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ABSTRACT

Expanding petroleum activities into northern marine areas have resulted in need for tools that specify Arctic specific characteristics when assessing environmental risks for these regions. In order to estimate the environmental impact of mechanically or chemically dispersed oil, on marine populations in sensitive northern environments, laboratory studies need to be developed. Such studies would be useful fir risk assessments and modelling potential effects of oil related pollution. The overall objective of the thesis was to establish an exposure study that would be useful for estimation of the effects of oil in ice or blowout scenarios on northern marine keystone organisms. Further, some basic parameters of growth and moulting of the Northern krill from the Stavanger area was established under different experimental conditions. A moult pre-study tested three different feeding regimes (Artemia nauplii + commercial shrimo larvae feed (EZ larvae), Thalassiosira algae paste + EZ Larvae and starved) studied over a period of two months. The length measurements recorded for moults and frozen animals only differentiated between the starved group and the fed treatments, and overall negative INC values at the end in all treatments were most likely caused by maintenance procedures. The study showed that the fed groups kept their moulting rhythm while the starved group did not.

The CFS exposure experiment conducted on *M. norvegica* revealed no significant difference between the Control (no oil), Low (0.54 mg/l) or Medium (1.6 mg/l) treatments of mechanically dispersed crude oil exposures in the moults frequency or animal lengths or weights. However, all individuals in the High (4.9 mg/l) exposure group died before first sampling, and all in Medium died before the second sampling. Still, the Low treatment was persistently lower than the Control in all measured parameters and visual observation of the moults revealed potential bacterial infection on oil exposed individuals. The second exposure experiment assessed the toxicity of crude oil (0.1%) and oil treated with chemical dispersant Corexit 9500A (2% of oil concentration), resulting in a Control, Oil and O+D treatment. Behavioural observations, respiration rates and moulting were recorded for the Northern krill, and egg production and mortality recordings for *C. finmarchicus* monitored over a period of fourteen days. The behavioural observations illustrated a significant difference both between and within the three treatments over the course of the experiment. The majority of the krill in the Oil and O+D treatments appeared hyperactive at the start of exposure, though later the activity level in a large fraction of the krill in the exposed treatments decreased to what appeared to be narcosis. The behavioural observations were highly informative, and preceded the increase in mortality among the krill. Respiration rates at the early and late experimental days correlated to the krill behavioural observations at the corresponding dates, with high oxygen consumption in the exposed treatments at first, then lower consumption towards the end. A significant difference was found between the Control and the exposed groups at the end of the experiment, but not between the Oil and O+D treatments. Moult recordings revealed no significant difference between the treatments. Yet, the animals in the oil and oil + dispersant appeared to have a slightly postpone moulting compared to the Control. The egg production rates in *C. finmarchicus* did not reveal a significant difference between the treatments. However, a reduction egg production in the oil exposed treatments (even more so in O+D) compared to the Control was observed. The morality recordings of *C. finmarchicus* clearly differentiated in both experimental days, treatments and the combination of the two. There were close to five times higher mortality in the O+D treatment than in the Control, and approximately 2 times higher mortality in the Oil than seen in the Control at the fourteenth and final day of exposure. As a result, caution should be taken when considering the direct application of dispersant in natural environments, even though it has the advantage of rapidly removing crude oil. These results may provide knowledge and tools to prepare for environmental management of future operations in sensitive boreal and sub-arctic environments.

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ACKNOWLEDGEMENTS

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1. INTRODUCTION

Oil pollution at sea remains a serious threat to the marine environments and continuous to get a great deal of attention by scientists and government.s There is evidence of a reduction in accidental oil spills over the last few decades (Huijer, 2005, Burgherr, 2007), but smaller spills and incidents still occur throughout the world on a daily basis. The petroleum industry has recently started to expand their activities northward into the European Arctic from the North Atlantic into Greenland, northern Norway and northwest Russia (AMAP, 2007). This creates a need for more information on the possible consequences oil spills may have in this area.

