ARTIC MARINE POTENTIAL OF MICROBIAL OIL DEGRADATION

Scientific Report from DCE – Danish Centre for Environment and Energy No. 271 2018

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Data sheet

Series title and no.: Scientific Report from DCE – Danish Centre for Environment and Energy No. 271

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Publisher: Aarhus University, DCE – Danish Centre for Environment and Energy ©
URL: http://dce.au.dk/en

Year of publication: April 2018
Editing completed: January 2018

Quality assurance, DCE: Jesper Fredshavn

Proof reading: Anne Mette Poulsen

Financial support: DANCEA

Please cite as: Wegeberg, S., Johnsen, A., Aamand, J., Lassen, P., Gosewinkel, U., Fritt-Rasmussen, J., Rigét, F., Gustavson, K. & Mosbech, A. 2018. Arctic marine potential of microbial oil degradation. Aarhus University, DCE – Danish Centre for Environment and Energy, 54 pp. Scientific Report from DCE – Danish Centre for Environment and Energy No. 271 http://dce2.au.dk/pub/SR271.pdf

Abstract: The literature review aims to collect and analyse relevant existing knowledge about microbial degradation of oil in the seawater around Greenland. Thereby to identify and recommend to what future research on the topic should focus on and to add to the knowledge base for developing contingency plans and perform Net Environmental Benefit Analyses (NEBAs) for Greenland in relation to oil spill from oil exploration activities and shipping in Arctic waters. The knowledge presently available about natural degradation of oil under Arctic conditions shows a complex picture depending on oil type/components and environmental conditions as reflected in the marine environments of Greenland, which is also complex and consist of different water bodies characterised by different temperatures and nutrient levels.

Keywords: Oil, microbial degradation, Arctic, marine

Layout: Graphic Group, AU Silkeborg
Front page photo: Blosseville Coast, East Greenland.

ISBN: 978-87-7156-329-0
ISSN (electronic): 2245-0203

Number of pages: 54

Internet version: The report is available in electronic format (pdf) at http://dce2.au.dk/pub/SR271.pdf

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Preface

Knowledge about the natural biodegradation potential of oil in Arctic seas is an important parameter in ecological risk evaluation of oil spill in Arctic areas as well as for developing contingency plans for oil spill response. In Greenland, combating of oil spill may include mechanical recovery and chemical dispersion, where the oil, by use of chemical dispersants, is broken up into small droplets and dispersed into the water column and hence removed from the sea surface, and *in situ* burning (ISB), where the oil is burned on the sea surface. In case of an acute oil spill, decisions on which methods to use for combating the oil spill must be taken, and the choice of oil spill response technique(s) should be carefully balanced with the presence of marine organisms in the sea and on the sea surface in a Net Environmental Benefit Analysis (NEBA).

Basic information needed to develop contingency plans and perform NEBA for Arctic water around Greenland is detailed knowledge of the distribution and sensitivity of Arctic organisms, the potential for environmental dispersal of the spilled oil and the toxicity of the oil in the seas. It is, however, also important to know the potential for biodegradation of the initial oil, the dispersed oil and *in situ* burning residues in Arctic waters.

Without this knowledge, important questions regarding the resilience of the Arctic to oil spill cannot be answered in depth. How sensitive is the Arctic to oil spills? Can a severe oil spill cause irreversible damage to the marine Arctic environment? How many years will the oil stay in the Arctic environment? Can degradation of the oil (or fractions of the oil) be expected under Arctic conditions when it is dispersed into the water or if beached on the shore? Will the low water temperature and low levels of nutrients slow down the biodegradation rate of oil in the Arctic and are the microorganisms in the Arctic capable of degrading the environmental hazardous oil components?

The knowledge presently available regarding natural degradation of oil under Arctic conditions shows a complex picture depending on oil type (light / heavy) and ambient conditions: temperature, nutrients, time of year etc. Degradation studies have primarily focused on the removal of selected oil components; thus, when using the knowledge that these studies provide, it is important to consider what analyses the results are based on, and how well these studies embrace the complexity of oil components and the Arctic ecosystems.

This review aims to collect and analyse existing knowledge of microbial degradation of oil relevant to the conditions in the sea around Greenland in order to add to the knowledge base for developing contingency plans and NEBAs for Greenland. The development of regional strategic NEBAs will support selection of the most environmental beneficial oil spill response techniques and facilitate that oil spill combat can be initiated fast and efficiently.

Knowledge of the microbial degradation of oil is also essential for the subsequent monitoring of the environment and assessing the potential negative environmental impacts and the rate of restitution.

Summary

This review aims to collect and analyse relevant existing knowledge about microbial degradation of oil in the seawater around Greenland to add to the knowledge base for developing contingency plans and perform Net Environmental Benefit Analyses (NEBAs) for Greenland in relation to oil spill from oil exploration activities and shipping in Arctic waters.

The main sources of oil pollution to the sea come from the offshore exploitation and transport of oil, but operational spills of oil from ships can also have a significant impact. Runoff from land, wastewater discharge and atmospheric deposition contribute to the load at regional scale. Furthermore, natural oil seeps in the ocean may act as a potential source at some locations. However, tanker accidents and blowouts are the most important sources of high-volume oil spills.

In the accident in 1989 in Price Williams Sound of the tanker *Exxon Valdez*, 42 million liters of crude oil were leaked into the environment. Due to the naturally low nutrient levels at the site, biodegradation was enhanced by adding nutrients to the spilled oil. However, despite of intensive oil combat and clean-up, a fraction of the spilled oil was buried in the shoreline sediment in between stones and pebbles. Slow seeping of non-weathered oil from these oil pockets is assessed to be the source of continued oil pollution in some of the impacted areas, now twenty-five years after the incident.

The *Deepwater Horizon* incident in the Mexican Gulf in 2010 is one of the world's largest offshore oil spill accidents; ca. 780,000 m^3 light crude oil were released into the marine environment from the seabed. It has been found that the oil dispersed in the water column was degraded by naturally occurring microorganisms in deep water (1,000 m) at temperatures of 4-5°C, which are not far from Greenland sea temperatures. The microbial flora needed for oil degradation was already present in the Mexican Gulf due to the numerous natural oil seeps in the seabed. Whether natural oil seeps in Greenland support a microbial flora capable of oil degradation is not yet known.

Existing studies on oil degradation have primarily focused on the total removal of oil within certain boiling point intervals. Some studies have been supplemented with analyses of a few oil components such as benzene, toluene, ethylbenzene and xylenes (BTEX) and EPA16 (Polycyclic Aromatic Hydrocarbons, PAHs). Of the oil components, alkanes have the lowest toxicity and a relatively high biodegradability. However, the heavy aromatic fraction is more toxic and is slowly degradable. Results based solely on total oil may underestimate the toxicity of oil and overestimate the degradation of potentially toxic oil components in the environment.

Oil consists of a very complex mixture of hydrocarbons, ranging from light gases to heavy residues. There can be millions of different hydrocarbons in oil. The hydrocarbon part of the crude oil mainly consists of straight and branched alkanes, cycloalkanes and aromatics, accounting for approximately 60-75%. These compounds are generally volatile and often easily degradable hydrocarbons.

For biodegradation of oil in the marine environment, a combination of factors is important:

- Presence of a microbial organism capable of degrading the oil
- Sufficient nutrients for the degradation process to occur
- Possibly temperature. A microbial flora may be adapted to low temperatures but the general expectation is that low temperatures will lead to lower degradation rates.

The presence of oil-degrading bacteria is a prerequisite for oil degradation to occur. The total number of bacteria in seawater is low compared with other environments such as soil or sediments. The number of oil-degrading bacteria constitutes only a fraction of the total number of bacteria in seawater.

Although the number of oil-degrading bacteria is low in pristine water, the number may increase following an oil spill. The absence of specific oil-degrading bacteria may, however, limit oil degradation as was seen in experiments with seawater from Disko Bay where, for instance, only simple PAH degraders were found and in very low densities. It is highly likely that also more complex PAH degraders occur naturally in Arctic seawater, but in extremely low densities.

The low temperatures along with low amounts of nutrients are limiting for oil degradation in the Arctic. However, in a recent laboratory study (5°C) with subsurface seawater from 150 m depth in Disko Bay, Greenland, rapid degradation of the alkane fraction of a crude oil was seen. The volatile alkanes were removed by both microbial degradation and abiotic processes, while the semi volatile and non-volatile alkanes were removed mainly by microbial degradation. In contrast, no degradation of either PAHs, dibenzothiophenes or alkyl-substituted homologue was observed during the 71-dayincubation period, which was explained by the pristine environment limited to bacteria adapted to degrade these structurally more complex molecules. However, slow degradation of the simpler PAHs naphthalene and 1-methyl naphthalene was recorded.

Rapid degradation of crude oil was seen in Arctic seawater from the Chukchi Sea, Alaska, at an even lower water temperature of -1°C. About 60% of the total hydrocarbons were degraded within two months, and also PAHs and substituted PAHs were degraded, although slower than *n*-alkanes and branched alkanes. It was not possible, though, to distinguish biological oil degradation from physical removal processes like evaporation as non-biological controls were not included in the study. More rapid degradation of straight chain alkanes followed by branched alkanes and larger and alkylated aromatics was also observed in in Arctic sediment from Spitzbergen, Svalbard, and cold unpolluted seawater (~6.5°C) from the Trondheim Fjord, Norway.

In seawater, one or more inorganic nutrients (e.g., nitrogen, phosphorus or iron) can become severely limiting to degradation processes, especially in the photic zone at times when the photosynthesising members of the trophic web sequester the limited nutrient supply. This may, however, to a certain extent be counterbalanced by grazing of the photosynthesizing organisms by other organisms by which the nutrients are recycled. Moreover, in general, mineralisation will take place and higher nutrient concentrations will exist below the photic zone. In some areas, nutrients can be brought to the photic zone by wind energy mixing and by upwelling.

The knowledge presently available about natural degradation of oil under Arctic conditions shows a complex picture depending on, for example, oil type/components and environmental conditions. The marine environments of Greenland are also complex and consist of different water bodies characterised by different temperatures and nutrient levels. For instance, the East Greenland Current is cold and poor in nutrients compared with the West Greenland Current.

In general, the potential for oil degradation in seawater depends on previous exposure of the environment to oil components. Following an oil spill, specific oil-degrading bacteria will proliferate until other factors such as nitrogen or phosphorus become limiting. Little is known about the time needed for growth of small degrader populations to the densities necessary for significant degradation to occur. This is especially the case for pristine environments such as the Arctic waters. A degradation potential may develop naturally due to exposure of oil components to hydrocarbons penetrating from the underground, the so-called natural oil seeps.

Natural oil seeps are found in Greenland, especially in central West Greenland. It is thought that the microbial community in areas with seeps may have an increased hydrocarbon-oxidizing potential and that areas with a relatively high potential for microbial degradation of oil are also areas with relatively high temperatures and nutrient supplies. However, this warrants further study.

The existing knowledge about biodegradation of oil in seawater in Greenland waters is presently limited to one study, which has direct focus on oil degradation in seawater in Disko Bay.

Hence, to enhance our knowledge about the marine oil biodegradation potential in Greenland, it is recommended that future research should focus on:

1. getting a more generalised picture of the oil degradation potential in the seawater around Greenland.
   In the seawater from Disko Bay, limited degradation of aromatics compared with aliphatic hydrocarbons was seen, but it is unknown whether this is also the case for other type of localities around Greenland.
   The sea around Greenland is pristine and in general characterised by low temperatures and low levels of nutrients. There is a gap in our knowledge about bacterial adaptation to oil degradation under such conditions as well as on the timeframe of the adaptation processes.

2. identifying Greenlandic localities showing similar oil-degrading characteristics.
   The broad picture of water masses around Greenland shows distinct masses of different origin with different temperature and salinity characteristics as well as different nutrient richness. The biodegradability of different oil components in different water bodies around Greenland needs further study.
   It is unknown whether there is a priming effect on the microbial degrader community in the sea around Greenland in areas with natural oil seeps.

3. linking the degradation potential with environmental parameters and toxic effects.
   We lack the mathematical models combining dispersal of oil with knowledge about degradation processes necessary for predicting the fate of oil in the Greenlandic marine environment.

An experiment has demonstrated that dispersants can exert a negative effect on microbial hydrocarbon degradation. There is a gap in our knowledge about the negative effects of dispersants on the degradation of oil in Arctic waters.

Finally, increased toxicity of oil exposed to photochemical reactions is area topic deserving more research as such photochemical processes may be much more critical in Greenland during summer with all-day sunlight.

1 Introduction

Susse Wegeberg & Anders Mosbech

Which oil spill response technique(s) will be most beneficial to the environment in the Arctic? To answer this question, a Net Environmental Benefit Analysis (NEBA) must be performed in each case of oil spill. In this analysis, the pros and cons of chemical dispersion of the oil into the water column or burning of the oil on the sea surface, *in situ* burning, should be assessed, either as sole combat strategies or as supplements to mechanical recovery of the oil from the surface – or in case action is logistically impossible due to harsh weather conditions, doing nothing (Wegeberg et al. 2016, Fritt-Rasmussen et al. 2015). The knowledge base for undertaking a NEBA in Greenland primarily consists of several environmental atlases for the west coast of Greenland developed by DCE – Danish Centre for Environment and Energy, Aarhus University – for the Greenland Government. Each atlas covers a shoreline interval and offshore areas for which a relative oil spill sensitivity index is calculated based on knowledge of the presence of important natural resources (http://bios.au.dk/en/knowledge-exchange/til-myndighederne-og-saerligt-interesserede/greenland-and-the-arctic/oil-and-environment/sensitivity-atlas/). The atlases also contain logistic information and can therefore be used operatively to prioritise oil spill protection zones.

However, the knowledge base for performing a NEBA in Greenland has gaps, among others on the effect of oil on key Arctic organisms by naturally or chemically dispersed oil or from burning of oil and the derived burn residues. Studies to elucidate toxic or smothering effects of oil and burn residue on fish eggs, birds, copepods and macroalgae in the Arctic have been performed or initiated (Gustavson et al. 2016, Wegeberg et al. 2016, Frits-Rasmussen et al. 2016). Moreover, knowledge is needed about the potential for natural degradation of oil and this knowledge should be integrated with studies on the long-term effects of oil and oil degradation products on Arctic organisms. This integrated knowledge will improve the possibilities for making an informed choice of oil spill response technique(s) (or mix of techniques).

Today, due to the potential oil exploration activities and increased shipping in the seas around Greenland and the consequent risk of oil spills, there is a great need for understanding the effect of oil on the marine organisms as well as the potential for natural degradation of oil in the different water bodies around Greenland.

More detailed knowledge of the natural potential for oil degradation in the Arctic will also aid in elucidating the resilience of the Arctic to oil spills.

The existing studies of oil degradation have primarily focused on the total removal of oil within certain boiling point intervals, and some studies have been supplemented with analyses of a few oil components such as, for example, benzene, toluene, ethylbenzene and xylenes (BTEX) and EPA16 (Polycyclic Aromatic Hydrocarbons, PAHs). Of the oil components, alkanes have the lowest toxicity and a relatively high biodegradability. The heavy aromatic fraction is, however, more toxic and is slowly degradable. Results based solely on measurements of total oil concentrations may underestimate the toxicity of oil and overestimate the degradation of oil in the environment.

