BASELINE STUDIES FOR ASSESSING ECOTOXICOLOGICAL EFFECTS OF OIL ACTIVITIES IN BAFFIN BAY

Scientific Report from DCE – Danish Centre for Environment and Energy No. 187 2016

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Data sheet

Series title and no.: Scientific Report from DCE – Danish Centre for Environment and Energy No. 187

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

Year of publication: July 2016
Editing completed: May 2016

Referee: Frank Rigét
Quality assurance, DCE: Kirsten Bang

Financial support: Bureau of Minerals and Petroleum, Greenland Government

Please cite as: Gustavson, K., Tairova, Z, Wegeberg, S. and Mosbech, A. 2016. Baseline studies for assessing ecotoxicological effects of oil activities in Baffin Bay. Aarhus University, DCE – Danish Centre for Environment and Energy, 42 pp. Scientific Report from DCE – Danish Centre for Environment and Energy No. 187.
http://dce2.au.dk/pub/SR187.pdf

Abstract: The study aimed to supplement the Arctic Oil and Gas Assessment 2007 report by AMAP that recommended research to be conducted to give a better understanding of short- and long-term effects of oil pollution on the Arctic marine ecosystem, evaluate Arctic species sensitivity to oil pollution compared to counterpart temperate species and assure integration of environmental monitoring and toxicological studies.

Keywords: Environmental monitoring, Integrated monitoring, Baffin Bay, oil pollution, biomarkers, high-arctic species.

Layout/proofreading: Anne van Acker
Drawings: The authors
Front page photo: Peter Lyngs

ISBN: 978-87-7156-207-1
ISSN (electronic): 2245-0203

Number of pages: 42

Internet version: The report is available in electronic format (pdf) at
http://dce2.au.dk/pub/SR187.pdf

Contents

Preface

This study is part of the Baffin Bay Environmental Study Programme 2011-2014 conducted by DCE - Danish Centre for Environment and Energy, Aarhus University and the Greenland Institute of Natural Resources (GINR) for the Bureau of Minerals and Petroleum, Greenland Government, and financed by license holders in the area.

Summary

The aim of the present study was to supplement the Arctic Oil and Gas Assessment 2007 report by AMAP (AMAP 2007) including 1) to give a better understanding of short- and long-term effects of oil pollution on the Arctic marine ecosystem, 2) to evaluate Arctic species sensitivity to oil pollution compared to counterpart temperate species and 3) to assure integration of environmental monitoring and toxicological studies.

The report includes:

- A review of current practices of using biological indicators for environmental monitoring of offshore oil exploration and exploitation
- A review of the sensitivity of Arctic species versus temperate species
- A review of suitable organisms for biomonitoring and biomarker responses for oil pollution in Baffin Bay
- A summary of experimental studies on high Arctic copepods performed as part of the project
- Recommendations for ecotoxicological monitoring and assessment of oil-related activities in Baffin Bay.

The risk assessments for polar marine species or ecosystems are in most cases based on toxicity data obtained for temperate species although it remains unclear whether toxicity data for temperate organisms are representative for polar organisms. In this report, various parameters that may have significance for differences in sensitivity are discussed. The report concludes that studies that specifically compare the sensitivity of arctic and temperate analogous species are not entirely conclusive.

The results from the experimental ecotoxicological studies in the project conclude, that for high arctic copepods (*Calanus Hyperbous*), a key species in the Arctic, larger oil spills have the potential to cause serious damage to the function of the Arctic pelagic food web, either at the surface water or chemically dispersed into deeper waters.

In the report it is recommended that assessment and monitoring follow an integrated approach, where biological and chemical analyses are performed within the same framework, e.g. time and space. It is recommended that the monitoring includes baseline surveys (before exploration and exploitation activities are started) and impact monitoring after the activities are started. It is recommended that the monitoring includes measurements in both "wild" biotas as well as caged. The report includes a review of suitable organisms for biomonitoring and biomarker responses for oil pollution in Baffin Bay

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Sammenfatning

Undersøgelsens formål var at supplere AMAP's assessment fra 2007 for 1) at give en bedre forståelse af korttids- og langtidseffekter af olie forurening i arktiske marine økosystem, 2) at vurdere arktiske arters følsomhed i forhold til tempererede arters følsomhed for olieforurening og 3) at integrere miljøovervågning og toksikologiske undersøgelser.

Rapporten indeholder:

- en gennemgang af den nuværende praksis ved brugen af biologiske indikatorer ved miljøovervågning af offshore olieefterforskning og olieindvinding
- en gennemgang af arktiske arters versus tempererede arters følsomhed i forhold til olieforurening
- en gennemgang af organismer, der kan anvendes som bioindikatorer og biomarkører for olieforurening i Baffin bugten
- et resumé af de eksperimentelle undersøgelser på højarktiske vandlopper gennemført som en del af projektet
- anbefalinger til miljøovervågning og vurdering af olieaktiviteter i Baffinbugten.

Selv om det er forbundet med usikkerhed, om toksicitetsdata for tempererede organismer er repræsentative for polare arter, er grundlaget for risikovurdering af kemikalie- og olie forurening i arktiske økosystemer typisk baseret på data for tempererede arter. I rapporten gennemgås en række parameter der kan have betydning for tempererede og arktiske organismer følsomhed i forhold til eksponering for toksiske stoffer. Rapporten sammenfatter, at datagrundlaget på nuværende tidspunkt ikke er tilstrækkeligt til at belyse forskelle mellem arktiske og tempererede organismers følsomhed i forhold til toksiske stoffer.

Resultaterne fra økotoksikologiske undersøgelser i projektet indikerer, at opløst såvel som dispergeret olie potentielt kan have store effekter på højarktiske vandlopper (*Calanus Hyperbous*), der er en nøgleorganisme i de arktiske økosystemer.

I rapporten anbefales det, at miljømonitering integrerede tilgang hvor biologiske og kemiske analyser sammenfattes. Det anbefales, at miljømonitering omfatter baseline-undersøgelser (før aktiviteter er startet), efterfulgt af monitering efter aktiviteterne er startet. Det anbefales at overvågningen dels foretages på organismer indsamlet i miljøet dels på organismer udsat i miljøet. I rapporten udpeges bioindikator- og biomarkør-organismer velegnet til miljøovervågning af olieefterforsknings- og indvindingsaktiviteter i Baffin Bugten.

1 Introduction

The purpose of offshore environmental monitoring is to provide an overview of environmental status and trends over time as a result of oil and gas activities. Monitoring programmes are intended to show whether the environmental status is stable, or adjustment of regulation is needed to prevent deterioration of the environment due to unexpected impacts from the operators' activities. Hence, the monitoring results are used by operators and authorities as a source of information and as basis for making decisions on new measures to be implemented offshore.

Biomarkers are used world-wide in monitoring exposure and effects of contamination in marine ecosystems. Biomarkers and assessment criteria have been and are under development in relation to oil offshore activities in temperate waters. Biomarkers and assessment criteria for Arctic species are in the process of being developed. Arctic species have distinct different physiology and biology compared to their temperate counterparts, such as higher lipid content, later sexual maturation, large seasonal variations, slow growth etc. (see Chapter 3). Biological markers and assessment developed for temperate areas may therefore not be suitable in the Arctic.

In relation to gas and oil exploration in Baffin Bay, which possesses environmental conditions considered to be high arctic, the aims of the study were to review and suggest:

- Suitable organisms for biomonitoring and biomarker responses for assessing ecotoxicological effects of contaminants related to offshore oil activity and
- Assessment criteria for assessing impact of contamination from offshore oil activity in Baffin Bay.

Hence, the aim of the present study was to supplement the Arctic Oil and Gas Assessment 2007 report by AMAP (AMAP 2007) that recommended research to be conducted to: 1) give a better understanding of short- and long-term effects of oil pollution on the Arctic marine ecosystem, 2) evaluate Arctic species sensitivity to oil pollution compared to counterpart temperate species and 3) assure integration of environmental monitoring and toxicological studies.

The report includes:

- A review of current practices of using biological indicators for environmental monitoring of offshore oil exploration and exploitation
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2 Biomarkers for environmental monitoring of exposure and effects of contaminants

2.1 Biomarkers

At present, environmental assessment (EA) for marine areas has developed to the stage where biomarkers for exposure and effects in selected key species/biomonitors are implemented together with the traditional methods of environmental chemistry.

Gestel & Brummelen (1996) defined "biomarker" as any biological response to an environmental chemical at the sub-individual level, measured inside an organism or in its products, indicating a deviation from its normal health status. "Bioindicator" is defined as an organism giving information about the environmental conditions by its presence/absence or its behaviour. However, there are still some conceptual inconsistencies in the definitions between "biological marker" and "biological indicator". In general, any biological responses, such as alterations in any of the biochemical, molecular, cellular and physiological processes occurring within the organism can be used as a biomarker response. Therefore, in this report, the term biomarker is used and related to exposure and effect measurements in an organism as well as the term biomonitor is used for an organism/species selected for the monitoring. To avoid any confusion, the term bioindicator is not used further in the report.

The biomarker research field has developed in response to the need for more sensitive indicators of sub-lethal ecological effects (Bickham et al. 2000). Rapidly responding biomarkers at the molecular and biochemical levels serve as short-term indicators/predictors of long-term biological/ecological effects (Martín-Díaz et al. 2004).

Biomarkers may be classified according to the information they provide, e.g. biomarker for exposure (diagnostic of the past and/or present exposure to contaminant), biomarkers for effects (diagnostic of both the occurrence of pollutant and demonstrating an adverse effect on the organism) and biomarkers for susceptibility. Biomarkers for susceptibility are normally not applied in EA and therefore are not further used in this report (Chambers et al. 2002; Hagger et al. 2006; Van der Oost et al. 2003).

2.2 Assessment criteria

Environmental Assessment Criteria (EACs) and Background Assessment Concentrations (BACs) are developed and adopted in the OSPAR Convention (The Convention for the Protection of the Marine Environment of the North-East Atlantic) and the International Council for the Exploration of the Sea (ICES) for a range of fish, mussels, amphipods and snails species. EACs are derived from toxicological data and indicate limits for concentration of contaminants for harmful effects at organism level or higher levels of organization. For instance, EACs can be derived from toxicological experimental data by linking oil exposure and PAH metabolite levels in fish with DNA adducts and fitness data. BACs are estimated from data for reference sites (remote and pristine areas) for concentration of contaminants in the organism or the biomarker values in the organism of the specific species. For instance, BACs for the biomarker EROD (activity of enzymes from cytochrome

P450) have been described as 90 percentiles of values from reference sites to distinguish between ″background″ and ″elevated″ response.

Development of assessment criteria (BACs and EACs) is still an ongoing process as data are reviewed and new data become available. A list of BACs and EACs for biomarkers adopted by the ICES and the OSPAR conventions is presented in *Table 2.1*.

Table 2.1. ICES and OSPAR background assessment criteria (BACs) and environmental assessment criteria (EACs) (Davies & Vethaak 2012; OSPAR Commission 2012a). "-"criteria have not been established.

