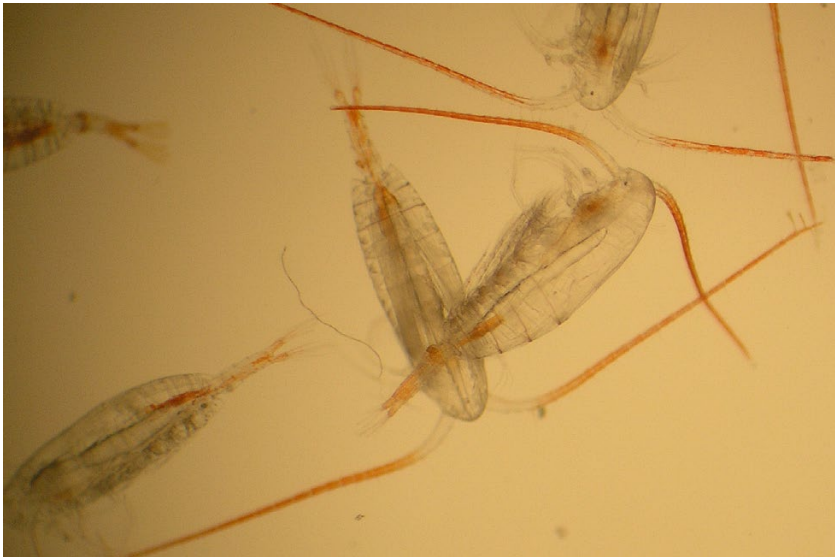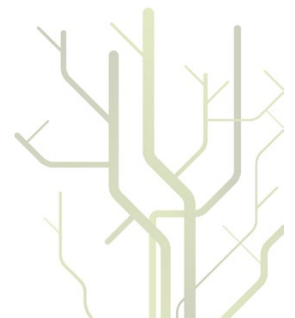# Acute and long-term effects from petroleum discharges on temperate and Arctic *Calanus* species

Louise Kiel Jensen

A dissertation for the degree of
Philosophiae Doctor

November 2011

Acute and long-term effects from petroleum discharges on temperate and Arctic *Calanus* species

By Louise Kiel Jensen

Department of Arctic and Marine Biology
Faculty of Biosciences, Fisheries and Economics

And

Akvaplan niva

Thesis submitted in partial fulfillment of the requirements for the Philosophiae Doctor degree

Tromsø, 2011

Doctoral thesis for the degree of Philosophiae Doctor

Supervisors:

Professor Paul Wassmann
Department of Arctic and Marine Biology
Faculty of Biosciences, Fisheries and Economics
University of Tromsø
Norway

Dr. JoLynn Carroll
Akvaplan niva
9296 Tromsø
Norway

Leader of the committee:
Professor Inger-Britt Falk Petersen
Department of Arctic and Marine Biology
Faculty of Biosciences, Fisheries and Economics
University of Tromsø
Norway

First opponent:
Dr. Ingela Dahllöf
Senior Scientist
Dept. of Marine Ecology,
National Environmental Research Institute,
University of Aarhus,
Denmark

Second opponent:
Dr. Tracy K. Collier
Science Advisor
Oceans and Human Health Program
National Ocean Service Mobile
National Oceanic and Atmospheric Administration
1315 East West Highway
Silver Spring, MD 20910 USA

Table of contents

Acknowledgements

Summary

Petroleum exploitation is emerging in the Arctic. In the Norwegian Arctic the southern Barents Sea is opened for development of oil and gas fields. This same area holds some of the world's largest and well managed commercial fish stocks such as Herring (*Clupea harengus*), Capelin (*Mallotus villosus*), Haddock (*Melanogrammus aeglefinus*), Atlantic Cod (*Gadus morhua*). For the interest of the fishing industry, as well as for maintaining a healthy ecosystem, petroleum related Environmental Risk Assessments (ERA) for the Barents Sea must be based on sound scientific knowledge of the special features of the Arctic ecosystem.

In colder areas the physical behavior of oil is different compared to warmer areas. The lower temperature and lack of sun light during the Arctic winter slows down the natural physical weathering process of oil. In ERA 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 life time body levels. Meanwhile higher lipid content implies a higher affinity of lipophilic contaminants such as polycyclic aromatic hydrocarbons (PAHs). So, Arctic species may be exposed to oil compounds for a longer time because of the prolonged phycical presence and may, due to special adaptive features, be more efficient at accumulating oil contaminants.

Today the knowledge of effects of oil contaminants on Arctic biota is limited, but growing. This thesis addresses a few of the research challenges in the field and provides knowledge on some effects of petroleum exposure to two copepod species; the sub-Arctic *Calanus finmarchicus* and the Arctic sibling species *C. glacialis.* First, *Calanus* was cultured for a generations under sub-Arctic conditions (Paper I). This work establishes that *Calanus* is suitable for ecological relevant long-term experiments. In an oil spill scenario, Arctic copepods and algae may be exposed to the more volatile fraction of the oil. Paper II provides

data showing that the Arctic diatom species, *Fragilariopsis oceanica*, was the most sensitive species to exposure to the volatile hydrocarbon xylene. Further, the smaller, less lipid rich copepod species *C. finmarchicus,* was more sensitive compared to the true Arctic *C. glacialis*. Extrapolating these results to a spill situation, *C. finmarchicus* and *C. glacialis* populations would be affected by xylene exposure through both direct exposure (mortality) and indirectly through a decline in prey organisms (algae).

Sub-lethal effects after long-term exposures to low concentrations of oil compounds may also be detected in *C. finmarchicus* and *C. glacialis*. Feeding was suppressed in *C. finmarchicus* after exposure to 7.0 µg PAHs l^{-1} for 11 days (Paper III). No significant effect on feeding was detected in *C. glacialis* exposed to 10.4 µg PAHs l^{-1} for 11 days. However, the hatching success of eggs laid by females exposed to 10.4 µg PAHs l^{-1} for two days was significantly reduced (Paper III).

The long term effects of lipophilic PAHs in biota depend on the bioaccumulation capacity and internal concentrations of the various PAHs. Accumulation of lipophilic compounds is particularly important for the lipid rich Arctic species. Paper IV examines the uptake kinetics of two PAHs in *C. finmarchicus* and show that the accumulation of the lighter and less lipophilic compound, phenanthrene, is faster that the uptake of the heavier and more lipophilic benzo[a]pyrene (B[a]P). Equilibrium was reached for phenanthrene within 192 h exposure while no equilibrium was detected for B[a]P. The bioconcentration factor (BCF) was higher for B[a]P, reflecting the higher affinity for lipids of B[a]P compared to phenanthrene (Paper IV). So the heavier, more lipophilic PAH accumulates slower but to a higher concentration in *C. finmarchicus.*

This thesis shows that not only acute but also long-term exposures may affect Arctic species. In addition, exposure duration may determine which compounds are accumulated by biota. Both indirect effects, such as decrease of food items, and direct effects, such as mortality, reduced feeding and reproduction failure may have implications for population development. These findings should be taken into consideration when developing ERA for the Barents Sea area.

List of acronyms and abbreviations:

ACF: Accumulation factor
ANOVA: Analysis of variance
B[a]P: Benzo[a]pyrene
BAF: Bioaccumulation factor
BCF: Bioconcentration factor
BTEX: Benzene, toluene, ethyl-benzene and xylene
CV: Copepodite stage V
DPM: Disintegration per minute
EC_{50}: The Concentration of a compound that cause a measurable Effect to 50 % of a population
EPA: The United States Environmental Protection Agency
ERA: Environmental Risk Assessment
EROD: Ethoxyresorufin-O-deethylase
GC/MS: Gas chromatography-mass spectrometry
GTS: glutathione S-transferase
K_{ow}: Octanol/water partitioning coefficient
LC_{50}: The Concentration of a compound that cause Lethality to 50 % of a population
NAC: Norwegian Atlantic Current
NCC: Norwegian Coastal Current
NOEC: No observed effect concentration
PAC: Polycyclic Aromatic Compounds
PAH: Polycyclic Aromatic Hydrocarbon
PEC: Predicted Environment Concentration
PNEC: Predicted No Effect Concentration
PW: Polar Water
ROS: Reactive Oxygen Species
WAF: Water accommodated fraction
WSF: Water soluble Fraction

List of papers

This dissertation is based on the following papers, which will be referred to in the text by their Roman numbers.

Paper I:

Jensen, L.K., Carroll, JL., Pedersen, G., Hylland, K., Dahle, S., Bakke, T. (2006) A multi-generation *Calanus finmarchicus* culturing system for use in long-term oil exposure experiments. J. Exp. Mar. Biol. Ecol. 333: 71-78.

Paper II:

Jensen, L.K., Carroll, JL (manuscript) Effects of the volatile petroleum component xylene on Arctic algae and zooplankton. Submitted to Marine Environmental Research, 12.08.11

Paper III:

Jensen L.K., Carroll JL (2010) Experimental studies of reproduction and feeding for two Arctic dwelling *Calanus* species exposed to crude oil. Aquatic Biology 10:261-271.

Paper IV:

Jensen, L.K., Jæger, I., Honkanen J., Carroll, JL (manuscript) Bioaccumulation of phenanthrene and benzo[a]pyrene in *Calanus finmarchicus.* Submitted to Ecotoxicology and Environmental safety, 20.06.11

1 Introduction

The Arctic is estimated to hold up to 25 % of undiscovered oil and gas reserves in the world and petroleum exploitation and transport will increase in the Arctic during the next decades (AMAP 2007). Management of these natural resources should be based on robust knowledge on the possible adverse effects to ecosystems from increasing human activities. Environmental risk assessment (ERA) is the regulatory framework used to evaluate the likelihood that chemicals and/or other stressors will have adverse impacts on ecological systems (Forbes et al. 2008). ERA is often used as a basis for environmental management decisions (e.g. as described in the European Committee's Technical Guidance Document for risk assessment (EC 2003)). However, standard ERA methods lack foundation on sufficient ecosystem knowledge. An additional problem is that in Norway, ERA procedures are based on the North Sea context and in particular on knowledge of temperate species sensitivity to petroleum compounds. However the life history traits of Arctic species differ from their sibling temperate species. Arctic species have longer life spans, larger body sizes and higher lipid contents compared to temperate sibling species (Maclean 1973, Koszteyn et al. 1995, Falk-Petersen et al. 2009). These adaptations may alter toxicant responses to chemical exposures in the Arctic compared to temperate species. Hence risk and impact assessment procedures should be calibrated for use in the Arctic. This dissertation focuses on the generation of baseline knowledge of Arctic species responses to petroleum exposures that will lead to improvements in the ecological relevance of environmental assessments and the development of improved ERA procedures for Arctic areas.
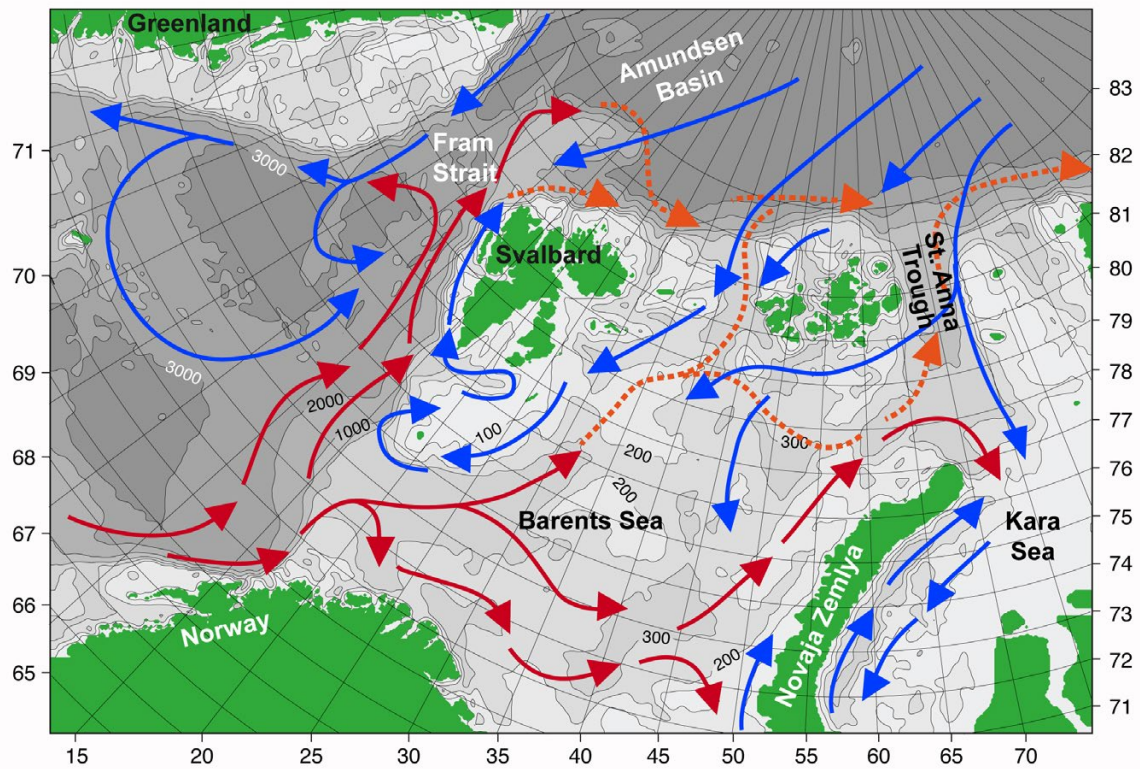
Figure 1: The hydrography of the Barents Sea. Warmer Atlantic propagates north into the Barents Sea and meets the cold Arctic water flowing south. For further explanation please see section 1.1.

1.1 The Barents Sea ecosystem

The main area of interest for this dissertation is the Barents Sea (Figure 1). The Barents Sea is a relatively shallow shelf area bordered by the Norwegian and Russian mainland to the south, Novaya Zemlya to the east, the continental shelf break to the Arctic Ocean to the north and the continental shelf break to the Norwegian Sea to the west (Wassmann et al. 2006, Loeng & Drinkwater 2007). The surface water masses in the Barents Sea consist of the Norwegian Coastal Current (NCC) running northward along the Norwegian coast. Parallel to NCC the Norwegian Atlantic Current (NAC) runs northward and splits at the entrance to the Barents Sea with one branch continuing northward along the west coast of Spitsbergen and the other branch entering the Barents Sea. The Barents Sea branch meets Polar Water (PW) running south from Franz Josef Land and the boundary between these two water masses is termed the Polar Front (Wassmann et al. 2006, Loeng & Drinkwater 2007).

The area is subjected to high seasonal fluctuations in light with 24 h of sunlight during the summer and continuous darkness for several months in winter. In addition, seasonal ice cover occurs in the northern and central Barents Sea with a maximum extent in April and a

minimum in August. Both light and ice cover regulate primary production, which starts in late March/early April in the southern Barents Sea and moves northwards as stratification and ice-melt proceeds over the season (Loeng & Drinkwater 2007, Wassmann et al. 2010, Reigstad et al. 2011). The phytoplankton biomass is grazed by zooplankton. Recent studies emphasize the role of smaller copepod species and proto-zooplankton for energy turn over (Pasternak et al. 2008, Seuthe et al. 2011). However, in the Barents Sea the main zooplankton genus is *Calanus*, which due to their effective synthesis of lipids are important in the energy transfer from lower trophic levels to fish populations (Wassmann et al. 2006, Loeng & Drinkwater 2007, Falk-Petersen et al. 2009).

The high harvestable production in the Barents Sea generates the basis for many commercial fish and shrimp stocks, such as Herring (*Clupea harengus*), Capelin (*Mallotus villosus*), Haddock (*Melanogrammus aeglefinus*), Atlantic Cod (*Gadus morhua*) and shrimp (*Pandalus borealis)* (Loeng and Drinkwater, 2007). These fish stocks represent a high and long lasting renewable income for Norway.

1.2 Composition of crude oil

1.2.1 Crude oil
The origin of the crude oil resources exploited today is marine and terrestrial plant material decaying under anoxic conditions in the late Jurassic period. During the degradation process, organic matter is converted to hundreds of different hydrocarbon based compounds, hence crude oil differs in composition in space and time according to the parent plant material and geological conditions. In general, terrestrial biological material produces gas while marine biological material produces oil (Nøttvedt & Johannessen 2008, Bjørlykke 2010).

1.2.2 Volatile aromatics
Benzene, toluene, ethyl-benzene and xylene (BTEX) are a group of one-ringed, light and highly volatile hydrocarbons which may comprise up to 5% of fresh crude oil (National Research Council 2003, Di Toro et al. 2007). The concentration of BTEX in produced water outlets from oil installations in the Norwegian Sea ranges between 2-11 mg l^{-1} (Utvik 1999). Lighter aromatics in crude oil are often associated with acute mortality (Donkin et al. 1989, Neff et al. 2000, National Research Council 2003), but as these hydrocarbons rapidly evaporate they are not commonly included in risk assessments. However, as evaporation of petroleum compounds will be slower in the Arctic due to lower temperatures, the exposure time for Arctic biota may be prolonged. Consequently, the Arctic Monitoring and Assessment

Program (AMAP) recommend inclusion of volatile organic compounds in monitoring programs (AMAP 2007).