The knowledge presently available about natural degradation of oil under Arctic conditions shows a complex picture depending on, for instance, oil type/components and environmental conditions. Also, the marine environments of Greenland are complex and consist of different water bodies characterised by different temperature and nutrient levels, which may affect the potential of microbial degradation of oil. Compared with the West Greenland Current, the East Greenland Current is cold and poor in nutrients.

In connection with the *Exxon Valdez* oil spill in Prince Williams Sound, Alaska, in 1989, natural biodegradation was limited due to insufficient availability of nitrogen and phosphorus, and oloephilic fertilizer was therefore added to enhance the biodegradation of the oil at sea (Margesin and Schinner 1999).

Despite of the intensive oil spill clean-up after the *Exxon Valdez* incident, a fraction of the spilled oil was buried in the shoreline sediment, in between stones and pebbles. Slow seeping of non-weathered oil from these oil pockets is assessed to be a source of the continued oil pollution observed in some of the impacted areas, today twenty-five years after the incident (Shigenaka 2014).

The *Deepwater Horizon* incident in the Mexican Gulf in 2010 was one of the world's largest offshore oil spill accidents;ca. 780,000 m^3 light crude oil were released into the marine environment from the seabed. Natural degradation of oil dispersed in the water column after this accident was degraded faster than expected by naturally occurring microorganisms in deep water (1,000 m) at temperatures of 4-5 °C (Hazen et al. 2010; Valentine et al. 2010), which are not far from Greenland sea temperatures. The relatively fast degradation observed in the Mexican Gulf has been related to the fact that the microbial flora needed for degrading the oil was already present here due to the numerous natural oil seeps in the seabed (Du and Kessler 2012). Whether natural oil seeps in Greenland support a microbial flora capable of oil degradation is unknown.

Thus, the rate of natural degradation of oil in the marine environment depends on a combination of factors:

- Presence of a microbial flora capable of degrading the oil.
- Sufficient nutrients for the microbial flora to grow and accelerate the degradation process.
- Temperature. A microbial flora may be adapted to low temperatures, but most often low temperatures will expectedly lead to lower degradation rates (Klungsøyr et al. 2007).

2 Background

2.1 Oil type characterization

Pia Lassen

2.1.1 Composition of oil

Crude oil is a very complex mixture of hydrocarbons, ranging from light gases to heavy residues. There can be millions of different hydrocarbons in a crude oil. The composition of crude oil is source specific and not two crude oils are identical. The main part of crude oil (90-98%) consists of hydrocarbons and the remaining part consists mainly of sulphur, oxygen and nitrogen. Trace metals can also be present in the oil, but in very small amounts. The elemental composition of crude oil is very much the same in all the oils, but the composition of the compounds in the oils varies, which makes the different oils unique. Generally, increasing molecular weight and increasing complexity of the compounds result in both decreased abiotic and biotic degradation rates.

The hydrocarbon part of the crude oil mainly consists of straight and branched alkanes, cycloalkanes and aromatics, accounting for approximately 60-75%. These compounds are generally volatile and often easily degradable hydrocarbons.

Cycloalkanes are dominant in oil, with up to 30-40%, and the main components are cyclopentane (C5H10) and cyclohexane (C6H12). Alkanes (paraffins), which normally range from C6 to C40, comprise 15-20 of the crude oil, predominantly in the range of C5-C10. About 10-15% of the crude oil consists of isoalkanes, which are branched alkanes, again with a predominant range of C6 to C8.

Steranes and triterpanes (hopanes) are complex cycloalkanes that are frequently used as markers to identify the source and age of the crude oil. They are often termed biomarkers. Their name refers to the fact that their present carbon skeleton is related to their originally functional precursors. These are the main compounds used to distinguish between different oils. Steranes is a class of four-ringed cyclic compounds; in oil, particularly those having a side chain at the C-24 position are present. Triterpenes are a class of chemical compounds composed of three terpene units with the molecular formula C30H48. Animals, plants and fungi all create triterpenes, the most important example being squalene that is the basis of almost all steroids.

Naphthenes are also referred to as cycloalkanes, which are saturated hydrocarbon compounds having the general formula of CnH2n. Apart from the smallest, naphthalene, with two rings, they are found in all fractions of petroleum crude oil. Naphthalene is not regarded as a PAH (see below), but is often referred to as such as it is analysed together with the PAHs.

The remaining part of the oil is a very large complex group of compounds that are more resistant to biodegradation and weathering. The dominant groups are aromatics and waxes, which will be described below. Generally, regarding the composition of crude oil, increasing molecular weight and increasing complexity of the compounds lead to a reduction of both abiotic and biotic degradation rates.

Polycyclic aromatic hydrocarbons (PAHs) consist of two or more benzene rings fused together. There are several hundred different PAH compounds and a large number of these are carcinogenic. PAHs are generated both in crude oil (petrogenic) and by burning (pyrogenic). One of the main differences is that in crude oil a large number of methylated PAHs are generated, whereas pyrogenic PAHs only consist of parent PAHs without methylation. In crude oil, the composition and numbers of PAHs are quite diverse, whereas PAHs generated by combustion are quite uniform and it is not possible to distinguish between sources.

Waxes from crude oil consist either of long chain, saturated hydrocarbons (linear alkanes / n-paraffins) with carbon chain lengths of C18 to C75+, called “macrocrystalline wax”, or naphthenic hydrocarbons (C18 to C36), called “microcrystalline wax.” Especially macrocrystalline waxes may cause paraffin problems in oil production and transport operations.

Different types of refined oil

The crude oil is divided into different oil types by refining such as distillation, hydrofining (hydrocarbons are hydrogenated and desulphurised catalytically) and cracking (where larger molecules are broken down to smaller molecules, typically catalytically). Below is a description of the main types of refined oil.

Gas oil (gasoline), diesel and light fuel oil constitute the vast majority of light fuels. They consist of “straight-run” gas oils, which are produced by atmospheric distillation (most commonly) or vacuum distillation. Light fuels consist of low-boiling hydrocarbons, mainly alkanes, cycloalkanes and smaller aromatic compounds, including some of the small PAHs. For gasoline, the compounds are in the range of C5 to C12, and for diesel and fuel oils they are in the range of C11-C18-27. Light fuel oils are low in biomarkers. They have good ignition characteristics and are therefore used as bunker oil in all sizes of ships.

Lubricating oils consist of residues from the atmospheric distillation, which have been further distilled under vacuum distillation and refined by solvent extraction and / or hydrofining in order to increase the viscosity index. This enhances the colour and converts unsaturated hydrocarbon and aromatics to less chemically reactive components. Finally, solvent dewaxing is also used to reduce the wax content of the oil in order to prevent formation of crystals under the normal working temperature of the oil. Lubricating oil is quite expensive to produce and is seldom used in pure form. Lubricating oil has a high content of biomarkers such as hopanes and steranes but does not contain aromatic steranes and PAHs.

Heavy fuel oil (HFO) and bunker oil are typically blended products containing residues from the various refinery distillations and cracking processes. They are highly viscous liquids that require heating for storage and combustion. The oil has to be cleaned for solid contaminants and water on board before use, for instance through fuel oil separation by centrifugation, which means that approx. 3% of the bunkered oil is waste that has to be discarded at harbour facilities. Heavy fuel oils are classified according to international standards and numbered relative to their viscosity at 50°C. Heavy fuel oils consist of high molecular weight compounds and have a high content of aromatic compounds such as PAHs and are low in alkanes and other saturated compounds.

2.2 Oil pollution sources

Pia Lassen

The main sources of oil pollution to the ocean come from the offshore exploitation and transport of oil, but operational discharges of oil from ships may also create local damage. Runoff from land, discharges in wastewater and atmospheric deposition contribute to the load on a regional scale. Furthermore, natural oil seeps in the ocean are a potential source of oil pollution at some locations.

2.2.1 Natural oil seeps

Crude oil and natural gas seeps are common in the ocean seabed and eroding sedimentary rock in areas with oil resources. These seeps are natural "springs" where liquid and gaseous hydrocarbons leak out of the ground. Natural oil seeps add to the load in the environment. In many instances, the seeps have led to the discovery of commercially recoverable petroleum reserves. Globally, oil seepage contributes between 0.02 and 2 million tons of oil per year to the environment (NRC US 2003). Of the total oil / hydrocarbon entering the marine environment from different sources, at least 15% comes from natural oil seeps, and some authorities have estimated that the contribution of natural seeps may constitute as much as 46% (NRC US 2003) of the total release. There are no estimates for the Arctic region, but as the region supposedly contains large gas and oil reserves (http://www.eia.gov/oiaf/analysispaper/arctic/), the amount of oil seeps could very well be higher than the global average. Although their rate of release may vary over time, the locations of seeps are consistent and predictable. Oil from underwater seeps generally behaves as oil spilled during or after extraction, forming large slicks that spread and drift with winds and currents. Slicks from larger seeps are visible by satellite, and some are persistent enough to be featured on navigation charts.

The environmental impact of oil seeps is somewhat uncertain. It is likely that they have been leaking for thousands of years and that the biological community close to the seeps has adapted to this, and most of the oil disappears quite fast upon release. A study by Farwell et al. (2009) revealed that oil from natural seeps normally stayed in the water between 10 hours and 5 days. During that time, the easily degradable compounds disappeared, either by dissolution, evaporation or degradation. The remaining heavier oil, which mainly consists of larger compounds, sank to the seabed.

Regarding natural oil seeps in Greenland, see Chapter 5.3.

2.2.2 Offshore activities and produced water

Practically all routine stages and operations of offshore oil and gas production and exploration are accompanied by undesirable discharges of liquid, solid and gaseous wastes.

The environmental concerns regarding discharges of produced waters dominate over other wastes in connection with oil and gas production. Produced waters include formation water, brine, injection water and other technological waters. Formation water and brine are extracted along with oil and gas. Hundreds of thousands of tons of injection water are pumped into the injection wells for maintaining the pressure in the system and pushing the hydrocarbons towards the producing wells. All of these waters are usually polluted by oil, natural low-molecular-weight hydrocarbons, inorganic salts, metals and added chemicals, such as biocides, corrosion inhibitors emulsion breakers etc. The production water is cleaned before its discharge into the sea and on the platforms separation units are used for oil separation. Depending on its quality, the produced water is either discharged into the sea or injected into the disposal well. Petroleum hydrocarbons are always present in produced waters; the levels of oil in discharges may vary but are regulated in the North Atlantic according to MARPOL and OSPAR (HELCOM 1997, OSPAR 2001). The main components of hydrocarbons in the cleaned production water are monocyclic aromatic hydrocarbon (BTEX), PAHs and related heterocyclic aromatic compounds (Somerville et al. 1987, Bakke et al. 2013).

The worst case scenario is a blowout, which may lead to high volume oil spills like the Macondo accident in the Mexican Gulf in 2010 of almost 800,000 m^3 oil.

2.2.3 Ship traffic

Ship traffic in the Arctic areas has increased significantly and is expected to increase even further: (http://www2.forsvaret.dk/nyheder/nationale_opgaver/Documents/FINAL%20report_Environmental%20Risk%20Assessment%20Greenland%20DNV%20GL.pdf), thereby enhancing the probability of oil pollution. The most common cause of oil pollution by ships is the so-called operational oil spills that are mainly due to human error or illegal disposal of oil waste. Bunkering of oil from one ship to another is the most common type of minor oil spills.

Also ship groundings may lead to oil spills, especially tanker accidents. Even though these constitute only a small percentage of the overall input of oil to the marine environment, they are mainly high volume oil spills that may cause severe environmental damage before the oil is dispersed, diluted and/or degraded. Usually, only a small fraction of the oil from large oil spills can be recovered by mechanical combat techniques.

2.3 Microbial degradation of oil

Anders Johnsen, Jens Aamand & Ulrich Gosewinkel

Several environmental factors control the rate by which oil is degraded in the environment or whether it is degraded at all. Although specific hydrocarbons have been shown to be degraded also under anaerobic conditions, oxygen is generally a prerequisite for oil degradation. Marine sediments are often anaerobic and degradation of oil may here be limited by oxygen availability. In the open water, oxygen is readily available, but rapid degradation of oil following a large oil spill may result in local oxygen deficiency. However, this largely depends on the dispersal of the oil, which is influenced by ocean currents and waves. Deficiency of nutrients, primarily nitrogen and phosphorous, may also limit oil degradation and nitrogen/phosphorus addition is consequently often used to combat marine oil spills. Most oil constituents have low water solubility, and oil is therefore often found dispersed in the seawater as oil droplets. Microbial degradation requires dissolution of the oil in the water , and the rate of dissolution thus often determines the rate of degradation. Temperature is also an important factor controlling the rate by which oil is degraded. In general, microbial processes, including degradation of oil, progress more rapidly at higher than low temperatures, and below the freezing point the processes almost stop. Finally, presence of microorganisms able to degrade the oil is required. As mentioned above, oil consists of thousands of compounds of which many are not easily degradable. Probably, bacteria capable of degrade alkanes and simple aromatics exist, although in low numbers, even in very pristine environments. However, complex hydrocarbons such as PAHs and dibenzothiophenes may require years of adaptation for degradation to occur, if at all.

2.3.1 Life in a cold environment

The most apparent difference between the Arctic marine environment and temperate and subtropical marine environments is the constantly low water temperature in the Arctic waters. For instance, the monthly mean temperature of surface water at Nuuk ranges from 0°C during the four coldest months to 4°C during the two warmest months (mitrejsevejr 2016). In contrast, the monthly means for Denmark, situated in a cold-temperate climate, range from 2°C during the two coldest months to 17°C in the two warmest months (mitrejsevejr 2016). It is important to notice that the temperature is low in most deep waters; in the Gulf of Mexico, for example, the temperature is 4°C at depths below 1,300 m (Wang et al. 2016), but such low temperatures are also found in Greenland waters, for instance at 500 m depth at Fylla Bank (Ribergaard 2013).

An important question is whether the low *in situ* temperatures in Arctic marine waters limit the potential for oil degradation. The so-called Q_{10} value is often used as a simple approach to describe the relation between temperature and the reaction rate. The Q_{10} value is defined as the multiplier by which degradation rates increase with a 10°C rise in temperature:

$$Q_{10} = k^{T+10} / k^{T} \qquad \text{Eq. 1}$$

where k^T *is* the reaction rate at temperature T and k^{T+10} is the reaction rate at a 10°C higher temperature. The Q_{10} can be calculated from two reaction rate measurements according to Eq. 2:

$$Q_{10} = \left(\frac{k_2}{k_1}\right)^{10/(T_2-T_1)} \qquad \text{Eq. 2}$$

where k is the reaction rate at temperature T. Bagi et al. (2013) reviewed studies of crude oil degradation in different seawater and oil-degrading enrichment cultures and calculated Q_{10} values ranging from 1.1 to 5.7, excluding a single Q_{10} of 16.2, and on the basis of their results they argued that factors other than temperature *per se* influence degradation processes. For example, an extremely high Q_{10} observed by Brakstad and Bonaunet (2006) was determined from 0°C where the used Statfjord crude oil was in a solid state and its bioavailability to degrading organisms thus very limited. A small increase in temperature may therefore change the physico-chemical state of the oil, leading to rapid degradation, and as a result of this a much higher Q_{10} value is obtained.