Numerous studies have been conducted, that indicate harmful impacts from oil spill incidences on the surrounding environment. In a review on the long-term environmental effects of oil spills by Kingston (2002), it was stated that environmental recovery could be considered complete within 2-10 years following an incident. There are however cases, such as when the *Florida* barge spilled 700 000 L No.2 fuel oil into the salt marsh sediments of Wild harbour (Buzzards Bay, MA), where petroleum-derived hydrocarbons continued to persist in the sediments (White et al., 2005). There were detectable sub-lethal biological impacts on fiddler crabs (Culbertson et al., 2007), impacts in salt mash grasses (Culbertson et al., 2008ba) and ribbed mussels (Culbertson et al., 2008ab) almost 40 years after the original spill. To recognize the potential effects from such pollution, both long and short term research should be done on keystone species in the area of concern. Key ecological species with important roles in the ecosystem stability are the most relevant organisms as changes to these may affect whole ecosystems (Primm, 1991).

Meganyctiphanes norvegica is a species that plays a significant role in the marine ecosystem (Youngbluth et al., 1989). It has a widespread distribution, vast densities and daily vertical migration in oceans and coastal waters. Krill are a link between the primary and secondary producers similar to copepods such as *Calanus finmarchicus.* A known minimum of 50 species have been reported to rely on these key species (Simard and Harvey, 2010), some of which are commercially important fish species such as herring, cod and capelin (Sakshaug et al., 2009). The Northern krill is therefore a highly suitable organism as an indicator species for ecological effects of oil pollution in the marine environment.

M. norvegica feed mostly on phytoplankton in the spring and summer but small zooplankton such as the copepod *Calanus finmarchicus* are dominant feed during autumn and winter (Tarling et al., 2010). While the Northern krill is considered important keystone specie in the sub-Arctic and boreal North Atlantic (Mauchline and Fisher, 1969, Parsons et al., 1984b), *C. finmarchicus* might be considered *the* most important organism in the same area. *C. finmarchicus* is one of the most abundant species in that region, and may constitute up to 70 % of the mesozooplankton biomass in the North Atlantic during summer (Fleminger and Hulsemann, 1977, Fransz et al., 1991)

Although several studies have been conducted on the consequences of oil pollution on these northern areas, much of the literature on spill effects on plankton is dedicated to the copepod *C. finmarchicus*. Very little information can be found on the possible impact an oil spill will have on the Northern krill. The effects chemically or mechanically dispersed oil has on either of these keystone species are even less known. Recent studies on Arctic cod larvae's show that cod cohorts are highly sensitive to possible zooplankton biomass reductions in the distribution area of the cod larvae, and point to a need for more knowledge about oil effects on zooplankton (Stige et al., 2011). *Meganyctiphanes norvegica* and *Calanus finmarchicus* are highly abundant and relevant species around oil producing areas in the Northern Hemisphere and the potential ecological effects of oil spill and or oil dispersal on the local ecosystems will be of great interest. The life history of *M. norvegica* and *C. finmarchicus*

should therefore be used to study such pollution scenarios that could then be reflected in mortality, growth and development of these species, oxygen uptake and reserves.

1.1. FATES AND EFFECTS OF ACCIDENTIAL OIL DISCHARGE

Spilled oil at sea may take several different pathways (collectively called weathering processes) entering the marine environment (Fig. 1.1). After a spill the oil will initially spread over the water surface as a thin slick a few millimetres thick by effects of gravity, friction, viscosity and surface tension (Scholz et al., 1999). Advection is a process similar to spreading, where the movement is caused by overlying winds and/or underlying currents (NRC, 1985). Neither spreading nor advection are uniform processes, and large variations in oil thickness can occur inside the slick. (ITOPF, 1987). As spreading increases the surface area of the slick it also increases the chance of direct contact between the oil and any biological resource on the surface on the water.