1.2.3 Polycyclic aromatic hydrocarbons (PAHs)

Polycyclic aromatic hydrocarbons (PAHs) are considered the most toxic component in crude oil. PAHs consist of two and up to ten fused benzene rings (Hylland 2006). The United States
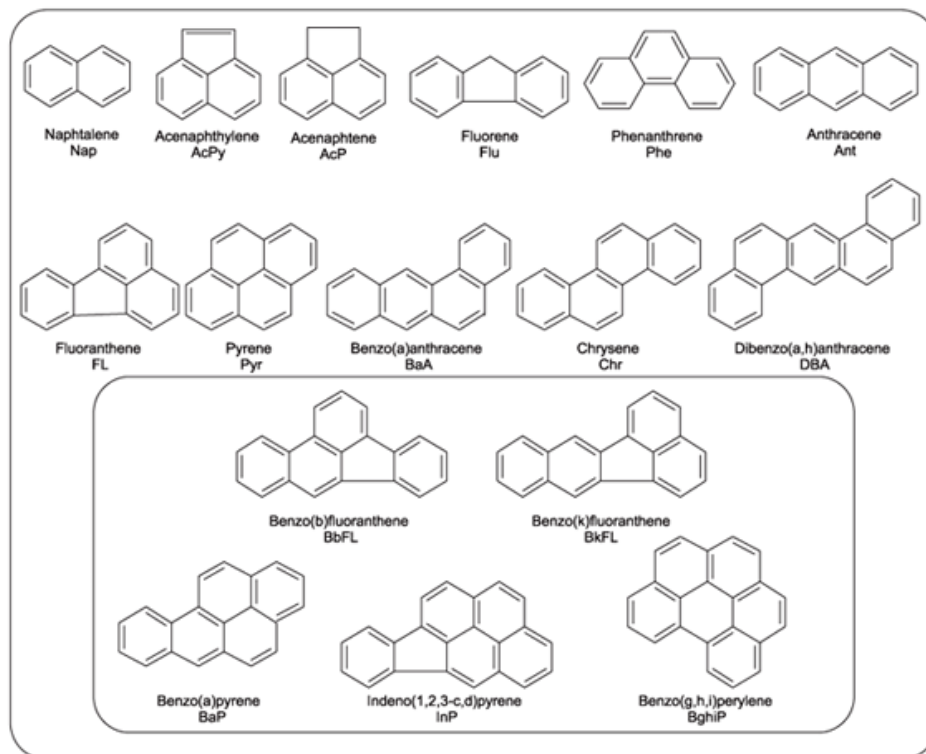
Figure 2: Structures of the 16 polycyclic aromatic hydrocarbons on the priority list of The United States Environmental Protection Agency (EPA). Figure adopted from Coelho et al. (2008).

Environmental Protection Agency (EPA) includes 16 PAHs on their priority list (EPA 1979) (see Figure 2). Effects differ considerably among PAH compounds due to the wide range of compound sizes and structures (Hylland 2006). Some PAHs are considered to be carcinogenic (causing cancer) (Harvey 1991), some mutagenic (may induce mutations in DNA) (Gil et al. 2000) and some are teratogenic (causing malformations of an embryo) (Billiard et al. 2008).

1.3 Mode of toxic action and the importance of lipids

Aromatic and polycyclic aromatic hydrocarbons are classified as type I narcotic chemicals (Veith & Broderius 1990, Di Toro & McGrath 2000). For such compounds the mode of toxic action is non-polar narcosis, which disturbs normal cell functioning through non-specific

binding to cell membranes. The binding of the chemicals are reversible, but under continuous exposure the effect on biota is observed as decreased activity, limited reaction to stimuli and eventually death. Non-polar narcosis is also termed "baseline toxicity" as it represents the minimum toxicity that may be caused by a range of chemicals (Van Wezel & Opperhuizen 1995). Specific modes of action, e.g. carcinogenic, mutagenic and teratogenic, are not examined in this dissertation.

A common chemical classification approach is to determine chemical affinities for lipids by measuring their partitioning in octanol and water, i.e. the K_{ow} partitioning coefficient. The K_{ow} value may be used for prediction of the accumulation of organic compounds into biota and a linear, near 1:1 relationship is expected between log K_{ow} and lipid based log bioconcentration factor (BCF) (Mackay 1982, Hoekstra et al. 2002, National Research Council 2003). All petroleum compounds used in this dissertation are considered lipophilic with a K_{ow} value exceeding 3. For lipophilic compounds, lipids in an organism act as a reservoir. Bioaccumulation in lipid reserves serves as a protective mechanism, reducing the fraction of chemicals available to act on cell membranes (Lassiter & Hallam 1990, Geyer et al. 1994, Jandacek & Tso 2001).

1.3.1 The *Calanus* species complex

The Barents Sea *Calanus* assemblage is dominated by three species, *C. finmarchicus*, *C. glacialis, and C. hyperboreus*. These species are considered to be mainly herbivores (Søreide et al. 2008, Falk-Petersen et al. 2009). Although they occupy similar positions in the Arctic food web and may be found at similar locations, they have different water temperature and depth preferences (Daase et al. 2007). Atlantic water masses contain the smaller and leaner *C. finmarchicus* (prosome length copepodite 5 (CV) ≤ 2.7 mm, (Arnkvaern et al. 2005)). This species has a one year life cycle at its northern limit of appearance (Arnkvaern et al. 2005). The distribution of *C. finmarchicus* overlaps with that of the true Arctic shallow water species, *C. glacialis,* and the latter dominates as we move into Arctic waters. *C. glacialis* is larger (prosome length copepodite 5 (CV) > 2.7-4.1 mm, (Arnkvaern et al. 2005)), has a higher lipid content and a 1-2 year life cycle (Scott et al. 2000). *C. hyperboreus* is the largest of the three species (prosome length copepodite 5 (CV) > 4.1 mm, (Arnkvaern et al. 2005)) and is found in Arctic and/or deep water areas (Hirche 1997). In this dissertation, *C. finmarchicus* and *C. glacialis* were used in experiments. *Calanus* spp. were chosen as test species in this dissertation due to their ecological relevance in Arctic food webs and the

possibility to compare responses between sibling species exhibiting overlapping distributions but with distinct ecological adaptations and different lipid contents.

1.4 Adaptation of risk assessment procedures

1.4.1 Oil development in the North

Continuous demand for petroleum related energy has led to increased interest in the development of oil and gas resources in the Arctic. Five percent of total global oil reserves are located in the Arctic, of which the majority are located in Russia. In addition, estimates of undiscovered oil and gas reserves place up to 25 % in the Arctic (AMAP 2007). In the Norwegian Arctic a "no physical discharge" policy is in place; thus emissions of produced water will in general not be allowed (Macdonald et al. 2008). However, later modifications to this policy open for some operational discharges (Miljøverndepartementet 2011). Increasing development activities result in an increased risk of accidents, for example due to increasing transport of petroleum products through Arctic marine waters (Bambulyak & Frantzen 2009). Hence, there is an increase in the exposure risk for Arctic dwelling organisms.

1.4.2 Environmental Risk Assessment

European legislation calls for ERA to be carried out when using known or introducing new potentially harmful compounds to the environment (EC 2003). In brief, an ERA must include hazard identification, dose-response assessment, exposure assessment and risk characterization. The main tool used to identify potential adverse effects from a chemical is the PEC/PNEC procedure. That is, the ratio of the Predicted Environmental Concentration (PEC) to the Predicted No Effect Concentration (PNEC). For a PEC/PNEC > 1, adverse effects for biota are likely to occur, while a PEC/PNEC < 1 indicates limited risk of adverse effects. The PEC value is either measured or derived from models and the PNEC is calculated based on short term toxicity metrics (LC_{50}, lethal chemical concentration that cause death to 50 % of the population) or longer term toxicity metrics (NOEC, no observed effect concentration). These toxicity metrics are derived through routine testing procedures carried out for single species exposed to a single compound. Alternatively, PNEC may be derived from Species Sensitivity Curves, a method that takes into consideration the sensitivity of species aggregates (Posthuma et al. 2002). Compounds with a log K_{ow} > 3.0 are characterized as bio-accumulative, and PNEC values for these compounds may be calculated based on experimentally derived BCFs. Alternatively, BCFs calculated from log K_{ow} values are used (EC 2003).

1.4.3 Oil spills in the Arctic

The Arctic is characterised by low temperatures and seasonal ice cover and light levels. These features will influence oil weathering processes after an oil spill event (Payne et al. 1991, Prince et al. 2002). In the marine environment, accidently discharged oil is naturally degraded by surface spreading, evaporation, emulsification and natural dispersion. These processes are influenced by temperature decreases, resulting in generally slower oil weathering (Payne et al. 1991, Macdonald et al. 2008). An important process during weathering of crude oil is evaporation of the lighter aromatic and polycyclic aromatic hydrocarbons. The more wide-spread and thinner the oil is at the sea surface, the higher the evaporation rate (Brandvik & Faksness 2009). Lower temperatures result in a higher density of the oil and denser oil spreads more slowly, limiting evaporation. In addition, the volatility of the lighter compounds decreases as temperature decreases, which further limits evaporation (Macdonald et al. 2008). Solubility also decreases in lower temperatures leading to greater persistence of water soluble components of oil in the environment (May et al. 1978). Concurrent with these physical processes, microbial degradation plays an important role in controlling oil spills. However, microbial degradation is less in colder areas (Atlas 1981, Prince et al. 2002, Macdonald et al. 2008). Crude oil will thus persist in the environment exposing biota to crude oil compounds over longer time periods.

Ice poses special challenges for oil spill mitigation and presence of sea ice may slow down the weathering process (Brandvik & Faksness 2009). Oil may be trapped in ice and released upon melting (Fingas & Hollebone 2002). In addition, water soluble fraction (WSF) of the oil may migrate within the sea ice brines causing long-term exposures to sympatic species (Fingas & Hollebone 2002, Faksness & Brandvik 2008). The presence of ice complicates the spill clean-up actions (Jensen & Mullin 2003). Even in areas without ice, the Arctic poses challenges for spill response and clean-up due to its remoteness, periods of darkness and frequently bad weather conditions (Macdonald et al. 2008).

To conclude, the behaviour of petroleum in cold waters will result in a longer exposure of the biota to the volatile fraction of crude oil. This fraction is normally neglected in risk assessments it is considered to evaporate before effects on the organisms occur. In addition, the exposures to PAHs are prolonged due to slower weathering processes.

1.5 Levels of effects in ecotoxicology studies

1.5.1 Ecotoxicological endpoints

The biological effects from exposure to xenobiotics are identified for different levels of biological organization as illustrated in Figure 3. On the cellular level of organization, exposure endpoints are assessed using a variety of biomarkers, e.g. EROD activity, glutathione S-transferase (GTS) activity, reactive oxygen species (ROS), PAH metabolites, lysosomal stability and DNA adducts (Hylland et al. 2008, Nahrgang et al. 2010). These biomarker assays are fast and cost effective, with biological responses often induced rapidly and at very low exposure concentrations. Hence, cellular biomarkers have been developed as a tool for routine monitoring programs (Hylland et al. 2008). To apply biomarkers as a part of a monitoring program, information is needed on biomarker baseline levels and how these levels may vary in response to other factors such as gender, season or through complimentary monitoring of individuals from a reference site. One limitation regarding the use of cellular biomarkers is the lack of consistency between cellular effects and individual level effects. This implies that one may measure effects that have no impact on individual performance. In addition, the linkage to ecological effects are poor (Clements 2000).

At the individual level, relevant endpoints include feeding ability, reproduction capacity, respiration, general condition and mortality. These measurements are often time consuming, labor intensive and require large numbers of test organisms. Individual level endpoints may also be applied in monitoring programs, but require considerable baseline environmental knowledge or the inclusion of complimentary monitoring at reference sites in the design of the monitoring program. The advantage of applying individual based endpoints is a more direct link between exposure and the effects for biota. Also, the ecological relevance is greater for exposure studies performed at the individual compared to the cellular level. Whether individual level effects propagate further to effects at the population or community level depends on the extent of the affected area and the proportion of exposed individuals within a population (Clements 2000).
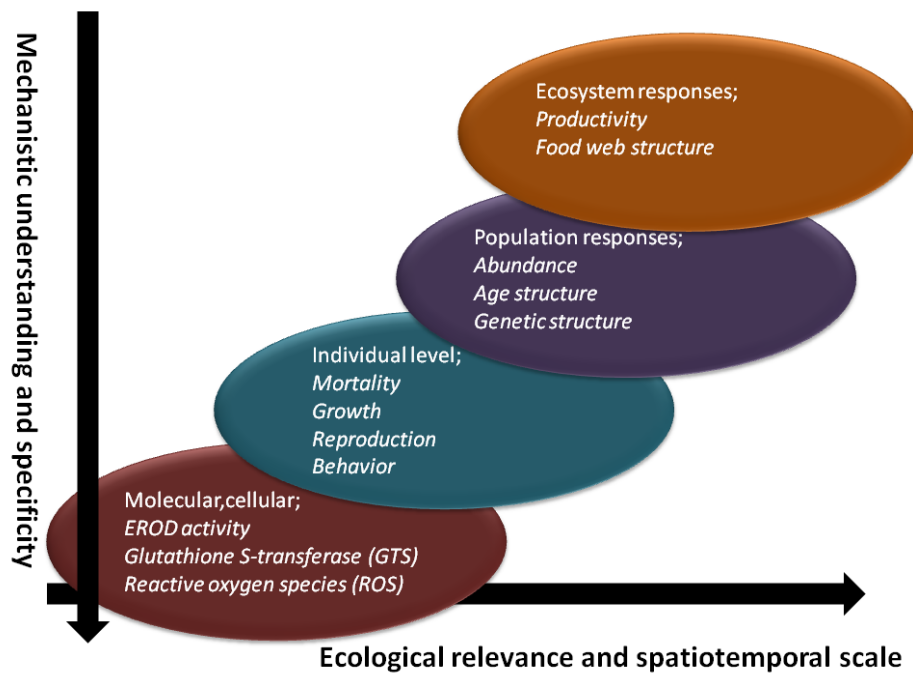
Figure 3: Show different biological levels and examples of ecotoxilogical end points that may be assessed at the different levels. From left to right, the ecological relevance of the end points increase. From top to bottom, the mechanistic understanding and specificity of the endpoints increase. Redrawn and modified from Clements (2000).

Population and community endpoints in environmental risk assessment depend on assessments of population size and the number of taxa in a given ecosystem. Documenting changes at this level are both labor and time intensive and the resulting linkage between the exposure source and population level effect will in most instances be weak (Clements 2000, Peterson et al. 2003). In the Arctic, documenting changes on an ecosystem level is further constrained by the lack of general knowledge of these ecosystems (Wassmann et al. 2011).

1.5.2 Endpoints used in our studies

All endpoints are associated with advantages and constraints in their application. Knowledge on all levels is important to develop risk assessment, but it is at all times important to focus on the applicability of the data achieved (Clements 2000). Within this project, focus has been given to individual level endpoints. This was chosen based on the wish to assess possible sub-lethal effects of long-term exposures on populations within a reasonable time interval. Following a population of *Calanus* spp. exposed to petroleum is, for natural reasons, not possible. Applying cellular biomarkers would possibly show effects of the exposure. However, how the cellular effects translate into individual performance is poorly established. So, for *Calanus* spp. we have assessed mortality, feeding ability, egg production as well as the accumulation and depuration of various petroleum related compounds.

Table 1: Peer-reviewed studies on the accumulation of various PAHs in pelagic copepod species.
Accumulation is assessed by either ^{14}C labelling or GC/MS detection technic.

	PAH	Experiment/ In situ	Method	Effect	Reference
Calanus helgolandicus	-Naphthalene	-Experiment -Up to 15 days	[14]C labeled	-Equilibrium reached after 7-9 days -Metabolism and excretion occurs	(Harris et al. 1977)
Eurytemora affinis	-Naphthalene	-Experiment -Up to 15 days	[14]C labeled	-Equilibrium reached after 7-9 days -Metabolism and excretion occurs	(Harris et al. 1977)
Calanus marshallae	-PAC (Polyaromatic compounds)	-Experiment -24 h	-GC/MS detection of water and tissue	-BCF=8000 -Similar composition as exposure water	(Duesterloh et al. 2002)
Metridia okhotensis	-PAC (Polyaromatic compounds)	-Experiment -24 h	-GC/MS detection of water and tissue	-BCF=2000 -Similar composition as exposure water	(Duesterloh et al. 2002)
Neocalanus spp.	-PAH	-In situ	-GC/MS detection of water and tissue	-Similar composition as water	(Carls et al. 2006)
Eurytemora affinis	-PAH	-In situ	-GC/MS detection of water and tissue	-ACF=17,000 -Lower relative accumulation of less hydrophobic PAHs	(Cailleaud et al. 2007)
Paracartia grani	-PAH	-Experiment -48 h exposure	-GC/MS detection of water and tissue	-BCF and BAF positively correlated with log K_{ow} -higher metabolism of less hydrophobic PAHs	(Berrojalbiz et al. 2009)
Eurytemora affinis	-Selected PAHs	-Experiment -86 h	-GC/MS detection of water and tissue	-A linear relation between individual PAH CF and their respective log K_{OW} -CF total PAH=1,230 -metabolism occurs	(Cailleaud et al. 2009)

1.6 Current knowledge of biological effects

1.6.1 Individual level effect studies on pelagic copepods

Many studies to assess effects of PAHs on pelagic copepods have been performed during the previous four decades. While the available literature is vast, studies are not easily comparable as they have been performed on many different copepod species exposed to a variety of PAHs. The available information from peer-reviewed publications is summarized in Table 1-4 which provides an overview of accumulation of PAHs and effects on mortality, feeding and reproduction in pelagic copepods exposed to PAHs.