The Q_{10} concept is based on the pre-condition that temperature is the only parameter that changes, and this may be a reasonable measure for assessing temperature effects on hydrocarbon degradation over short-term periods. However, what if we contemplate scenarios where the microbial communities have adapted *in situ* over long-term periods to permanently cold conditions? We have not been able to find reports of *in situ* hydrocarbon degradation rates in Arctic marine environments, but we might get an indication of these through the temperature effect on the general elemental cycles and the turnover of organic carbon. A study of carbon input, sulphate reduction and oxygen uptake in Antarctic coastal sediments at Signy Island conducted over a 16-month period by Nedwell et al. (1993a) showed that the sediment oxygen uptake reached 80-90 mmol O_2 m^{-2} day^{-1} during summer, even though the water temperature was only 0.5 °C. Nedwell et al. (1993a) also demonstrated that the oxygen uptake rates were just as high, or even higher, than the rates in sediments from warmer climates, including North Sea sediment with rates ranging between 10 and 30 mmol O_2 m^{-2} day^{-1} at 20-24°C (Nedwell et al. 1993b), Long Island Sound sediment where the rates ranged between 81 and 137 mmol O_2 m^{-2} day^{-1} at 20-24 °C (Mackin and Swider 1989), Aarhus Bay sediment where the rate was 27 mmol O_2 m^{-2} day^{-1} at 22 °C (Jørgensen and Revsbech 1985) and Thai mangrove sediment where the rates varied between 17 and 61 mmol O_2 m^{-2} day^{-1} at 28-33 °C (Kristensen et al. 1991). This suggests that the microbial activity was not limited by temperature but rather by the seasonal availability of organic matter. Another study of carbon mineralisation pathways in Arctic marine sediments from fjords in Svalbard and northern Norway undertaken by Kostka et al. (1999) came to similar conclusions: “Microbial communities in permanently cold Arctic sediments exhibited mineralization rates and pathways comparable to temperate nearshore environments” and “microbial communities in cold sediments exposed to relatively high C deposition appear to respond to the input or availability of organic matter rather than to temperature”.

If oil degradation in Arctic marine environments follows the general trends for organic carbon turnover, the potential for oil degradation at low temperatures may be higher than previously anticipated, but this is only (1) if the microbial degraders are present in sufficiently high numbers, (2) if they are cold-adapted, (3) if they have the metabolic capacity to degrade the multitude of structurally different compounds that are present in oil products, (4) if mineral nutrients are available and, finally, (5) if the bioavailability of oil compounds is not severely limited by the low temperature. These aspects are further discussed in the following sections.

2.3.2 Physiological adaptation to cold conditions

Microorganisms that grow and thrive in cold environments are called psychrophiles. Obligate psychrophiles require cold conditions, whereas facultative psychrophiles (also called psychrotrophs) tolerate cold conditions but have their growth optima at higher temperatures. There are several definitions, but a simple way to differentiate between the two is by testing growth at 4°C and 20°C. The idea is that obligate psychrophiles will grow only at 4°C, whereas the facultative psychrophiles will grow at both 4°C and 20°C (Gow and Mills 1994). Though easy to apply, these criteria may not be adequate for detecting all obligate psychrophiles as some may grow even at 20°C (Gow and Mills 1994). An alternative definition is based on bacterial growth rates as a function of temperature, where obligate psychrophiles have growth optima of about 15°C or lower, a maximum temperature for growth of about 20°C and a minimum temperature for growth at 0°C or lower (Morita 1975). In this context, facultative psychrophiles were defined as those microorganisms that could grow at low temperatures, but where the optimal growth temperature is above 15°C and the maximum temperature for growth is above 20°C (Morita 1975). Morita (2002) noted that: “The abnormal thermosensitivity of psychrophilic bacteria indicates the adaptation of cold-loving bacteria to their cold environment. Microbes do not have thermoregulatory mechanisms. When exposed to temperatures above their maximal growth temperatures, they expire; in some psychrophiles, this temperature can be between 10°C and 20°C.”

Facultative psychrophiles tolerate large variations in temperature and can therefore be found both in constantly cold environments and in environments with varying temperatures. In contrast, obligate psychrophiles are found only in constantly cold environments as even short heating may inactivate them, but the obligate psychrophiles may grow faster than the facultative psychrophiles at low temperatures due to physiological adaptation to life in the cold (Harder and Veldkamp 1971). It might therefore be hypothesised that obligate psychrophiles would dominate in constantly cold environments such as the deep sea and the Arctic Ocean, whereas facultative psychrophiles would dominate in environments with changing temperatures. Facultative psychrophiles may, however, be far more common than obligate psychrophiles in cold environments (Baross and Marita 1978).

The effect of temperature on a reaction rate can in many cases be described by the Arrhenius equation (Eq. 3):

$$ln(k) = \ln(A) - \frac{E_a}{R}\left(\frac{1}{T}\right) \qquad \text{Eq. 3}$$

where T is the temperature in Kelvin (°K), k is the reaction rate at temperature T, A is called the pre-exponential factor, E_a is the activation energy and R is the gas constant. In its strict sense, the Arrhenius equation applies only to the temperature effect on physico-chemical reactions, but it is also a good approximation of many microbial-mediated reactions; in this case the slope of the Arrhenius plot (E_a / R) is called the temperature sensitivity. It is seen from eq. 3 that a straight line with the slope E_a / R will be obtained if ln(k) is plotted against ($1 / T$). This is only as long as the bacterial activity follows simple thermodynamics. Deviation from the straight line at high or low temperatures indicates physiological changes like enzyme denaturation or critical changes of membrane fluidity. Arrhenius plots are therefore perfectly suited for characterising mesophiles, facultative psychrophiles and obligate psychrophiles.

Two strategies for cold adaptation are easily distinguishable in Arrhenius plots; either the slope may be different from that of the facultative psychrophile, i.e., the obligate psychrophile is less temperature sensitive so that it is more active than the facultative psychrophile at low temperature (Figure 2. 1A), or the slope may be the same, but the temperature range where the slope is constant is different so that the obligate psychrophile is more active at low temperature (Figure 2.1B) (Harder and Veldkamp 1971). In this case, the facultative psychrophile may deviate from linearity already at 4 to 5°C, which means that the decrease in growth rate at low temperatures is much greater than can be expected from the simple temperature effect on the chemical reactions within the cells (Harder and Veldkamp 1971).

A study of 143 Antarctic, marine, non-degraders showed that most were facultative psychrophiles (Delille and Perret 1989). On the other hand, a study of sulphate reducers in Arctic marine sediments found predominance of obligate psychrophiles as MPN (Most Probable Number) counts of sulphate reducers were higher at 10°C than at 20°C (Knoblauch et al. 1999). Characterisation of 19 isolated psychrophiles showed that: "as a physiological adaptation to the permanently cold Arctic environment, psychrophilic sulfate reducers have considerably higher specific metabolic rates than their mesophilic counterparts at similarly low temperatures" (Knoblauch et al. 1999). In a related study, five psychrophilic Arctic sulphate reducers exhibited relative growth rates of 25-41% of those at their optimum growth temperature at 0°C, and three of the five isolates were obligate psychrophiles with optimum growth temperatures of 7-10°C (Knoblauch and Jørgensen 1999). Whereas the sulphate reduction rates generally increased exponentially with temperature, the growth yield of three strains ranged almost constantly between -1.8°C and their optimum temperature, and the growth yield of two other strains was actually highest around 0°C (Knoblauch and Jørgensen 1999). These results indicate that: "psychrophilic sulphate-reducing bacteria are specially adapted to permanently low temperatures by high relative growth rates and high growth yields at *in situ* conditions" (Knoblauch and Jørgensen 1999).

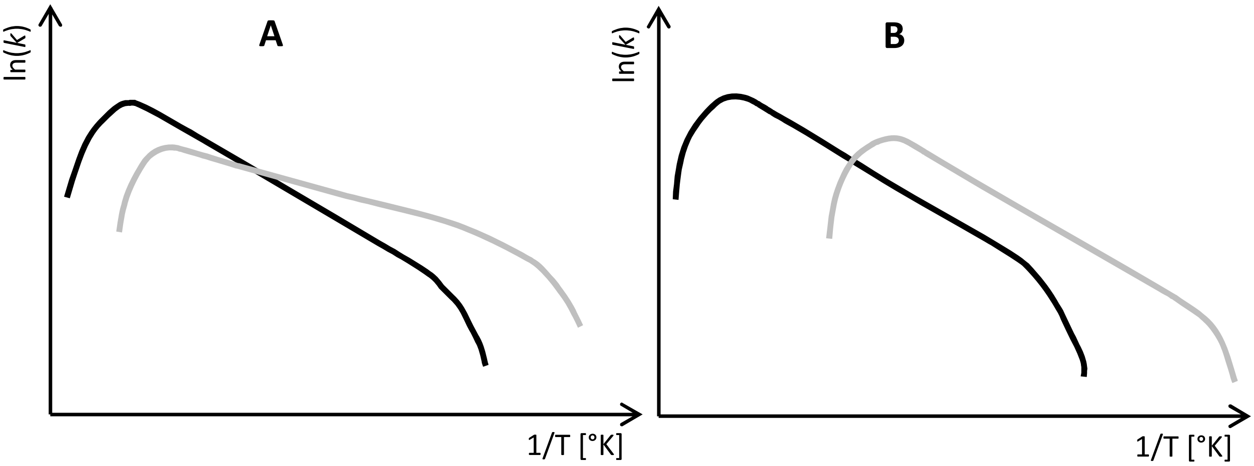


Figure 2.1. Hypothetical Arrhenius plots showing two different strategies for cold adaptation (A and B). Obligate psychrophiles are shown in grey, facultative psychrophiles in black, *k* is the maximum specific growth rate at temperature *T*, redrawn after Harder and Veldkamp (1971).

It is currently unknown whether Arctic hydrocarbon degraders are mostly obligate psychrophiles, and thus similarly efficient and adapted to life at very low temperatures, or merely facultative psychrophiles that may show some activity at low temperature but are not specially adapted to the cold conditions.

2.3.3 Microbial population dynamics

Total absence at a location of bacterial strains capable of hydrocarbon degradation is unlikely (e.g., Roubal and Atlas 1978), but the metabolic diversity of the hydrocarbon degrader community may range from strains capable of degrading only simple alkanes to very diverse communities capable of degrading a multitude of structurally different oil compounds. The presence or absence of degrader cells with the metabolic ability to degrade specific oil components in Arctic marine environments is discussed in detail in section 2.3.3. The *in situ* activity of microbial degraders in the marine environment does not depend only on the presence of specific metabolic functions, it is just as important how these degrader populations are modulated by the availability of nutrients and by predation, for instance by bacteriophages. Bacteriophages, or simply phages, are viruses that infect bacterial cells and replicate within them. Phages are very common in the marine environment (Børsheim 1993, Fuhrman and Suttle 1993) where there are few places for a bacterial prey cell to hide. The phages may also come from inside of the hydrocarbon degrader cells as phages may hide in the bacterial genome as pro-phages that can be activated upon an environmental stimulus. A study of pro-phages in marine bacteria showed that 43% of the isolates contained inducible pro-phages (Jiang and Paul 1994). One of the stimuli that can trigger activity of these pro-phages is actually exposure to oil compounds (Jiang and Paul 1996). As is known now, the population dynamics between phages and bacteria is determined by strain-specificity, i.e., phage strains *versus* bacterial strains. This means that the proliferation of individual bacterial strains is controlled by phages, not by the total amount of bacterial biomass and not by the density of species or functional groups (Thingstad 2000, Rodrigues-Valera et al. 2009, Thingstad et al. 2014). The same should apply to phages in relation to the diversity of bacterial hydrocarbon degraders, where phages in principle may wipe out entire subpopulations of degraders. There is no apparent reason why this should be any different under Greenlandic / Arctic conditions. Functional redundancy is probably high in environments where hydrocarbon degrader populations are diverse, but if the diversity of hydrocarbon degraders is low, for instance in the water column of non-exposed pristine waters, a specific oil component may be degraded by a specific strain that could be wiped out by phages, resulting in loss of the ability to degrade that specific compound.

2.3.4 Mineral nutrients and trophic interactions

In seawaters, one or more inorganic nutrients (e.g., nitrogen, phosphorus or iron) can become severely limiting to degradation processes, especially at times when photosynthesising members of the trophic web sequester the limited nutrient supply in the photic zone. However, to a certain extent, this can be counterbalanced by grazing of the photosynthesising organisms by other organisms through which the nutrients are recycled. In Greenland, this situation can occur after the spring bloom when copepods (mesozooplankton) come to the surface where they will graze heavily on ciliates, thus reducing ciliate density. As ciliates graze on photosynthesising autotrophic flagellates and on heterotrophic flagellates, reduced ciliate density results in a highly increased density of autotrophic and heterotrophic flagellates. Thus, the limiting nutrient, incorporated into the flagellate biomass, will not be mineralised, resulting in a serious lack of nutrients within the system (Thingstad et al. 2008). Larsen et al. (2015) observed a complete lack of bacterial response to addition of glucose in an Arctic mesocosm experiments in such a trophic situation. In surface waters of Disko Bay, Hansen et al. (2004) observed a comparable lack of bacterial response, where the effect of nitrogen deficiency indirectly affected other members of the trophic web during spring bloom.

2.3.5 Physico-chemical constraints

Bacteria that use hydrocarbons as sources of carbon and energy are faced with the problem of limited bioavailability of the compounds; the bacteria live in the water phase, but their growth substrate, the hydrocarbons, are mostly insoluble in water. Some oil compounds are slightly soluble but tend to partition between the oil and the water phase so that the aqueous equilibrium concentration may be very low. Hydrocarbon-degrading bacteria have developed strategies to compensate for the low bioavailability. These strategies include high-affinity uptake systems (Hua et al. 2013) and enzymes that ensure efficient degradation of hydrocarbons at very low aqueous concentrations (Harms and Bosma 1997, Wick et al. 2002), as well as the ability to form biofilm directly on the surface of their hydrocarbon substrate to reduce the diffusive distance and thereby maximise the uptake (Whyte et al. 1999, Vaysse et al. 2009, Wick et al. 2002). A third strategy is to produce surface-active compounds (Whyte et al. 1999, Zhang and Miller 1992) that may disperse the oil phase so that the surface-to-volume ratio is greatly enhanced, thereby facilitating increased transfer from the oil phase to the water phase as well as large surface areas for biofilm growth. There is no reason why these strategies should be different under Arctic conditions.

However, physical non-availability of oil may result from formation of large aggregates exposing hydrophobic surfaces. Photochemical reactions induced by solar radiation can lead to the formation of amphoteric hydrocarbon molecules whose hydrophobic end is larger than their hydrophilic head (Thingstad and Pengerud 1983). These molecules will form anti-micelles enclosing water droplets and exposing hydrophobic films on the outside, which is called a water-in-oil emulsion. This means that oil will behave like soft butter, forming large aggregates with a limited surface for microbial attack and thereby degradation. During an oil spill, water-in-oil emulsions can be facilitated by wave and current actions, frequently called "chocolate mousse," which may complicate the clean-up.