Biological effect	**BAC**	**EAC**
VTG in plasma	+	-
Reproductive success in fish (Eelpout, *Zoarces viviparous*)	+	+
EROD/CYP1A activity	+	-
PAHs Bile metabolites	+	+ (not all fish species)
DR-Luc	+	+
DNA adducts	+ (not all fish species)	+
Bioassays (% mortality)	+	+
Bioassays (% abnormality)	+	+
Bioassays (% growth)	+	+
Lysosomal stability	+	+
Micronuclei	+	-
Comet Assay	+	-
Stress on Stress (SoS)	+	+
AChE activity	+	+
Externally visible diseases	+	+
Liver histopathology-non specific	-	+
Liver histopathology- contaminant-specific	+	+
Macroscopic liver neoplasms	+	+
Intersex in fish (% prevalence)	+	-
Scope for growth	+	+
Hepatic metallothionein	+	-
Histopathology in mussels	+	+ (except S/VLYS)
Imposex/intersex in snails	+	+

For some of the biological effect measurements there are no data available to make a linkage to the deleterious effects to higher organisational levels. Therefore the EACs (levels of response below which unacceptable responses at higher, e.g. organism or population, levels would not be expected) for some of the biomarkers are not set, and hence these biomarkers can only be employed as biomarker for exposure (Davies & Vethaak 2012).

2.3 Integrated monitoring

Historically within marine pollution research and monitoring, chemical and biological studies have often remained largely independent of each other. One of the more recent insights is the beneficial approach of combining chemical and biological measurements into an integrated assessment of environmental quality (Davies & Vethaak 2012). The combination of chemical and biological measurements increases the interpretive value of the individual measurements and the potential for identification of the substances contributing to the observed effects.

Integrated monitoring has been adapted for environmental monitoring by international institutions like the OSPAR Commission (managing the Convention for the Protection of the Marine Environment of the North-East Atlantic) and HELCOM (Baltic Marine Environment Protection Commission - Helsinki Commission), and is required under the Descriptor 8 of "Good Environmental Status" (concentrations of contaminants are at levels not giving rise to pollution effects) within the Marine Strategy Framework Directive (MSFD) (Davies & Vethaak 2012; European Commission 2008; OSPAR Commission 2012b). Integrated monitoring approach is employed within the frames of national programmes in EU countries, e.g. Denmark and Sweden.

The ecosystem components of the integrated assessment include three main environmental matrices: *water*, *sediment* and *biota* (see *Figure 2.1*). Integrated monitoring consists of simultaneous measurement of contaminant concentrations in all three matrices, and biological effects parameters, using the same species/population/individual for both biological and chemical measurements and sampling in the same area and within the same time frame. It is recommended that sentinel species of fish and/or mussel and/or gastropod representing key ecosystem components are selected for such integrated assessment (Davies & Vethaak 2012).

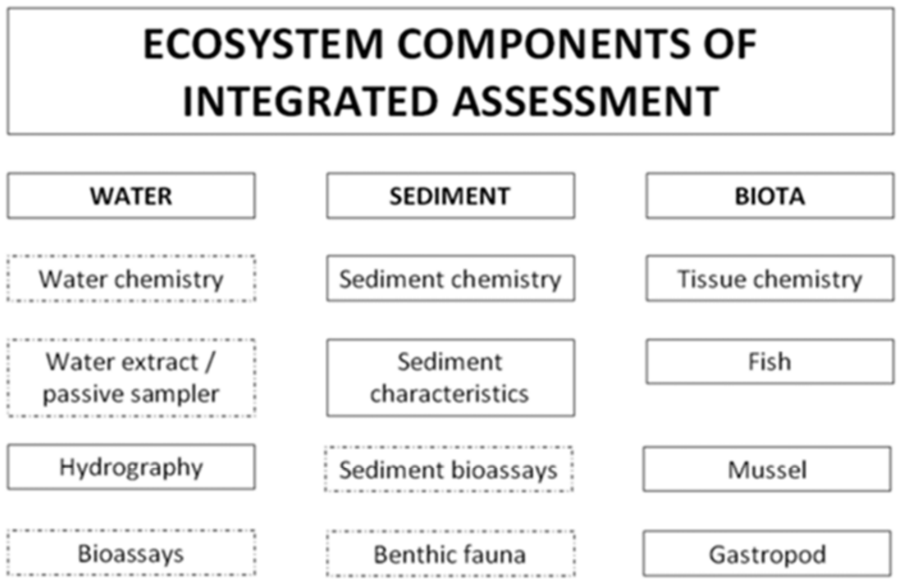

Figure 2.1. Overview of components in a framework for an integrated monitoring programme for chemical contaminants and their biological effects. An integrated monitoring programme should always include descriptions, samples and analyses as stated in the boxes with solid lines. Measurements suggested in boxes with broken lines are additional measurements to be considered (Davies & Vethaak 2012).

Biological effect methods to be included in an integrated programme have the following requirements (Davies & Vethaak, 2012):

- The ability to separate contaminant-related effects from natural variability (e.g. natural variability, food availability)
- Sensitivity to contaminants (i.e. providing "early warning")
- A suite of methods that covers a range of mechanisms of toxic reactions (e.g. oestrogenicity/androgenicity, carcinogenicity, genotoxicity and mutagenicity)
- The inclusion of at least one method which measures the "general health" of the organism.

2.4 Norwegian guidelines for offshore environmental monitoring

Guidelines for environmental monitoring for offshore activities on the Norwegian shelf are specified by the Norwegian authorities (KLIF 2011). The guidelines were developed in a corporation between the Climate and Pollution Agency (KLIF) (now Norwegian Environment Agency), an expert advisory group appointed by the former KLIF, the Norwegian Radiation Protection Authority (NRPA), the Norwegian Oil and Gas Association, oil and gas companies and consultancy firms. The guidelines specify monitoring of the water column and benthic habitats.

2.4.1 Monitoring of the water column

The Norwegian guidelines (KLIF 2011) state that monitoring must be carried out in a way that makes it possible to verify that the actual environmental impacts of the activities do not exceed the accepted level for the activity. The scope of the monitoring programme must be proportional to the expected risk.

There are two main elements of the monitoring programme for the water column on the Norwegian shelf:

- Condition monitoring: applies to fish, and surveys are required every three years. The monitoring is intended to document the extent of the effects in fish caused by discharges/potential pollution from the oil and gas industry. The analyses are required to include measurements of hydrocarbons and selected biomarkers in fish. The geographical priority is given to areas close to the activity and to fish nursery areas. Condition monitoring should include the representative fish species from the area. Other species may also be included in the surveys. In addition to the chemical parameters (see below), analyses should include a selection of biomarkers that are indicative of exposure to pollutants and any adverse effects on fish. Condition monitoring must include analyses of the content of contaminants in fish fillet because of the food safety implications of these pollutants.

- Impact monitoring must as a minimum include fish and mussels. Impact monitoring is based on exposing organisms placed in cages in the environment. To obtain better information on dispersal of pollutants, a minimum number of cages should be placed in different directions from the discharge point. The preferred monitoring species is the blue mussel (*Mytilus edulis*), because it is naturally stationary and robust for handling, but fish species may also be suitable in some cases. Biomarkers for assessing exposure and possible impacts are constantly being developed.

A set of key methods should be included in the impact monitoring programmes, but adaptation of the methodology to include new knowledge should be taken into account during the planning process. *Table 2.2*, from KLIF 2011, gives an overview of current analytical methods. The chemical parameters to be investigated under impact monitoring must be specified in the draft programme, discussed in the planning process and listed in the final monitoring programme. For the impact monitoring, the organisms and biomarkers to be employed can be specified according to the monitoring programme. The experience from Norway shows that blue mussels should be used. So far, the only fish species used has been cod.

Table 2.2. Overview of the relevant analyses to include in a water column monitoring programme (from KLIF 2011).

Method	Tissue type/matrix	Substance/group of substances	Organisms
PAH metabolites (FF/GCMS)	Bile	PAHs	Fish
Alkyl phenol (AP) metabolites	Bile	APs	Fish
PAHs (body burden)	Soft tissue	PAHs	Mussels
Histology	Gills	Different sources of stress	Fish
DNA adducts	Liver	PAHs (+)	Fish
CYP 1A	Liver	PAHs (+)	Fish
Vitellogenin (VTG)	Blood plasma	Xenoestrogens	Fish
Pyrene hydroxylase	Digestive gland	PAHs	Mussels
Micronucleus formation	Cells	Genotoxic stress	Mussels
Lysosomal membrane stability	Haemocytes (blood cells)	Metals and organic contaminants	Mussels
Lipofuscin	Histological sections	Different sources of stress	Mussels
Neutral lipid	Histological sections	Different sources of stress	Mussels

3 Sensitivity of Arctic species compared to temperate species

3.1 Differences in sensitivity between Arctic and temperate marine species

A reliable risk assessment approach for Arctic environment requires toxicity data specifically for Arctic species. Currently, the database for toxicity of physically and chemically dispersed oil to Arctic species, compared to the substantial body of data for sub-Arctic, temperate and tropical species, is insufficient (Chapman & Riddle 2005; De Laender et al. 2011; Camus et al. 2014). A recent review of data that could be used for toxicity effect modelling for oil compounds for a selection of cold-water marine species of fish and plankton associated with the Barents Sea showed that data coverage was limited to only a sub-set of the required endpoints (Olsen et al. 2013). The review concluded that there is a need for new experimental studies for zooplankton and for larvae and juvenile fish focused on growth and development.

An extensive recent review of the environmental impacts from Arctic oil spills by the Arctic Oil Spill Response Technology – Joint Industry Programme, JIP (Camus et al. 2014), indicated that the copepod dataset is among the most complete for Arctic species, particularly for acute lethality; the emerging dataset regarding sub-lethal endpoints for copepods and cod is still limited. The report also concluded that: other pelagic valuable ecosystem components (VECs) that have little or no data (e.g. capelin, hyperiid amphipods, *Calanus hyperboreus* and *Arctogadus glacialis*) should be a focus of future efforts; the data regarding benthic communities are limited; for acute-lethal endpoints, datasets could be expanded with qualified data from boreal or temperate datasets; additional work is necessary on the effects and exposures to sub-lethal effects concentrations of oil in near sea-ice communities (Camus et al. 2014).

The risk assessments for polar marine species or ecosystems are mostly based on toxicity data obtained for temperate species although it remains unclear whether toxicity data of temperate organisms are representative for polar species and ecosystems. In order to assess the applicability of existing risk tools for Arctic areas, basic knowledge on the sensitivity of Arctic species has to be developed (Camus et al. 2015).

Despite some clear differences, it is not yet clear whether species from the Arctic, or generally polar regions, are more sensitive to and susceptible to chemical pollution than those from temperate and tropical areas. Fragility of polar species depends on many factors, including how do these species interact with their environment and respond to stressors as individual organisms, populations and communities (Chapman & Riddle 2005).