Accumulation of PAHs has been examined in 6 species in 6 different publications (Table 1). Water and tissue concentrations of PAHs were assessed by either gas chromatography-mass spectrometry (GC/MS) or by applying ^{14}C labelled compounds. These studies confirm that copepods accumulate PAHs (Table 1), and show that different times are needed to achieve equilibrium between water and biota (Harris et al. 1977). BCFs of chemical compounds should be estimated at equilibrium. BCF values determined from short term exposures (24-48 h), where equilibrium has not been reached, may therefore be underestimated (Duesterloh et al. 2002, Berrojalbiz et al. 2009). This is also evident when comparing accumulation studies on *Eurytemora affinis* performed *in situ* and in the laboratory. The *in situ* BCF (accumulation factor (ACF) in paper) for PAHs was 17,000 (Cailleaud et al. 2007) while an 86 h laboratory experiment gave a BCF value for total PAHs of only 1,230 (Cailleaud et al. 2009). Copepods accumulate PAHs according to their respective log K_{ow} values with higher accumulation of PAHs for those with higher log K_{ow} values (Berrojalbiz et al. 2009, Cailleaud et al. 2009). However, copepods are also able to metabolize and excrete PAHs (Harris et al. 1977, Berrojalbiz et al. 2009, Cailleaud et al. 2009). There is also evidence that copepods may selectively metabolize high log K_{ow} PAHs (Berrojalbiz et al. 2009). With regard to effect studies, the extent of bioaccumulation is important since it determines the internal body concentrations of toxic compounds. And it is the internal quantity, and not what is found in the surroundings, that cause biological effects (Tamis et al. 2009).

Table 2: Peer-reviewed studies on the mortality in pelagic copepod species exposed to various PAHs.

	PAH	Experiment/ In situ	Method	Effect	Reference
Eurytemora affinis	-Naphthalene -2-methyl-naphthalene -2,6-dimethyl-naphthalene -2,3,5-trimethyl-naphthalene	-Experiment -24 h	-Mortality -LC_{50}	-Naphthalene LC_{50}=3798 µg l^{-1} -2-methylnaphthalene LC_{50}=1499 µg l^{-1} -2,6-dimethylnaphthalene LC_{50}=852 µg l^{-1} -2,3,5-trimethylnaphthalene LC_{50}=316 µg l^{-1}	(Ott et al. 1978)
Acartia hudsonica	-Venezuelan crude oil in sea water	-Experiment -19 days	-Mortality	-Exposure to $5.8*10^3$ µg l^{-1} for >8 days gave increased mortality	(Hebert & Poulet 1980)
Calanus marshallae	-PAC (Polyaromatic compounds) - 2 µg l^{-1}	-Experiment -24 h	-Mortality	-Oil only: no mortality -Oil+UV light: 20 % mortality	(Duesterloh et al. 2002)
Metridia okhotensis	-PAC (Polyaromatic compounds) - 2 µg l^{-1}	-Experiment -24 h	-Mortality	-Oil only: 5 % affected -Oil+UV light: 100 % mortality	(Duesterloh et al. 2002)
Oithona davisae	-10 PAHs -Single or mixture	-Experiment -48 h	-Mortality -LC_{50}	-Naphthalene LC_{50}=56.1 µmol l^{-1} (least toxic of 10) -Pyrene LC_{50}=0.8 µmol l^{-1} (most toxic of 10) -Additive toxicity of PAHs in mixture	(Barata et al. 2005)
Eurytemora affinis Nauplii	-Benzo(*a*)-pyrene	-Experiment -96 h	-Mortality -LC_{50}	-LC_{50}= 58 (C.I. 52-64) µg l^{-1}	(Forget-Leray et al. 2005)
Acartia tonsa	-Fluoranthene -Phenanthrene -Pyrene	-Experiment -48 h	-Mortality -LC_{50}	-Fluoranthene LC_{50}=594 nM -Phenanthrene LC_{50}=2,366 nM -Pyrene LC_{50}= >640 nM	(Bellas & Thor 2007)
Paracartia grani	-Naphthalene -1,2-dimethyl-naphthalene	-Experiment -48 h	-Mortality -LC_{50}	-Naphthalene LC_{50}= 2535 (95 %C.I. 2163-2907) µg l^{-1} -1,2-dimethyl-naphthalene LC_{50}= 161 (95 %C.I. 153-168) µg l^{-1}	(Calbet et al. 2007)

Table 2 continued

Microsetella spp.	-Pyrene	-Experiment -96 h	-Mortality	-Significantly lower survival at 0.1 nmol l^{-1} (0.02 µg l^{-1}) and higher	(Hjorth & Dahllöf 2008)
Oithona davisae Nauplii	-Naphthalene -Dimethyl-naphthalene	-Experiment -24 h	-Mortality -LC_{50}	- Naphthalene LC_{50}= 4422 (95% C.I. 3942-4961) µg l^{-1} -Dimethylnaphthalene LC_{50}= 1346 (95% C.I. 1047-1732)µg l^{-1}	(Saiz et al. 2009)
Oithona davisae Adults	-Naphthalene -Dimethyl-naphthalene	-Experiment -24 h	-Mortality -LC_{50}	- Naphthalene – no mortality -Dimethylnaphthalene LC_{50}= 771 (95% C.I. 759-784) µg l^{-1}	(Saiz et al. 2009)
Calanus finmarchicus	-Water accommodated Fraction of crude oil	-Experiment -96 h	-Mortality -LC_{50}	-LC_{50} = 0.817 (95 % C.I.: 0.784-0.853) µg total hydroarbons l^{-1}	(Hansen et al. 2011)
Calanus glacialis	-Water accommodated Fraction of crude oil	-Experiment -96 h	-Mortality -LC_{50}	-LC_{50} = 1.037 (95 % C.I.: 0.883-1.217) µg total hydroarbons l^{-1}	(Hansen et al. 2011)

Ten studies have examined mortality in pelagic copepod species (see Table 2). PAHs with higher log K_{ow} values seem to induce mortality at lower exposure concentrations (Barata et al. 2005, Bellas & Thor 2007) and methylated compounds are relatively more toxic than their associated parent compounds (Ott et al. 1978, Barata et al. 2005, Calbet et al. 2007, Saiz et al. 2009). The toxicity of PAHs is enhanced during simultaneous exposure to UV radiation, i.e. photo induced toxicity. Duesterloh et al. (2002) examined photo induced toxicity of weathered crude oil on two copepod species and found increased mortality in the exposed specimens relative to specimens exposed to oil or UV radiation alone. Due to the presence of UV radiation in the environment, *in situ* PAH toxicity will be higher than estimates derived in laboratory facilities. In the study of Barata et al. (2005) the acute toxicity of single PAH compounds was compared to mixtures of several compounds. These authors conclude that the toxicity of individual PAHs is additive. In practice, single compound estimates of toxicity may thus be converted to toxicity of mixtures, e.g. as found in oil spills.

Sub-lethal doses of PAHs may induce change in feeding of copepods (Table 3). In these studies, feeding is assessed by gut fullness, fecal pellet production, algae cell reduction and filtering rates. Most studies indicate suppression of feeding at some exposure concentration (see Table 3), but Vandermeulen (1986) report increased feeding in *Calanus finmarchicus* at higher concentrations of naphthalene and WSF of Kuwait crude oil. The flagellate *Pavlova lutheri,* which was the prey species offered *C. finmarchicus* in the Vandermeulen (1986) study, was probably immobilized by the PAHs. *C. finmarchicus* is a passive filter feeding copepod that will have a higher encounter rate of non-motile prey species compare to motile prey species. Thus, immobilization of the prey led to higher feeding rates in *C. finmarchicus.* No direct effects were observed for the copepods exposed to 0.5 and 1.0 µg naphthalene l^{-1} for 48 h (Vandermeulen 1986).

Interspecific differences between copepod species are expected to lead to differences in species' vulnerability to PAH exposures. Hjorth and Dahllöf (2008) observed a reduction in feeding by the small *Microsetella* spp. after 4 days exposure to 0.1 nM of pyrene. Jensen et al. (2008) and Hjorth and Nielsen (2011) used fecal pellet production as an alternative indicator of feeding responses to pyrene exposure. These authors observed a reduction in cumulative specific fecal pellet production for *C. finmarchicus* at pyrene exposure concentrations of 100 nM. However, exposures to lower concentrations of pyrene (< 10 nM) in some cases caused increased cumulative specific fecal pellet production in both *C. finmarchicus* and *C. glacialis* (Hjorth & Nielsen 2011). Depending on the timing and duration of PAH exposures at sub-

lethal doses, reduced feeding may influence population dynamics through increased mortality as a result of starvation or reduced reproduction. The energy demands of reproduction are high. Copepods rely on either lipid stores or contemporary food uptake as their energy source for reproduction (Varpe et al. 2009).

Direct effects on reproductive success have been assessed in six different copepod species and reported in eight studies (Table 4). Indicators of success used in these studies are: egg production, brood size, hatching success and survival of the nauplii. The reproductive capacity of *E. affinis* was reduced both by chronic exposure to low PAH concentrations and short term exposure to high PAH concentrations (Berdugo et al. 1977, Ott et al. 1978, Forget-Leray et al. 2005). For other species, only high PAH concentrations resulted in reduced egg production (Bellas & Thor 2007, Calbet et al. 2007, Jensen et al. 2008, Hjorth & Nielsen 2011). Concentrations which did not affect egg production may however cause lower egg hatching success (Cowles & Remillard 1983). The lower hatching success may have been induced by the presence of lower molecular weight compounds in the WSFs of crude oil used in this study, as the hatching success of eggs from exposed females and directly exposed eggs was not affected by exposure to the higher molecular weight compound pyrene (Jensen et al. 2008, Hjorth & Nielsen 2011). Thus, the effect of PAH exposure on reproductive success in copepods is a function of both the concentration and composition of PAHs.

Table 3: Peer-reviewed studies on the feeding in pelagic copepod species exposed to various PAHs.

	PAH	Experiment/ In situ	Method	Effect	Reference
Eurytemora affinis	-WSF of heating oil -Naphthalene	-Experiment -24 h	-Ingestion rate	-WSF at 520 µg l^{-1}: sig. lower ingestion -Naphthalene 1000 µg l^{-1}: sig. lower ingestion	(Berdugo et al. 1977)
Calanus helgolandicus	-2 and 10 ppm dispersed Kuwait crude oil	-Experiment -20 h	-Fecal pellets production	-No effect of 2 ppm, but significantly lower fecal pellets production at 10 ppm + 2 ppm dispersants	(Spooner & Corkett 1979)
Acartia clausi	-WAF of No. 2 fuel oil	-Experiment	-Filtering rate	-exposure to 250 ppb WAF caused suppression of feeding and altering of feeding mode	(Berman & Heinle 1980)
Acartia tonsa	-WAF of No. 2 fuel oil	-Experiment	-Filtering rate	-exposure to 250 ppb WAF caused suppression of feeding and altering of feeding mode	(Berman & Heinle 1980)
Centropages hamatus	-crude oil/seawater dispersions 10, 20 and 80 ppb	- Experiment -48-64 h exposure	-Ingestion rate	-Decline in ingestion rates when exposed to > 10 ppb crude oil/seawater dispersion	(Cowles & Remillard 1983)
Calanus finmarchicus	-0, 0.1, 0.5 and 1.0 µg l^{-1} of naphthalene	-Experiment -48 h exposure	-Cell reduction	-Exposure for 48 h to 0.5 and 1.0 µg l^{-1} enhanced feeding efficiency; probably due to immobilization of prey species	(Vandermeulen 1986)
Calanus finmarchicus	-0, 0.1, 0.5 and 1.0 ppm WSF of Kuwait crude oil	-Experiment -120 h exposure	-Cell reduction	-Exposure for 24 h to 0.5 and 1.0 ppm enhanced feeding efficiency; probably due to immobilization of prey species	(Vandermeulen 1986)
Paracartia grani	-Naphthalene -1,2-dimethyl-naphthalene	-Experiment -48 h exposure	-Fecal pellets production -EC_{50}	-Naphthalene EC_{50}: 1264 (95% C.I.:1060-1468) µg l^{-1} -1,2-dimethylnaphthalene EC_{50}: 146 (95% C.I.:135-157) µg l^{-1}	(Calbet et al. 2007)
Microsetella spp.	-Pyrene; 0 (solvent only), 0.01, 0.1, 1, 10 and 100nM (nominal values)	-Experiment -96 h exposure	-Registration of gut contents	-Significant reduction in proportion of specimens with full guts after 96 h in 0.1, 10 and 100 nM exposures	(Hjorth & Dahllöf 2008)

Table 3 continued

Calanus finmarchicus	-Pyrene; 0 (solvent only), 0.01, 0.1, 1, 10 and 100nM (nominal values)	-Experiment -9 day exposures	-Specific fecal pellets production (SPP)	-Significantly lower SPP from day 2 in 100 nM exposure.	(Jensen et al. 2008)
Calanus glacialis	-Pyrene; 0 (solvent only), 0.01, 0.1, 1, 10 and 100nM (nominal values)	-Experiment -9 day exposures	-Specific fecal pellets production (SPP)	-Significantly lower SPP on day 9 in 10 nM exposure.	(Jensen et al. 2008)
Oithona davisae Nauplii	-Naphthalene -Dimethyl-naphthalene	-Experiment -24 h exposure	-Clearance rate	-Naphthalene; decline observed at exposure to 2000 $\mu g\ l^{-1}$ -Dimethylnaphthalene; decline observed at exposure to 200-250 $\mu g\ l^{-1}$	(Saiz et al. 2009)
Oithona davisae Adults	-Naphthalene -Dimethyl-naphthalene	-Experiment -24 h exposure	-Clearance rate	-Naphthalene; decline observed 2000 $\mu g\ l^{-1}$ -Dimethylnaphthalene; decline observed at 200-250 $\mu g\ l^{-1}$	(Saiz et al. 2009)
Calanus finmarchicus	-Pyrene; 0 (solvent only), 0.01, 0.1, 1, 10 and 100nM (nominal values)	-Experiment -9 day exposures	-Specific fecal pellets production (SPP)	-Decreased SPP at 100 nM pyrene at 0.5 and 5 °C, but not at 8 °C -At 5 °C, 0.1 and 1 nM pyrene exposure caused increase in SPP	(Hjorth & Nielsen 2011)
Calanus glacialis	-Pyrene; 0 (solvent only), 0.01, 0.1, 1, 10 and 100nM (nominal values)	-Experiment -7 day exposures	-Specific fecal pellets production (SPP)	-Exposure to lower the concentrations of pyrene gave increased SPP at all temperatures	(Hjorth & Nielsen 2011)

Interspecific differences between copepod species are expected to lead to differences in species' vulnerability to PAH exposures. Hjorth and Dahllöf (2008) observed a reduction in feeding by the small *Microsetella* spp. after 4 days exposure to 0.1 nM of pyrene. Jensen et al. (2008) and Hjorth and Nielsen (2011) used fecal pellet production as an alternative indicator of feeding responses to pyrene exposure. These authors observed a reduction in cumulative specific fecal pellet production for *C. finmarchicus* at pyrene exposure concentrations of 100 nM. However, exposures to lower concentrations of pyrene (< 10 nM) in some cases caused increased cumulative specific fecal pellet production in both *C. finmarchicus* and *C. glacialis* (Hjorth & Nielsen 2011). Depending on the timing and duration of PAH exposures at sub-lethal doses, reduced feeding may influence population dynamics through increased mortality as a result of starvation or reduced reproduction. The energy demands of reproduction are high. Copepods rely on either lipid stores or contemporary food uptake as their energy source for reproduction (Varpe et al. 2009).

Direct effects on reproductive success have been assessed in six different copepod species and reported in eight studies (Table 4). Indicators of success used in these studies are: egg production, brood size, hatching success and survival of the nauplii. The reproductive capacity of *E. affinis* was reduced both by chronic exposure to low PAH concentrations and short term exposure to high PAH concentrations (Berdugo et al. 1977, Ott et al. 1978, Forget-Leray et al. 2005). For other species, only high PAH concentrations resulted in reduced egg production (Bellas & Thor 2007, Calbet et al. 2007, Jensen et al. 2008, Hjorth & Nielsen 2011). Concentrations which did not affect egg production may however cause lower egg hatching success (Cowles & Remillard 1983). The lower hatching success may have been induced by the presence of lower molecular weight compounds in the WSFs of crude oil used in this study, as the hatching success of eggs from exposed females and directly exposed eggs was not affected by exposure to the higher molecular weight compound pyrene (Jensen et al. 2008, Hjorth & Nielsen 2011). Thus, the effect of PAH exposure on reproductive success in copepods is a function of both the concentration and composition of PAHs.