3 Present knowledge state of oil degradation in cold waters (<5°C)

Anders Johnsen & Jens Aamand

3.1 Arctic waters

3.1.1 PAH degradation potential in Arctic water sediments

The deep sea is by far the largest marine cold-water environment. However, most of the area has not been investigated with respect to oil degradation; in fact, most of it has not been investigated at all. A notable exception is a study of the distribution of PAHs and PAH-degrading bacteria in deep-sea sediments of the high latitude Arctic Ocean (Dong et al. 2015). Here, sediment cores were taken at four places along a north-south transect north of Canada where the water depth ranged from 2,500 m to 4,000 m. The sediment samples were analysed for the content of PAHs (Σ16PAHs: 2-42 ng g^{-1}), the general bacterial communities were characterised by high through-put DNA sequencing, and PAH-degrader enrichment cultures were established with naphthalene, phenanthrene and pyreen as carbon sources. Interestingly, the supposedly obligate hydrocarbon-degrader genera *Alkanivorax* and *Cycloclasticus* were detected in all of the sequenced sediments. The overall conclusion was that PAHs and PAH-degrading bacteria are widespread in the deep-sea sediments of the Arctic Ocean. A limitation of the study was, however, that both the PAH analyses and the enrichment focused on pyrogenic PAHs (EPA16 PAHs) that are found only in very small concentrations in oil. It is therefore difficult to generalise the results with respect to degradation of petrogenic oil PAHs, since these comprise a high number of different alkylated isomers. The potential for biodegradation generally decreases with increasing number of alkyl substituents within classes of PAHs, implying that, for instance, the phenanthrenes with zero to four methyl groups are degraded in the following order: phenanthrene > C1-phenanthrenes > C2-phenanthrenes > C3-phenathrenes > C4 phenanthrenes (Wang and Fingas 1995, Wang et al. 1998, Kristensen et al. 2015). Accordingly, extrapolation of the phenanthrene degradation potential in the sediments would greatly overestimate the general potential for sediment degradation of the alkyl-substituted petrogenic phenanthrenes.

3.1.2 Degradation of oil in the Arctic pelagic waters

The low temperatures along with low availability of nutrients (nitrogen and phosphorus) limit oil degradation in the Arctic. However, in a recent laboratory study conducted at 5°C with subsurface sea water collected at 150 m depth in Disko Bay, Greenland, rapid degradation of the alkane fraction of a crude oil was seen (Kristensen et al. 2015). The volatile alkanes (nC_8 to nC_{14}) were removed by both microbial degradation and abiotic processes, while the semi-volatile (nC_{15} to nC_{24}) and non-volatile ($nC_{>15}$ to nC_{24}) alkanes were removed mainly by microbial degradation. In contrast, in this study, no degradation of either PAHs, dibenzothiophenes or alkyl-substituted homologue was observed during the 71-day incubation period, which was explained by the pristine environment inhabited by only bacteria species adapted to degrade these structurally more complex molecules. Only slow degradation of the simpler PAHs naphthalene and 1-methyl naphthalene was seen. Based on these results, the authors provided the following order of degradability in pristine seawater from

Disko Bay: *n*-alkanes > alkyltoluenes (para->meta->ortho-isomers) > PAHs and dibenzothiophenes. Surprisingly, in seawater from the North Sea the degradation order of the same crude oil was opposite: PAHs and dibenzothiophenes > alkyltoluenes > n-alkanes (Kristensen et al. 2015). The more rapid degradation of PAHs and other cyclic compounds compared with the alkanes may be explained by the fact that the North Sea is more polluted than the Arctic Ocean and thereby better adapted to degradation of complex molecules.

A similar rapid degradation of crude oil was seen in Arctic seawater from the Chukchi Sea, Alaska, at an even lower water temperature of -1°C (McFarlin et al. 2014). About 60% of total hydrocarbons were degraded within two months, and in this study also PAHs and substituted PAHs were degraded, although at a slower rate than n-alkanes and branched alkanes. It was not possible to distinguish biological oil degradation from physical removal processes such as evaporation as non-biological controls were not included in the study. Faster degradation of straight chain alkanes than of branched alkanes and larger and alkylated aromatics was also seen in sediments in Arctic sediment from Spitzbergen, Svalbard (Garrett et al. 2003), and cold unpolluted seawater (~6.5°C) from Trondheimsfjord, Norway (Brakstad et al. 2004).

3.1.3 Limiting factors in the Arctic

Temperature, including Q_{10}

As mentioned above, temperature is a key factor determining oil degradation rates. The Q_{10} value (see section 2.3.1) is often used as input in predictive modelling of oil degradation in seawater in order to simulate degradation processes at changing temperatures (Bagi et al. 2013). It is generally accepted that a Q_{10} value of 2-3 may be valid in many environments for calculating biodegradation rates at one temperature to another temperature, and a Q_{10} value of 2 is often used as input to predictive modelling, although higher values often are reported – especially at low temperatures (Bagi et al. 2013, Debbe et al. 2005). Assuming a Q_{10} of 2.5, degradation rates derived from lab experiments carried out at 5°C will, for instance, overestimate rates at *in situ* temperatures of 2°C by a factor of 1.3 according to Eq. 3 (section 2.3.1). However, in most cases, the effect of this will be minor.

Degradation of oil depends on other environmental factors than merely temperature such as, for example, availability of nutrients, salinity and presence of cold-adapted degrader bacteria (as described in section 3.2). These factors may differ from location to location and thereby hamper the use of the Q_{10} approach and degradation rates obtained from the literature for predicting degradation rates in other environments such as Arctic seawater. On the other hand, if degradation rates are available from a specific marine environment, then the Q_{10} approach is important for modelling the degradation of oil where water temperatures fluctuate (Bagi et al. 2013). Unfortunately, geographically and climatically distinct marine environments with similar inherent biodegradation capacities are still to be defined (see Chapter 5), implying that appropriate degradation rates for predictive modelling are not readily available.

N, P and O_2

Oxygen is a prerequisite for effective microbial degradation of hydrocarbons, and following heavy oil spills oxygen may limit degradation (Hazen et al. 2010). In most cases, however, ocean currents and mixing by waves secure rapid spreading of the oil over large areas, thereby preventing that oxygen becomes limiting. The situation is different at the sea bottom, where heavy oil fractions deposit and degradation may become limited by oxygen.

Oil-degrading bacteria are ubiquitous in most environments and following an oil spill, oil-degrading bacteria may bloom to a level where nitrogen and phosphorus limit their growth (Head et al. 2006, Roubal and Atlas 1978, Atlas and Bartha 1972). Around Greenland, it is typically nitrogen that becomes limiting as the seawater here has a low nitrogen / phosphorus ratio. In addition, the anthropogenic input of nitrogen to the seawater around Greenland is generally low and what is more, the seawater originates from the North Pacific where it has been depleted of nitrogen through denitrification caused by anaerobic degradation of organics during subsidence on the way to the Arctic.

Oil-degrading bacteria

The presence of oil-degrading bacteria is a prerequisite for oil degradation to occur. The total number of bacteria in seawater is low compared with other environments such as soil or sediments. By use of direct counting in the microscope (DAPI), 2-8 × 10^5 bacteria ml^{-1} was counted in North Sea seawater (Zweifel and Hagström 1995), and the number of bacteria was found to be within the range measured for the Mediterranean (Bianchi and Giuliano 1996). Similar low bacterial numbers were measured at the cooler latitudes of Trondheimsfjord, Norway (90 m depth), where 3.3 to 7.2 × 10^5 bacteria ml^{-1} was counted (DAPI). The numbers of viable bacteria measured by growth-based methods (CFU or MPN) are typically 10 to 100 times lower than those achieved by the direct counting procedures. The number of oil-degrading bacteria constitutes only a fraction of the total number of bacteria in seawater. The level of hexadecane degraders measured in seawater from Disko Bay, Greenland, reached 30 bacteria ml^{-1} using a MPN technique as a proxy of the number of alkane degraders, while no specific PAH-degrading bacteria were recorded (Kristensen et al. 2015). In contrast, both alkane and PAH degraders were measured in water from the North Sea and in somewhat higher numbers (30-320 bacteria ml^{-1}). Similarly, Roubal and Atlas (1978) counted 1-1,000 hexadecane degraders ml^{-1} in seawater from the north coast of Alaska and found a correlation between the distribution of hydrocarbon degraders and the occurrence of hydrocarbons in the environment. The potential for biodegradation of ^{14}C-labelled model-hydrocarbons varied over the season, probably due to seasonal depletion of available nutrients. The generally recalcitrant compounds, pristane and benz-anthracene, were not mineralised, probably due to absence of degraders with the appropriate enzyme systems.

Although the number of oil-degrading bacteria is low in pristine water, their numbers may increase following an oil spill (Atlas et al. 1976). The absence of specific oil-degrading bacteria may, however, limit oil degradation as was seen in experiments with seawater from Disko Bay where neither PAH degraders nor PAH degradation was observed.

Whether, and from where, a population of PAH degraders will appear following an oil spill in Greenland is today unknown. It cannot be excluded that PAH degraders are present naturally in Arctic seawater but in extremely low densities of, for instance, a few bacteria km^{-3} seawater.

Little is known about the time required for growth of small degrader populations to densities necessary for significant degradation to occur.

A degradation potential may develop naturally due to exposure of oil components to hydrocarbons penetrating from the underground, the so-called seeps. Seeps of ethane and propane may, for example, support a natural population of alkane degraders, which can jump-start microbial degradation of an oil spill

(Valentine et al. 2010). Such natural hydrocarbon seeps also exist in Greenland (see section 5.3).

A deeper understanding of the adaptation processes leading to oil degradation following an oil spill is further complicated by the pollutants (and bacteria) rapidly being transported over long distances. In a fjord or another confined area with little discharge of water, an oil spill may with time lead to an increased degradation potential, but in open waters both hydrocarbons, oil degrading bacteria and with them the degradation potential, are rapidly being dispersed in the environment.

3.2 Other relevant cold water areas

3.2.1 Lessons from the Macondo Deep Water Horizon disaster

In 2010, an explosion on the oil rig Macondo Deep Water Horizon (DWH) in the Gulf of Mexico led to a blowout of oil on the sea floor at 1,500 m depth, and it is so far the largest offshore oil spill in history (Camilli et al. 2010). The official estimate of the oil discharge was 4.9 million barrels, equivalent to 780,000 m^3, of which only 25% was skimmed from the surface, burned or recovered from the wellhead (On Scene Coordinator Report 2011). Surface temperatures in this area are high, but since the spill took place at large depth, a significant proportion of the oil ended up in a cold subsurface environment. In the northern Gulf of Mexico, there is a thermocline in the upper 800 m, whereas the temperature gradually decreases from 6°C to 4°C when depth increases from 1,000 to 1,300 m (Wang et al. 2016). This means that the temperatures in the deeper parts of the Gulf of Mexico are only slightly higher but still comparable with those of the sea around Greenland. An estimated amount of >300,000 m^3 of oil was trapped in deep-sea, horizontal intrusion layers (McNutt et al. 2012) at depths of 1,000-1,300 m (Camili et al. 2010). At least 4-31% of the oil in the intrusion layers was deposited on the deep-sea floor, covering an area of minimum 3,200 km^2 (Valentine et al. 2014).

Based on methane and oxygen depth profiles from 207 stations, Kessler et al. (2011) calculated that a large amount of oxygen ($3.0\text{-}3.9\times10^9$ moles) was consumed within the polluted plume, primarily by a vigorous bacterial bloom of methane degraders. All of the released methane was trapped in subsurface waters (McNutt et al. 2012), but oxygen concentrations did not fall to anoxic levels (Hazen et al. 2010), suggesting high dispersion of the oil pollution. This agrees well with an estimated transport velocity of 5.7 km day^{-1} (Camilli et al. 2010). However, dispersion depends heavily on the local currents, and the results from the Mexican Gulf may therefore not comply with other regions.

One of the most cited studies on the microbial potential for degradation of oil in the water column after the DWH disaster showed that the cells that were enriched in the plume included many cold-adapted (obligate psychrophiles) or cold-tolerant (facultative psychrophiles) bacteria species known from other cold marine environments (Hazen et al. 2010). More than 90% of the cells from samples within the plume belonged to a single taxonomic unit of *Oceanospirillales* (Hazen et al. 2010). This taxon was also identified as a dominant group in water samples from the plume in another study (Redmont and Valentine 2012), whereas *Colwellia* and *Cycloclasticus* dominated in other water samples. Species from the groups *Oceanospirillales* and *Cycloclasticus* are connected to oil degradation capacity, where *Cycloclasticus* seems to be a common marine degrader of aromatic compounds (Dyksterhouse et al. 1995, Wang et al. 2008, Sauret et al. 2014, Dong et al. 2015). *Colwellia*, on the other hand, are generally not considered as oil degraders, and laboratory studies performed by Redmont and Valentine (2012) demonstrated that especially *Colwellia* was stimulated by the natural gas components ethane and propane, suggesting that ethane and propane co-released with the crude oil played an important role in stimulating the growth of *Colwellia*. Another interesting conclusion from the study of Redmont and Valentine (2012) is that the low temperature selected for cold-tolerant degrader strains as the main hydrocarbon degraders from the taxa *Oceanospirillales* and *Cycloclasticus* were closely related to known psychrophile species, suggesting that similar strains might become enriched during Arctic subsurface oil spills. For a more detailed review of the microbial dynamics following the DWH blowout, see Joye et al. (2014). It has been suggested that the deep-sea microbial community in the Gulf of Mexico has adapted over long time to degradation of oil leaked from natural seeps (Hazen et al. 2010). This seems plausible as the natural oil input to the Gulf of Mexico from seeps has been estimated to 140,000 tons per year (Kvenvolden and Cooper 2003).

The currents also induced an autoinoculation process where oil-polluted water bodies enriched in oil-degrading microorganisms later returned to the spill site with hydrocarbon-degrading bacteria, thereby accelerating the oil degradation process (Valentine et al. 2012). This effect was caused by the specific oceanography of the Gulf of Mexico and will probably be very limited in case of a subsurface blowout offshore of Greenland.

During the DWH disaster, 7,000 m^3 of the chemical dispersant Corexit were added at the wellhead (Kleindienst et al. 2015). The intended effect of adding dispersants was to increase the surface of the oil drops to enhance the area for bacterial oil degradation, possibly leading to faster oil removal. Another expected effect was that the oil dispersed in deeper layers would remain there, thus avoiding its emergence on the sea surface. The effect of dispersants has, however, been questioned, and experiments have demonstrated that dispersants can exert a negative effect on microbial hydrocarbon degradation (Kleindienst et al. 2015).

One major lesson learned from the DWH blowout was that knowledge of the intrinsic potential for oil degradation in water bodies is crucial in case of subsurface accidents where biodegradation is the only true removal process for oil that is dispersed (naturally or chemically) within the water columns.

4 Assessment of existing knowledge in a Greenland context

Anders Johnsen, Jens Aamand, Ulrich Gosewinckel

4.1 Abiotic degradation (weathering, including, for instance, evaporation and photo-transformation)

Abiotic weathering of oil comprises different physical and chemical processes, including evaporation, dissolution, emulsification, photo-oxidation and sedimentation. As these processes have been intensively studied for decades, knowledge of abiotic degradation is substantial, particularly under temperate conditions but limited under Arctic conditions. Even though most of the processes are the same regardless of where in the world a spill happens, low temperature, ice coverage and lack of daylight may slow down these processes significantly under these cold temperate conditions. Knowledge of abiotic degradation of oils around Greenland is limited but a few studies from other Arctic areas exist (e.g., Brandvik et al. 2009, Faksness et al. 2011, Prince et al. 2002). However, in most cases only a few abiotic processes, and only to a limited extent, are investigated in detail.

Ice coverage is of main importance when predicting the abiotic weathering and transport of oil spill in the Arctic. Oil can be absorbed in the ice/snow, and investigations have shown up to 20-25% absorption (Fingas and Hollebone 2003). The ice can also cause delayed release of ice-covered oil. In spring and summer when the ice melts, oil can be released to the surface of the ice through brine channels and by simple ice ablation (melting of the ice). Further, it has been demonstrated that the ice coverage slows down the emulsification rate (water uptake) of the oil (Brandvik et al. 2009).