The efforts of the recent toxicity studies evaluating sensitivity of aquatic Arctic species to oil pollution (Chapter 6) attempt to answer the following questions:

- Can data from tests with temperate species be transferred to Arctic species?
- Can data from short-term tests be used in risk assessment for Arctic species?

3.2 Physiological and biochemical differences between polar and temperate species

Aquatic polar species have several specific physiological/biochemical adaptations to their environment that make them different from temperate species.

The main differences between the polar species and their temperate counterparts, relevant to the toxicity testing, are presented in *Table 3.1*.

Table 3.1. Overview of the physiological characteristics between Arctic and temperate species (see text below). The comparison of different parameters between Arctic and temperate species is presented as "Arc </> Temp", showing "higher" or "lower" rate of a selected parameter in either Arctic or temperate species.

Parameter	Comparison	References
Metabolism	Arc < Temp	Chapman & Riddle 2005; Karamushko 2001; Camus et al. 2014
Lipid fraction	Arc > Temp	Chapman & Riddle 2005; Hansen et al. 2011; Jensen & Carroll 2010; Lee et al. 2006; Swalethorp et al. 2011
Energy usage	Arc < Temp	Chapman & Riddle 2005
Developmental time periods	Arc > Temp	Camus et al. 2015; Chapman & Riddle 2005; King & Riddle 2001
Acute response time	Arc > Temp	Chapman & Riddle 2005; Gardiner et al. 2013a; Hansen et al. 2011; Hansen et al. 2014; Olsen et al. 2011; Camus et al. 2014
Lipid composition	Arc > Temp	De Hoop et al. 2011
Resistance to freezing	Arc > Temp	Christiansen et al. 1996; Duman 2015; Ingebrigtsen et al. 2000
Antioxidants	Arc > Temp	Camus et al. 2005.

3.2.1 Metabolism

Arctic species have *lower metabolic rates* – therefore slow uptake kinetics for contaminants. In all cases, relatively low temperatures result in slow uptake kinetics in polar species (Chapman & Riddle 2003). Generally, organisms with lower metabolism accumulate contaminants in tissues at slower rates, however, as an adaptation to the seasonal availability of food, polar biota has relatively high lipid content for energy storage, which, as a consequence, means higher uptake of lipophilic contaminants (Chapman & Riddle 2005). The study of metabolic adaptation of fish at high latitudes by Karamushko (2001) showed that the metabolic rate in polar organisms was lower than in the same organisms living at temperate latitudes. The study showed that there was no evolutionary metabolic adaptation to low temperature in the studied marine fish, while the low annual rate of energy consumption in polar organisms was the most probable mechanism of their adaptation to low temperature. The author concluded, based on the analysis of the published data on the metabolic rate in representatives of some groups of marine organisms that the rate of energy metabolism in polar species was significantly lower than in tropical species (Karamushko 2001).

3.2.2 Lipid fraction

Arctic species have *high lipid content* and this may result in a greater uptake of lipophilic contaminants. Populations of arctic species are often dependent on a brief period of maximum planktonic growth followed by plankton decline, thus the exposure potential varies with season. Lipid accumulation of Arctic copepods is closely linked to phytoplankton blooms, during which they feed and accumulate large lipid stores leading to that more than half of the dry mass can be lipid (Lee et al. 2006). The feeding season is followed by long starvation periods at which they migrate to deep waters for diapause (Lee et al. 2006). Storage lipids have different functions depending on the different life history stages of zooplankton, i.e. during reproduction, ontogeny and diapause. Lipid droplets in zooplankton ovaries can be transferred to developing oocytes, while developing embryos use their lipovitellin (yolk) and lipid droplets for energy and materials until feeding begins (Lee et al. 2006; Swalethorp et al. 2011).

The strict seasonality of Arctic species makes the pelagic animal populations more vulnerable to acute exposures of the pollutants, when comparing to temperate populations. Sea-ice limits wind-driven mixing of the water column creating a strong stratification which restricts nutrient exchange. Light penetration is also restricted when sea-ice cover is present limiting photosynthesis. In general, due to these factors, together with the physical abrasion of the intertidal zones, growth of plants and animals in polar marine waters is very seasonal (Chapman & Riddle 2005). Hansen et al. (2011), in the study of the effects of biodegraded oil on copepods, has shown for both Arctic and temperate species that animals with higher lipid content survived longer. The authors explained this inter-relation with the large lipid reservoir that may protect against short-term acute toxicity if the oil compounds accumulate directly in the lipid reservoir and therefor are immobilized here. Additionally, a study of reproduction and feeding for two Arctic-dwelling Calanus species exposed to oil showed that the smaller species *C. finmarchicus* was more sensitive than *C. glacialis* to effects of oil (Jensen & Carroll 2010). This was linked to the higher amount of storage lipids in *C. glacialis* which may bind lipophilic pollutants such as PAHs, and further lead to a prolonged time interval until observable effects.

3.2.3 Developmental time periods

In Arctic species, *the early life stages have longer developmental times* that may result in longer periods of increased sensitivity. Therefore, in case of chronic exposures, comparisons between development stages are more ecologically relevant than exposures times. Long times of ontogenetic development of Arctic invertebrate species mean that the toxic effect depends on the particular life stage tolerance of the organisms under comparison, and hence that tolerance to toxic substances has to be evaluated on comparable life stages. A study that investigated effects of metal contaminant to Antarctic invertebrate species (King & Riddle 2001) showed that the sensitivity of a polar echinoid to copper and cadmium in tests based on development to hatched blastulae (6 to 8 days) was generally comparable to results of tests on echinoids from tropical and temperate regions that use development to 2-arm plutei (2 to 4 days) as the end-point. However, the Antarctic species were more sensitive to copper and cadmium than the tropical and temperate species if the exposure was continued to the same stage of development, the pluteus larva (20 to 23 days for the Antarctic species). Comparing the tolerance of a key developmental stage common to all planktotrophic sea urchins may be more ecologically relevant than simply comparing exposure over a fixed period of time, because for an embryo to survive to adulthood it must successfully complete all development stages. The authors concluded that based on these results, polar species could be more sensitive to contaminants than species from warmer regions (King & Riddle 2001).

3.2.4 Acute response time

Arctic species have *longer acute response times* but have similar ultimate responses compared with temperate species. Similar portions of toxicity curves for Arctic and temperate species need to be compared. For example, it is clear at this point that polar marine organisms have consistently longer acute response times and therefore, comparisons between the acute response of polar and temperate marine biota should be based on similar portions of the toxicity curve, which can mean comparing 14-day LC50 results for polar organisms with 4-day LC50 results for temperate organisms (Chapman & Riddle 2005; Camus et al. 2014).

3.2.5 Lipid composition

The lipid composition is also different between Arctic and temperate species. As a particular adaptation of polar fish and invertebrates to low temperatures, the elevated levels of polyunsaturated fatty acids (PUFA) in cell membranes may indirectly contribute to a higher sensitivity of polar species to oil. PUFA are primary targets for reactive oxygen species (ROS) and additional production of ROS stimulated by the biotransformation of oil constituents taken up by organisms may lead to an imbalance and thus oxidative damage (De Hoop et al. 2011).

3.2.6 Resistance to freezing

Resistance to freezing is higher in Arctic species. One of the evolutionary adaptations in Arctic species is antifreeze proteins that not only affect water crystallization in body fluids, but also stabilize cell membranes at low temperature. These proteins increase permeability as membranes are cooled through their phase transition temperature (Duman 2015). Furthermore, studies showed that a glomerular kidney of polar cod, evolved to retain antifreeze biomolecules in the blood, may prevent excretion of xenobiotics via urine, with bile being the major pathway. This suggests that polar fish could be more susceptible to contaminants by retaining and slower excretion of toxic compounds, although no evidence showed polar fish being more vulnerable than other fish species (Christiansen et al. 1996; Ingebrigtsen et al. 2000; Camus et al. 2014).

3.2.7 Antioxidants

Arctic organisms have *higher levels of antioxidants* than their temperate counterparts. In order to counteract reactive oxygen species (ROS), organisms developed an adaptation by possessing a suite of antioxidant defences (AOX), which comprise enzymes and low-molecular weight molecules, such as glutathione, beta-carotene and vitamins A, E and C. Despite the fact that the mitochondrial ROS generation is low due to the lower metabolic rate in Arctic species, studies have reported elevated AOX levels in polar pectinid bivalves compared with temperate congeners (Camus et al. 2005). The study showed higher levels of AOX in Arctic bivalves, and the authors proposed that high AOX is required in environments characterized by low food availability as AOX efficiently protects biomolecules, also, high AOX may explain the relatively long lifespan of most polar ectotherms.

3.3 Other conditions unique for the Arctic

Additionally, clear waters and midnight sun are polar conditions that may increase the rate of phototoxicity of PAHs. Due to this potentially enhanced formation of reactive oxygen species, Arctic organisms developed an evolutionary adaptation resulting in higher levels of antioxidant species in their organisms. The exposure to increased phototoxicity of PAHs may be especially actual for sea-ice communities, due to the high transmission of the light through the ice and clear waters (Chapman & Riddle 2003).

Arctic species may be subjected to longer exposure periods for eponic (under ice) communities, as water under the ice is protected from wind-driven mixing. Since presence of ice may slow down weathering of oil, e.g. ice can encapsulate oil, making containment and recovery difficult, eponic species can be subjected to longer exposure periods to petroleum compounds than their temperate counterparts.

Environmental fluctuations may enhance the overall susceptibility of polar species to contaminant exposure, since pollution can act as an additional stress factor. However, in environments where conditions are constants, biota could be less adaptable to unexpected stressors. The range of polar water temperature fluctuations is narrower than in the most temperate regions. But since the temperatures are close to freezing point, meaning that the system is close to a phase change, i.e. from water to ice phase, which affects kinetics of many chemical reactions, slight temperature differences in this range may have significant influences on biological processes, such as properties of cell membranes (Chapman & Riddle 2005).

3.4 Review of recent studies that compared toxicity effects between temperate and polar/Arctic species

Presently, only a limited number of studies specifically compared toxicity metrics between temperate and polar/Arctic species.

3.4.1 Crude oil and PAH toxicity

The study of De Hoop et al. (2011) compared sensitivities of polar and temperate marine species to crude oil and two PAHs, 2-methyl-naphthalene and naphthalene. Species sensitivity distributions (SSDs) were constructed for polar and temperate species based on acute toxicity data from scientific literature, reports and databases. This comparison study used acute toxicity data, i.e. LC50, EC50 and TLm (median tolerance limit) to derive SSD and hazardous concentration for 5 % and 50 % of the species (HC_5 and HC_{50}), and only toxicity data with short-term test durations (1-8 days) were included. Overall, only for naphthalene there was a significant difference in both means ($p = 0.002$) and variances ($p = 0.02$) of the SSDs, with a factor 3 difference between the HC_{50} values and a factor 1.2 between the HC_5 values of the temperate and polar species groups. Except for chordates and naphthalene, polar and temperate species sensitivities did not differ significantly. The authors conclude that acute toxicity data obtained for from temperate organisms may serve to obtain a first indication of risks in polar regions. It should, however, be noticed that the study only applied short-term acute toxicity test data. It is thus recommended, due to the difference in physiology and developmental time periods, to compare chronic toxicity data when evaluating differences between Arctic and temperate species (Chapman & Riddle 2005).