Today, it is difficult to assess the overall effect of crude oil exposure to copepods given the variety of species and experimental approaches used. However, the available evidence on effects of exposure to copepods suggests that a) copepods do accumulate, but also metabolize

PAHs, b) mortality of PAHs increases with log K_{ow}, methylation and simultaneous exposure to light, c) feeding is often suppressed by PAH exposure and d) reproductive success is reduced in exposed specimens.

1.6.2 Arctic studies

Only five of the above mentioned effect studies have been conducted on Sub-Arctic and Arctic copepod species. Duesterloh et al. (2002) measured PAH body burdens in the Sub-Arctic copepods *Calanus marshallae* and *Metridia okhotensis* and found similar composition as in the exposure water, indicating passive partitioning of PAHs in these two species. The same authors report a bioaccumulation coefficient of 8000 in *C. marshallae* and 2000 in *M. okhotensis*, a difference they attributed to lipid content differences between these two species.

Three studies compare the effects of oil compounds on the less lipid rich, sub-Arctic *C. finmarchicus* and the more lipid rich, true Arctic *C. glacialis* (Jensen et al. 2008, Hansen et al. 2011, Hjorth & Nielsen 2011). Hansen et al. (2011) compare the mortality in these *Calanus* species exposed to water accommodated fraction (WAF) of crude oil and found higher LC_{50} value for *C. glacialis*. Within each species, an earlier death of the specimens with the lowest lipid contents was observed (Hansen et al. 2011). Both Jensen et al. (2008) and Hjorth & Nielsen (2011) compare the feeding and reproduction in the two *Calanus* species under exposure to pyrene. While only a few sub-lethal responses of *C. glacialis* were observed, the effects were more pronounced in *C. finmarchicus* (Jensen et al. 2008, Hjorth & Nielsen 2011). In addition, *C. finmarchicus* are more affected by the combination of warmer water and pyrene exposure (Hjorth & Nielsen 2011). An effect study on the small harpacticoid copepod *Microsetella* spp. use the same nominal concentrations of pyrene as in the two *Calanus* studies (Hjorth & Dahllöf 2008). Effects of the pyrene exposure are seen at lower concentrations in *Microsetella* spp. compared to effects recorded in the larger calanoid species. This implies more severe effects on the pelagic food web in late summer, when *Calanus* are descending and smaller species like *Microsetella* spp. become the dominating copepods found in the pelagic food web (Hjorth & Dahllöf 2008).

Table 4: Peer-reviewed studies on the reproduction of pelagic copepod species exposed to various PAHs.

	PAH	Experiment/ In situ	Method	Effect	Reference
Eurytemora affinis	-WSF of heating oil	-Experiment -240 minute exposures	-Egg production	-Exposure to 3000 µg l^{-1} for more than 120 minutes the life time egg production was significantly reduced	(Berdugo et al. 1977)
Eurytemora affinis	-Naphthalene -2-methylnaphthalene -2,6-dimethyl-naphthalene -2,3,5-trimethylnaphthalene	-Experiment -Chronic exposure to 10 µg l^{-1}	-Egg production -Brood size -Length of life	Effect of each exposure: -Egg production – significantly reduced -Brood size – significantly reduced -Length of life – significantly reduced	(Ott et al. 1978)
Centropages hamatus	-crude oil/seawater dispersions 10, 20 and 80 ppb	-Experiment -48-64 h	-Egg production -Egg hatching	-Egg production unaffected by exposures -Egg hatching decrease due to exposures	(Cowles & Remillard 1983)
-*Eurytemora affinis* Nauplii	-Benzo(*a*)pyrene	-Experiment -Chronic exposure to NOEC	-Life length	-Did not moult into copepodites	(Forget-Leray et al. 2005)
Acartia tonsa	-Fluoranthene -Phenanthrene -Pyrene	-Experiment -48 h exposures	-Egg production	-Fluoranthene EC$_{50}$=433 nM -Phenanthrene EC$_{50}$=1,245 nM -Pyrene EC$_{50}$=306 nM	(Bellas & Thor 2007)
Paracartia grani	-Naphthalene -1,2-dimethyl--naphthalene	-Experiment -48 h exposures	-Egg production	-Naphthalene EC$_{50}$: 2096 (95% C.I.:1640–2552) µg l^{-1} -1,2-dimethylnaphthalene EC$_{50}$: 254 (95% C.I.:195–313) µg l^{-1}	(Calbet et al. 2007)
Calanus glacialis	-Pyrene; 0 (solvent only), 0.01, 0.1, 1, 10 and 100nM (nominal values)	-Experiment -9 day exposures	-Cumulative specific egg production (SEP)	-No effect	(Jensen et al. 2008)
Calanus finmarchicus	-Pyrene; 0 (solvent only), 0.01, 0.1, 1, 10 and 100nM (nominal values)	-Experiment -9 day exposures	- Cumulative specific egg production (SEP)	-Significantly lower SEP on day 8 and 9 in 100 nM exposure.	(Jensen et al. 2008)

Table 4 continued

Calanus finmarchicus	-Pyrene; 0 (solvent only), 0.01, 0.1, 1, 10 and 100nM (nominal values)	-Experiment -9 day exposures	-Cumulative specific egg production (SEP) -Hatching success	-Reduced SEP at exposures > 1 nM -No effect of pyrene exposure on hatching success	(Hjorth & Nielsen 2011)
Calanus glacialis	-Pyrene; 0 (solvent only), 0.01, 0.1, 1, 10 and 100nM (nominal values)	-Experiment -7 day exposures	-Cumulative specific egg production (SEP) -Hatching success	-At 0.5 °C SEP reduced by pyrene, except for 100 nM. -At 5 and 8 °C SEP induced by pyrene at low concentrations -No effect of pyrene exposure on hatching success	(Hjorth & Nielsen 2011)

1.6.3 Biological responses – gaps of knowledge

Arctic dwelling biota is adapted to variable environmental conditions such as light availability, nutrients, presence/absence of sea ice, and food availability throughout the year. Arctic species have longer life spans compared to their southern sibling species (Maclean 1973, Koszteyn et al. 1995), and thus have a longer time to accumulate contaminants in their environment. In addition, lipid contents for Arctic species are often high which allows organisms to survive long winters without food (Lee 1974, Falk-Petersen et al. 1990). However the high lipid contents of Arctic species facilitates storage of lipophilic contaminants in the lipid sac of organisms. These characteristics, concerning Arctic species' adaptations to their environment, have led to more emphasis on comparing responses of Arctic and non-Arctic dwelling organisms to petroleum hydrocarbons. This is a relatively new and important research field given the recent increase in the interest of exploration, extraction, and transport of petroleum reserves in this unique region of the world. Hence, many knowledge gaps exist with this thesis addressing only a select few of the research challenges in this field and adding to the small but growing available scientific information on Arctic ecotoxicology.

Calanus species are of interest as test organisms in ecotoxicology experimental studies addressing species' sensitivities to petroleum pollutants. This is because the *Calanus* species complex consists of sibling species with similar ecological niches but unique adaptations to their individual habitats (see also section 1.3.1). To use copepods such as *Calanus* species as test organisms, they must be easily cultured and maintained in laboratory settings. Temperate copepod species such as *Acartia tonsa, Temora longicornis* and *Pseudocalanus* spp. (Klein Breteler 1980, Klein Breteler et al. 1982, Støttrup et al. 1986), have been used in this way since the 1980's but it is only within the last decade that *Calanus* spp. began to be viable as test species (Campbell et al. 2001a, Hansen et al. 2007). This advance required establishing appropriate laboratory routines for *Calanus*.

For longer lived organisms such as *Calanus* spp., mortality is a practical end point for laboratory experiments performed to assess individual level effects resulting from exposure to short lived volatile hydrocarbons. One of the expected dissimilarities between oil spills in Arctic vs. temperate areas is the increased and prolonged exposure times by organisms to the most volatile fraction of petroleum. This is due to lower evaporation rates in colder areas. However, even under Arctic conditions volatile hydrocarbons will only persist in an area for a

short time. Today, data on the mortality of pelagic copepods due to the volatile hydrocarbon compounds is almost non-existent (Table 2).

Weathering processes for oil are also slower in the Arctic leading to increased exposure to oil over time for Arctic organisms. In the event of an oil spill, the more persistent PAHs remain longer in the environment leading to increased exposure to these more lipophilic compounds by organisms. To produce realistic exposure conditions in long-term laboratory experiments techniques to mimic weathering are needed (Carls et al. 1999, Camus & Olsen 2008). There is little information on effects of long-term, sub-lethal exposure to hydrocarbons by *Calanus* as well as most Arctic species. Possible effects are suppressed feeding and lower reproduction capability which may alter the population dynamics of *Calanus* and thereby the competition capacity of a given species towards other species. To evaluate to what extent a petroleum spill may affect areas where habitats of sibling species overlap, experiments on the different *Calanus* sibling species are therefore needed.

Biological responses are not a function of hydrocarbon concentrations in the sea water surrounding an organism but are the result of the accumulated amount of hydrocarbons in biota. In lipid rich organisms a part of the accumulated hydrocarbons will be bound in lipids and will not cause adverse effects until their release during lipid mobilisation. This makes the prediction of harmful concentrations of PAHs more difficult for the Arctic. The various PAHs have specific affinities for lipids and may accumulate to different equilibrium concentrations in biota dependent on lipid concentration and ability of the species to metabolize the PAHs. In addition, uptake rates of PAHs may differ. The accumulation of PAHs in pelagic copepods has been examined before, but the uptake kinetics of PAHs has not previously been examined (Table 1). To improve predictions of the effects of petroleum exposure to northern residing copepods, better information on the uptake kinetics of different hydrocarbons must become available.

2 Main objectives of dissertation

The main goal of this dissertation is to provide data on effects of petroleum discharges on Arctic and sub-Arctic *Calanus* species to support the development of Arctic risk assessment by the petroleum industry. Currently there are only limited knowledge on the sensitivity of Arctic species towards petroleum discharges but it is hypothesized that Arctic and temperate species will differ in sensitivity due to differences in their life cycle. Thus, extensive knowledge on Arctic species vulnerability is needed before ERA can be developed and applied in the Arctic. *C. finmarchicus* and *C. glacialis* are key copepod species in Sub-arctic and Arctic water masses, respectively. The experimental studies performed as part of this dissertation provide needed knowledge to assess species' responses and effects of petroleum compounds. The following specific objectives are addressed:

Objective 1: Evaluate if *C. finmarchicus* is a species suited for culturing and if it is possible to manipulate egg production in culture by food quality and quantity (**Paper I**)

Objective 2: Provide baseline mortality data on Arctic algae and copepod species based on short term exposures to volatile petroleum components (**Paper II**)

Objective 3: Assess the impact of long-term exposure to the water soluble fraction of crude oil on feeding and egg production of *Calanus* species (**Paper III**)

Objective 4: Examine bioaccumulation in *C. finmarchicus* for two selected PAHs (**Paper IV**)
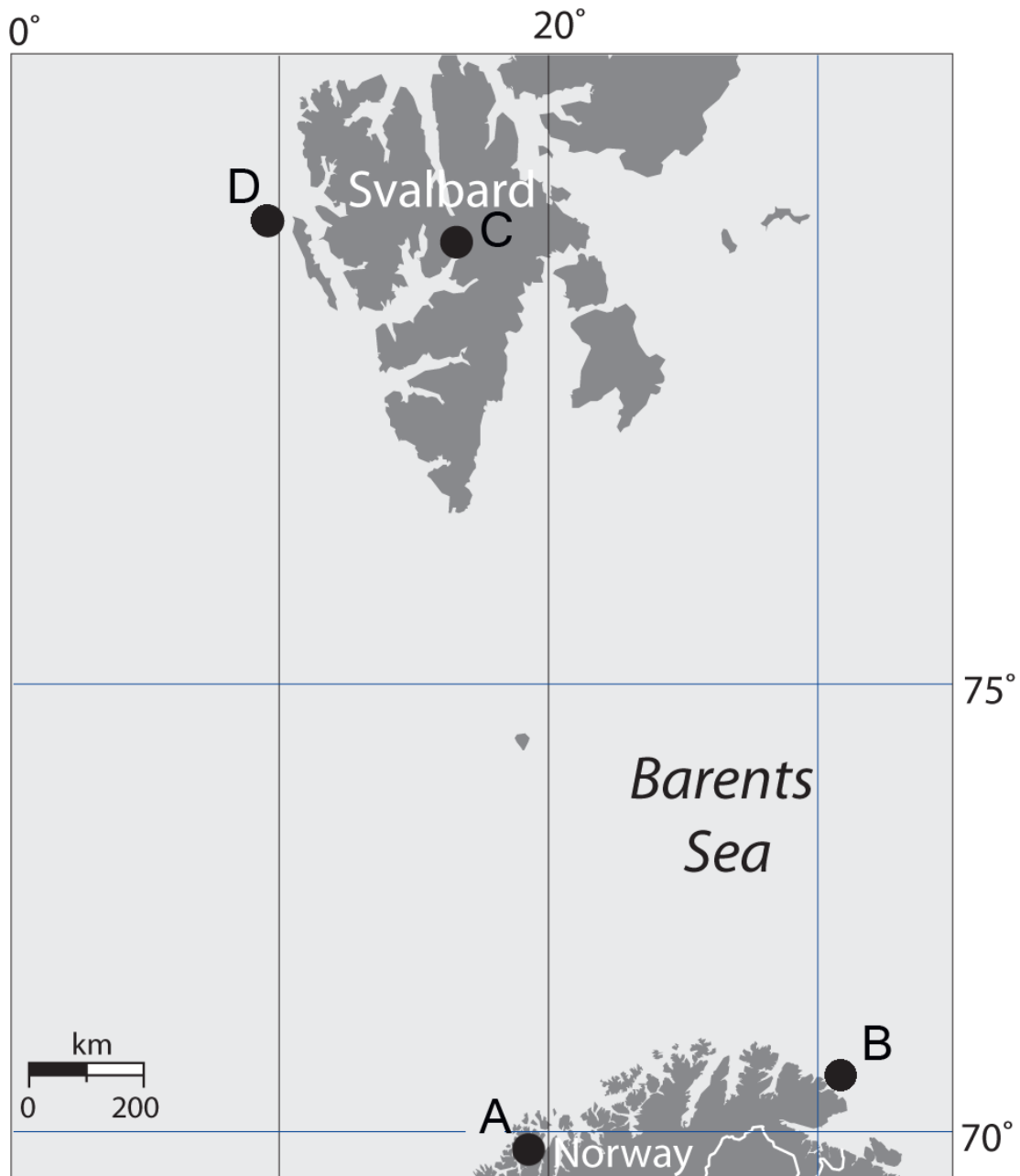
3 Methods and main results

Figure 4: Map showing all sampling areas. A: Vicinity of Tromsø, *Calanus finmarchicus* sampled for paper I, II and IV. B: Syltefjorden, *C. finmarchicus* sampled for paper III. C: Billefjorden, *C. glacialis* sampled for paper II and III. D: Prins Karls Forland, *Fragilariopsis oceanica* sampled for paper II.

3.1 Culturing *Calanus* – Paper I

3.1.1 Material and methods

A culturing system for *Calanus finmarchicus* was designed and tested for a full generation (adults-adults) to demonstrate that healthy specimens of *Calanus* can be maintained in the laboratory. A multi-stage culturing system is needed to perform long-term laboratory exposure experiments. As part of this work, feeding experiments were performed to optimize *Calanus* egg production as a function of feeding conditions. Specimens were collected in the vicinity of Tromsø, Northern Norway, just before their spring ascent from deep to shallow waters (March 2004). The life stage distribution of specimens was 75 % adults and 25 % copepodite stage V (CV). The specimens were transferred to 100 l containers; spawned eggs were collected and transferred to new containers for hatching. The generation development was monitored every third week by aliquot sampling and microscopic determination of specimen numbers and stage composition.

Figure 5: Egg production chambers used in experiments in Paper I and III. Left panel shows a vertical view of the two beakers stacked within each other with a lid on top. Right panel shows the inner cup lifted up showing the plankton netting used to separate the females from produced eggs and fecal pellets.

In two separate experiments egg production in *C. finmarchicus* was assessed as a function of food quality and quantity. Both experiments were run in single female egg production beakers (on Figure 5). The set-up allowed for daily inspection of egg and fecal pellet production. The first experiment contrasted the egg production of females fed *Chaetoceros socialis* to females fed *Thalassiosira weisflogii* at three different food concentrations. The second experiment

tested differences in egg production and feeding in females fed steadily on a medium concentration diet against a group that experienced a shift from a low to high concentration diet.