Evaporation is the most rapid and important weathering process, also in Arctic areas. In general, evaporation may account for approximately 20-40% of the oil mass balance (Fingas and Holleborne 2003). However, several studies have shown that ice coverage reduces evaporation significantly by diminishing the spreading and the surface area of the oil. Furthermore, the cold temperature increases the viscosity of the oil, leading to lower evaporation. Another important abiotic process in the long term is photo-oxidation. Photo-oxidation primarily affects the aromatic compounds in oil, especially PAHs, resulting in a higher toxicity of the oil (King et al. 2014, Baron and Ka'Aihue 2001). Long-term studies of oil pollution have revealed that after the short-term weathering process from evaporation, photo-oxidation and biodegradation are the two most dominant weathering processes in Arctic areas (Prince et al. 2002).

Predictive models are important tools to assess the dispersion and fate of oil pollution. An extensive review by Afenyo et al. (2015) evaluated the existing models for fate and transport of oil in ice-covered areas. The authors found that ice-specific algorithms do not exist for most abiotic processes, mainly because the processes are poorly understood due to lack of experimental data.

4.2 Biotic degradation of oil (microbial flora, degradation potential of oil components)

Knowledge about biodegradation of oil in seawater around Greenland is very sparse. In fact, only one study can be found in the international scientific literature with direct focus on degradation of oil in Greenlandic seawater (Kristensen et al. 2015). The study compared degradation of crude oil in seawater from Disko Bay and the North Sea and surprisingly revealed that degradation of alkanes only occurred in the samples from Disko Bay, while PAHs only degraded in the North Sea samples. Generally, alkanes are considered to be more easily degraded than PAHs, and probably adaptation to PAH degradation did not occur in the pristine Disko Bay water. The lack of PAH degradation in Disko Bay contradicts the findings in other studies on Arctic waters in which degradation of PAHs was observed (McFarlin et al. 2014). It is important, though, to caution against drawing general conclusions from a single study. More research focusing on oil degradation in the Artic seas in general is needed, aiming at defining Arctic type localities representing degradation characteristics in the region and defining the environmental factors that determine the degradation potential.

Although the total number of bacteria in seawater is approximately the same whether the samples are taken in the North Sea, the Mediterranean or a fjord in Norway, there may be great differences in the number and type of oil-degrading bacteria. The number of degrading bacteria inevitably depends on previous exposure to the hydrocarbons, but the specific degrader communities that develop in cold waters may differ. For example, the alkane-degrading bacterium *Oleispira antarctica* is found to dominate in many cold waters where it has developed physiological features typical for psychrotolerant organisms (Kube et al. 2013).

Very little is known about both how bacteria in the Arctic adapt to oil degradation following exposure to oil and the time frame of such adaptation. The situation is complicated by the constant movement of the seawater. Assuming a sudden pollution from an oil rig, part of the pollutants will settle to the sediments, but a large fraction will be present as dissolved or dispersed small oil droplets in the water phase. In the sediment, the highest pollutant concentrations may be found close to the pollutant source where the degrading bacteria will adapt to the conditions and with time degrade the oil at a rate depending also on the environmental conditions. In the open sea, the highest oil concentrations will similarly be found close to the pollutant source where the oil is mixed with the non-exposed seawater. In the pelagic, however, the pollutants are rapidly dispersed and diluted, and degrader bacteria do not have the time to adapt to oil degradation. Development of mathematical models that combine dispersal of the pollutants with knowledge about degradation processes is urgent in order to better predict the fate of the pollutants in the marine environment.

Are the marine Arctic hydrocarbon degrader adapted to the cold environment and hence suggesting a potential for fast turnover of oil pollution or are the degraders merely mesotrophic strains that are severely inhibited by the cold conditions? The results from the DWH disaster demonstrated that efficient microbial hydrocarbon degradation is possible at great depth at low temperature. However, it also seemed that the continued release of oil from natural subsurface seeps had primed the microbial population with a high capacity for oil degradation. Natural seeps exist in Greenland, but it is, questionable whether a similar priming effect on the microbial degrader community can be expected in the seas around Greenland as the amount of oil leaked into the marine environment is quite low compared with the Mexican Gulf (see Chapter 5). Thus, the microbial response to oil pollution will expectedly be much slower here than in the Gulf of Mexico and the bacterial oil metabolism is likely to be less diverse, leaving more oil components resistant to degradation. These expectations are supported by the conclusion drawn by Kristensen et al. (2015) in their study of both the metabolic diversity of marine hydrocarbon degraders and the metabolic potential for degradation of different classes of oil compounds in the water column of Disko Bay. Their study of the topic is the only one available, and they found that the metabolic potential for degradation of different classes of oil compounds is very limited.

It is not known whether the results of Kristensen et al. (2015) showing low degradability of some, especially aromatic oil compound classes, are representative for other marine regions around Greenland. The study by Dong et al. (2015) indicated that there is a potential for degradation of the smaller pyrogenic PAHs in sediments of the Arctic Ocean, but it is unknown how the microbial communities will respond to alkylated oil PAHs that are more relevant in an oil spill scenario.

It is also unknown how the hydrocarbon degrader communities in the sediment relate to degrader bacteria in the water column. Do microbial sediment communities represent a general trend in the Arctic Ocean or are the degraders and the PAHs enriched only in the sediments, but almost absent in the water column where the turnover of the bacterial communities presumably is much faster? Assessment of the potential for degradation of different oil components in different compartments (sediments, water column and surface waters) of the marine ecosystem around Greenland is needed to clarify this issue. This is especially relevant for the toxic aromatic oil fraction that covers the various alkylated mono-aromatics, the alkylated PAH series and the series of alkylated NSO (nitrogen, sulphur, oxygen) aromatics.

A potential limiting factor for oil biodegradation is the possibly increased toxicity of the oil due to photochemical reactions; for instance, North Sea crude oil becomes increasingly toxic through photo-oxidation (Pengerud et al. 1984), which may be inhibitory to bacterial hydrocarbon degraders.

The ice coverage in the Arctic will, if oil is spilled in the ice, delay the degradation of the oil until spring. The extent to which the physical transformation processes (weathering) are also delayed by freezing temperatures remains to be clarified. Possibly, it is a specific Arctic situation: when spring arrives, the physical processes may have altered the oil, making it less (or more?) degradable by bacteria.

Some medium and long chain aliphatic hydrocarbons become solids at low temperature (Verbruggen et al. 2000). This is a challenge for oil biodegradation at any temperature. Whether this problem is exacerbated by Arctic temperatures remains to be investigated; degradation of long chain aliphatic hydrocarbons will, to a larger degree, depend on dissolution by short chain aliphatic hydrocarbons that tend to evaporate or be faster degraded, leaving some of the long chain aliphatic hydrocarbons as a residue problem.

The availability of nitrogen and other mineral nutrients may be severely limited by the spring bloom of algae in the photic zone. An oil spill on ice will delay the degradation of the oil until spring, i.e. the possible springtime limitation of mineral nutrients in the photic zone applies not only to spring spills but also to winter spills. In many parts of Greenland, these two periods together may cover more than half of the year during which oil degradation in the photic zone would be very slow or even non-existing.

5 Physical and biological characterisation of the regional water bodies in relation to natural oil biodegradation around Greenland

Susse Wegeberg, Frank Rigét, Janne Fritt-Rasmussen, Kim Gustavson

Distinct marine environments with similar inherent biodegradation capacities are still to be defined, as already mentioned in section 3.2. This chapter will thus review the present knowledge about the characteristics and biology of Greenland water bodies in relation to the natural oil biodegradation potential around Greenland. Hydrography will be described to delimitate the water bodies and general productivity will be used as a proxy for the nutrient state in these distinct marine environments.

Furthermore, occurrence of natural oil seeps are described for Greenland, as these areas may provide substratum for an oil-degrading microflora.

These potential differences between water bodies and areas with natural oil seepage can be used in a focused and coordinated research effort directed at mapping and assessing the microbial floras and oil degradation potential.

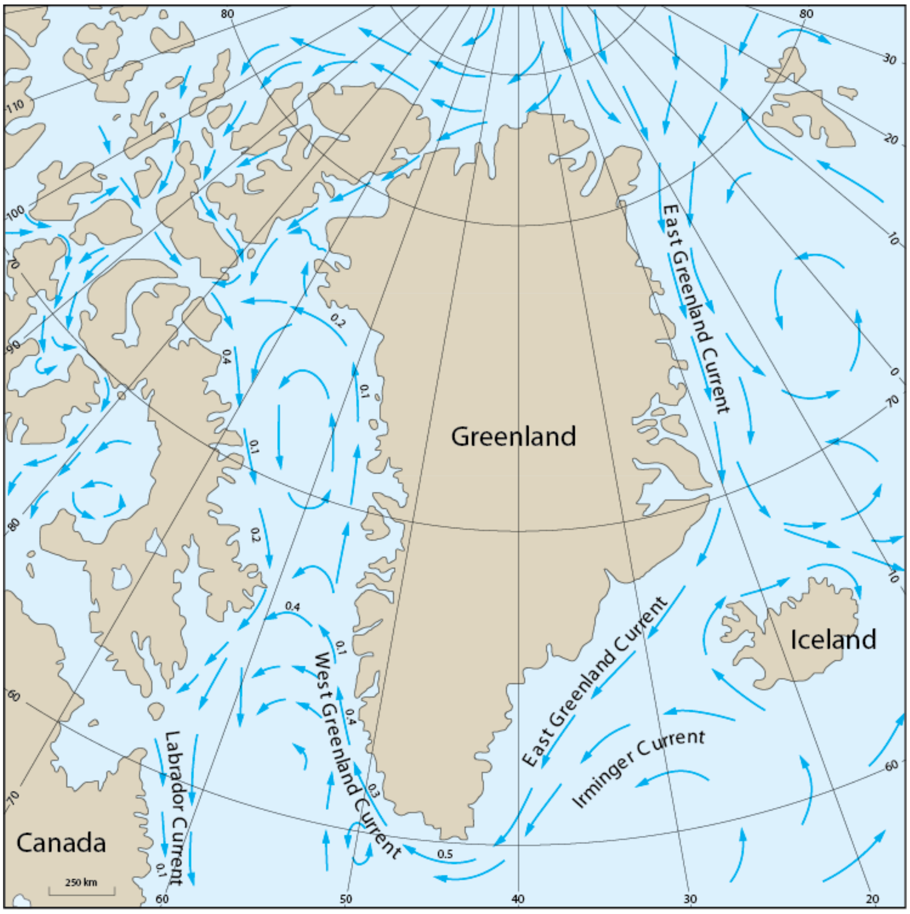
5.1 Regional water bodies – physical and biological characterisation

Greenland is surrounded by the Arctic Ocean, the Fram Strait, the Greenland Sea, the Denmark Strait, the Cape Farewell area, the Davis Strait, Baffin Bay and the Nares Strait. Inflow of water derives from the North Atlantic, but also inflow of cold water from the Arctic Ocean occurs. Variations in the North Atlantic Current due to climate fluctuations impact the circulation in the waters surrounding Greenland (Buch 2002). Such variations are related to the North Atlantic Oscillation (NOA); thus, a positive NOA index results in warmer conditions in Europe and the Arctic region, while a negative NAO index has the opposite result (Buch 2002, Liord et al. 2007).

Figures 5.1 shows the dominant ocean surface currents around Greenland.

The broad picture of the sea around Greenland shows distinct water masses of different origin, exhibiting different temperature and salinity characteristics as well as varying nutrient levels. At a regional scale, the areas with a relatively high potential for oil biodegradation may coincide with areas with relatively high temperature and upwelling areas and thereby higher levels of nutrients and primary production. In general, the input of nitrogen to the marine environment around Greenland is small. In addition, some of the ocean waters around Greenland originate from the North Pacific where nitrogen depletion through denitrification has occurred. This means that the N / P-ratio typically is low so that nitrogen is commonly the limiting factor. Furthermore, at low temperatures N_2 fixation is low, meaning that nitrogen remains limiting. However, this mainly applies to the photic zone of the waters, whereas nitrogen may not become limiting in the waters below the photic zone due to the absence of photosynthesis. In the Gulf of Mexico, for instance, the concentration of nitrogen and phosphorus is high at the sea bottom. However, whether the situation is the same in Greenland waters requires investigations at specific locations using available data or, if necessary, undertaking new measurements.

Figure 5.1. General ocean surface circulation around Greenland. (Modified from Oceanographic Atlas of the Polar Seas (US Navy Hydrographic Office 1958)).



The following marine areas have been identified, which may possess similar physical properties and nutritional level (Figure 5.2):

- Polynyas
- Fram Strait / Greenland Sea affected by the East Greenland Current
- Denmark Strait / Cape Farewell affected by East Greenland Current + Irminger Current into the West Greenland Current
- Davis Strait affected by East Greenland Current + Irminger Current running into the West Greenland Current
- Baffin Bay affected by inflow from the Arctic Ocean and the West Greenland Current
- Fjords affected by runoff from land.

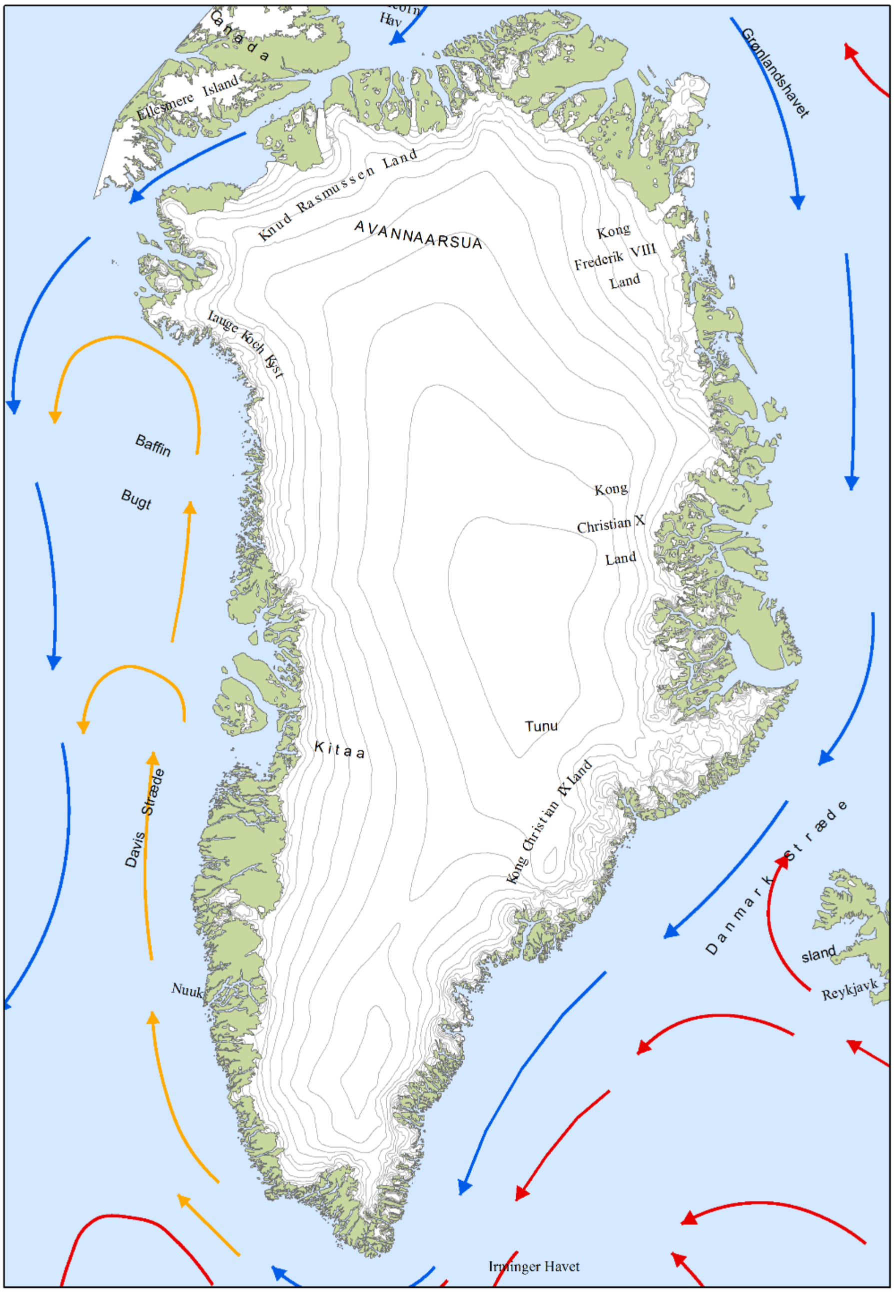


Figure 5.2. Marine areas, which may possess similar physical properties, and which are indicated by same color arrows.