Another study performed toxicity tests on Arctic and temperate species exposed to the narcotic acting oil component 2-methyl naphthalene (Olsen et al. 2011). The experimental results were used to quantify LC50 and no-effect concentration (NEC). For estimates at community level, the HC_5 and HC_{50} were calculated from sensitivity distribution curves. The same experimental protocols were employed for comparable Arctic and temperate species. Toxicity tests for all species were run for 96 h or until 100 % mortality was achieved. Then the uncertainty was calculated using DEBtox model to compare species sensitivities. The study showed that when taking data uncertainty into consideration, there was no regional difference in tolerances to 2-

methyl naphthalene either at the species level or at the community level. The authors concluded that values of survival metrics for temperate regions are transferrable to the Arctic for the chemical 2-methyl naphthalene, as long as extrapolation techniques are properly applied and uncertainties are taken into consideration (Olsen et al. 2011). Uncertainties about the results, as discussed in the article, include the temperature influencing chemical partitioning in the experimental chambers as well as degradation rates for 2-methyl naphthalene. Higher rates for these processes in temperate tests compared to Arctic tests consequently caused the effective exposure concentration in Arctic experiments possibly being higher than in temperate experiments. It was also discussed in the articles whether the chosen test organisms from seven different taxonomic groups would be 100 % representative of the sensitivity for the ecosystems being compared.

3.4.2 Produced water toxicity

A recent study by Camus et al. (2015) compared chronic toxicity of produced water for six Arctic and six temperate species. Acute and chronic toxicity data were used to calculate species sensitivity distribution curves (SSDs) and the hazardous concentrations affecting 5 % and 50 % (HC_5 and HC_{50}). Hazardous concentrations were compared to elucidate whether temperate toxicity data used in risk assessment are sufficiently representative for Arctic species. Both SSDs, HC_5 and HC_{50} values were overlapping indicating that based on the single toxicity data, no differences can be observed in species assemblages for the Arctic and non-Arctic regions. The authors discussed, however, different sources of uncertainty in the toxicity testing, including e.g. insolubility of chemicals, since the measured water concentrations clearly indicated higher solubility in the temperate tests than in the Arctic test. The authors add that the pairwise comparison may be difficult and the direct comparison of toxicity data between temperate and Arctic species of the same taxa may be irrelevant as the selected Arctic species were chosen due to their key role in the Arctic ecosystem and not as comparable homologues in terms of habitat, ecology and biology. The authors concluded, however, that the manner in which Arctic and non-Arctic populations and communities respond to exposure levels above established thresholds remains to be investigated. Hence, responses at higher levels of biological organization should be studied to reveal potential differences in sensitivities to produced water between Arctic and non-Arctic ecosystems.

3.4.3 Comparison of benthic communities

Marine benthic communities from the Arctic area have been shown to respond differently to oil-related compounds than communities from temperate areas (Olsen et al. 2007). The authors compared two soft-bottom benthic communities from the Barents Sea, depth 312 m, temperature 1.5 °C and Oslofjord, depth 214 m, temperature 4 °C, as Arctic and temperate areas, respectively. In a series of controlled laboratory experiments, sediment cores were subjected to different concentrations of crude oil contaminated sediments and water-based drill cuttings. Polychaetes and bivalves were the most abundant infauna in both areas. The results demonstrated that benthic communities from an arctic location responded differently to oil-related compounds compared to communities from a temperate location. Enhanced respiration rates in the high-end oil concentrations and drill cuttings in treatments compared to controls were only observed in the benthic communities from the Arctic area. The authors related the observed differences in respiration response to the treatments between the Arctic and temperate locations to different factors, e.g. differences in total biomass, faunal composition, functional groups, temperature, differences in susceptibility to oil-related compounds among individual taxa. Additionally, PAH levels in oil-treated cores from both areas were much lower than PAH concentrations generally found after an oil spill, thus, the observed response in respiration to the generally low PAH levels indicated that benthic communities in the Arctic can be impacted by low doses of crude oil (Olsen et al. 2007).

3.5 Discussion

Although several comparative studies have shown that the toxicity data can be transferred between Arctic and temperate species and applied for ecological risk assessment studies, there are still certain factors that have to be taken into account when comparing animals in these two regions.

For example, it is clear at this point that polar marine organisms have consistently longer acute response times and, therefore, comparisons between the acute response of polar and temperate marine biota should be based on similar parts of the toxicity curve, which can mean comparing 14-day LC50 results for polar organisms with 4-day LC50 results for temperate organisms (Chapman & Riddle 2005). A study of the effects of biodegraded crude oil on copepods has shown that the toxic effects and mortality occurred later in the high Arctic species than in temperate species (Hansen et al. 2011). Gardiner et al. (2013a) in an acute toxicity of oil study with Arctic copepods *C. glacialis*, based on an evaluation of control mortality and dose responses in preliminary tests, copepod tests were conducted as 12-days tests (a standard 96-h spiked exposure followed by an additional 8-day observation period) to detect responses exhibited over a longer period of time. Another recent study by Hansen et al. (2014) that compared acute toxicity of eight oil spill response chemicals between temperate, boreal and Arctic copepod species observed that it was necessary to extend exposure time for the Arctic and boreal copepods.

It is generally hypothesized that the fact that polar species have generally higher lipid content may potentially result in a greater uptake of lipophilic contaminants. The seasonality of lipid stores mobilization during periods of e.g. reproduction or starvation may result in increased critical internal concentration of lipophilic contaminants. The toxicity tests comparing sensitivity between polar and temperate aquatic species have to take this seasonality of lipid storage into account. It is therefore also important to consider whether field or laboratory cultured animals were used in the exposure studies, since field animals can have different feeding season/status and thus different metabolism of the lipid storage. In the reviewed study of De Hoop et al. (2011), the available toxicity data from the databases were used, thus it is impossible to validate the life stage of the animals and the homogeneousness of the animal cultures used in tests. Camus et al. (2015), comparing chronic toxicity of produced water for six Arctic and six temperate species, used cultured animals for the temperate species, while Arctic species were collected in the field. Arctic copepod *C. glacialis* was collected in May and the exposure tests were initiated 48 h after the collection. However, previously, Swalethorp et al. (2011) has shown that in May, during the post algal bloom period, *C. glacialis* has increased its body weight several fold due to accumulation with respect to carbon and total lipid content, which could mean that potentially accumulated oil-compounds went into lipid growing reserves during the acute exposure experiment by Camus et al. (2015). In the study by Olsen et al. (2011) comparing 2-methyl naphthalene effects in Arctic and temperate species, the authors used field collected animals for both geographical groups. However, it is not reported which season animals were collected. De Hoop et al. (2011) discuss that since polar organisms generally have higher lipid contents than temperate organisms, the binding of lipophilic toxicants may leave a smaller amount of oil components to interfere with cell membranes and exert toxic effects. This conclusion, though, does not include the whole life span of the Arctic organism, during which the lipid resources are consumed and therefore releasing the lipophilic substances. Interestingly, Hansen et al. (2011), in the study of the effects of biodegraded crude oil on two closely related species of copepods, the temperate *C. finmarchicus* and the high Arctic *C. glacialis*, showed that the Arctic species was somewhat less sensitive to oil than *C. finmarchicus*, but the difference was small. When the authors related acute toxicity to lipid content, it was shown that the two species were not very different in terms of lipid content and results also did not indicate a difference between the two species in terms of the relationship between lipid content and acute toxicity. The season of the Arctic copepods collection from the field as opposed to the cultured animals used for temperate species was not indicated, leaving room for uncertainty of the life stage development of the Arctic species, which could explain their metabolic rates and related acute toxicity results.

Younger life stages are typically more sensitive than adult organisms (Green et al. 1996; Verriopoulos & Moraitou-Apostolopoulou 1982). Since the early life stages in Arctic species have longer developmental periods than in temperate species, the sensitivity to pollutants for Arctic species may be more life-stage dependent than in temperate regions, also as many polar species grow slowly and are relatively long-lived. Thus, sensitive, early-development stages persist for longer periods of time resulting in greater sensitivities over longer exposure periods (Chapman & Riddle 2003).

Uncertainties in the laboratory testing may obstruct the objective comparison between Arctic and temperate groups. For instance, homogeneity of laboratory cultures with regard to age and sex groups is an important factor in toxicity testing, since results can give an under- or overestimation of the sensitivity (Camus et al. 2015). Both cultures, Arctic and temperate species' groups, should be homogenous, which is difficult to achieve since many Arctic species are not available year around. Another obstacle is adaptation of test protocols for developmental assays. The fact that Arctic species have longer developmental times, may affect exposure conditions in the laboratory, i.e. water oxygen and chemical concentrations, making laboratory assay hard to compare (Camus et al. 2015).

Additionally, on the ecosystem level, polar populations, including Arctic, are generally less diverse than those from temperate or tropical regions (Chapman & Riddle 2005; Sanders 1968), and therefore also the genetic diversity reflecting on molecular responses, e.g. enzymatic activity, including enzymes that metabolise oil-derived compounds, may be less diverse. Consequently, this means limited "buffer" for the biochemical defence mechanisms in case of pollution accidents and therefore increased general sensitivity of polar/Arctic species. Therefore, Arctic populations may exhibit increased vulnerability to pollution due to the vulnerability of certain life stages of Arctic species and generally lower biodiversity. In polar organisms many benthic species have shortened or eliminated the pelagic larval phase, have generally longer development times, and have fewer developmental stages, hence, more fully developed young are released. This reduced pelagic phase means that dispersion and long-distance recolonization are restricted leading to a longer time needed for re-establishment of population (Chapman & Riddle 2005).

In colder areas the physical behaviour of oil is different compared to warmer areas. The lower temperature and lack of sun light during the Arctic winter slow down the natural physical weathering process of oil. In temperate areas, the effects of exposure to the most volatile fraction of the oil are neglected due to short exposure time. However, in the Arctic, the exposure to biota of this volatile fraction may be prolonged due to lower evaporation rates and the volatile fraction may be an important contributor to the overall adverse effect of an oil spill. Likewise, the exposure to the heavier oil fractions may be prolonged and knowledge on the long-term effects of exposure is essential.

As an adaptation to the Arctic environment, species found here have longer life spans, larger body sizes and higher lipid contents compared to temperate equivalent species. Thus Arctic species may accumulate contaminants over longer time and reach higher lifetime body levels. Meanwhile higher lipid content implies a higher affinity of lipophilic contaminants such as polycyclic aromatic hydrocarbons (PAHs). Thus, Arctic species may be exposed to oil compounds for a longer time because of the prolonged physical presence and may, due to special adaptive features, be more efficient at accumulating oil contaminants.