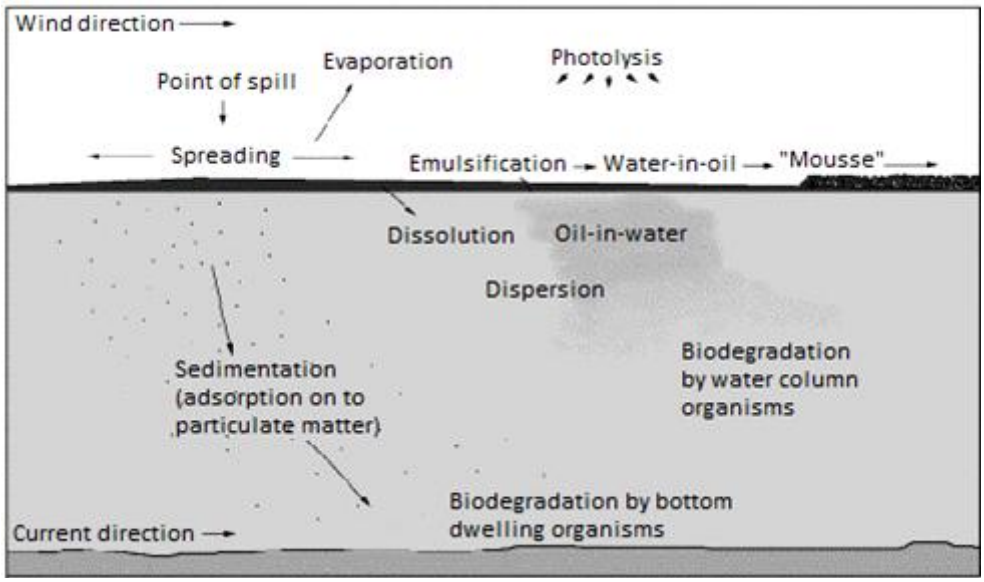

Figure 1.1 *Pathways spilled oil may enter the marine ecosystem (Kingston, 2002).*

The single largest volume reduction of an oil spill happens within the first 24-48 hours of the spill and is caused by evaporation (Payne and McNabb Jr., 1984). Lighter oils (components with low boiling points which are more volatile) will evaporation more than heavier ones (ITOPF, 1987, Mielke, 1990), vaporising most of the oils toxic components (Lewis and Aurand, 1997). Evaporation can lead to biological exposure by creating a toxic vapour which can be inhaled; however, the time of exposure is relatively short. Exposure to photolysis (photo-oxidation) often occurs in association with evaporation of the oil film. In the presence of oxygen, UV radiation in the sunlight will oxidize some of the components in the oil (Mielke, 1990). A number of these compounds may be even more toxic than the original hydrocarbons (Mielke, 1990, Neff, 1990). Both water column and surface organisms may come in contact to these by-products by inhalation, direct contact, adsorption, and direct and indirect ingestion.

A minute quantity (2- 5 % at the most) of the hydrocarbons (Neff, 1990), mostly low molecular weight, volatile compounds that are fairly toxic (NRC, 2003), are transferred into solution in the seawater by a process called dissolution. Even though dissolved concentrations are very low, resources in the water column may be exposed by direct contact, indirect

digestion and adsorption through the body surface. Somewhat similar to dissolution is the weathering process of natural dispersion, which is second only to the volume reduction caused by evaporation (Neff, 1990). The slick is then broken up into small oil droplets by waves and becomes incorporated into the water column in the form of dilute oil-in-water suspensions (Exxon Corporation, 1985). Dispersion has the same contact routes as dissolution, though the organisms are now exposed to whole oil droplet and not individual compounds.

Under certain sea conditions emulsifications, a mixing of water droplets in floating oil, may form. These water-in-oil emulsions are highly viscous and may have densities approaching that of seawater (Mackay and McAuliffe, 1988), forming a substance referred to as "chocolate mousse" (Mielke, 1990, Neff, 1990), which can increase the persistence of the slick (ITOPF, 1987, Neff, 1990). Organisms on the surface can be exposed to the emulsions by direct contact or via direct and indirect ingestion.