5.1.1 Polynyas

Polynyas are large expanses of open water in seas that are otherwise covered by ice. The open water is caused by hydrographic features together with the use by animals of the area. The most important polynyas in Greenland are the North Water in Baffin Bay, the Northeast Water off Northeast Greenland and the polynya at the mouth of Scoresby Sound (Frederiksen and Johansen 2011, 2012). Polynyas differ from the surrounding ice-covered areas by upwelling of nutrients at the ice edge, creating a greater nutrient influx. The resulting relatively large primary production favours biological diversity, and hence probably also the microbial flora. More details about the specific polynyas are found in the sections below.

5.1.2 Fram Strait/Greenland Sea

The water masses in the Greenland Sea can, according to Richardson et al. (2005) following Swift and Aagaard (1981), be classified as Atlantic water, Polar water, Polar intermediate water, Arctic surface water and Upper Arctic intermediate water based on temperature and salinity characteristics (Table 5.1).

In the Fram Strait, large amounts of cold water from the Arctic Ocean enter the waters around Greenland. The Arctic Ocean is the water surrounding the North Pole between North America, Asia and the Arctic Circle. It is the smallest ocean in the world and is covered with floating ice during winter. The Arctic Ocean differs from other marine environments by having low temperatures and ice coverage for several months during winter with limited sunlight. Although temperatures are low, they fluctuate at the surface during the year, typically from 4°C to the freezing point, which for Arctic seawater is about -1.8°C. At these low temperatures, the density of seawater increases and the cold water tends to sink, which contributes to generally low temperatures also at greater depths. However, at depths of >200 m, the temperature may be more stable, typically around 2°C.

Overall, the Fram Strait / Greenland Sea is a region where the dominant flow is that of cold nutrient-poor water from the Arctic Ocean and it generally has a brief and intense phytoplankton bloom in connection with ice break-up (Frederiksen and Johansen 2013, and references herein). However, in connection with presence of large polynyas, such as the North East Water or the marginal ice zone, upwelling of nutrient-rich water may locally lead to very high production (Gradinger and Baumann 1991, Frederiksen and Johansen 2013). Gradinger and Baumann (1991) thus found that: “three main domains could be distinguished [in the Fram Strait area] in terms of both hydrography and biology: (1) the East Greenland shelf polynya with a high biomass of mainly produced chain-forming diatoms, (2) the ice-covered East Greenland Current with extremely low standing stock dominated by flagellates and (3) the marginal ice zone with biomass maximum in 20 to 40 m depth formed diatoms, dinoflagellates…” Hence, these areas must be considered as seasonally nutrient rich.

Also, during five cruises in the Greenland Sea, between 71° and 80° N, in March-April, May, June, August and November-December, Richardson et al. (2005) found that inorganic nutrients (nitrogenous and phosphorous) were measurable in the sea surface; only in August, indications of nutrient depletion was observed.

Regarding temperature (and salinity) data, the differences between the different water masses (as classified by Swift and Aagaard (1981) and followed by Richardson et al. 2005)) are presented in Table 5.1, modified from Richardson et al. (2005).

Richardson et al. (2005) found that there were significant differences in primary production between all cruises, but for the March-April 1993 cruise, significant difference was recorded in primary production between the different water masses. The primary production was significantly higher in the Arctic surface water than in the Polar intermediate water, likely due to the more than 2°C higher temperatures in the Arctic surface water. In the June 1999 cruise, significant differences in nitrogen concentrations were recorded for the three water masses sampled (Atlantic water, Arctic intermediate water and Arctic surface water).

Hence, there may be reasons to believe that the differences between water bodies reflected in primary production and nitrogen concentrations may reflect differences also in microflora abundance and diversity / composition.

Furthermore, the vertical distribution of water masses mapped by Richardson et al. (2005) (Table 5.1) should be taken into account when designing a sampling program for investigating the potential for natural oil degradation (see also the section on Baffin Bay).

Table 5.1. Classification of water masses of the Greenland Sea including related temperature and salinity. Following Richardson et al. (2005).

Water mass	Temperature (°C)	Salinity (psu)
Atlantic water	>3	>35.0
Polar water	<0	<34.4
Polar intermediate water	<0	34.4-34.7
Arctic surface water	>0	34.4-34.7
	>2	34.4-34.9
Upper Arctic intermediate water	<2	34.4-34.9

5.1.3 Denmark Strait/Cape Farewell

The East Greenland Current flows southward at the eastern side of Greenland and the current is tightly trapped to the shelf break (Pickart et al. 2001). The East Greenland Current brings cold and low saline water as well as ice floes from the Arctic Ocean to Cape Farewell and thereby influences the area. However, the area around Cape Farewell is also affected by the inflow of water masses from other parts of the North Atlantic (Buch et al. 2004). The warm and saline North Atlantic Current branches off and follows the southern coastline of Iceland as the Irminger Current, and part of this current flows southward along the East Greenland coast. Here, it meets the cold and low salinity East Greenland Current, and the two currents flow parallel southward to Cape Farewell where they turns under mixing and continue as the West Greenland Current.

Therefore, as South Greenland is hydrographically connected with the Northeast Atlantic through the North Atlantic drift and the Irminger Current, this may facilitate transport and introduction of northern temperate organisms the Cape Farewell area, which may thus act as a potentially critical gateway for species propagules (dispersal units) (Høgslund et al. 2014).

Furthermore, the turn of the current from south to north over the Eirik Ridge, the most southern ridge of Greenland, causes mixing of the water masses from the warmer Irminger Current with the West Greenland Current (Holyday et al. 2009), which may also lead to more nutrient-rich waters.

Therefore, the Denmark Strait / Cape Farewell area may be of particular interest in a survey of the microbial flora in Greenland.

5.1.4 Davis Strait

The West Greenland Current flows northward along the coast. Therefore, the hydrographic conditions along West Greenland depend greatly on the relative strengths of the two currents, the East Greenland Current and the Irminger Current. Closest to the shore is the polar water derived from The East Greenland Current. The polar component loses its momentum on the way northward, and at the latitude of Fylla Bank it turns westward and joins the south-flowing Labrador Current along the Canadian coast. The West Greenland component originates from the North Atlantic Sea and the Irminger Sea, which is relatively warm and salty and can be traced all the way from Cape Farewell to Thule. On its way northward, the West Greenland Current is diluted by runoff waters from the fjords.

The Davis Strait area holds several fishing banks with a special biology and hydrology due to the relatively low water levels (Merkel et al. 2012). Accordingly, the bank areas along the mid-western coast of Greenland are highly productive as revealed by the high chlorophyll concentrations, but also reflected in the high abundance and biomass of zooplankton and sandeel (Figure 5.3, Hansen and Hjort 2013, Dünweber 2013, Box 1, figure 3; Frederiksen et al. 2008, figure 3.14, Munk et al. 2003, figure 10).

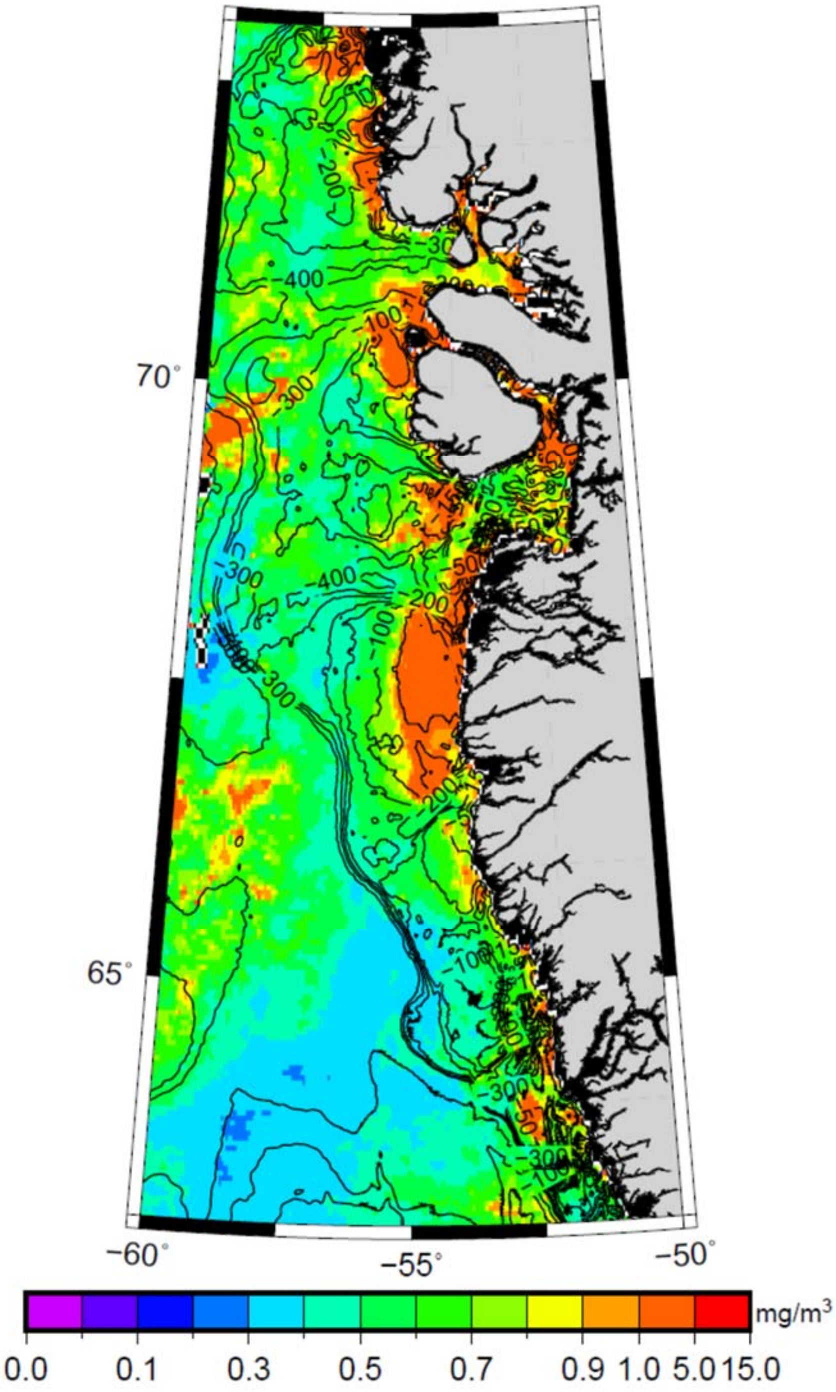


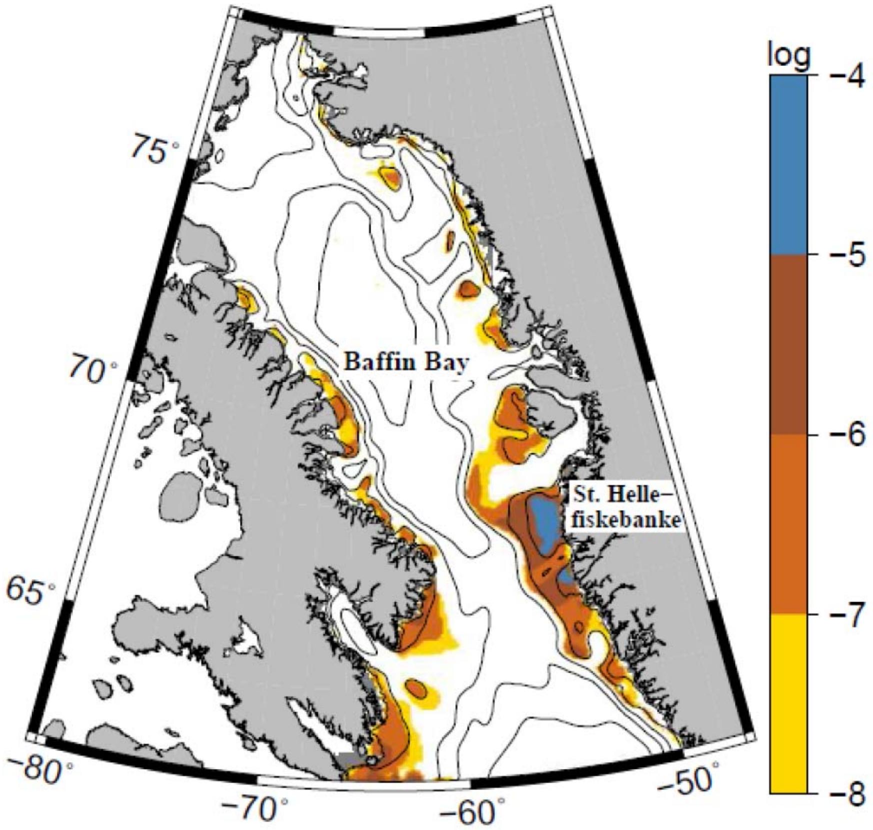
Figure 5.3. Chlorophyll concentrations in June (2003-2014) (ClimateLab 2014).

Regarding Store Hellefiskebanke, the modelling of the tidal dynamics shows that the water mass above the bank is fully vertically mixed (Wegeberg et al. 2016) (Figure 5.4). Hence, the production is not delimited to certain areas of upwelling. If the tidal dissipation is compared with the chlorophyll distribution in June, i.e., after the spring bloom, the areas defined by depth isobars coincide (Figure 5.5). The mixing of water masses in the area seems to explain some of the production, and the waters on and around Store Hellefiskebanke must be considered nutrient rich.

Also the other fishing banks, Disko Banke, Lille Hellefiskebanke, Sukkertop Banke and Fyllas Banke, show high abundance of zooplankton species as well as of fish and shrimp larvae (Munk et al. 2003, Pedersen et al. 2002). However, Munk et al. (2003) found that "differences between hydrographic characteristics and plankton distribution differed among species, and apparently specific plankton communities were established in different areas of the shelf".