3.6 Conclusions

Considering the issues related to general differences in physiology and ecology between Arctic and temperate species, the studies that specifically compare the sensitivity of Arctic and temperate analogous species are not entirely conclusive on whether the existing temperate toxicity database could be applied in the risk assessment for Arctic communities. Factors such as strict seasonality resulting in particular physiological characteristics of Arctic species that may affect their toxic response have to be taken into consideration. Since Arctic marine organisms have consistently longer acute response times, the short-term tests used in the risk assessment of oil pollution for Arctic species must be adjusted accordingly, i.e. test duration should be extended in order to evaluate acute response on similar portions of the toxicity curve. Due to differences in ecology between Arctic and temperate species, i.e. increased rate of phototoxicity of PAHs, longer exposure periods of eponic communities, environmental fluctuations and strict seasonality, these factors should be used during the interpretation of the results of the comparative studies, and animals representing wide range of developmental stages as well as ecological life strategies should be selected.

4 Suitable biomonitor species in Baffin Bay

4.1 Description of the Baffin Bay ecosystem and species

The following description is based on the summary in "Eastern Baffin Bay - A strategic environmental impact assessment of hydrocarbon activities" (SEIA, Eastern Baffin Bay) by Boertmann & Mosbech (2011) unless otherwise is stated.

The ecosystem of Baffin Bay is determined by the physical and climatic factors characteristic for the Arctic region. Sea ice and icebergs are present throughout the year, with open water zones as polynyas (ice-free or almost ice-free areas surrounded by sea ice) and shear zone between the dynamic drift ice and the coastal fast ice that become free of ice during summer periods. Due to these factors, ecological/biological properties of Baffin Bay are typical for this climatic region: low biodiversity but often numerous and dense animal populations; a relatively simple food web from primary producers to top predators and with a few species playing a key role in the ecology of the region.

The most significant ecological event in the marine environment is the spring bloom of planktonic algae, and subsequent grazing by zooplankton, including the important copepods *Calanus* spp., which is one of the key species groups in the marine ecosystem. The higher trophic levels are represented by fish, seabirds, marine mammals and humans, where polar bear and humans are the top predators. The fish fauna is low in diversity, but some species are very important in the food web. The polar cod, bottom dwelling Greenland halibut and capelin are important species that are abundant, widely distributed and constitute a major food resource for marine mammals and seabirds.

Information on the benthic communities of the eastern Baffin Bay is limited, though. The sympagic flora and fauna are a specialised ecosystem living in and on the underside of sea ice and mainly consisting of microalgae (diatoms) and crustaceans.

Seabirds, both residing in the area and the migrating populations, are locally abundant with several species present in the study area in spring and summer. Some of the most important species are northern fulmar, common eider, thick-billed murre, little auk, black-legged kittiwake and the rather rare ivory gull. The most important species of marine mammals in the area are narwhal, white whale (or beluga), bowhead whale, walrus, ringed seal, bearded seal and polar bear.

The ecosystem interactions between different biological components in the Arctic marine environment are represented as a general scheme in *Figure 4.1*.

Human use of natural resources such as subsistence hunting of marine mammals and seabirds and subsistence fishery occurs throughout the eastern Baffin Bay, except for the most offshore parts. Commercial fishery for Greenland halibut and northern shrimp takes place in the southern part of the eastern Baffin Bay.

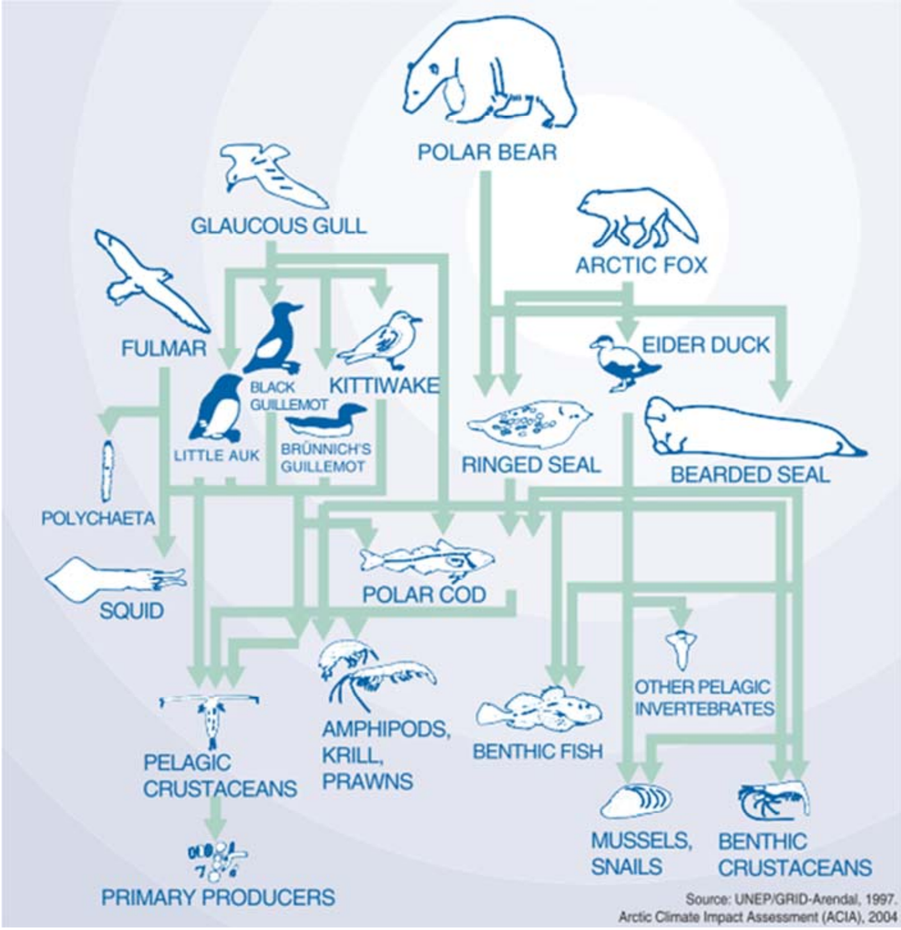

Figure 4.1. A schematic description of the food web interactions in the marine Arctic environment (from Boertmann & Mosbech 2011).

4.2 Valuable Ecosystem Components in Baffin Bay

The first step of an impact assessment is identification of the potential interactions between petroleum activities and ecological components in the area both in time and space, as well as evaluation of these interactions to cause impacts (Boertmann et al. 2011).

Since it is not possible to evaluate all ecological components in the area, the concept of Valuable Ecosystem Components, VECs, has been developed. VECs are flora and fauna, habitats which may be temporary and dynamic like the marginal ice zone and polynyas, and processes such as the spring bloom in primary production, or other environmental features that are important to the human population, nationally and internationally. These VECs can act as indicators of environmental change, or can be the focus of management or other administrative tools (Boertmann et al. 2011).

VECs are defined as those species that possess the following qualities (Gardiner et al. 2013b):

- Taxa that are important to the function of Arctic food webs
- Taxa that are representative of pelagic, benthic and sea-ice realms
- Taxa that are relatively abundant
- Taxa that may have cultural or commercial importance and
- Taxa that are well suited to impact analysis.

The VECs recommended by Word & Clark (2013) for the recovery and resiliency assessment of ecosystem in case of oil spill are:

- Seabirds
- Marine mammals
- Nearshore populations of capelin, herring, salmonids and coregonids.

The food web components that support those taxa types include:

- Calanoid copepods and krill from the water column
- Algae
- Amphipods; *Gammarus wikitzkii*, *Apherusa glacialis*, *Onisimus nanseni* and *O. glacialis* and the idotheids (isopods) at the underside of ice or ice floes and polynyas
- Amphipods, polychaetes and Mollusca in sediment
- Corals and sponges on deep water hard substrate
- Kelp on shallow water hard substrate
- Age classes of Arctic cods, *Boreogadus saida* and *Arctogadus glacialis*, using different parts of the environment from the near surface layers to deep waters.

In the strategic environmental impact assessment of the eastern Baffin Bay area of activities related to oil pollution (Boertmann & Mosbech 2011), VECs, which potentially can be impacted by oil activities, were selected. Below the VECs selected for the Arctic area in general (Gardiner et al. 2013b) and eastern Baffin Bay specifically (Boertmann & Mosbech 2011), are described.

Marine communities of the Arctic can be divided into three spatial compartments: the pelagic, benthic and sea-ice.

4.3 Pelagic and water realms

Pelagic communities relevant as VECs are zooplankton, phytoplankton and pelagic fish communities. In relation to primary producers, Boertmann & Mosbech (2011) state that due to lack of data and large variability it is not possible to point out particularly important, recurrent areas for primary productivity, except for a general designation of polynyas and ice edges.

The zooplankton community in the Arctic is dominated by copepods, particularly the Calanoid copepods, *Calanus glacialis* and *C. hyperboreus*. The third main copepod species, *C. finmarchicus*, was first characterised as boreal but is now generally regarded as a North Atlantic species. The life cycle duration for this species is still debated, but *C. finmarchicus* is known to overwinter in diapause in deep water. *C. finmarchicus* is imported into Baffin Bay by the inflow of Atlantic water (Dünweber & Schiedek 2011). While the three dominant calanoid copepods co-occur, they differ significantly in their life histories (Gardiner et al. 2013b). According to SEIA, Eastern Baffin Bay (Boertmann & Mosbech 2011), it is not possible to designate specific important areas for zooplankton, but the key species of zooplankton are designated as VECs, which include *Calanus hyperboreus*, *C. glacialis* and *Parathemisto libellula*.

According to SEIA, Eastern Baffin Bay (Boertmann & Mosbech 2011), VECs among the pelagic fish include the polar cod, capelin and Arctic char. The fishing grounds for Greenland halibut, the rivers utilised by Arctic char and the near-shore habitat used by capelin to spawn are important VECs; however, it is not possible to designate important areas for polar cod or other fish species due to lack of data (Boertmann & Mosbech 2011). Capelin is also an important secondary consumer throughout the Arctic, particularly in the Barents Sea where they are the primary link between copepods and Atlantic cod (Gardiner et al. 2013b). Arctic and polar cod, capelin and herring are important food resources for higher trophic levels including larger fish, e.g. Atlantic cod (Gardiner et al. 2013b).

Arctic cod and Polar cod represent a critical link between the zooplankton community and higher trophic levels throughout the Arctic. Both fish species are pan-Arctic and are widely distributed in the Arctic, occupying nearshore, pelagic and sea-ice habitats, residing both at depth and near the surface waters, depending upon age and season (Gardiner et al. 2013b).

4.4 Benthic and sediment realms

Benthic macro fauna in the Arctic is dominated by polychaetes, bivalve molluscs, crustaceans, e.g. amphipods and isopods, as well as echinoderms while in the nearshore and inner shelf communities, Arctic mollusc species often define the benthic communities and are a key prey item for higher trophic levels, e.g. walrus and bearded seals (Gardiner et al. 2013b).