3.1.2 Main results

The culture was maintained in a cold room under controlled light and temperature conditions. The light and temperature regime in the cold room was adjusted continuously to resemble the natural yearly cycle for the Tromsø region. *Calanus finmarchicus* was successfully maintained for one generation. The median generation time (time at which 50% reached adulthood) was on average 105 days (See Figure 6). This generation time is within the 91-128 day range derived in a mesocosm study performed near Tromsø (Hansen et al. 2003).
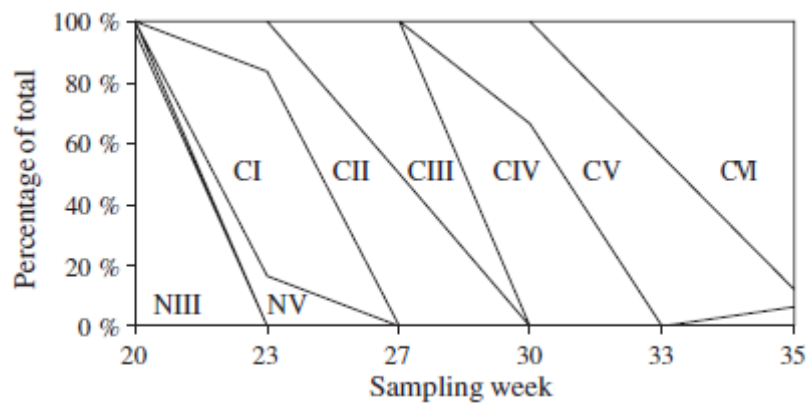
Figure 6: As an example of an average development of a generation of *Calanus finmarchicus* the development of eggs collected in week 18 through nauplius (III-V) and copepodites (I-VI) stages are shown.

An increase in food concentration from 10 to 80 µg C l^{-1} did not alter the egg production in *C. finmarchicus*. Both algae species, *Chaetoceros socialis* and *Thalassiosira weisflogii,* were equally suitable as food items in relation to egg production by *C. finmarchicus.* If food concentrations remain low, both hatching success and egg production will be negatively impacted. Hatching success may decrease and egg production may cease. With the reintroduction of higher food concentrations, egg production may be reinitiated with a corresponding increase in hatching success after some days.

3.2 Growth inhibition and mortality under xylene exposure – Paper II

3.2.1 Material and methods

A series of short term exposure experiments with xylene were conducted on the Arctic diatom species *Fragilariopsis oceanic* and two *Calanus* species; *C. finmarchicus* and *C. glacialis*. The algae experiment was run for 96 h in 250 ml Nunc © bottles at 3.2 °C with algae in an exponential growth phase. Throughout the experiment, algae cell numbers were determined in subsamples taken every 24 h in each of 6 treatments (*n=3*). The end point was growth inhibition.

The copepod experiments were run in 100 ml polypropylene containers with 10 specimens in each container. Each exposure concentration was run in five replicates for a total of 96 h; mortality was recorded every 24 h by visual inspection in each container. The experiments were conducted at 2 °C for both *C. glacialis* and *C. finmarchicus.* The end point in the copepod experiments was mortality.

For all three experiments, water samples were collected at time 0 and 96 h for determination of xylene concentrations. These samples were analysed by headspace extraction, with detection and quantification performed by GC/MSD by ALS Scandinavia.

3.2.2 Main results

The growth of *Fragilaria oceanica* was inhibited after 24 h in treatments $\geq$ 2.8 mg l^{-1}, and in all treatments > 1.4 mg l^{-1} after 48 h (1 way ANOVA followed by Tukey post hoc test, p < 0.05) (Paper II, Figure 7).
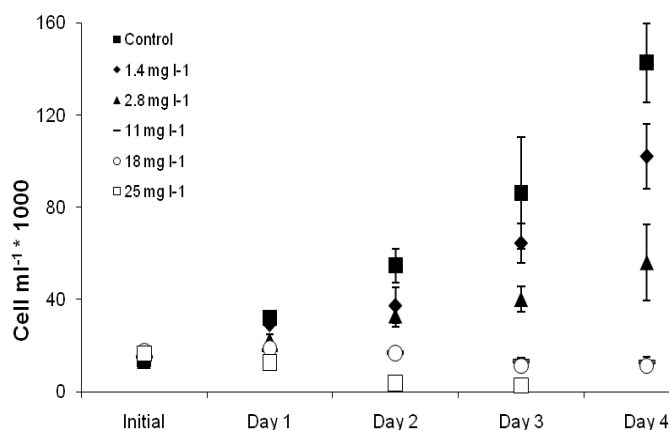
Figure 7: Cell count of *Fragilariopsis oceanica* on four successive days at 6 different concentrations of xylene (average ± S.D., n=3).

Figure 8: The percentage mortality of *Calanus finmarchicus* at different xylene concentrations accessed each day (average ± S.D., n=5).
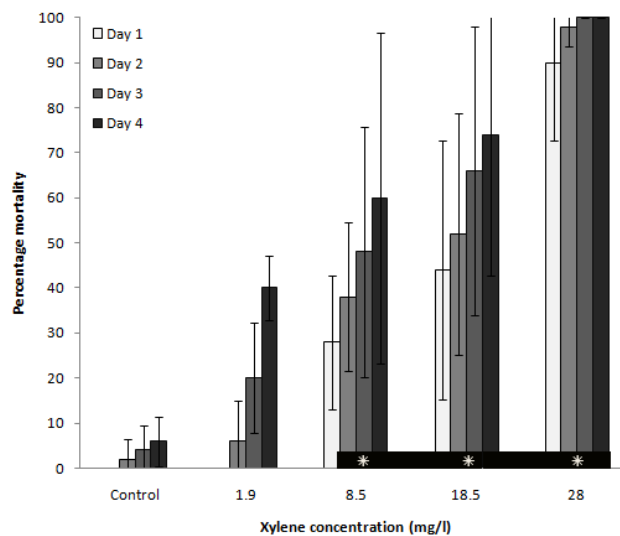
Calanus finmarchicus had a significantly higher mortality after 24 h when exposed to ≥ 18.5 mg l⁻¹ xylene, and after 48 h exposure to 8.5 mg l⁻¹ xylene also caused significantly higher mortality (1 way ANOVA followed by Tukey post hoc test, $p < 0.05$) (Paper II, Figure 8). *C. glacialis* had a significantly higher mortality at exposures ≥ 20 mg l⁻¹ for all time points (1 way ANOVA followed by Tukey post hoc test, $p < 0.05$) (Paper II, Figure 9).
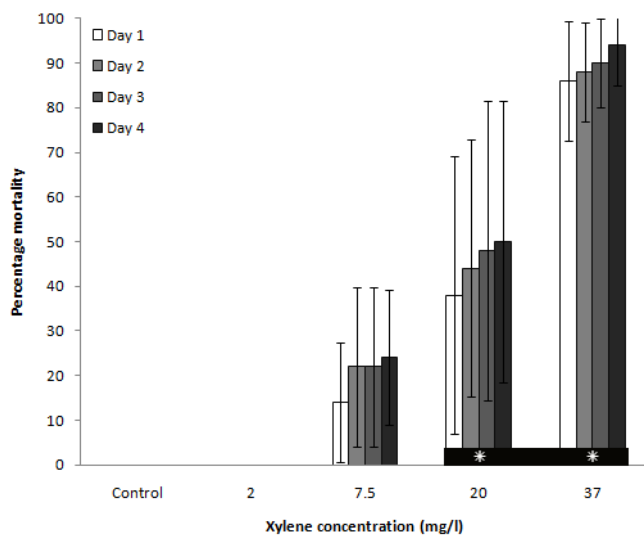
Figure 9: The percentage mortality of *Calanus glacialis* at different xylene concentrations accessed each day (average ± S.D., n=5).

29

These findings indicate that Arctic algae are more sensitive to xylene exposure compared to the tested copepod species. *C. finmarchicus* was more sensitive than *C. glacialis* to xylene. These results provide insight into the possible effects of xylene during an oil spill.

3.3 Effect of Water Soluble Fraction of crude oil on *Calanus* spp. – Paper III

In paper III, a flow-through exposure system was employed to separate the Water Soluble Fraction (WSF) from crude oil. The system is a modification (downscaling) of a system constructed to mimic the Exxon Valdez oil spill in an experimental setting (Carls et al. 1999). The system is well established through numerous applications both in Alaska and Norway (e.g. Carls et al. 1999, Duesterloh et al. 2002, Camus & Olsen 2008, Olsen et al. 2008, Nahrgang et al. 2010). Different concentrations of North Sea crude oil WSF were prepared by pumping filtered sea water through columns containing oil covered glass beads. The oil had been weathered to reduce the most soluble aromatics and PAHs. However, initially, the concentration of the lightest and most soluble PAHs was high in the filtered seawater passed through the columns. Over time, the concentration of the most soluble fraction decreased leading to a relatively higher concentration of the less soluble PAHs. Thus both the concentrations and relative composition of PAHs varied throughout the experiment. For this reason, samples for PAH quantification were taken at various times.

3.3.1 The effect of WSF on feeding and egg production in *Calanus glacialis*

Longer term exposure to sub-lethal doses of PAHs may have adverse effects on the feeding and egg production of copepods. This was examined in paper III where females of *Calanus glacialis* were exposed to water soluble fraction (WSF) of North Sea crude oil for 11 days. Feeding and egg production was monitored on a daily basis.

Specimens of *C. glacialis* were caught in Billefjorden, Spitsbergen, and 20 replicates consisting of single females were prepared for each of three exposure concentrations (CT: control treatment (0 µg PAHs l^{-1}), LT: low treatment (3.6 µg PAHs l^{-1}) and HT: high treatment (10.4 µg PAHs l^{-1})). Exposures were run in individual female egg production chambers. These were constructed by two stacked polypropylene beakers where the bottom of the inner beaker consists of plankton netting (300 µm) see Fig 5. The plankton netting separates females from fecal pellets and eggs which sink through the netting. Each day fresh test solution from the flow through system was mixed with the diatom prey species

Thalassiosira sp. to achieve a final cell concentration of 4000 cells ml^{-1}. Test solution (100 ml) was added empty outer beakers of the egg production chambers and copepods were moved to the new chambers by moving the inner beaker. Eggs and fecal pellets were collected by sieving the spend test solution onto plankton netting (80 µm) and enumerated under the microscope. During the first two days, eggs were transferred to uncontaminated water and incubated for 5 days to count hatched nauplii and determine hatching success.

3.3.2 Main results for *Calanus glacialis*

The females exposed to the highest concentration of WSF of crude oil had a lower cumulative egg production and lower cumulative feeding over the 11 day experiment, but the differences were not significant (Paper III). The hatching success of eggs laid by females exposed to the highest WSF concentration was significantly lower compared to the other two treatments (Paper III, Figure 10). Thus, we found no effects of the WSF exposure on the adult specimens of *Calanus glacialis*, but a lower hatching success may have implications for the population development.
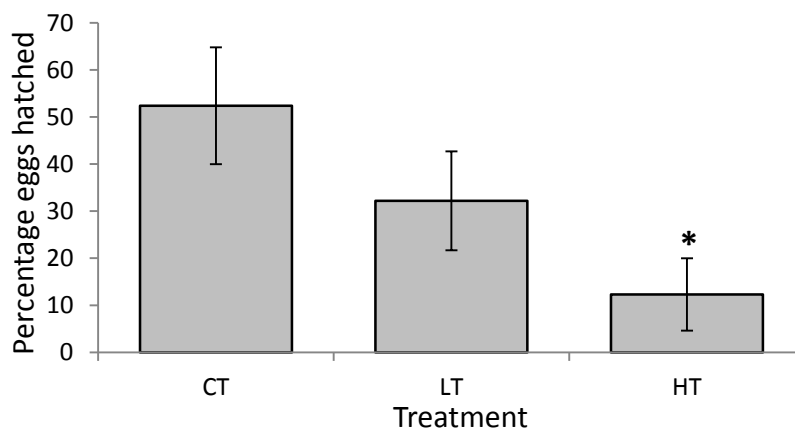
Figure 10: The hatching success of eggs from *Calanus glacialis* females exposed to WSF of crude oil. CT: control treatment (0 µg PAHs l^{-1}), LT: low treatment (3.6 µg PAHs l^{-1}) and HT: high treatment (10.4 µg PAHs l^{-1}). *Significant difference from control ($p < 0.05$).

3.3.3 The effect of WSF exposure on feeding by *Calanus finmarchicus*

Calanus finmarchicus accumulate lipids during summer and descend to deeper waters to overwinter. To examine if *C. finmarchicus* may be affected by long term exposure to WSF of

crude oil, stages CV copepodites were exposed to WSF for 11 and 18 days in a flow through system, and feeding was assessed. The experiment is described in paper III.

Specimens of *C. finmarchicus* were caught near Syltefjorden, Finnmark (See Figure 4). The described flow-through system was constructed as a continuous flow-through system by connection to 1 l blue cap bottles containing copepods. Three different concentrations were produced by pumping filtered water through different oil containing cylinders by means of a peristaltic pump (flow rate = 1.7 ml min^{-1}). The system was kept in a cold room at 5.5 °C. The copepods were kept undisturbed and not fed for 11 and 18 days. After exposures, 6 specimens from each treatment were moved to individual 300 ml amber bottles with known algae concentrations and incubated for 24 h. Feeding was assessed by algae enumeration of initial, control (without copepods) and copepod containing bottles.

3.3.4 Main results for *Calanus finmarchicus*

A decrease in feeding, revealed as differences in algae concentrations after 24 h incubation and fecal pellets produced by the incubated *C. finmarchicus*, was found for specimens exposed to the highest concentration of WSF, but the difference between treatments was not significant (Paper III, Figure 11).

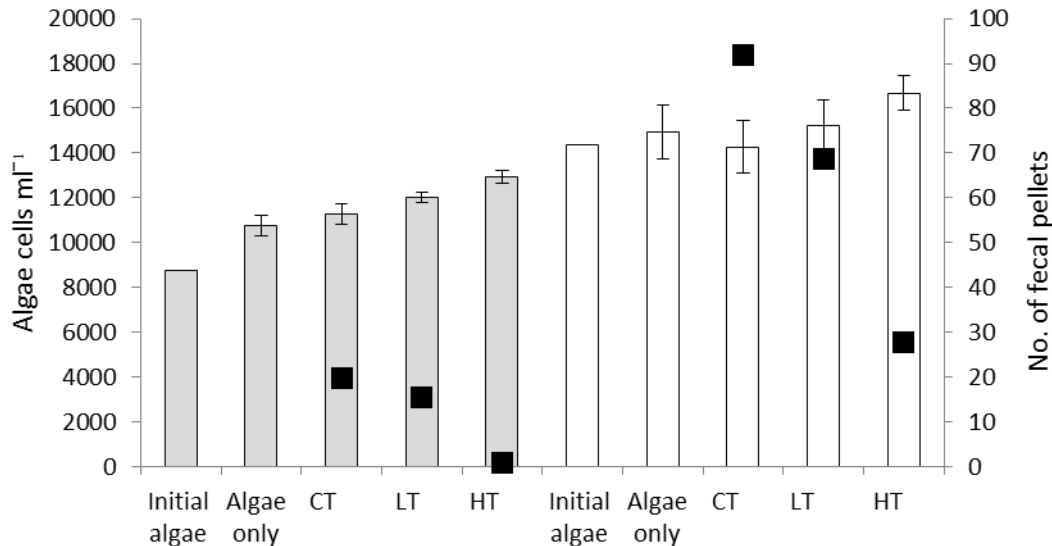Figure 11: Left panel, grey bars: 11 day exposure study. Right panel, white bars: 18 day exposure study. The bars are initial algae counts and algae counts after 24 h (grey) and 72 h (white) (Mean ± SE). Black squares: mean number of fecal pellets produced in each treatment. CT: control treatment (0 µg PAHs l^{-1}), LT: low treatment (3.4 µg PAHs l^{-1}) and HT: high treatment (7.0 µg PAHs l^{-1}).

3.4 Bioaccumulation of selected PAHs – Paper IV

3.4.1 Material and methods

The accumulation of PAHs differs among species and as a function of the molecular size of individual PAH compounds. Paper IV examines how the accumulation of two PAHs, phenanthrene and benzo[a]pyrene (B[a]P), differ in *Calanus finmarchicus.*

Stage CV *C. finmarchicus* collected in the vicinity of Tromsø was exposed to ^{14}C labelled phenanthrene or B[a]P in a semi static experiment run in glass beakers. The water was renewed daily and exposures lasted 8 days (192 h) followed by a depuration period in uncontaminated water for 4 days (96 h). Sampling of animals was done at times 0, 6, 12, 24, 96, 192 h (exposure) and further at 198, 204, 216 and 288 h (depuration). Water samples were collected every sixth hour on day 1 and 8, otherwise once every day. In the phenanthrene experiment, n=5 and the B[a]P experiment was replicate 4 times.

Accumulated ^{14}C and water concentrations were counted on a liquid scintillation counter and copepod (µg g^{-1}_{lipid}) and water (µg l^{-1}) concentration of phenanthrene and B[a]P were calculated from the measured disintegration per minute (DPM) using the specific activity of the compounds.