As oil, especially the heavier components, is sticky, it tends to adhere to particles in the water column, on shorelines and on the seabed. Sedimentation is the process of incorporating the oil into both suspended and bottom sediments (ITOPF, 1987, Neff, 1990). This process is especially important in shallow, rough sea conditions where bottom sediments are repeatedly resuspended (Exxon Corporation, 1985). Shoreline stranding is a process that may contribute to sedimentation on shorelines instead of sediments, where the oil is visibly accumulated after a spill. Both of these processes expose water-column, bottom-dwelling and intertidal organisms to the oil through direct contact and via direct and indirect ingestion.

While sedimentation may occur when oil droplets pass through organisms undigested and are eliminated in the faeces, biodegradation is when microbes consume the hydrocarbons as a food source. By doing so, carbon dioxide and water are excreted as waste products (Atlas, 1981). This is a slow, but significant process (Neff, 1990). Biodegradation take place in the water column, on the surface, in the sediments and on the shore (Lewis and Aurand, 1997) and produces bi-products which may be either more or less harmful than the original oil, exposing other organisms by all previously mentioned contact routes. These weathering processes do not occur separately, but will overlap and interact during the course of a spill, and consequently affect the properties of the spilled oil and thereby the effect the oil has on the environment.

1.2 BLOW OUT AND OIL IN ICE SCENARIOS

Deep water exploration for oil is increasing as onshore reserves are staring to dwindle and the world's oil demand is ever growing. This brings with it possible accidental oil discharges from well blowouts and pipeline or riser ruptures. Deep water blowouts are particularly undesirable from an environmental point of view, as they are difficult to handle. Deep water blowouts principally have lower temperatures and much higher pressure than shallow or surface water have. Thermal stratification and underwater currents also tend to complicate the situation. Operators therefore need to know how the oil will disperse as it moves up through the water column and how to clean it up when it reaches the surface.

In June 2000, the DeepSpill experiment was conducted as a field study of a simulated oil and gas blowout in deep water (Johansen et al., 2003). Four controlled discharges amounting to a total of 120 m^2 of oil was released on 844m depth, roughly 125km off the central coast of Norway. Results showed that the oil started reaching the surface within a few hundred meters of the release site after approximately an hour after the release began. Oil continued to surface for several hours after the release stopped. The slick was much thinner

and dispersed than slick oil released as a point source on the surface would have created. Emulsions were observed at the surface with increasing water content over time.

In arctic conditions, sea ice contains a highly porous bottom layer which is directly connected to the ocean beneath, allowing for fluid exchange (i.e. Eide and Martin, 1975) in addition to serve as a biological habitat (Cota and Smith, 1991, Krembs et al., 2000, Gradinger et al., 2009). This layer can also provide for possible entrainment and retention of oil spilled under the ice, causing environmental concern. Oil advancing on the underside of sea ice spreads as a film or as discrete droplets. The spread is limited by the bottom topography of the ice, giving rise to pooling capacity (i.e. Wilkinson et al., 2007). Once the oil is stationary, a layer of ice will grow over the oil lens, encapsulating and immobilizing the oil. Ice above the lens entrains the oil into the connected brine pore space, extending the oil through the porous lowermost layer of the ice into brine channels and the ice above.

One of the first field experiments on the subject investigated the fate of oil released under sea ice from winter through spring in the Canadian Arctic (NORCOR, 1975, Martin, 1979). The project confirmed that most of the oil spilled in the fall was entrained as lenses under the ice and then encapsulated in the ice. When the ice started to warm in the spring and brine channels expanded, the oil began to migrate upward. As the ice continued to deteriorate, the oil gradually saturated the spaces within and between the ice crystals. Eventually, the oil reached the surface through discrete channels in May. Oil-saturated sea ice contained and average of 4.5% oil, with a maximum of 7% in a 4 cm section.

Blow out scenarios in Arctic environment are in other words more unpredictable and need more preparation, than in many other environments. As a result of this, more laboratory and field studies under similar conditions are needed to enhance future preparedness should an oil spill occur.