Due to the nutrient-rich characteristics of the water masses of these bank areas, future research investigating the potential for natural degradation of oil in the Davis Strait area should in particular focus on the water masses above the banks, separately, though, as they show diverse patterns in plankton abundance linked to the hydrographic characteristics (Munk et al. 2003). Additionally, the low water depth at the banks, together with their importance as production areas, support future research focus on natural biodegradation of oil. These characteristics make the banks extra sensitive to oil pollution as well as restrict combat of oil spill due to potential less environmental benefit from using, e.g., chemical dispersion and *in situ* burning(Wegeberg et al. 2016).

Figure 5.4. Logarithmic ($\log_{10}$) illustration of energy dissipation (W kg^{-1}). The highest values are found along the West Greenland banks, especially St. Hellefiskebanke, indicating relatively high vertical mixing of water masses caused by tide dynamics (ClimateLab 2014).



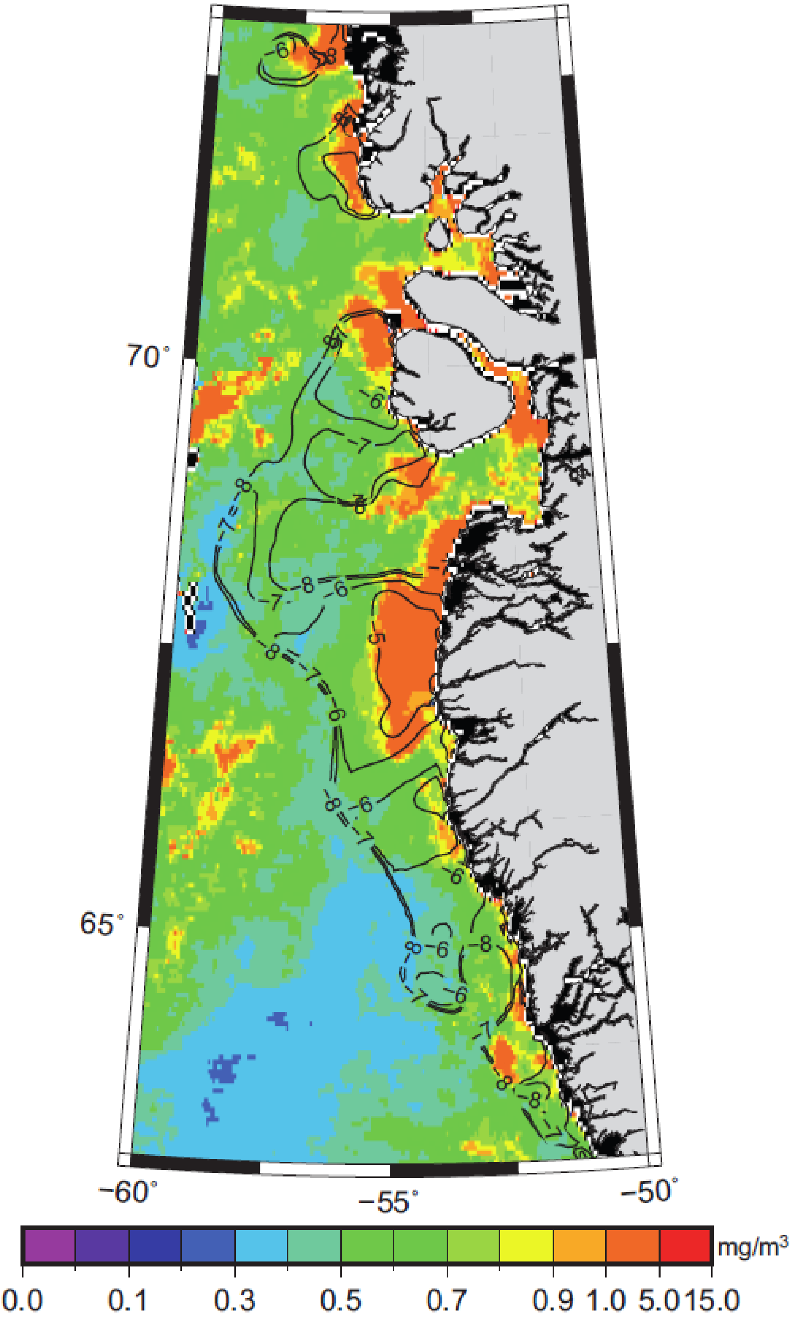


Figure 5.5. Overlay illustration of modelled mix of water masses (isobars) and climatology of chlorophyll from the MODIS-satellite in June (2003-2014). From Wegeberg et al. (2016).

5.1.5 Baffin Bay

Along West Greenland, the flow of the West Greenland Current is characterised by two principal components – closest to the shore, cold polar water from East Greenland moves northward and is diluted on its way by runoff waters from the various fjord systems. The other component, from the North Atlantic, derives from the Irminger Sea (Boertman and Mosbech 2011).

The heavy and long periods of ice cover in the Baffin Bay area drive the availability of nutrients in the upper part of the water masses due to the prevention of water column mixing, which, in turn, may be driven by temperature- and salinity-induced stratification (Frederiksen and Johansen 2011).

Sameoto (1984) found that the vertical distribution of copepod species correlated with the temperature and salinity profile in the northeastern Baffin Bay. The water column was divided into an upper subarctic zone (>0°C, salinity <

32 psu), a lower subarctic zone (0°C, salinity 34 psu), and a deep zone of Atlantic water (>0°C, salinity 34 psu). He also reported a stratification layer at approximately 15 m depth (figures 5.2-5.7).

As exchange of nutrients across the stratification layer is minor, depletion of nutrients may occur during the phytoplankton spring bloom into early summer, where stratification is consolidated, as observed by Tremblay et al. (2002) for the northern area of Baffin Bay.

Hence, this vertical stratification of the water masses, as also observed for the Greenland Sea (Richardson et al. 2005), should be included in the considerations when designing a sampling programme for investigating the potential for natural degradation of oil in the Baffin Bay water masses.

Figure 5.6 shows salinity profiles along transects in Southeast and Southwest Greenland illustrating the two water bodies of the East Greenland Current (relatively low salinity) and the Irminger Current components (relatively high salinity).

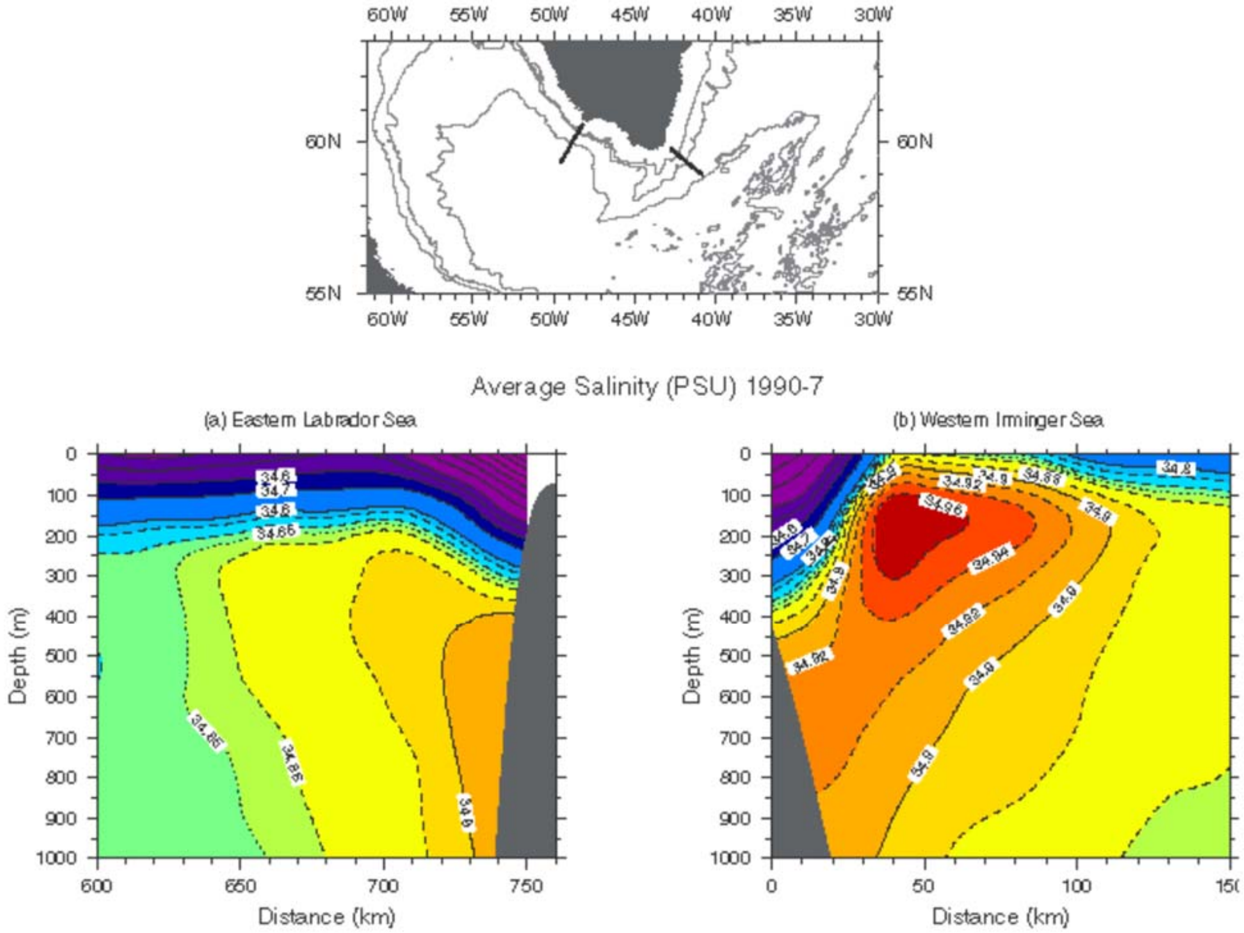


Figure 5.6. Mean upper-layer salinity for the period 1990-97. Eastern Labrador Sea, b) western Irminger Sea (source: Pickart et al. 2001).

5.1.6 Fjords affected by runoff from land.

Most fjords in Greenland are sill fjords. The inflow of fresh water to the fjord drives the large-scale circulation as it generates a higher water level in than outside the fjord. Thus, a surface current with brackish water flows out of the fjord, and a deeper current of more saline water flows into the fjord. Almost no inflow is found during winter when the lakes and rivers are frozen, and the precipitation on land often occurs as snow. Therefore, in winter, the surface salinity in the fjords increases and reaches the same level as outside the fjord, implying reduced circulation in the fjord (Buch 2002). In the fjords, the relatively warmer and brackish surface water may also favour microbial activity relative to the more cold and saline water below as well as surplus of nutrients with runoff from land.

5.2 Natural oil seeps in Greenland

Areas with natural oil seeps may be of particular interest for mapping of the local microbiological flora, its taxonomy and ability to degrade oil since the permanent natural input of oil may have created a flora capable of degrading oil as a carbon source, as known from the Gulf of Mexico (Hazen et al. 2010)

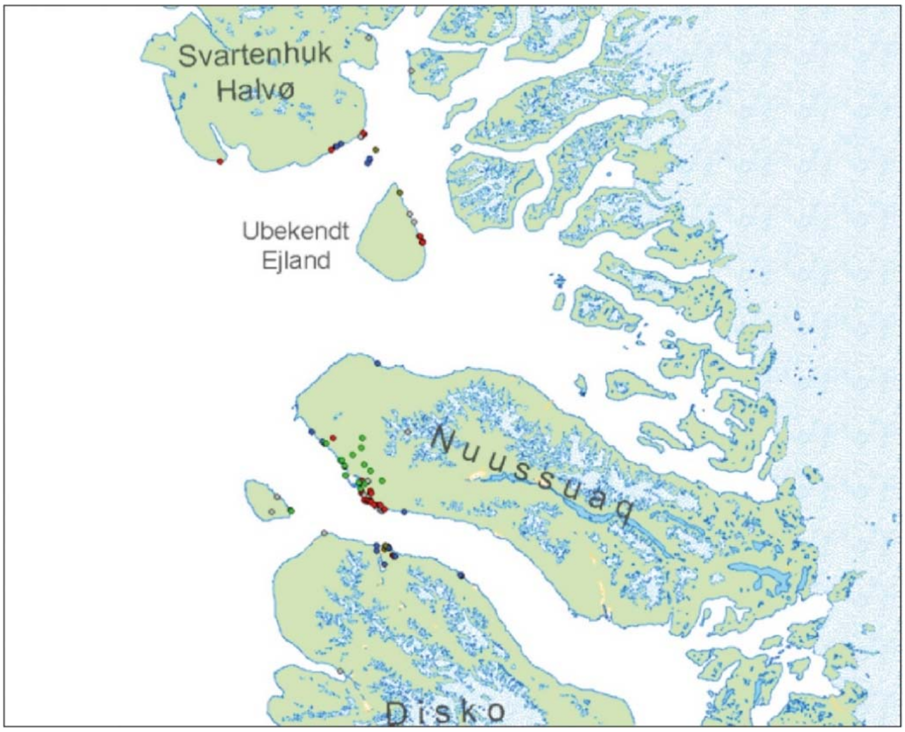
In an Arctic context, sources of anthropogenic PAHs to the environment were reviewed and held against the background level from natural sources of the contaminants (Klungsøyr et al. 2007, Macdonald et al. 2005). Often contamination with PAHs from natural oil seeps is difficult to detect with conventional monitoring methods, such as measurement PAH concentrations in mussels, fish species and sediment, due to low fluxes or pulse fluxes combined with potentially strong currents in rivers and along coastlines (Klungsøyr et al. 2007). However, even small concentrations of PAHs may be able to sustain a microbiological flora capable of using the environmental oil as a carbon source. Klungsøyr et al. (2007) provide an overview of known natural oil seeps in the Arctic and the natural contamination of these areas:

In Alaska, natural seeps are known from sandstone, while no marine seeps have yet been identified. In Russia, oil seeps and tar sand are well-known in and around the Timan-Pechora basin. In Canada and Greenland, coastal oil and submarine seeps are known, in particular in the area of Baffin Island and at the coast of the Nuussuaq peninsula.

Natural oil seeps and submarine gas seeps have been studied, especially in central West Greenland (Bojesen-Kofoed 2011; Nielsen et al. 2014), and Bojesen-Koefoed (2011) concluded that oil seepage and staining are widespread in this area. Figure 5.7 shows locations of oil seepage on land. In the coastal rocks of the western and outer part of the Nuussuaq peninsula, natural oil seeps have been located. Both fresh oil, weathered and degradation products are present, indicating active seeping of oil (Bojesen-Koefoed et al. 1999).

Nielsen et al. (2014) studied methane occurrences in the marine area around Disko. Four locations were selected for study (Vaigat, Ilulissat, Egedesminde Dyb and Aasiat) because the seabed features and psychical conditions here made seepages of gas most likely and thus for formation of gas hydrates. Natural gas hydrates are a mix of gas and water, which is not chemically bound, and constitute a solid solution where gas is the solute and water the solvent. The formation of gas hydrates is favoured by low temperature and high pressure. For all four locations, the conclusion was that gas seepage and the occurrence of gas hydrates was highly probable (Nielsen et al. 2014).

Figure 5.7. Location of sampling sites where samples were taken and analysed for oil staining. Colour coding refers to petroleum types (from Bojesen-Koefoed 2011).



Several studies have investigated background levels of PAH in sea sediment in order to discover potential natural petroleum seeps (Mosbech et al. 2007). In 2005, DCE conducted a study of potential environmental effects of the local natural oil seepage at Marraat on the Nuussuaq peninsula (Mosbech et al. 2007). The studies, performed in 2005, included investigations of sediment, blue mussels (*Mytilus edulis*) and fish species, but no local seepage or environmental effects of such seepage could be detected. However, the PAH composition in the organisms showed traces of both oil of terrestrial origin and of local pollution from fuel oil.