To compare uptake kinetics of phenanthrene and B[a]P, the lipid based concentrations of PAHs were fitted to a first order kinetic model (Landrum et al. 1992b);

$$C_a = \frac{k_u\, C_w}{k_e}\,(1 - e^{-k_e t})$$

where C_a is the concentration of PAH in the copepods (µg g_{lipid}^{-1}), k_u is the conditional uptake clearance rate (ml g^{-1} h^{-1}), k_e is the conditional depuration rate (h^{-1}), C_w is the concentration of PAH in water (µg ml^{-1}) and t is the time in (h).

Bioaccumulation Factors (BCF) at 192 h, i.e. when accumulation was terminated, was calculated as $BCF_{192h}= C_{a(192h)}/C_w$ and BCF at steady state were calculated based on the model derived k_u and k_e values, eg. $BCF_{ku/ke} = k_u /k_e$.
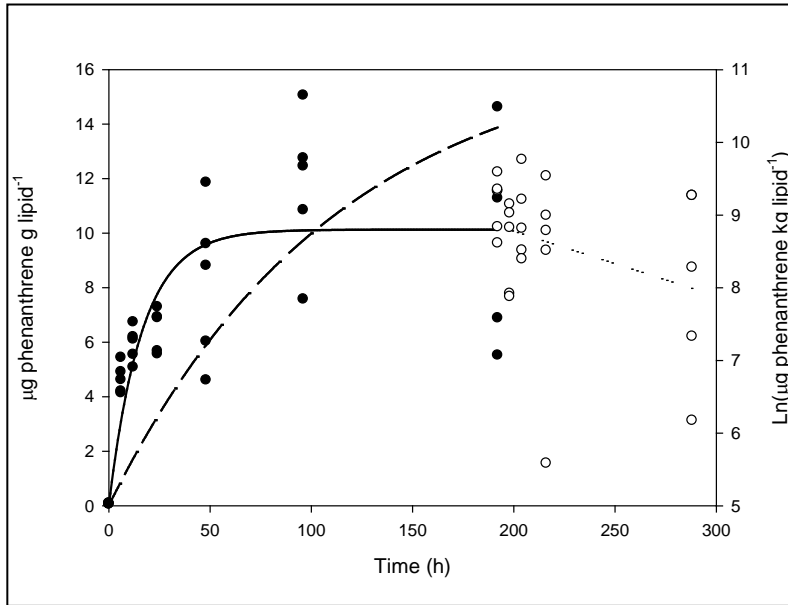
Figure 12: Lipid normalized accumulation of phenanthrene (^{14}C equivalents) in *Calanus finmarchicus.* Solid circles are the measured accumulation over a 192 h experiment (*n*=5) and solid line represents the toxicokinetics model run. Dashed line show the toxicokinetics model run based on the measured depuration rate. Open circles refer to right y-axis and are concentration of phenanthrene in *C. finmarchicus* during depuration (ln(µg phenanthrene kg lipid^{-1})). Dashed & dot line is the linear regression (r^2=0.1) of the depuration data. Please note that the y-axis differ between Figure 12 and 13.

Figure 13: Lipid normalized accumulation of benzo[a]pyrene (^{14}C equivalents) in *Calanus finmarchicus.* Solid circles are the measured accumulation over a 192 h experiment (*n*=4) and solid line represents the toxicokinetics model run. Dashed line show the toxicokinetics model run based on the measured depuration rate. Open circles refer to right y-axis and are concentration of B[a]P in *C. finmarchicus* during depuration (ln(µg B[a]P kg lipid^{-1})). Dashed & dot line is the linear regression (r^2=0.68) of the depuration data. Please note that the y-axis differ between Figure 12 and 13.
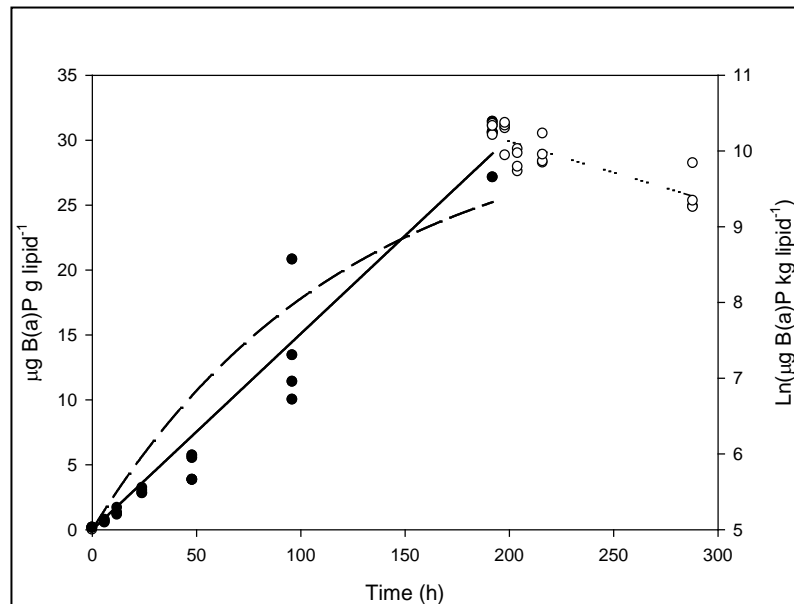
3.4.2 Main results

Accumulation of phenanthrene was fast and equilibrium was reached at 10 µg g_{lipid}^{-1} within the 192 h of exposure (Figure 12). Accumulation of B[a]P was slower, and equilibrium was not reached within the 192 h of exposure. Equilibrium was calculated to be 32 µg g_{lipid}^{-1} (Figure 13). Bioconcentration factor (BCF) was approximately 5,300 for phenanthrene, while the achieved BCF for B[a]P was approximately 43,000 (Table 2 in Paper IV). Log BCF values for both PAHs were lower than their respective log K_{ow} values (Paper IV).

4 Discussion

4.1 Culturing *Calanus*

When assessing the effects of long-term exposure to sub-lethal concentrations of crude oil it is necessary to maintain healthy test specimens over time. Often, tests on sub-lethal concentrations of contaminants are done at room temperature on copepod species with a short generation time, i.e. less than 20 days (Bengtsson 1978, Lotufo 1997). However, as mentioned earlier, results from tests at higher water temperatures performed on shorter lived copepod species may not be applicable to longer lived cold water species. Therefore the ability to perform long-term ecotoxicological testing on cold water species with alternative life history traits is a necessary research development (Breitholtz et al. 2006).

A literature review of culturing systems for pelagic copepods revealed the success of using static systems in large (100-200 l) containers (Klein Breteler 1980, Støttrup et al. 1986, Campbell et al. 2001a, Campbell et al. 2001b, Rey-Rassat et al. 2002). In Paper I, we showed that also for the sub-Arctic species *Calanus finmarchicus*, this system allowed for healthy specimens over a long time period. Concurrently with the development of our culturing system, a similarly designed continuous *C. finmarchicus* culture was initiated by a laboratory in Trondheim (Hansen et al. 2007). This culture is still running (Hansen et al. 2010).

The natural generation time of *C. finmarchicus* in waters around Tromsø is one year. These copepods exploit the seasonal phytoplankton production cycle for their development to stages IV and V and their accumulation of lipids. This period in their life cycle prepares them for the descent to deeper waters in June-August and survival during the winter period without food (Halvorsen & Tande 1999, Falk-Petersen et al. 2009). In the cultures, generation 1 developed further to adults and reproduced (Paper I). This indicates a flexible life strategy in *C. finmarchicus* and that overwintering may be avoided even at low food concentrations (9 µg C l^{-1}). The cultures of *C. finmarchicus* were successfully maintained on a mixed diet of *Chaetoceros socialis* and *Thalassiosira weisflogii* and additional experiments showed that these algae species were equally well suited as food items for *C. finmarchicus*. Manipulation of algae concentrations altered egg production and hatching success. It is therefore possible to postpone or increase egg production as needed to achieve larger quantities of test specimens for experiments.

The knowledge gained from Paper I has been applied in the execution of the ecotoxicology tests on *Calanus* spp. and we have been able to maintain healthy specimens with low natural mortality and sufficient egg production during exposure experiments.

4.2 Growth inhibition and mortality from xylene

At low concentrations of xylene *Calanus* spp. may not suffer from acute mortality but they will experience a decline in food availability (Paper II). The adverse effect of starvation will be more severe for younger compared to older stages (Campbell et al. 2001b) and the overall effect will depend on the timing of a decrease in food supply. When food limitation occurs during the egg production period, egg production will cease (Paper I, Hirche et al. 1997, Campbell et al. 2001b, Niehoff 2004). However, as xylene evaporates fast, the algae production and subsequent egg production will be resumed. Due to their larger lipid reserves, the later stages of *Calanus* are better suited to survive shorter time periods of low food availability; nauplii stages may thus be the most vulnerable to short term decreases in food availability. Examining the impact of starvation on *C. pacificus* nauplii stages, Lopez (1996) observed increased mortality and prolonged developmental time when these copepods were starved for short periods (< 24 h). The effects of sudden mortality events in the reproduction period on population development in *C. finmarchicus* and *C. glacialis* have been examined by using a population model (Skarðhamar et al. 2011). The model predicted an almost complete recovery of biomass in *C. finmarchicus* population during the next reproduction event. In contrast, *C. glacialis* were not able to fully compensate for lower overwintering population by increased reproduction (Skarðhamar et al. 2011). In conclusion, the long-term indirect effects of petroleum exposure may be more severe for the Arctic *C. glacialis*.

The direct effect of short term exposure to xylene was more severe for *C. finmarchicus* compared to *C. glacialis* (Paper II), a difference that may be due to the different lipid contents in the two species. The lipid content of *C. finmarchicus* in the xylene experiment was 30 % while *C. glacialis* contained 49 % lipids of dry weight (Paper II). As mentioned, the mode of action of xylene is by non-specific binding to the cell membranes, but a part of the hydrophobic chemicals may bind to storage lipids (Jandacek & Tso 2001). This means that a greater part of the xylene will accumulate in the storage lipids in *C. glacialis* with lower concentrations free to bind to cell membranes. Thus for *C. glacialis* larger concentrations of xylene will be needed to induce acute mortality. This is in accordance with the results of Hansen et al. (2011) who found mortality of *C. finmarchicus* and *C. glacialis* exposed to dispersed crude oil to vary with individual lipid contents.

Based on the mortality determined for *Calanus* spp. exposed to xylene in Paper II, LC_{50} values may be calculated. However, because the concentration of xylene decreased during the experiment (Paper II) LC_{50} values are upper limit estimates. For this reason, comparison with other LC_{50} values should be carried out with care. The 24 h LC_{50} values were 19.1 (95 % C.I. 15.5-23.6) mg l^{-1} for *C. glacialis* and 13.7 (95 % C.I. 11.8-15.8) mg l^{-1} for *C. finmarchicus*. These values correspond well with 24 h LC_{50} values (14.0 ppm) determined for Daggerblade Grass Shrimps (*Palaemonetes pugio*) (Tatem et al. 1978). However, compared to all LC_{50} values reported for other PAHs on copepods (see Table 2), the xylene LC_{50} values are high. This indicates that higher concentrations of xylene are needed to induce mortality in copepods, which is in line with the observation that PAHs with higher log K_{ow} values induce mortality at lower concentrations (Table 2, Barata et al. 2005, Bellas & Thor 2007).

4.3 The use of WSF vs model compounds in experiments

Choosing between exposure systems is not trivial, and all have their advantages and disadvantages. The advantages of using WSF as applied here is that it produces concentrations and compositions that are close to what may be expected following an oil spill. In long-term exposures, WSF will include additive effects of the various PAHs and specific toxic actions of the individual PAHs. The disadvantage is the lack of control of composition and concentration, which is to some extent controlled for by collecting samples for later analysis throughout the experiment. However, it is extremely difficult to reproduce exposure measurements when repeating WSF experiments and comparison of data between experiments must be carried out with caution.

Model compounds are either single compound or mixtures of PAHs prepared in the laboratory and used in exposure experiments. This is an alternative to WSF as it allows for better control of the composition and concentration of compounds used in exposure experiments. It provides valuable complimentary information on effects from mixtures of compounds compared to single compound effect studies and allows for easy comparison with similar experiments. However, caution should be taken when extrapolating from "effects of one or mixtures of PAHs" to "effects of crude oil." Barata et al. (2005) have demonstrated additive acute toxic effects of different PAHs, indicating that extrapolation from one PAH to combined toxic effects may be possible. However, possible carcinogenic, mutagenic or teratogenic effects will not be accounted for by this approach. In addition, control measurements of concentrations should always be included as nominal concentrations are seldom equivalent to actual concentrations.

4.4 Effect of WSF on *Calanus finmarchicus* and *Calanus glacialis*

Differences in lipid contents for copepods is the most likely explanation for observed differences in the effect of long-term exposure to petroleum compounds between *Calanus finmarchicus* and *C. glacialis* (Paper III, Jensen et al. 2008, Hjorth & Nielsen 2011). In Paper III which examined feeding and egg production of *C. glacialis* and feeding of *C. finmarchicus* under exposure to WSF, no direct comparison between the two species is made as two separate experiments were conducted. However, we can conclude that *C. finmarchicus* was more readily affected by WSF than *C. glacialis* (Paper III). These results are in line with the results of Jensen et al. (2008) and Hjorth & Nielsen (2011) who, in similar experiments with pyrene, examined changes in egg production and feeding in the two *Calanus* species. These studies also concluded that the more lipid rich *C. glacialis* was more resistant to pyrene exposure.

Many studies have examined copepods feeding efficiency after exposure to PAHs, generally concluding that feeding is reduced after exposure to PAHs (Table 3). *C. finmarchicus* had a significant reduction of cumulative specific fecal pellet production when exposed to 100 nM (20.23 µg l^{-1}) of pyrene (Jensen et al. 2008). Algae counts after 24 h incubations of *C. finmarchicus* previously exposed to 7.0 µg l^{-1} of WSF for 11 days gave indications of suppressed feeding (Paper III). However specimens exposed over a longer time period (18 days), did not show the same significant difference in algae number grazed between the control and exposure treatments (Paper III). For *C. glacialis*, Jensen et al. (2008) reported reduced feeding after nine days exposure to 10 nM (2.02 µg l^{-1}) pyrene, but not in specimens exposed to 100 nM. In Paper III, feeding by *C. glacialis* was not reduced by exposures to 3.6 and 10.4 µg l^{-1} WSF of crude oil. The inconsistency of effect concentrations among these studies, i.e. for *C. finmarchicus* in Paper III and *C. glacialis* in Jensen et al. (2008), may indicate that the concentrations applied here are at the threshold level of effects for these species. The concentrations applied in Paper III and Jensen et al. (2008) are low compared to most of the other studies reported in Table 3 where effects on feeding are induced by concentrations of WSF and naphthalenes at least 10 times higher. In addition most of the other studies are also restricted in time with exposures run for 2-5 days (Table 3).

The large scale distribution of *C. glacialis* and *C. finmarchicus* is to a large extent governed by temperature (Daase et al. 2007). The projected warming of the Arctic (ACIA 2004) may alter the distribution and relative abundance of *Calanus* species as has been recorded in the North Sea where the more southern species *C. helgolandicus* spread northward into areas

previously dominated by *C. finmarchicus* (Helaouet & Beaugrand 2007). In the Barents Sea an exchange of the more lipid rich *C. glacialis* to the leaner *C. finmarchicus* is expected

(Karnovsky et al. 2010, Slagstad et al. 2011). Such changes in copepod composition will have implications for plankton predators such as cod larvae (*Gadus morhua*) and little Auk (*Alle alle*) (Beaugrand et al. 2003, Karnovsky et al. 2010). In addition, it has implications for the response of the plankton community to petroleum discharges, as exposure to PAHs may have a more severe direct effect on a community based on *C. finmarchicus* (Paper II, III and Jensen et al. 2008).

4.5 Sub-lethal end point detection

Today, ERAs are based on measured LC_{50} values and calculated PEC/PNEC ratios i.e. short term exposures to lethal doses of contaminants. Paper III indicates that we may detect sub-lethal effects in *Calanus* spp. caused by long-term exposure to PAHs. Due to low temperatures and ice, weathering of oil in the Arctic is slow compared to temperate areas (Siron et al. 1993, Brandvik & Faksness 2009). Prolonged exposures to sub-lethal concentrations of PAHs have the potential to impact biota and ecosystems (Peterson et al. 2003). To fully evaluate the impact of crude oil on ecosystems one must expand on todays ERA approach to include longer term exposures and sub-lethal endpoints (National Research Council 2003, Peterson et al. 2003). Evaluation of sub-lethal endpoints is needed to provide insight into concentrations that may cause changes in population dynamics.