1.3. CHEMICAL DISPERSION

Natural dispersion of heavy crude oil is relatively slow. However, by adding a chemical dispersant to an oil slick, the oil is rapidly dispersed into the bottom layers and diluted into the water column. Such dispersants are composed of surface-active agents (surfactants), solvents and stabilizing agents. The surfactants reduce the interfacial tension on the water-oil interphase and thereby enhance the break-up of the slick into fine droplets, transferring the oil into the water column. Surfactants also prevent re-coalescence of suspended, chemically dispersed oil droplets and reduce the energy necessary for dispersion (ITOPF, 1987, Neff, 1990, NRC, 1989). Usages of dispersants are optimally within a narrow window less than 72 hour after the spill, though some variations are expected according to the oil type spilled. This is because the dispersant will work best on oil that has not weathered, or not weathered much (Pond et al., 1997). Chemical dispersants are normally applied at a 1:20-25 dispersant:oil ratio to oil spills at open sea (NRC, 2005). Chemical dispersion will also affect each of the individual weathering processes previously mentioned, though the extent of the impacts will vary on the oil type and the environmental conditions. Table 1 give a short summary of these effects.

By removing the oil from the water surface and thereby dilute the oil concentration in the water column, chemical dispersion will (NRC, 1989, IT Corperation, 1993) :

- Reduce the fouling of shorelines and other economically important resources
- Reduce the potential damage to birds, marine mammals and other national resources
- Enhance microbial degradation by increasing the surface area of the oil droplets
- Reduce the formation of tar balls and mousse

- Provide a clean-up option when other response resources are not effective (i.e. too high waves for booms and skimmers)

However, as with many response options, there are also issues to consider when applying dispersants:

- There is a narrow window of response, so that it is usually not possible to make a decision on their use unless some pre-spill planning has been done beforehand
- Chemical dispersion requires some wave action to mix the dispersant into the oil slick, making it unattractive in arctic environments with sea ice
- Low water temperatures may increase the oil viscosity, making chemical dispersion more difficult
- The oil should have an API gravity between 17 and 45 (generally, oil's with higher API's are non-persistent and will evaporate, while lower ones are highly viscous and not dispersible)
- Ecosystem trade-offs (protect surface resources over water-column and bottom-dwelling ones)
- Type of dispersant used (availability, quantity, application rate etc.)
- The shape and size of the spill (potential formation of "windows" in the slick)

Table 1.1 *Summary of the effects chemical dispersion has on the oil's weathering processes.*

Weathering process	**Effect of chemical dispersion**
Spreading	Enhanced
Evaporation	Some conflicting results, but generally reduced
Natural dissolution	Enhanced
Natural dispersion	Enhanced
Emulsification	Reduced
Photolysis	Reduced
Sedimentation & shoreline stranding	Reduced
Biodegradation	Enhanced

1.4 KEYSTONE SPECIES - *MEGANYCTIPHANES NORVEGICA*

Following an oil spill, effects on the ecosystems keystone species can give us basis for the overall potental impact the spill can have on the ecosystem. In terms of abundance and biomass, *M. norvegica* (Northern krill) is one of the two most dominant Euphausiid species in the North Atlantic (Einarsson, 1945, Lindley, 1978), with the Norwegian coast as a hotspot.

M. norvegica is an omnivore and preys upon other small zooplankton such as the copepod *Calanus finmarchicus* and graze on phytoplankton and detritus (Båmstedt and Karlson, 1998). They perform diel vertical migration (DVM) with residence at depth during daytime and ascent into upper waters after sunset. The migration ranges from less than 100m in shallow waters (Liljebladh and Thomasson, 2001) to more than 500m in the Ligurian Sea (Tarling et al., 1999). Due to the species DVM, its enormous densities and widespread distribution in coastal waters, the Northern krill plays a significant role in the exchange of nutrients and material between the bentic and pelagic food webs (Youngbluth et al., 1989).