The difficulty in tracing the natural pollution from oil seeps in marine sediment and/or organisms may be due to factors such as natural degradation, metabolism and dilution, not least in the Vaigat (the water body between Disko and the Nuussuaq peninsula) with strong currents, and if the oil is released in pulses, tracing may be even more complicated.

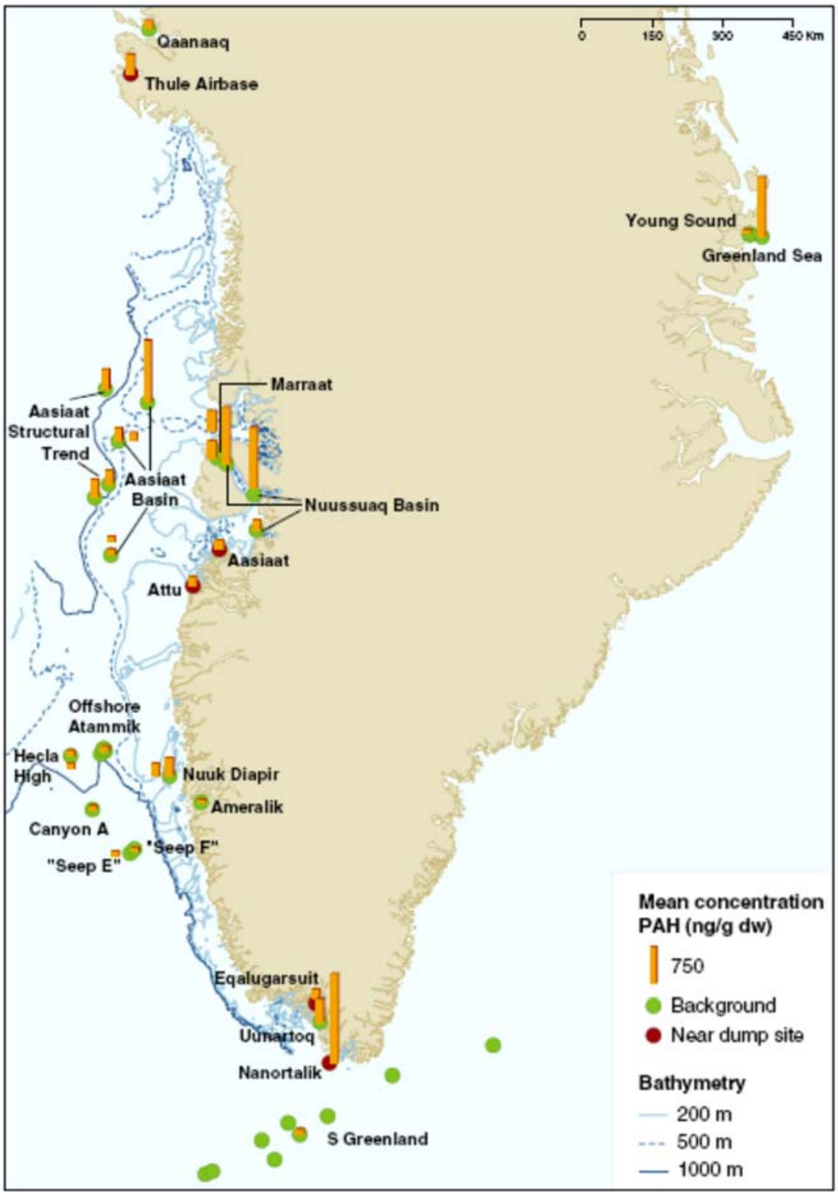
In 2015, DCE visited Sikillingi at the south coast of the Nuussuaq peninsula to collect samples of naturally occurring oil in rocks and stones. When the stones are broken, fresh, viscous and smelling oil drops are visual to the eye (Figure 5.8).

Figure 5.8. Natural occurrence of oil in rocks and stones at Sikillingi on the southern coast of the Nuussuaq peninsula. When the stones are broken, fresh, viscous and smelling oil drops are visible.

In 2004, PAH concentrations in sea sediment were analysed from areas showing indications of natural petroleum seeps and backgrounds areas. In total, six different areas in Southwest Greenland and two areas in East Greenland were investigated. In none of the areas, petrogenic sources were revealed, and no indication of natural petroleum seeps were found.

DCE stores the data on PAHs in marine sediments obtained from the above studies as well as from, for instance, baseline surveys performed by oil companies (Figure 5.9, 5.10, 5.11).

Figure 5.9. Map showing PAH pollution and background levels around Greenland.



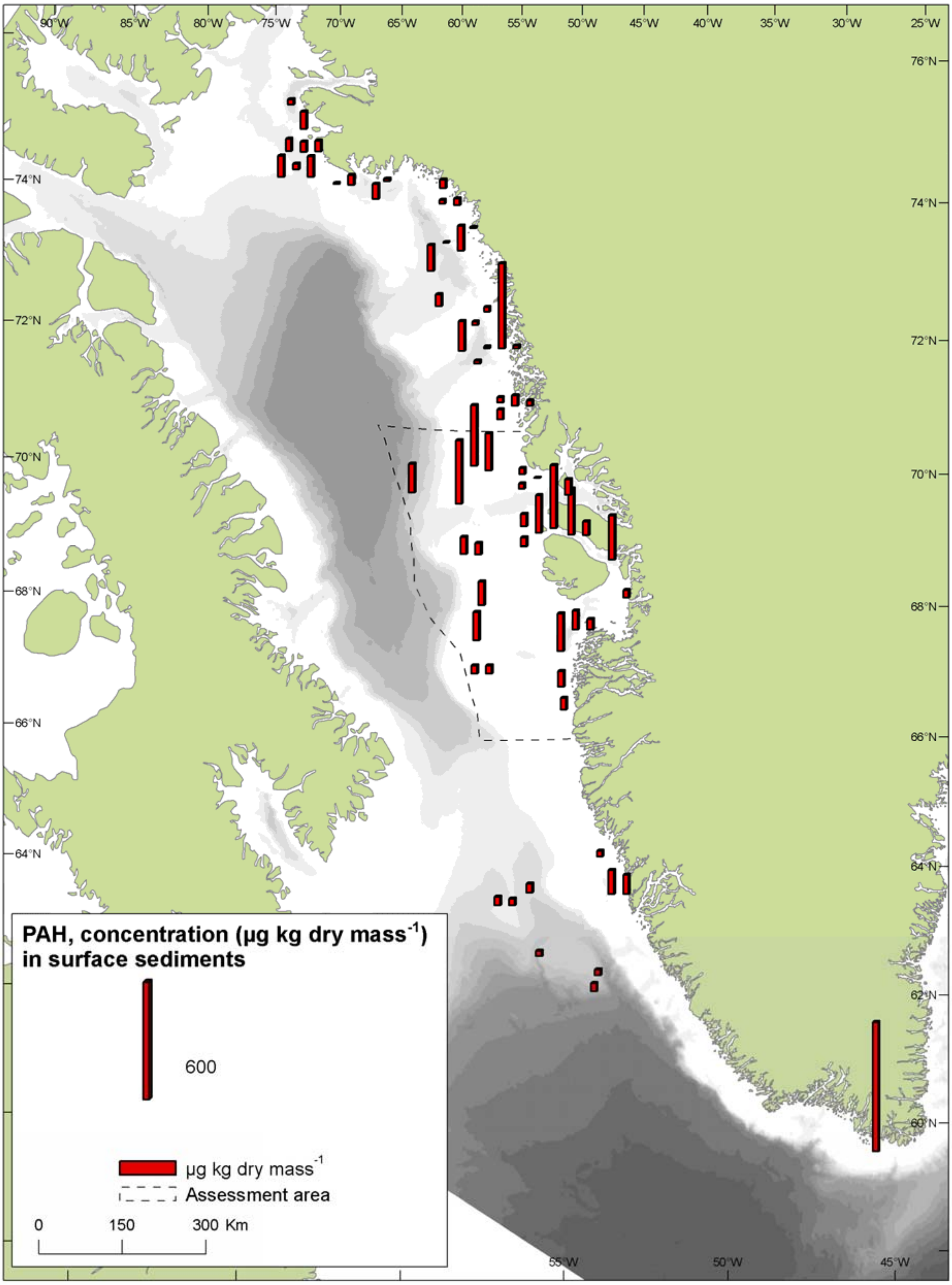


Figure 5.10. PAH concentrations in surface sediments on the west coast of Greenland.

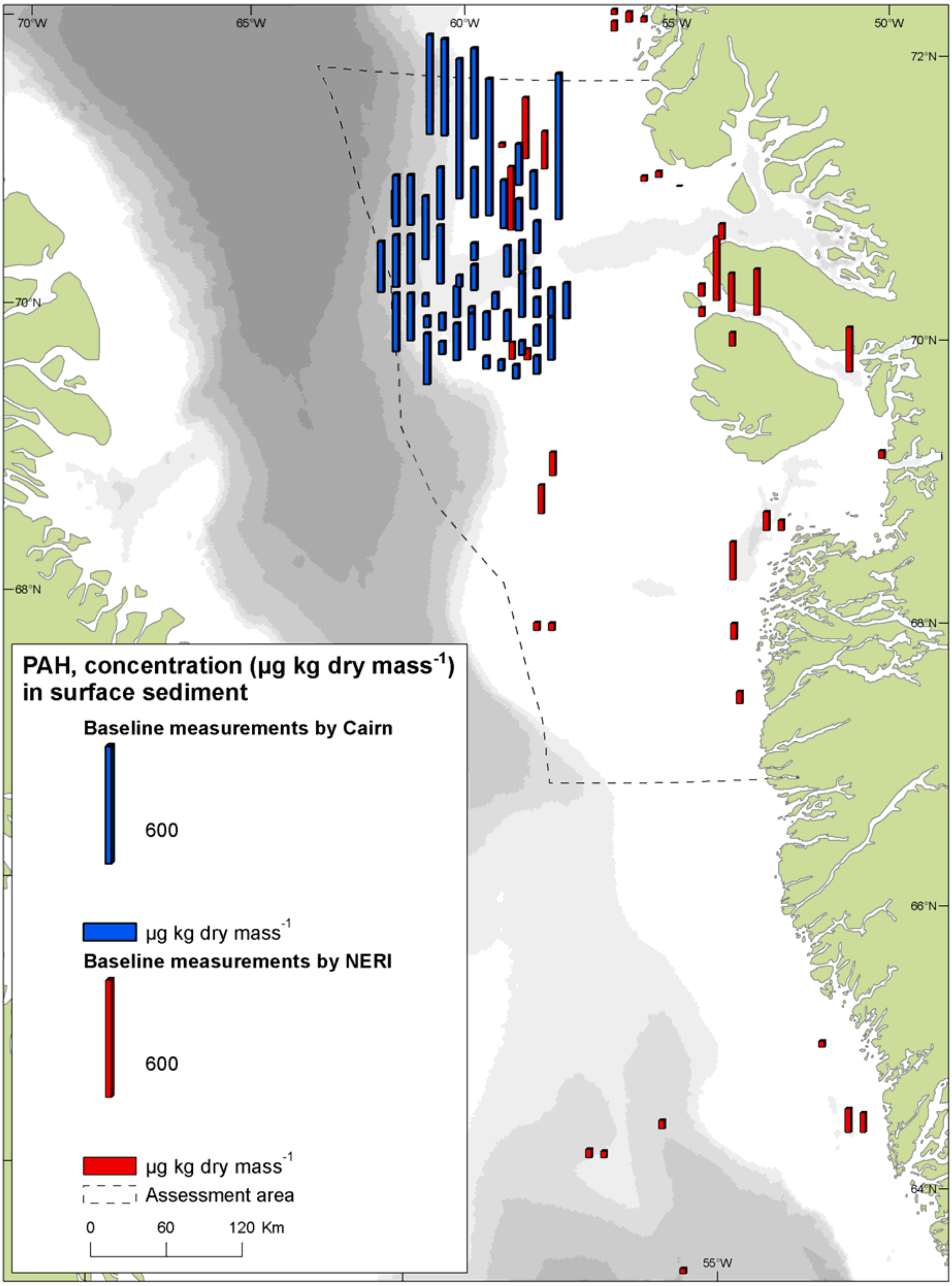


Figure 5.11. PAH concentrations in surface sediments determined by the oil company Cairn Ltd. in connection with baseline surveys as well as background concentrations measured by DCE (formerly National Environmental research Institute, NERI).

6 Knowledge gaps

More research is needed to 1) get a more generalised picture of the oil degradation potential in the seawater around Greenland, 2) identify Greenlandic localities with comparable oil-degrading characteristics and 3) link degradation potentials with environmental parameters and toxic effects.

In the seawater from Disko Bay, limited degradation of aromatic compared with aliphatic hydrocarbons was seen, but it is unknown whether this is also the case for other localities. Furthermore, it is unknown how microbial communities respond to alkylated oil PAHs, which are more relevant in an oil spill context but has so far received little attention.

The sea around Greenland is pristine with little input of organic and inorganic nutrients. There is a gap in our knowledge about bacterial adaptation to oil degradation under such conditions as well as of the timeframe of the adaptation processes. To obtain such knowledge is urgent in order to predict the fate of oil from an accidental spill.

An experiment has demonstrated that dispersants may have a negative effect on microbial hydrocarbon degradation. There is a gap in our knowledge about the negative effects of dispersants on the degradation of oil in Arctic waters.

Furthermore, we lack mathematical models that combine dispersal of oil with knowledge about degradation processes, but such models are necessary in order to predict the fate of oil in the Greenlandic marine environment.

Observations from the *Deep Water Horizon* disaster demonstrated that efficient microbial hydrocarbon degradation occurred at great depths at low temperature. The presence of hydrocarbon seeps in the Mexican Gulf probably contributed to this high natural oil degradation potential and to the rapid adaptation and ability of the microbial community to degrade the oil pollutants. It is unknown whether a similar priming effect on the microbial degrader community exists in the sea around Greenland where the oil volume from natural seeps may be more limited.

Environmental factors play an important role for the degradation of oil spills. In cold and icy waters biodegradation may be lower than at higher temperatures, which also may be the case for a surplus of nutrients, which again favour biodegradation. The water masses around Greenland are of different origin and hence demonstrate different temperature and salinity characteristics as well as different levels of nutrient richness. The biodegradability of oil components in water bodies around Greenland thus warrants further study.

Finally, increased toxicity of oil exposed to photochemical reactions is an area deserving more research as these photochemical processes may be much more critical in Greenland during summer with all-day sunlight.

7 References

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ARCTIC MARINE POTENTIAL OF MICROBIAL OIL DEGRADATION

The literature review aims to collect and analyse relevant existing knowledge about microbial degradation of oil in the seawater around Greenland. Thereby to identify and recommend to what future research on the topic should focus on and to add to the knowledge base for developing contingency plans and perform Net Environmental Benefit Analyses (NEBAs) for Greenland in relation to oil spill from oil exploration activities and shipping in Arctic waters. The knowledge presently available about natural degradation of oil under Arctic conditions shows a complex picture depending on oil type/components and environmental conditions as reflected in the marine environments of Greenland, which is also complex and consist of different water bodies characterised by different temperatures and nutrient levels.

ISBN: 978-87-7156-329-0
ISSN: 2245-0203