Paper III shows that feeding of *C. finmarchicus* may be affected by sub-lethal exposure to WSF of crude oil. Depending on the seasonal timing and length of exposure this may lead to measurable effects at the population level (Skarðhamar et al. 2011). Egg production and hatching success in *C. finmarchicus* rely partly on successful feeding of gravid females (Paper I, Niehoff 2004). Reduced feeding in the egg production period may thus lead to lower recruitment. However, population modelling indicates that *C. finmarchicus* fully compensates for population losses through the next reproduction season (Skarðhamar et al. 2011) and recruitment is further enhanced by advection from adjacent areas (Willis et al. 2006, Kosobokova & Hirche 2009). Thus, while the *C. finmarchicus* population within a particular area may be reduced due to sub-lethal exposure to petroleum compounds, recovery, facilitated by recruitment, is to be expected. Reduced hatching success as evident in *C. glacialis* (Paper III) may also cause a reduction in overall population size and the ability of *C. glacialis* to compensate for population loss within a year (i.e. by the next reproduction period) is more

limited (Skarðhamar et al. 2011). Thus, the long lasting effects of sub-lethal exposures may be more severe on the true Arctic *Calanus* species.

4.6 Bioaccumulation of selected PAHs

The long-term effects of lipophilic PAHs in biota depend on the bioaccumulation capacity and internal concentrations of the various PAHs. Accumulation of lipophilic compounds is particularly important for the lipid rich Arctic species. Future ERAs for Arctic areas should include measurements of bioaccumulation (Tamis et al. 2009).

In Paper IV the difference in the uptake and depuration kinetics, bioaccumulation and equilibrium concentrations of phenanthrene and B[a]P are described. The two PAHs differ in molecular weight (178.2 and 252.3 for phenanthrene and B[a]P, respectively) and log K_{ow} values (4.5 and 6.0 for phenanthrene and B[a]P, respectively).

The lipid based tissue levels of phenanthrene and B[a]P were plotted against time and the kinetic model of Landrum et al. (1992b) was used to fit to the data. Comparison of the uptake curves for phenanthrene and B[a]P reveal that the uptake of phenanthrene was fast and equilibrium concentration (10 µg g_{lipid}^{-1}) was reached within the 192 h exposure. The uptake of B[a]P was slower and equilibrium was not reached within the exposure time. The equilibrium concentration, calculated by means of the kinetic model, was 32 µg g_{lipid}^{-1} (Paper IV). Slower accumulation and longer time to reach equilibrium of compounds with higher log K_{ow} is also found in oligochaetes (Leppanen & Kukkonen 2000, Van Hoof et al. 2001, Ingersoll et al. 2003). This finding highlights the importance of knowledge on crude oil composition and exposure times if *in situ* measurements of PAH tissue levels are used in exposure assessments. Shortly after a spill event the tissue levels of higher log K_{ow} PAHs may be low and considered insignificant, while after prolonged exposures threshold levels may be reached with associated biological effects.

If copepods were merely a lipid pool where uptake and elimination of chemical compounds was governed by passive partitioning alone we would expect a positive linear 1:1 relationship between lipid based log BCF and log K_{ow} (Mackay 1982, Hoekstra et al. 2002). In our experiment we do find a positive relationship between log BCF and log K_{ow} as the log BCF of *Calanus finmarchicus* for B[a]P is higher than log BCF for phenanthrene (Table 2 in Paper IV). However, the log BCF values determined in Paper IV are lower than the corresponding log K_{ow} values. This deviation from the 1:1 relationship may be due to insufficient time to achieve equilibrium (Leppanen & Kukkonen 2000, Van Hoof et al. 2001, Ingersoll et al.

2003), active uptake via food items, (Magnusson et al. 2007, Magnusson & Tiselius 2010) or metabolism and excretion of compounds (Barron 1990).

For B[a]P, the experiment duration was not long enough for *C. finmarchicus* to reach equilibrium. However, the log BCFs calculated for B[a]P after 192 and at the model estimated equilibrium was equal (log BCF_{192} = log $BCF_{ku/kd}$ = 4.6) and thus does not reflect that a higher accumulation may be achieved after longer exposure time. Still, the model predicted equilibrium log BCF value for this compound (log $BCF_{ku/ke,}$) is lower than the corresponding log K_{ow} value for B[a]P and other factors besides duration of experiment must account for this discrepancy.

In a natural environment there are a number of pathways of contaminant accumulation in marine organisms. For some PCB congeners, food uptake has been identified as one of these with higher accumulations detected in fed compared to unfed specimens (Magnusson et al. 2007, Magnusson & Tiselius 2010). However, Landrum et al. (1992a) showed that feeding *reduced* the accumulation of the readily metabolized B[a]P but *increased* accumulation of hexachlorobiphenyl (HCB) which *Mysis relicta* are not able to metabolize. In addition, no difference in BCF values between fed and unfed copepods exposed to various PAHs was found (Berrojalbiz et al. 2009). Whether feeding would affect the accumulation of PAHs is uncertain but our accumulation estimates based on unfed specimens should be considered as baseline concentrations. Thus, the derived BCF values are realistic for overwintering *C. finmarchicus* in a non-feeding diapausing state. Repeated experiments on actively fed specimens would probably produce alternative BCF values.

Metabolism and excretion are important mechanisms governing the levels of PAHs in copepods (Harris et al. 1977, Berrojalbiz et al. 2009, Cailleaud et al. 2009). These specific processes were not examined in Paper IV, but it cannot be excluded that concurrent PAH metabolism and excretion provides an alternate explanation for the reported lower than expected BCF values for phenanthrene and B[a]P .

5 Main conclusions

- Healthy *Calanus finmarchicus* may be maintained in culture for multiple generations and egg production may be manipulated by food quantity (Paper I)

- Arctic algae was most sensitive to exposure to the volatile petroleum compound xylene while the smaller and leaner copepod species *C. finmarchicus* was more sensitive than the larger and more lipid rich *C. glacialis* (Paper II)

- Long-term exposure to sub-lethal doses of WSF of crude oil have an impact on egg hatching success of eggs from *C. glacialis* and suppresses feeding in *C. finmarchicus* (Paper III)

- Uptake kinetics and accumulation of phenanthrene and B[a]P differs in *C. finmarchicus*. The duration of exposure and composition of the oil will affect bioconcentration in copepods (Paper IV).

6 Further development of good environmental practices for the petroleum industry

Findings in this dissertation reveal the importance of indirect effects compared to direct effects from petroleum exposures. Thus, effects of petroleum discharges on natural populations are not fully assessed using classical dose-response measurements (LC_{50}) and individual based end points like feeding and egg production. Today population models may be applied to examine population development under various scenarios taking into consideration both direct and indirect effects of petroleum exposures (Skarðhamar et al. 2011, Stige et al. 2011) and models may provide useful knowledge for future ERA development (Forbes et al. 2010, Carroll & Smit 2011, De Laender et al. 2011).

Direct short-term effects of petroleum discharges will be more pronounced in *Calanus finmarchicus* compared to *C. glacialis* (Paper II). This contradicts the suggestion of Arctic species being more sensitive to petroleum discharges and thus, implies that ERA developed for temperate areas can be applied for Arctic areas. However, to control for differences in species sensitivity more research, including additional species, should be conducted. In addition, the direct effect of temperature on species sensitivity should be examined to reveal if e.g. the sub-Arctic species *C. finmarchicus* shows the same response for ambient temperatures characteristic for its southern limit of distribution.

The bioaccumulation experiment in Paper IV showed a difference in the uptake kinetics of two PAHs with a higher accumulation of the more lipophilic PAH. This difference in accumulation is in line with what is generally found in invertebrates. Compared to lighter PAH compounds, higher molecular weight PAHs accumulate at a slower rate, but to higher concentrations. In the Arctic environment, longer term exposures are expected due to slower natural degradation of oil. Thus, Arctic dwelling organisms may accumulate a larger proportion of the PAHs with carcinogenic, mutagenic and teratogenic properties. This should be taken into consideration when assessing the long-term risk of petroleum exposures.

The Barents Sea is considered unique due to the large standing stocks of commercial fish species. Moving the petroleum industry into a new area provides an opportunity to strengthen and further develop environmental risk assessment tools. Improved risk assessment tools combined with regulations of petroleum related operations in the Artic may allow for healthy management of the Arctic ecosystems. This will secure the benefit of future generations from the recreational and economical value of the Arctic.

7 References

ACIA (2004) Impacts of a Warming Arctic: Arctic Climate Impact Assessment, Cambridge University Press

AMAP (2007) Arctic oil and gas 2007, Arctic Monitoring and Assessment Programme, Oslo

Arnkvaern G, Daase M, Eiane K (2005) Dynamics of coexisting *Calanus finmarchicus*, *Calanus glacialis* and *Calanus hyperboreus* populations in a high-Arctic fjord. Polar Biology 28:528-538

Atlas RM (1981) Microbial degradation of petroleum hydrocarbons - An environmental perspective. Microbiological Reviews 45:180-209

Bambulyak A, Frantzen B (2009) Oil transport from the Russian part of the Barents Region. Status per January 2009 The Norwegian Barents Secretariat and Akvaplan-niva, Norway

Barata C, Calbet A, Saiz E, Ortiz L, Bayona JM (2005) Predicting single and mixture toxicity of petrogenic polycyclic aromatic hydrocarbons to the copepod *Oithona davisae*. Environmental Toxicology and Chemistry 24:2992-2999

Barron MG (1990) Bioconcentration. Will water-borne organic chemicals accumulate in aquatic animals? Environmental Science & Technology 24:1612-1618

Beaugrand G, Brander KM, Lindley JA, Souissi S, Reid PC (2003) Plankton effect on cod recruitment in the North Sea. Nature 426:661-664

Bellas J, Thor P (2007) Effects of selected PAHs on reproduction and survival of the calanoid copepod *Acartia tonsa*. Ecotoxicology 16:465-474

Bengtsson B-E (1978) Use of a harpacticoid copepod in toxicity tests. Marine Pollution Bulletin 9:238-241

Berdugo V, Harris RP, O'Hara SC (1977) The effect of petroleum hydrocarbons on reproduction of an estuarine planktonic copepod in laboratory cultures. Marine Pollution Bulletin 8:138-143

Berman MS, Heinle DR (1980) Modification of the feeding behavior of marine copepods by sub-lethal concentrations of water-accommodated fuel oil. Marine Biology 56:59-64

Berrojalbiz N, Lacorte S, Calbet A, Saiz E, Barata C, Dachs J (2009) Accumulation and Cycling of Polycyclic Aromatic Hydrocarbons in Zooplankton. Environmental Science & Technology 43:2295-2301

Billiard SM, Meyer JN, Wassenberg DM, Hodson PV, Di Giulio RT (2008) Nonadditive effects of PAHs on Early Vertebrate Development: mechanisms and implications for risk assessment. Toxicological Sciences 105:5-23

Bjørlykke K (2010) Petroleum geoscience: From sedimentary environments to rock physics, Springer Verlag, Berlin Heidelberg

Brandvik PJ, Faksness L-G (2009) Weathering processes in Arctic oil spills: Meso-scale experiments with different ice conditions. Cold Regions Science and Technology 55:160-166

Breitholtz M, Ruden C, Hansson SO, Bengtsson BE (2006) Ten challenges for improved ecotoxicological testing in environmental risk assessment. Ecotoxicology and Environmental Safety 63:324-335

Cailleaud K, Budzinski H, Le Menach K, Souissi S, Forget-Leray J (2009) Uptake and elimination of hydrophobic organic contaminants in estuarine copepods: an experimental study. Environmental Toxicology and Chemistry 28:239-246

Cailleaud K, Forget-Leray J, Souissi S, Hilde D, LeMenach K, Budzinski H (2007) Seasonal variations of hydrophobic organic contaminant concentrations in the water-column of the Seine Estuary and their transfer to a planktonic species *Eurytemora affinis* (Calanoida, copepoda). Part 1: PCBs and PAHs. Chemosphere 70:270-280

Calbet A, Saiz E, Barata C (2007) Lethal and sublethal effects of naphthalene and 1,2-dimethylnaphthalene on the marine copepod *Paracartia grani*. Marine Biology 151:195-204

Campbell R, Wagner M, Teegarden G, Boudreau C, Durbin E (2001a) Growth and development rates of the copepod *Calanus finmarchicus* reared in the laboratory. Marine Ecology Progress Series 221:161-183

Campbell RG, Runge JA, Durbin EG (2001b) Evidence for food limitation of *Calanus finmarchicus* production rates on the southern flank of Georges Bank during April 1997. Deep Sea Research Part II: Topical Studies in Oceanography 48:531-549

Camus L, Olsen GH (2008) Embryo aberrations in sea ice amphipod *Gammarus wilkitzkii* exposed to water soluble fraction of oil. Marine Environmental Research 66:223-224

Carls MG, Rice SD, Hose JE (1999) Sensitivity of fish embryos to weathered crude oil: Part I. Low-level exposure during incubation causes malformations, genetic damage, and mortality in larval Pacific herring (*Clupea pallasi*). Environmental Toxicology and Chemistry 18:481-493

Carls MG, Short JW, Payne J (2006) Accumulation of polycyclic aromatic hydrocarbons by *Neocalanus* copepods in Port Valdez, Alaska. Marine Pollution Bulletin 52:1480-1489

Carroll J, Smit MGD (2011) An integrated modeling framework for decision support in ecosystem-based management: case study Lofoten/Barents Sea. SPE International

Clements WH (2000) Integrating effects of contaminants across levels of biological organization: an overview. Journal of Aquatic Ecosystem Stress and Recovery (Formerly Journal of Aquatic Ecosystem Health) 7:113-116

Coelho E, Ferreira C, Almeida CMM (2008) Analysis of polynuclear aromatic hydrocarbons by SPME-GC-FID in environmental and tap waters. Journal of the Brazilian Chemical Society 19:1084-1097

Cowles TJ, Remillard JF (1983) Effects of exposure to sublethal concentrations of crude oil on the copepod *Centropages hamatus*. 1. Feeding and egg production. Marine Biology 78:45-51

Daase M, Vik JO, Bagøien E, Stenseth NC, Eiane K (2007) The influence of advection on *Calanus* near Svalbard: statistical relations between salinity, temperature and copepod abundance. Journal of Plankton Research 29:903-911

De Laender F, Olsen GH, Frost T, Grøsvik BE, Grung M, Hansen BH, Hendriks AJ, Hjorth M, Janssen CR, Klok C, Nordtug T, Smit M, Carroll J, Camus L (2011) Ecotoxicological Mechanisms and Models in an Impact Analysis Tool for Oil Spills. Journal of Toxicology and Environmental Health, Part A: Current Issues 74:605 - 619

Di Toro DM, McGrath JA (2000) Technical basis for narcotic chemicals and polycyclic aromatic hydrocarbon criteria. II. Mixtures and sediments. Environmental Toxicology and Chemistry 19:1971-1982

Di Toro DM, McGrath JA, Stubblefield WA (2007) Predicting the toxicity of neat and weathered crude oil: Toxic potential and the toxicity of saturated mixtures. Environmental Toxicology and Chemistry 26:24-36

Donkin P, Widdows J, Evans SV, Worrall CM, Carr M (1989) Quantitative structure-activity relationships for the effect of hydrophobic organic chemicals on rate of feeding by mussels (*Mytilus edulis*). Aquatic Toxicology 14:277-293

Duesterloh S, Short JW, Barron MG (2002) Photoenhanced toxicity of Weathered Alaska North Slope Crude Oil to the Calanoid Copepods *Calanus Marshallae* and *Metridia Okhotensis*. Environmental Science and Technology 36:3953-3959

EC (2003) Technical Guidance Document on Risk Assessment in support of Commission Directive 93/67/EEC on Risk Assessment for new notified substances Commission Regulation (EC) No 1488/94 on Risk Assessment for existing substances Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market., European Commission Joint Research Centre. EUR 20418 EN/2

EPA US (1979) Water-Related Enviromental Fate of 129 Piority Pollutants., Washington

Faksness L-G, Brandvik PJ (2008) Distribution of water soluble components from oil encapsulated in Arctic sea ice: Summary of three field seasons. Cold Regions Science and Technology 54:106-114

Falk-Petersen S, Hopkins C, Sargent J (1990) Trophic relationships in the pelagic, Arctic food web. In: Barnes M, Gibson R (eds) 24th European Marine Biology Symposium, p 315-333

Falk-Petersen S, Mayzaud P, Kattner G, Sargent J (2009) Lipids and life strategy of Arctic *Calanus*. Marine Biology Research 5:18-39

Fingas MF, Hollebone BP (2002) Review of behaviour of oil in freezing environments. Marine Pollution Bulletin 44:333-340

Forbes VE, Calow P, Grimm V, Hayashi T, Jager T, Palmqvist A, Pastorok R, Salvito D, Sibly R, Spromberg J, Stark J, Stillman RA (2010) Integrating population modeling into ecological risk assessment. Integrated Environmental Assessment and Management 6:191-193

Forbes VE, Calow P, Sibly RM (2008) The extrapolation problem and how population modeling can help. Environmental Toxicology and Chemistry 27:1987-1994

Forget-Leray J, Landriau I, Minier C, Leboulenger F (2005) Impact of endocrine toxicants on survival, development, and reproduction of the estuarine copepod *Eurytemora affinis* (Poppe). Ecotoxicology and Environmental Safety 60:288-294