M. norvegica's wide distribution stretches across the North Atlantic; from the coasts of USA and Canada, over the shelf of Greenland and Iceland, to the British Isles and the Norwegian coast. Its northern limits are the Barents Sea and the Greenland Sea, while in the

south it has been spotted as far as the Mediterranean Sea and Canary Islands (Mauchline and Fisher, 1969). The Northern krill is one of the largest of the 86 described euphausiid species, and reaches a total body length of 40-50mm (Baker et al., 1990). The adult body can be divided into two main regions – the cephalothorax and the abdomen (Figure 2). The abdomen consists of six segments terminated by the telson. The cephalothorax extends about one third of the krill's body, is covered by carapace and holds the head and seven pairs of thoracic segments (the eight being absent in Northern krill) (Mauchline and Fisher, 1969).

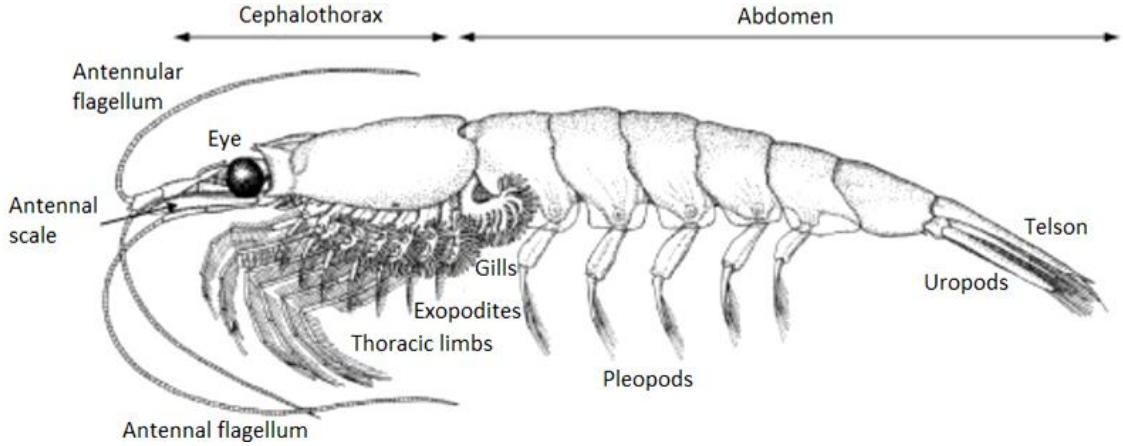

Figure 1.2 *Illustration of an adult Meganyctiphanes norvegica (total body length around 40mm) indicating the main morphological features (Tarling et al., 2010).*

It takes about 1 year for *M. norvegica* to mature and spawning of the first krill occurs in March–July in the northern areas (reaching approximately 25mm), relative to February–April (27-35mm) in the more southern parts (Einarsson, 1945). As the sub-arctic environment represents the northern boundary of successful spawning, populations in the Barents Sea and sub-arctic waters north of Iceland where spawning are sporadic at best, recruitment is most likely to have been migrations from more southern populations (Dalpadado and Skjoldal, 1991, Astthosson and Gislason, 1997). Once hatched, the larvae develop through a series of stages, punctuated by moults, exchanging the rigid exoskeleton for a larger or differently equipped one. A larva is usually referred to as 'adolescent' once it has five pairs of setose pleopods and all luminescent organs and numbers of lateral and terminal spines on the telson are reduced to the adult number (Mauchline and Fisher, 1969).

At a superficial level, growth in euphausiids may appear discontinuous, given the abrupt increments which occur at each ecdysis. It can be characterised by two parameters: The intermoult period (IMP) and the growth increment at moult (INC). The IMP has been found to be consistent at around 13 days in adult krill of a total length of approx. 30mm in a number of separate studies carried out at 10˚C (Cuzin-Roudy and Buchholz, 1999, Buchholz et al., 2006). INC is more variable, ranging from –6% to +6% per moult in krill taken from Gullmarsfjord (Buchholz and Buchholz, 2010), while Cuzin-Roudy et al. (2004) state that INC was not statistically different from zero with variance (–0.1% to +0.1%). However, such variations may be caused by maintenance procedures (Buchholz, 1991).

