatom cell will germinate from the spore. How long diatom resting spores can remain viable under a PAH exposure is not known and should be studied experimentally using algal cultures.

Our experiment indicates also, that the application of any of the compounds (crude oil, Inipol EAP 22, fish food) in any combination (solely or in mixtures) onto ice will cause notable damage to the ice community. The consequences of exposure of crude oil, Inipol EAP 22 or fish food are indicated as a decline in the species diversity and the abundance of organisms. The use of fish food only favoured heterotrophic flagellates that are capable of using organic compounds as energy source. In all treatments, apart from the application of oil only, the effects of a treatment on ice biota were pronounced in the ice surface layers, and less in ice interior and the bottom layers. Later in spring, if the experiment had been continued, also the ice interior and bottom would assumably have become affected by the ice surface melting processes, meltwater migration and thus effects of oil weathering, and also the presence of Inipol EAP 22 and fish food.

Microbial degradation is important in oil weathering and elimination from the biological system, and can be facilitated by numerous bacteria and fungi (Jordan & Payne 1980, Floodgate 1984, Leahy & Colwell 1980). The actual hydrocarbon degradation rates are, however, low in the Arctic. Despite the potential for notably higher degradation, i.e. the presence of oil degrading bacteria, the physical and chemical environment does not favour biodegradation. Limiting factors are primarily the higher viscosity and lower evaporation rate, thus reduced availability of oil for biodegradation, and the limited availability of oxygen, nitrogen and phosphorous (Atlas 1985, Robertson 1988).

Results of the detailed biodegradation study during our experiment will be published in spring 2005. However, the analysis of hydrocarbon concentrations in our experiment showed clearly, that the addition of nutrients, either in the form of Inipol EAP 22 or fish food, accelerates notably the degradation of hydrocarbons in sea ice. The addition of Inipol EAP 22 showed the best results, as concentrations of both the aliphatic and aromatic hydrocarbons decreased the most. When no nutrients were added, hydrocarbon concentrations decreased only very little. However, Inipol EAP 22 is no longer manufactured. Also, this chemical is moderately toxic if absorbed through the skin, and can cause skin irritation. It is slightly toxic if inhaled (www.ilpi.com/msds/osha/). High vapour concentrations of Inipol EAP 22 may irritate the eyes and respiratory tract, and cause central nervous system effects, such as headaches, dizziness, and nausea. Toxic - and even lethal - effects on animals (*Menidia beryllina* (Inland silverfish, Menidiinae) and *Mysidopsis bahia* (mysid shrimp, Mysidacea) has been documented in laboratory experiments (www.epa.gov/oilspill/ncp/inipolea.html). As the difference in the use of Inipol EAP 22 and fish food for accelerating the bioremediation of hydrocarbons was only minor in our experiment, fish food would be a safer option to use even if a chemical corresponding to Inipol EAP 22 would be available in the future. Although fish food has the potential to increase the abundance not only of bacteria, but also heterotrophic flagellates, the negative effect is only temporary. Flagellates will become consumed by larger protozoans, such as rotifers and planktonic crustaceans, and finally fish when released into the water column.

Dispersants are often used during oil spills. These, however, are even more toxic to organisms than hydrocarbons. Bergueiro-Lopéz et al. (1997) studied the degradation of ionic dispersant (FINASOL OSR 51) with and without the presence of Inipol EAP 22. There was no notable difference between the two treatments, and thus the use of Inipol EAP 22 together with dispersants is not justified even here.

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APPENDIX I

TABLE 4.

	blanks			**no oil**	**Inipol**	**fish meal**	**oil only**			**oil + Inipol**			**oil + fish meal**		
	0-25 cm	50-75 cm	95-120 cm	0-25 cm	0-25 cm	0-25 cm	0-25 cm	50-75 cm	103-128 cm	0-25 cm	50-75 cm	95-120 cm	0-25 cm	50-75 cm	98-123 cm
Dinoflagellata															
Gymnodinium sp.	x	x				x									
Katodinium sp.	x	x													
Chrysophyta															
Paraphysomonas sp.							x								
Bacillariophyceae															
Achnantes spp.			x						x					x	
Bacillaria paxillifera	x	x	x	x	x	x					x	x		x	x
Banquisia belgicae	x	x	x	x			x	x	x		x			x	x
Cocconeis sp.		x													
Coscinodiscus oculus-iridis				x											
Cylindrotheca closterium	x	x	x	x	x	x		x		x	x				
Gyrosigma sp./*Pleurosigma* sp.												x			
cf. *Haslea wawrikae*															
Navicula spp.			x	x	x	x		x	x	x		x		x	
Nitzschia frigida	x	x	x	x	x	x									x
N. longissima				x											
Pseudonitzschia cf. *cuspidata*	x														
P. cf. *delicatissima*	x														
Pseudonitzschia sp.		x	x		x	x					x				
Thalassionema nitzschioides			x	x											
Thalassiosira sp.	x			x											
unidentified centric diatoms	x	x	x	x											
unidentified pennate diatoms	x	x		x			x								
diatom resting spores		x					x		x			x			
Euglenida															
Anisonema sp./*Dinema* sp.						x									
Euglena sp.						x									
Chlorophyta															
Polytoma papillata						x									
Protista Incertae Sedis															
Metromonas simplex						x									
Protaspis sp.					x										
Telonema subtile				x		x									
Thaumatomastix sp.	x														
unident. phototrophic nanoflagellates			x												
unident. heterotrophic nanoflagellates	x	x		x		x		x							