Geyer HJ, Scheunert I, Bruggemann R, Matthies M, Steinberg CEW, Zitko V, Kettrup A, Garrison W (1994) The relevance of aquatic organisms lipid-content to the toxicity of lipophilic chemicals - toxicity of lindane to different fish species. Ecotoxicology and Environmental Safety 28:53-70

Gil L, King L, Adonis M (2000) Trends of polycyclic aromatic hydrocarbon levels and mutagenicity in Santiago's inhalable airborne particles in the period 1992-1996. Inhalation Toxicology 12:1185-1204

Halvorsen E, Tande KS (1999) Physical and biological factors influencing the seasonal variation in distribution of zooplankton across the shelf at Nordvestbanken, northern Norway, 1994. Sarsia 84:279-292

Hansen B, Marker T, Andreasen P, Arashkewich E, Carlotti F, Lindeque P, Tande KS, Wagner M (2003) Differences in life-cycle traits of *Calanus finmarchicus* originating from 60 ^{o}N and 69 ^{o}N, when reared in mesocosms at 69 ^{o}N. Marine Biology 142:877-893

Hansen BH, Altin D, Booth A, Vang S-H, Frenzel M, Sørheim KR, Brakstad OG, Størseth TR (2010) Molecular effects of diethanolamine exposure on *Calanus finmarchicus* (Crustacea: Copepoda). Aquatic Toxicology 99:212-222

Hansen BH, Altin D, Nordtug T, Olsen AJ (2007) Suppression subtractive hybridization library prepared from the copepod *Calanus finmarchicus* exposed to a sublethal mixture of environmental stressors. Comparative Biochemistry and Physiology Part D: Genomics and Proteomics 2:250-256

Hansen BH, Altin D, Rørvik SF, Øverjordet IB, Olsen AJ, Nordtug T (2011) Comparative study on acute effects of water accommodated fractions of an artificially weathered crude oil on *Calanus finmarchicus* and *Calanus glacialis* (Crustacea: Copepoda). Science of the Total Environment 409:704-709

Harris RP, Berdugo V, Ohara SCM, Corner EDS (1977) Accumulation of ^{14}C-1-naphthalene by an oceanic and an estuarine copepod during long-term exposure to low level concentrations. Marine Biology 42:187-195

Harvey RG (1991) Polycyclic aromatic hydrocarbons: chemistry and carcinogenicity. In. Cambridge University Press, Cambridge, p 396

Hebert R, Poulet SA (1980) Effect of modification of particle size of emulsions of venezuelan crude oil on feeding, survival and growth of marine zooplankton. Marine Environmental Research 4:121-134

Helaouet P, Beaugrand G (2007) Macroecology of *Calanus finmarchicus* and *C. helgolandicus* in the North Atlantic Ocean and adjacent seas. Marine Ecology Progress Series 345:147-165

Hirche H, Meyer U, Niehoff B (1997) Egg production of *Calanus finmarchicus*: Effect of temperature, food and season. Marine Biology 127:609-620

Hirche HJ (1997) Life cycle of the copepod *Calanus hyperboreus* in the Greenland sea. Marine Biology 128:607-618

Hjorth M, Dahllöf I (2008) A harpacticoid copepod *Microsetella* spp. from sub-Arctic coastal waters and its sensitivity towards the polyaromatic hydrocarbon pyrene. Polar Biology 31:1437-1443

Hjorth M, Nielsen TG (2011) Oil exposure in a warmer Arctic: potential impacts on key zooplankton species. Marine Biology 158:1339-1347

Hoekstra PF, O'Hara TM, Teixeira C, Backus S, Fisk AT, Muir DCG (2002) Spatial trends and bioaccumulation of organochlorine pollutants in marine zooplankton from the Alaskan and Canadian Arctic. Environmental Toxicology and Chemistry 21:575-583

Hylland K (2006) Polycyclic aromatic hydrocarbon (PAH) ecotoxicology in marine ecosystems. Journal of Toxicology and Environmental Health-Part a-Current Issues 69:109-123

Hylland K, Tollefsen K-E, Ruus A, Jonsson G, Sundt RC, Sanni S, Røe Utvik TI, Johnsen S, Nilssen I, Pinturier L, Balk L, Barsiene J, Marigòmez I, Feist SW, Børseth JF (2008) Water column monitoring near oil installations in the North Sea 2001-2004. Marine Pollution Bulletin 56:414-429

Ingersoll CG, Brunson EL, Wang N, Dwyer EJ, Ankley GT, Mount DR, Huckins J, Petty J, Landrum PE (2003) Uptake and depuration of nonionic organic contaminants from sediment by the oligochaete, *Lumbriculus variegatus*. Environmental Toxicology and Chemistry 22:872-885

Jandacek RJ, Tso P (2001) Factors affecting the storage and excretion of toxic lipophilic xenobiotics. Lipids 36:1289-1305

Jensen HV, Mullin JV (2003) MORICE - new technology for mechanical oil recovery in ice infested waters. Marine Pollution Bulletin 47:453-469

Jensen MH, Nielsen TG, Dahllöf I (2008) Effects of pyrene on grazing and reproduction of *Calanus finmarchicus* and *Calanus glacialis* from Disko Bay, West Greenland. Aquatic Toxicology 87:99-107

Karnovsky N, Harding A, Walkusz W, Kwasniewski S, Goszczko I, Wiktor J, Routti H, Bailey A, McFadden L, Brown Z, Beaugrand G, Gremillet D (2010) Foraging distributions of little auks *Alle alle* across the Greenland Sea: implications of present and future Arctic climate change. Marine Ecology-Progress Series 415:283-293

Klein Breteler WCM (1980) Continuous breeding of marine pelagic copepods in the presence of heterotrophic dinoflagellates. Marine Ecology Progress Series 2:229-233

Klein Breteler WCM, Fransz HG, Gonzalez SR (1982) Growth and development of four calanoid copepod species under experimental and natural conditions. Netherlands Journal of Sea Research 16:195-207

Kosobokova K, Hirche HJ (2009) Biomass of zooplankton in the eastern Arctic Ocean - A base line study. Progress in Oceanography 82:265-280

Koszteyn J, Timofeev S, Weslawski JM, Malinga B (1995) Size structure of *Themisto abyssorum* Boeck and *Themisto libellula* (Mandt) populations in european Arctic seas. Polar Biology 15:85-92

Landrum PF, Frez WA, Simmons MS (1992a) The effect of food consumption on the toxicokinetics of benzo(a)pyrene and 2,2',4,4',5,5'-hexachlorobiphenyl in *Mysis relicta*. Chemosphere 25:397-415

Landrum PF, Lee H, Lydy MJ (1992b) Toxicokinetics in aquatic systems - model comparisons and use in hazard assessment. Environmental Toxicology and Chemistry 11:1709-1725

Lassiter RR, Hallam TG (1990) Survival of the fattest - implications for acute effects of lipophilic chemicals on aquatic populations. Environmental Toxicology and Chemistry 9:585-595

Lee RF (1974) Lipid composition of copepod *Calanus hyperboreas* from Arctic ocean - changes with depth and season. Marine Biology 26:313-318

Leppanen MT, Kukkonen JVK (2000) Fate of sediment-associated pyrene and benzo(a)pyrene in the freshwater oligochaete *Lumbriculus variegatus* (Muller). Aquatic Toxicology 49:199-212

Loeng H, Drinkwater K (2007) An overview of the ecosystems of the Barents and Norwegian Seas and their response to climate variability. Deep-Sea Research Part II-Topical Studies in Oceanography 54:2478-2500

Lopez MDG (1996) Effect of starvation on development and survivorship of naupliar *Calanus pacificus* (Brodsky). Journal of Experimental Marine Biology and Ecology 203:133-146

Lotufo GR (1997) Toxicity of sediment-associated PAHs to a estuarine copepod: Effects on survival, feeding, reproduction and behavior. Marine Environmental Research 44:149-166

Macdonald C, Lockhart L, Gilman A, Baker T, Bakke T, Cantin D, Dam M, Davies I, Forbes BC, Hoydal K, Hylland K, Ikavalko P, Mararevich P, Meier S, Mosbeck A, Pawlak J, Peltonen K, Titov O, Zhilin A, MMBI, PINRO (2008) Effects of oil and gas activity on the environment and human health. In: Assessment of Oil & Gas Activities AMAP (www.amap.no), Oslo

Mackay D (1982) Correlation of bioconcentration factors. Environmental Science & Technology 16:274-278

Maclean SF (1973) Life cycle and growth energetics of Arctic crane fly *Pedicia hannai antenatta*. Oikos 24:436-443

Magnusson K, Magnusson M, Ostberg P, Granberg M, Tiselius P (2007) Bioaccumulation of C-14-PCB 101 and C-14-PBDE 99 in the marine planktonic copepod *Calanus finmarchicus* under different food regimes. Marine Environmental Research 63:67-81

Magnusson K, Tiselius P (2010) The importance of uptake from food for the bioaccumulation of PCB and PBDE in the marine planktonic copepod *Acartia clausi*. Aquatic Toxicology 98:374-380

May WE, Wasik SP, Freeman DH (1978) Determination of the aqueous solubility of polynuclear aromatic hydrocarbons by a coupled column liquid chromatographic technique. Analytical Chemistry 50:175-179

Miljøverndepartementet (2011) Oppdatering av forvaltningsplanen for det marine miljø i Barentshavet og havområdene utenfor Lofoten. St.Meld. 10. *In norwegian*

Nahrgang J, Camus L, Carls MG, Gonzalez P, Jönsson M, Taban IC, Bechmann RK, Christiansen JS, Hop H (2010) Biomarker responses in polar cod (*Boreogadus saida*) exposed to the water soluble fraction of crude oil. Aquatic Toxicology 97:234-242

National Research Council US (2003) Oil in the sea III: inputs, fates, and effects. In. National Academy of Sciences, Washington

Neff JM, Ostazeski S, Gardiner W, Stejskal I (2000) Effects of weathering on the toxicity of three offshore australian crude oils and a diesel fuel to marine animals. Environmental Toxicology and Chemistry 19:1809-1821

Niehoff B (2004) The Effect of Food Limitation on Gonad Development and Egg Production of the Planktonic Copepod *Calanus finmarchicus*. Journal of Experimental Marine Biology and Ecology 307:237-259

Nøttvedt A, Johannessen E (2008) The making of a land - geology of Norway. In: Ramberg I, Bryhni I, Nøttvedt A, Rangnes K (eds). Norsk Geologisk Forening, Trondheim

Olsen GH, Carroll J, Sva E, Camus L (2008) Cellular energy allocation in the Arctic sea ice amphipod *Gammarus wilkitzkii* exposed to the water soluble fractions of oil. Marine Environmental Research 66:215-216

Ott FS, Harris RP, Ohara SCM (1978) Acute and sublethal toxicity of naphthalene and 3 methylated derivatives to estuarine copepod, *Eurytemora affinis*. Marine Environmental Research 1:49-58

Pasternak A, Arashkevich E, Reigstad M, Wassmann P, Falk-Petersen S (2008) Dividing mesozooplankton into upper and lower size groups: Applications to the grazing impact in the Marginal Ice Zone of the Barents Sea. Deep-Sea Research Part Ii-Topical Studies in Oceanography 55:2245-2256

Payne JR, McNabb GD, Clayton JR (1991) Oil-weathering behaviour in Arctic environments. Polar Research 10:631-662

Peterson CH, Rice SD, Short JW, Esler D, Bodkin JL, Ballachey BE, Irons DB (2003) Long-term ecosystem response to the Exxon Valdez oil spill. Science 302:2082-2086

Posthuma L, Suter GW, Traas TP (eds) (2002) Species sensitivity distributions in ecotoxicology. Lewis Publishers, Boca Raton, Fla.

Prince RC, Owens EH, Sergy GA (2002) Weathering of an Arctic oil spill over 20 years: the BIOS experiment revisited. Marine Pollution Bulletin 44:1236-1242

Reigstad M, Carroll J, Slagstad D, Ellingsen I, Wassmann P (2011) Intra-regional comparison of productivity, carbon flux and ecosystem composition within the northern Barents Sea. Progress in Oceanography 90:33-46

Rey-Rassat C, Irigoien X, Harris R, Head R, Carlotti F (2002) Growth and development of *Calanus helgolandicus* reared in the laboratory. Marine Ecology Progress Series 238:125-138

Saiz E, Movilla J, Yebra L, Barata C, Calbet A (2009) Lethal and sublethal effects of naphthalene and 1,2-dimethylnaphthalene on naupliar and adult stages of the marine cyclopoid copepod *Oithona davisae*. Environmental Pollution 157:1219-1226

Scott CL, Kwasniewski S, Falk-Petersen S, Sargent JR (2000) Lipids and life strategies of *Calanus finmarchicus*, *Calanus glacialis* and *Calanus hyperboreus* in late autumn, Kongsfjorden, Svalbard. Polar Biology 23:510-516

Seuthe L, Rokkan Iversen K, Narcy F (2011) Microbial processes in a high-latitude fjord (Kongsfjorden, Svalbard): II. Ciliates and dinoflagellates. Polar Biology 34:751-766

Siron R, Pelletier E, Delille D, Roy S (1993) Fate and effects of dispersed crude oil under icy conditions simulated in mesocosms. Marine Environmental Research 35:273-302

Skarðhamar J, Reigstad M, Carroll J, Eiane K, Wexels Riser C, Slagstad D (2011) Mortality rates of *Calanus* and simulated responses to pertubations in the Barents Sea. Aquatic Biology 12:129-145

Slagstad D, Ellingsen IH, Wassmann P (2011) Evaluating primary and secondary production in an Arctic Ocean void of summer sea ice: An experimental simulation approach. Progress in Oceanography 90:117-131

Spooner MF, Corkett CJ (1979) Effects of Kuwait oils on feeding rates of copepods. Marine Pollution Bulletin 10:197-202

Stige LC, Ottersen G, Hjermann DØ, Dalpadado P, Jensen LK, Stenseth NC (2011) Environmental toxicology: Population modeling of cod larvae shows high sensitivity to loss of zooplankton prey. Marine Pollution Bulletin 62:395-398

Støttrup JG, Richardson K, Kirkegaard E, Pihl NJ (1986) The cultivarion of *Acartia tonsa* Dana for use as a live food source for marine fish larvae. Aquaculture 52:87-96

Søreide JE, Falk-Petersen S, Hegseth EN, Hop H, Carroll ML, Hobson KA, Blachowiak-Samolyk K (2008) Seasonal feeding strategies of *Calanus* in the high-Arctic Svalbard region. Deep-Sea Research Part II-Topical Studies in Oceanography 55:2225-2244

Tamis J, de Vries P, Karman C (2009) Risk assessment of bioaccumulative substances. Part II: Description of a model framework, Institute for Marine Resources and Ecosystem Studies, report C107b/09, Den Helsen

Tatem HE, Cox BA, Anderson JW (1978) Toxicity of oils and petroleum hydrocarbons to estuarine crustaceans. Estuarine and Coastal Marine Science 6:365-373

Utvik TIR (1999) Chemical characterisation of produced water from four offshore oil production platforms in the North Sea. Chemosphere 39:2593-2606

Van Hoof PL, Kukkonen JVK, Landrum PF (2001) Impact of sediment manipulation on the bioaccumulation of polycyclic aromatic hydrocarbons from field-contaminated and laboratory-dosed sediments by an oligochaete. Environmental Toxicology and Chemistry 20:1752-1761

Van Wezel AP, Opperhuizen A (1995) Narcosis due to environmental pollutants in aquatic organisms: residue-based toxity, mechanisms, and membrane burdens. Critical Reviews in Toxicology 25:255-279

Vandermeulen JH (1986) Altered grazing patterns in an experimental copepod-alga ecosystem exposed to naphthalene and kuwait crude oil. Bulletin of Environmental Contamination and Toxicology 36:260-266

Varpe Ø, Jørgensen C, Tarling GA, Fiksen Ø (2009) The adaptive value of energy storage and capital breeding in seasonal environments. Oikos 118:363-370

Veith GD, Broderius SJ (1990) Rules for distinguishing toxicants that cause type I and type II narcosis syndromes. Environmental Health Perspectives 87:207-211

Wassmann P, Duarte CM, Agustí S, Sejr MK (2011) Footprints of climate change in the Arctic marine ecosystem. Global Change Biology 17:1235-1249

Wassmann P, Reigstad M, Haug T, Rudels B, Carroll ML, Hop H, Gabrielsen GW, Falk-Petersen S, Denisenko SG, Arashkevich E, Slagstad D, Pavlova O (2006) Food webs and carbon flux in the Barents Sea. Progress in Oceanography 71:232-287

Wassmann P, Slagstad D, Ellingsen I (2010) Primary production and climatic variability in the European sector of the Arctic Ocean prior to 2007: preliminary results. Polar Biology 33:1641-1650

Willis K, Cottier F, Kwasniewski S, Wold A, Falk-Petersen S (2006) The influence of advection on zooplankton community composition in an Arctic fjord (Kongsfjorden, Svalbard). Journal of Marine Systems 61:39-54

Paper I

Paper II

Paper III

Paper IV