From the benthic fauna species, SEIA, Eastern Baffin Bay (Boertmann & Mosbech 2011) has designated the northern shrimp *Pandalus borealis* as VEC for oil pollution assessment, since it is an important species for commercial significant fishery in Greenland. Demersal fish species, such as sculpins (Cottidae) and eelpouts (Zoarcidae) comprising over 50 % of the species in polar waters. Both taxa are well adapted for living in the soft substrates found in the Arctic (sand, silt and mud), as well as rocky bottoms, with most species spending the majority of their life cycle in close association with the seabed. Adults often deposit eggs directly on benthic substrates or on marine vegetation. Larvae and early juvenile forms may remain at the bottom near the adults or move into the water column or into vegetation before descending to the bottom (Gardiner et al. 2013b). Deepwater demersal fish taxa include Zoarcids (eelpouts) and Macrourids (grenadiers). The Greenland halibut *Reinhardtius hippoglossoides* is typically associated with deep waters of the Arctic (200-1600 m), and is being recommended as VEC by SEIA, Eastern Baffin Bay (Boertmann & Mosbech 2011). The Greenland halibut is epibenthic and feeds on epibenthic crustaceans, demersal fish and other invertebrates. The midwater and deepwater fish species common in other oceanic basins have been observed in the Arctic, including Myctophids (eks.) and Gonostomidae (eks) (Gardiner et al. 2013b).

4.5 Sea ice

Several sympagic copepod species are designated as VECs in the review on the Arctic ecosystems and valuable resources (Gardiner et al. 2013b) (see *Table 4.1*). However, according to SEIA, Eastern Baffin Bay, due to the lack and wide variability of data it was not possible to point out particularly important, recurrent areas for sympagic flora and fauna (Wegeberg & Boertmann 2011).

Table 4.1. Valuable Ecosystem Components for Arctic communities adopted from the review on the Arctic ecosystems and valuable resources (Gardiner et al. 2013b). Boxes marked in red: VECs identified for Baffin Bay in relation to monitoring of the pollution from offshore oil activities (Boertmann & Mosbech 2011).

Valuable Ecosystem Components	Associated Communities			
	Pelagic	Benthic	Sea-Ice	Deepwater
Phytoplankton	●		●	
Sympagic copepods				
Gammarus wilkitzkii			●	
Apherusa glacialis			●	
Onismus spp.			●	
Calanoid copepods				
Calanus hyperboreus	●		●	●
Calanus glacialis	●		●	
Calanus finmarchicus	●			●
Euphausiids				
Thysanoessa spp	●		●	
Hyperiid amphipods				
Themisto libellula	●		●	
Cephalopods				
Gonatus fabricii	●	Ⓟ		●
Pelagic Fish				
Arctic cod - *Boreogadus saida*	●	Ⓟ	●	
Polar cod - *Arctogadus glacialis*	●	Ⓟ	●	
Capelin - *Mallotus villosus*	●	Ⓟ		
Myctophids	●			●
Gonostomids	●			●
Clams				
Serripes groenlandica	Ⓟ	●		
Macoma sp.		●		
Benthic and Epibenthic Amphipods				
Ampelisca sp.		●		
Anonyx nugax		●		●
Eurythenes gryllus		●		●
Decapod crustaceans				
Shrimp - *Pandalus borealis*				
Crab - *Chionoecetes* spp.				
Echinoderm				
Urchin - *Strongylocentrotus droebachiensis*				
Epibenthic Fish				
Sculpin – *Myoxocephalus* spp.	Ⓟ	●		
Eelpout – *Lycodes* spp.	Ⓟ	●		●
Greenland halibut - *Reinhardtius* sp.	Ⓟ	●		●
Mammals				
Ringed seal – *Phoca hispeda*	●		●	
Walrus - *Odobenus rosmarus*		●		
Narwhal – *Monodon monoceros*	●	●	●	
White whale – *Delphinaptrus leucas*	●		●	
Bowhead whale – *Balaena mysticetus*	●			
Polar bear – *Ursus maritimus*	Ⓟ		●	
Seabirds	●		●	

● Integral component of the community
Ⓟ Prey item or predator, but not an integral component of the community

4.6 Birds

The following bird species are considered VECs for the Baffin Bay area as they occur in the area at different seasons, at dense populations, for either breeding or migrating through the area and are considered to be vulnerable to oil spills (Boertmann & Mosbech 2011). Great cormorant occurs in the southern part of the Baffin Bay. Common eider is an important species for hunting and is breeding in colonies throughout the coastal parts of the Baffin Bay. King eider occurs in the area in late summer in large moulting concentrations along the coasts, kittiwake breeds in large and dense colonies, and ivory gull migrates through the Baffin Bay, and occurs as summer visitor. Most of the thick-billed murre in Greenland breeds in large colonies in the

Baffin Bay. Most of the worlds' Little auk population is breeding in the northern Baffin Bay. Atlantic puffins are also breeding in the Baffin Bay. Arctic tern breeds in colonies along the coast of West Greenland.

4.7 Selected VECs

Based on VECs appointed for Eastern Baffin Bay (Boertmann & Mosbech 2011) and the review on the Arctic ecosystems and valuable resources (Gardiner et al. 2013b), VECs selected for the Arctic area in general have been selected. The selected species are described in *Chapter 6* and presented in *Table 4.1*, together with their ecological habitats and migration patterns in the area.

5 Experimental studies performed as part of the project

Two experimental ecotoxicological studies had been performed under this project, investigating effects and accumulation of oil compounds into the high Arctic copepod species *Calanus hyperboreus*.

The studies are:

1) Nørregaard, R.D., Nielsen, T.G., Møller, E.F., Strand, J., Espersen, L. & Møhl, M. (2014). Evaluating pyrene toxicity on Arctic key copepod species *Calanus hyperboreus*. Ecotoxicology 23(2): 163-174
http://link.springer.com/article/10.1007%2Fs10646-013-1160-z

2) Nørregaard, R.D., Gustavson, K., Møller, E.F., Strand, J., Tairova, Z. & Mosbech, A. (2015). Ecotoxicological investigation of the effect of accumulation of PAH and possible impact of dispersant in resting high Arctic copepod *Calanus hyperboreus*. Aquatic Toxicology 167: 1-11
http://www.sciencedirect.com/science/article/pii/S0166445X15300114#

5.1 Unique environmental aspects of Arctic waters

The Arctic aquatic environment is characterized by low water temperatures, marked seasonal variations of solar radiation, high prevalence of sea ice, and consequently effects such as increased rates of photo toxicity, water column stratification and restricted rates of nutrients exchange, and has led to evolution of certain adaptive mechanisms in Arctic marine species. Therefore, Arctic aquatic species are characterized by general slow metabolism, lower energy usage, high lipid content, longer developmental time and simple structure of food chains (see *Chapter 4* for detailed review). The relatively high levels of lipids serve partially as an energy reserve to withstand long periods of starvation during hibernation and partially as body thermo-insulation. These factors, together with the strict seasonality in activity related to reproduction and feeding, and consequently seasonality in metabolic activity of Arctic organisms may infer with the responses of these organisms to an additional environmental stressor as chemical pollution (Chapman & Riddle 2005; Word 2013).

Copepods are keystone species in the Arctic marine ecosystem, transferring energy from lower to higher trophic levels of the Arctic and sub-Arctic food web and calanoid copepods comprise the largest zooplankton biomass in the Arctic. Of the three dominant calanoid copepods, i.e. *Calanus hyperboreus*, *C. glacialis* and *C. finmarchicus*, *C. hyperboreus* is the most polar species and is most common in the deep sea areas of the Arctic. *Calanus* spp. feed through planktonic diatom blooms, converting low energy sugars into high energy lipid reserves (Word 2013). These plankton species have strict seasonal activity patterns, where they re-fill lipid reserves during summer and migrate to the deeper waters for the following period of spawning and hibernation. This strict seasonality in activity can have consequences in their response to oil exposure, especially due to the seasonal variability in body lipid content. Therefore, it is necessary to study biological effects of oil-derived compounds, polycyclic aromatic hydrocarbons (PAHs), on different life-stages of these copepods.

Additionally, since only limited amount of chemical dispersant were tested in exposure studies with Arctic copepods (Word 2013) and as, to our knowledge, no studies have yet tested the dispersant product AGMA DR372, extension of studies on toxicity data applying dispersants is necessary.

5.2 Aims of the studies of effects and bioaccumulation of PAHs in high Arctic key species of copepods

- Evaluate specific biological effects of one of the representative PAHs, pyrene, in two separate dose–response experiments, one prior to the spring bloom when female copepods are still at their hibernation depths; and one during the bloom when females have stopped spawning and migrated to the surface waters. The first experiment evaluated grazing rate of exposed copepods, and therefore animals were fed increasing doses during the whole time of exposure, while the latter one evaluated egg production of exposed copepods and therefore animals were not fed. Both groups of organisms, fed and not fed, were used in order to determine internal concentrations of pyrene and its metabolite composition.

- To study the effect of the dispersant, AGMA DR372, which is planned to be applied in the Arctic, by passive uptake of three representative PAHs (phenanthrene, pyrene and benzo-a-pyrene) in resting/hibernating high Arctic copepods. Since copepods were collected during the resting life stage, they were not fed while exposed in the laboratory. Additionally, mortality rates and reproduction success were monitored for two months following the exposure to determine long-term effects of PAH uptake in copepods.

5.3 Model organism

Arctic copepod *Calanus hyperboreus*, from Western Greenland, was chosen as subject for both studies, since it is a key species in the Arctic regions and because of its large size, abundance and role in the Arctic food web. In the Disko Bay and Disko West area – the sampling site for both studies – *Calanus spp.* constitute > 90 % of the zooplankton biomass in the upper 50 m of the water column during the summer period, and during the winter these copepods are at deep waters hibernating. During the phytoplankton spring bloom, copepods are migrating to the surface waters re-filling their energy reserves for the next winter. *Calanus hyperboreus* produce most of their eggs while in the deep waters utilizing the lipid stores they build up during the previous summer. Oil exposure during winter may therefore affect the egg production and the offspring directly through body surface exposure while exposure later in the year also may be through the food in-take. Preliminary results indicated that there is a very high risk of accumulation of oil components in Arctic copepods, probably due to the high lipid content (> 60 % of body mass at the end of summer) and a slow metabolism. There is also an indication, i.e. minor or no significant differences, that exposure to oil compounds can affect hatching of *Calanus spp*. A high accumulation rate increases the risk of toxic effects on the copepods and offspring as well as the risk for potential exposure and transfer of oil compounds further in the Arctic food chain.

5.4 Model oil-derived compounds

Polycyclic aromatic hydrocarbons (PAHs) are oil-derived hydrophobic contaminants with mutagenic and carcinogenic characteristics. Sublethal effects of PAHs include reduced reproduction and feeding rates and disturbance of cell membrane fluidity resulting in non-polar narcosis leading to decreased activity and ability to react to stimuli. Several different PAHs were used in these exposure studies as proxy for oil-derived compounds.

5.5 Study 1 - Evaluating pyrene toxicity on Arctic key copepod species *Calanus hyperboreus*

This study evaluated the biological effect of oil exposure on *C. hyperboreus* females using pyrene as a proxy PAH for an oil-derived compound. The specific effects of pyrene under investigation were:

- On egg production
- On faecal pellet production as proxy for grazing rate
- On hatching success,
- Internal pyrene concentrations in the exposed animals
- Pyrene metabolite composition in the exposed animals.

In this study two separate dose–response experiments were conducted: one immediately before the spring bloom (March) for the egg production experiment; and one during the bloom (April) where copepods that migrated to the surface for feeding were collected for the faecal pellet production experiment. *C. hyperboreus* females were exposed to concentrations of 0, 0.1, 1, 10 and 100 nM pyrene and saturated concentrations measured to approx. 300 nM. Egg and faecal pellet production experiments lasted 14 days and hatching experiment lasted 4 days. Daily quantification of egg and faecal pellet production showed significant decreases in the pellet production, while the egg production, despite observed decrease in egg production at higher pyrene doses, did not show the significant statistical difference due to high inter-individual variance across all treatments on the day-to-day basis. The hatching success was generally unaffected by pyrene, although the total reproductive output was reduced with increased pyrene concentrations. The only significant difference in hatching success was observed between control groups and the highest pyrene concentration. Accumulation of pyrene in the copepods was higher in feeding than starving females and only trace amounts of the pyrene metabolite, 1-hydroxypyrene, were found in the feeding females. Lowered reproductive output, reduced grazing rate and reduced ability to metabolize pyrene suggest that oil contamination may constitute a risk to *C. hyperboreus* recruitment, energy transfer in the food web and also a potential risk for biomagnification of PAHs.

5.6 Study 2 - Ecotoxicological investigation of the effect of accumulation of PAH and possible impact of dispersant in resting high Arctic copepod *Calanus hyperboreus*

The aim of this study was to increase the knowledge on how the chemical dispersion during an oil spill may affect the passive uptake of PAHs in resting high Arctic copepods using *C. hyperboreus* as an Arctic model organism. The common concerns regarding the use of dispersants are the dispersant-increased bioavailability of oil in the water column and the potentially added toxicity caused by the dispersant. The small oil droplets produced via chemical dispersion are within the size range for *Calanus* spp. ingestion, making the oil and dispersant bioavailable through the digestive system of the copepods.

To study the effects of chemical dispersion on oil bioavailability and accumulation of PAHs in Arctic copepods, resting high Arctic *C. hyperboreus* were collected in the field at > 250 m depth and exposed in laboratory to mixtures of PAHs and oil with and without the addition of dispersant, followed by quantification of the PAH concentrations in the copepods.

C. hyperboreus females were incubated in phenanthrene, pyrene and benzo(a)pyrene for three days in treatments with and without corn oil and dispersant (AGMA DR372). The exposure to PAHs lasted for five days. At the end of exposure, some exposed animals were sampled to measure PAH concentrations and bio-concentration factors. Some animals from the exposure treatments were then transferred to a clean water environment for two months. These latter organisms were used to study mortality rate, depuration rates and reproduction success, measured as egg production rate.

At the end of the exposure experiment, the highest measured concentrations of phenanthrene, pyrene and benzo(a)pyrene in the copepods were 129, 30 and 6 nmol PAH g female $^{-1}$, respectively. Results showed that with addition of oil (corn oil) and dispersant to the water, the accumulation of PAH was significantly reduced, due to the deposition of the PAHs in the oil phase, decreasing the available PAHs for copepod uptake. While PAH metabolites and a depuration of the PAHs were observed, the copepods still contained PAHs after 77 days of incubation in clean seawater. Differences of treatments with and without oil and dispersant on the egg production were not statistically conclusive, although there is most likely an effect of the highly variable day-to-day egg production between individual copepods. When investigating the effects on egg production, the number of dead copepods was quantified for a mortality rate estimation over a period of two months and although there was an indication that the addition of dispersant and oil increased the mortality rate, no statistically significant effects were found.

5.7 Comparison of PAH concentrations observed during oil spill events to effect concentrations observed in presented studies

Oil spill events can lead to high local PAH concentrations, frequently ranging from 1-150 µg L^{-1} (Barbier et al. 1973; Short et al. 1993; Neff et al. 1995; Law et al. 1997). Numbers reported during the Deepwater Horizon oil spill, USA, for the water concentrations of PAHs were in the range of 0-189 µg L^{-1} , while pyrene concentrations within 1 km off the blow-out from the well in the Mexican Gulf were 0.34 µg L^{-1} or 1.68 nM (Diercks et al. 2010). These pyrene concentrations are lower than the effect concentrations (≥ 100 nM) observed in the first study described here “Evaluating pyrene toxicity on Arctic key copepod species *Calanus hyperboreus*”.

The total PAH concentration, i.e. $[PAH_{total}]$ = [phenanthrene] + [pyrene] + [benzo(a)pyrene], in the high end PAH treatments of the second study “Ecotoxicological investigation of the effect of accumulation of PAH and possible impact of dispersant in resting high Arctic copepod *Calanus hyperboreus*” was 40.57 µg L^{-1}, which is well within the range of the concentrations seen at Deepwater Horizon or even the frequent range of PAHs in oil spills. The level of dispersant in this study for all the PAH treatments was 42.5 mg L^{-1} or 50 ppm. This is above the normal range measured in field experiments during oil spills. Concentrations up to 13 ppm dispersant have been reported during oil spills (Bocard et al. 1984), but general concentrations during such events are thought to be < 1-10 ppm (Mackay & Hossain 1982; Wells 1984).

Although the effect concentrations of pyrene were above the levels found during the historic oil spills, PAH concentration at 189 µg L^{-1} measured during the Deepwater Horizon oil spill is almost three times the maximum concentration of pyrene used in the first study: 317 nM or 64 µg L^{-1}. Considering the additive nature of PAH toxicity (Barata et al. 2005) and the fact that pyrene has a moderate toxicity compared to other PAHs (Law et al. 2002), it is unlikely that the effects documented in this study are overestimated.

5.8 Conclusions and perspectives

The presented studies showed that exposure to a representative oil-derived PAH, pyrene, can have adverse biological effects, i.e. grazing rate and reproduction of *C. hyperboreus*. Also it was shown that feeding copepods accumulated higher rates of PAHs than resting animals. Due to the strict seasonality of the life-cycle stages with feeding and reproductive activities in Calanus populations in the Arctic, accidental oil spills have the potential to cause serious damage to the function of the Arctic pelagic food web, either at the surface water or chemically dispersed into deeper waters. A spill can affect feeding copepods during seasonal migration to the surface water for grazing and can affect their reproduction rates during spawning in the deep waters. These results demonstrate the need to evaluate long-term effects of exposure to PAHs on both grazing rate and reproduction of one of the dominating Calanus species in the Arctic seas, *C. hyperboreus*.

PAH accumulation in resting copepods was significantly lower when both oil and dispersant were present. This result indicates that while copepods are in diapause life-stage resting at the deeper waters and not feeding, in case of accidental oil spill event, addition of dispersant will not lead to elevated levels of toxic effects, due to increased bioaccumulation rates of oil compounds from feeding.

Both studies demonstrated the ability of *C. hyperboreus* to metabolize PAHs, although to a lower degree. Decreased ability to biotransform oil-derived compounds together with high-lipid fraction in these copepods, may potentially lead to increased rate of bioaccumulation of PAHs. Elevated levels of bioaccumulation of PAHs in this dominating species of zooplankton in the Arctic can further mean potential exposure of oil-derived compounds through the food web. Further studies are necessary to determine the biotransformation capacities of these Arctic species.

6 Recommendations for ecotoxicological monitoring and assessment of oil-related activities in Baffin Bay

6.1 Baseline and impact monitoring

It is recommended that assessment and monitoring follow the integrated approach, where biological and chemical samples and analyses are performed within the same framework, e.g. time and space. An integrated approach is defined by international institutions and conventions, e.g. OSPAR and HELCOM. This approach implies that the monitoring includes the key environmental matrices: water, sediment, biota and an appropriate combination of biological effects and chemical measurements. Furthermore, the design of sampling programmes should allow the chemical concentrations, the biological effects data and other supporting parameters to be combined for assessment.

It is recommended that the monitoring includes baseline surveys (before exploration and exploitation activities are started) and impact monitoring after the activities are started. It is recommended that the monitoring includes measurements in both "wild" biotas as well as caged.

Due to significant differences in physiology and biology between Arctic and temperate species, toxicity and assessment criteria for temperate species can not directly be applied in the risk assessment for Arctic organisms. Against this background it is recommended that assessment criteria for the selected biomonitor species are established in a baseline survey (before exploration and exploitation activities are started) as well as in reference areas (before and after the activities are started, before-after-control-impact (BACI design)). It is suggested that the assessment criteria are defined from statistical variation of the specific chemical and biological parameter.

Baseline surveys are the first environmental surveys of an area or specific field location to obtain information on its natural environmental status, which includes chemical and biological measurements, prior to the start of an oil-related activity in the area. Baseline surveys are required before exploration and production drilling in the assessment area. Baseline surveys are intended to establish the background levels of selected chemical compounds in environmental matrices (water, sediment and biota) as well as determine baseline values for the individual biomarkers in the specific biomonitor species. Impact monitoring programmes should be conducted after the activities have started and are aimed to document to what extent the area is affected by pollution from the oil and gas industry. The impact is assessed against the baseline values. Such programmes are within the frames of the KLIF (2011) guidelines. The number and location of the instrument rigs deployed for impact monitoring must be able to provide the best possible picture of the situation of the selected field in the region.

6.2 Recommendations for selection of species to biomonitor

In relation to environmental assessment of oil spill impact, the VECs should represent taxa that are important to the function of Arctic food webs, are representative of all the environmental compartments, e.g. pelagic, benthic, deep-sea and sea-ice, relatively abundant in the area that may have cultural and commercial importance and taxa that are well suited to impact analysis, e.g. species suited for laboratory conditions and to experimental approaches for evaluating the effects, species for which there are toxicity testing methods that can be or have been used to identify effects at the individual or population level (Word 2013).

Our recommendation for selection of biomonitor species is based on those principles and the combination of recommended VECs species selection from the OGP/JIP report and SEIA for Baffin Bay.

Calanoid copepods, *Calanus hyperboreus*, *C. glacialis* and *C. finmarchicus*, are key species of zooplankton in the Arctic. Decreased ability to biotransform oil-derived compounds and ability to bioaccumulate oil compounds within the organism were demonstrated in both studies performed within the present project. High-lipid content and key positions within the Arctic food chain make these zooplankton species valuable biomonitoring species in order to evaluate exposure to oil-derived compounds and potential ecological effects of oil spill. It is recommended that these Calanoid copepods should be collected according to seasonal availability at different depths of the water columns and analysed for PAH content and for biological effects (i.e. CYP1A activity).

Of pelagic fish species, polar cod is recommended for biomonitoring. It should be collected in the field for baseline values and also exposed in deployed cages at different distances to the sites with hydrocarbon exploration or exploitation activities.

Additionally, blue mussels (*Mytilus edulis*) are recommended for monitoring purposes. It can be transferred from the reference sites for which the baseline values are known and exposed to the potential contaminants at the assessment area in cages.

Since a fraction of oil may sink to the seabed or beach at the shore and subsequently be mixed with the marine sediments, it is necessary to evaluate chemical impacts and biological effects on benthic organisms. It is hence recommended to include the northern shrimp (*Pandalus borealis*) and the Greenland halibut in the monitoring programme. For both species, chemical measurements and biological effect measurements are applicable.

When in contact with oil and oil-derived compounds in water, bird feather tissue may take up the compounds and the feather contents reflect the pollution of the sea surface. Therefore, bird feather contents of oil and oil-derived compounds are considered as a suitable biomonitoring parameter. It is thus recommended to incorporate feather content analyses from sea birds species that are breeding locally and seasonally residing in the assessment areas. Based on these parameters and local and seasonal occurrence of birds, it is recommended to include the thick-billed murre and the Little auk as VECs into the monitoring programme.

6.3 Recommendations for chemical monitoring

Our recommendation is that the chemical measurement programme follows “Guidelines for offshore environmental monitoring” developed for Norway (KLIF 2011). The guidelines prescribe chemical measurements in three environmental matrices: water, sediment and biota. The types of chemical measurements in each matrix are presented in *Table 6.1*.

Table 6.1. Recommended chemical analyses based on the "Guidelines for offshore environmental monitoring" (KLIF 2011).

Chemical analysis	Environmental matrix	Organism
Hydrocarbon analysis (NPDs, PAHs)	Water, sediment	Fish, mussels, zooplankton, bird feathers
Synthetic drilling fluids	Sediment	Fish, mussels, zooplankton, bird feathers
Metals	Sediment	Fish, mussels
Radioactivity	Water, sediment	Fish, mussels, zooplankton, bird feathers

The following analyses are required for samples from all stations in baseline surveys and impact monitoring surveys in connection with hydrocarbon exploration/exploitation activities:

- THC: Total hydrocarbon content; content of all hydrocarbons in the samples within a particular range of carbon chain length (n-C12 – n-C35), both those formed biologically and those originating from oil and other sources of pollution.

- NPD: The sum of naphthalene, phenanthrene, dibenzothiophene and their C1-, C2- and C3- alkyl homologues.

- PAHs: Polycyclic aromatic hydrocarbons; all hydrocarbons in which the molecule contains three or more aromatic rings. Hydrocarbons with only two aromatic rings are often included as well. The list of 16 main PAH compounds identified in relation to the presence of pollution by the US Environmental Protection Agency is presented in Appendix I of KLIF (2011). This list should be consulted to include all relevant PAH monitoring species.

- For both types of monitoring surveys, analyses of the following elements are required for all stations: Barium and other weighing agents if used in the area, Cd, Cr, Cu, Pb, Zn and Hg.

- Analyses of Ra-226, Ra-228 and Pb-210 are required for all sediment samples. The Th-228 content must also be analysed in samples from platforms where mechanical or chemical removal of deposits from processing equipment was followed by discharges to the sea.

- Analyses of Ra-226 and Ra-228 are required for all water samples from all stations and the recommended sample size for all water samples is 25 litres.

6.4 Recommendations for biomarker monitoring

It is recommended that biomarkers listed in *Table 6.2* are included in baseline surveys as well as in the impact monitoring for fish and mussels, including tissues type/biological matrix. The recommended list of biomarkers is based on the "Guidelines for offshore environmental monitoring" (KLIF, 2011) and the report of the joint ICES/OSPAR study group on the "Integrated marine environmental monitoring of chemicals and their effects" (Davies & Vethaak 2012). Detailed description of the biomarkers and protocols are available in the technical annexes to the above-mentioned references.

Table 6.2. Recommended biomarkers for assessing exposure and effects of oil contamination I. List are compiled from the "Guidelines for environmental monitoring for offshore activities" (KLIF, 2011) and the report on "Integrated marine environmental monitoring for chemicals and their effects" by the ICES/OSPAR study group (Davies & Vethaak, 2012).

Method	Tissue type/matrix	Substance/group of substances	Organism	Priority analysis
PAH metabolites	Bile	PAHs	Fish	+
Alkyl phenol (AP) metabolites	Bile	APs	Fish	+
Histology	Gills	Different sources of stress	Fish	
DNA adducts	Liver	PAHs (+)	Fish	
CYP1A activity	Liver	PAHs (+)	Fish	+
Vitellogenin (VTG)	Blood plasma	Xenoestrogens	Fish	
Liver histopathology - contaminant specific	Liver	PAHs	Fish	+
Macroscopic liver neoplasms	Liver	PAHs (+)	Fish	+
PAHs (body burden)	Soft tissue	PAHs	Mussels	+
Pyrene hydroxylase	Digestive gland	PAHs	Mussels	
Micronucleus formation	Cells	Genotoxic stress	Mussels	
Lysosomal membrane stability	Haemocytes (blood cells)	Metals and organic contaminants	Mussels	
Lipofuscin	Histological sections	Different sources of stress	Mussels	
Neutral lipid	Histological sections	Different sources of stress	Mussels	
Comet assay	Haemocytes (blood cells), gill cells, digestive gland	PAHs (+)	Mussels	
Histopathology in mussels	Gonad, gills, mantle, digestive gland, kidney, foot	PAHs (+)	Mussels	+
CYP1A activity	Whole organism, pooled sample	PAHs (+)	Crustaceans (zooplankton, shrimp)	+

In *Table 6.2*, all types of recommended biological effect measurements that can be applied in evaluation of exposure and effects of contaminants are included. Several types of effect measurements are accentuated as "Priority analysis", which represent the minimum programme requirements for biological measurement.

In relation to biological effect measurements, due to natural seasonal variability of biomarker responses because of e.g. reproduction periods, the following important considerations have to be taken into consideration in order to collect data comparable across the geographical areas and different years:

- Collection and sampling of biomonitor organisms have to be conducted at the same season, every year.
- Within species, organisms of the same age group should be used at every station and during every sampling campaign.
- The species should be collected outside their specific reproduction seasons.

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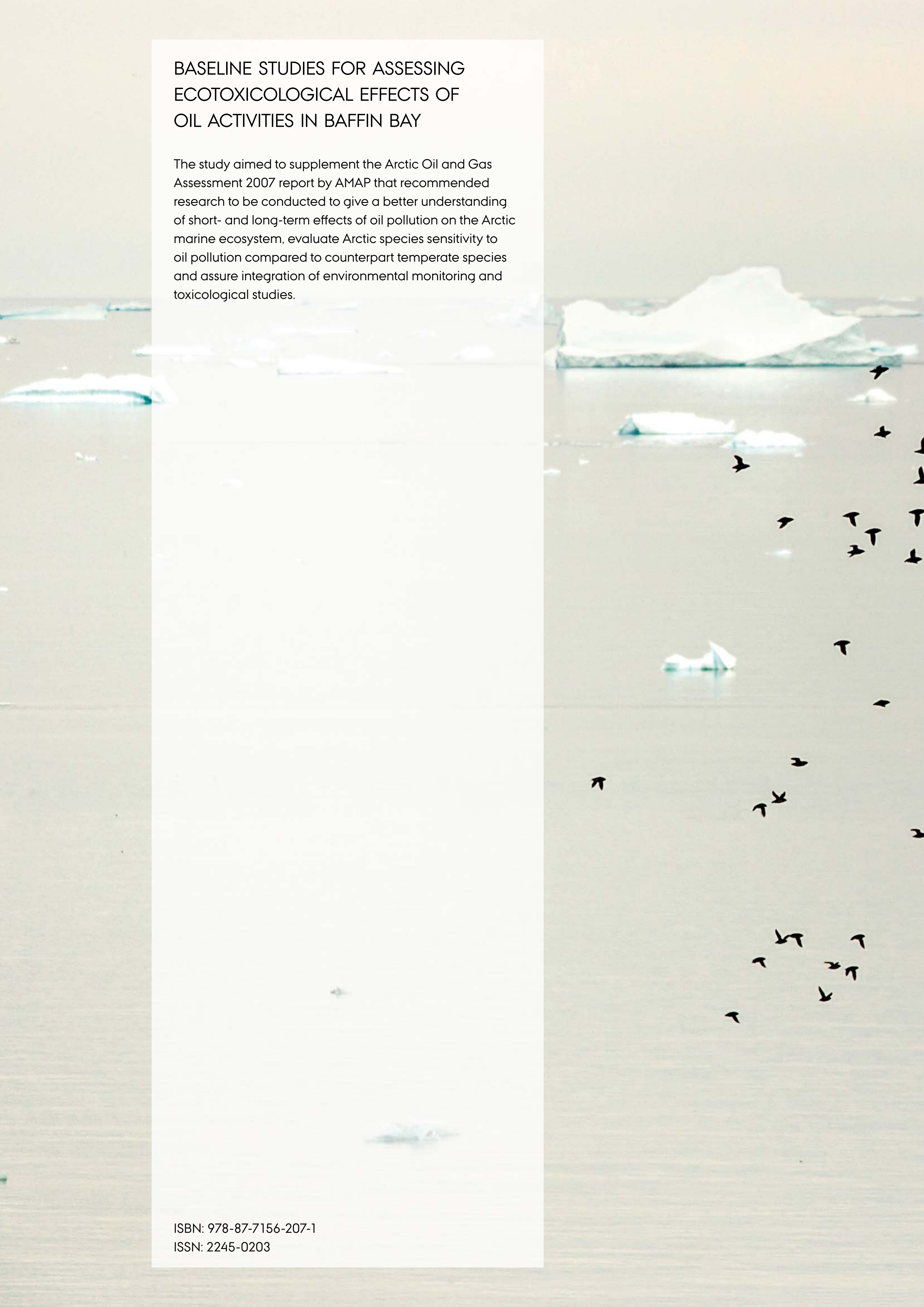

BASELINE STUDIES FOR ASSESSING ECOTOXICOLOGICAL EFFECTS OF OIL ACTIVITIES IN BAFFIN BAY

The study aimed to supplement the Arctic Oil and Gas Assessment 2007 report by AMAP that recommended research to be conducted to give a better understanding of short- and long-term effects of oil pollution on the Arctic marine ecosystem, evaluate Arctic species sensitivity to oil pollution compared to counterpart temperate species and assure integration of environmental monitoring and toxicological studies.

ISBN: 978-87-7156-207-1
ISSN: 2245-0203