Under favourable conditions, one moult cycle may be immediately linked to the next, and under harsh conditions the krill may even shrink at moult (Marinovic and Mangel, 1999, Buchholz, 2003). As soon as the new exoskeleton is completed the water, which was used to burst open the old shell at fixed seams to reveal the new one, is replaced continuously by tissue and lipid stores. The timing of the moult cycle is controlled by hormones which are the functional interphase to environmental signals, such as tropic input temperature and phytoperiod. While growth is relatively undefined, moulting is cyclical and mostly uniform in

its phases, and in turn has a large influence on rates such as growth, reproduction and physiology. Based on this, the life history of the Northern krill can be used to study environmental change which can be clearly reflected in the growth and moult cycle of the species.

1.5 KEYSTONE SPECIES – *CALANUS FINMARCHICUS*

Copepods are probably the most abundant multicellular organisms on the planet (Parsons et al., 1984b, Mauchline, 1998). They are a key route of energy transfer in the marine ecosystem, as they graze on phytoplankton and are themselves an important prey for larvae, juveniles and adults of commercial fish (Runge, 1988). In Arctic waters, the copepods of the *Calanus* genus are chiefly responsible for this link (Falk-Petersen et al., 1990).

A marine planktonic crustacean of the order Calanodia. *C. finmarchicus* plays a very significant functional role being the main prey for larva and juveniles of many commercially important fish species, such as cod, haddock (Kane, 1984, Buckley and Durbin, 2006), herring (Cohen and Lough, 1983, Kiørboe et al., 1988, Purcell and Grover, 1990) and shellfish, like the shrimp *Pandalus borealis* (Savenkoff et al., 2006). Thus *C. finmarchicus* is of primary importance for the survival and abundance of these populations. *Calanus* spp. are one of the most abundant groups in the Arctic and North Atlantic latitudes and can constitute up to 70 % of the mesozooplankton biomass in the North Sea during the summer (Fleminger and Hulsemann, 1977, Fransz et al., 1991).

C. finmarchicus is distributed all over the North Atlantic and the eastern Arctic Ocean, from the mid-Atlantic Shelf off the US east coast to the Barents Sea north of Norway (Jaschnov, 1970, Conover, 1988, Hirchea and Kosobokova, 2007). Because of the North Atlantic current, high numbers of the species is transported into sub-arctic and arctic areas. Its ability to live long periods of time with little energetic effort makes *C. finmarchicus* able to enter into regions of expatriation.

C. finmarchicus develops through six nauplii stages, followed by five copepodite stages before it becomes sexually mature. Figure 3 show the two last copepodite developmental stages of both males and females. Each stage is transitioned by production of a new exoskeleton before the old one is shred (moulting). The later copepodite stages of the *Calanus* copepods accumulate lipids during the productivity season and survive the winter by diapausing in deeper waters (Falk-Petersen et al., 1990). The lipid reservoirs in *Calanus* form visible elongated structures inside the cephalothorax, normally with a reddish colour due to the presence of the carotenoid, astaxanthin (Sakshaug et al., 2009). Description of overwintering for copepods, including *C. finmarchicus* is explained by the classification of dormancy for insects. Diapause in the animal is under endocrine control and is a suppression of growth and development, maintained for some time irrespective of the environment. It may intervene at any of the major life stages of the full life cycle which is fixed in the specie (Mansingh, 1971). Several results indicate that the reproduction and development of copepods from March to July coincide with the main phytoplankton spring bloom (Melle and Skjoldal, 1998, Skjoldal et al., 2004). This happens in the upper 200 meters of the water column (Nicholls, 1933, Marshall et al., 1934, Conover, 1988), while from late June, *C. finmarchicus* is found in increasing numbers at depth (Østvedt, 1955, Hirche, 1984), where they do not do not perform diurnal vertical migration (Marshall and Orr, 1955).

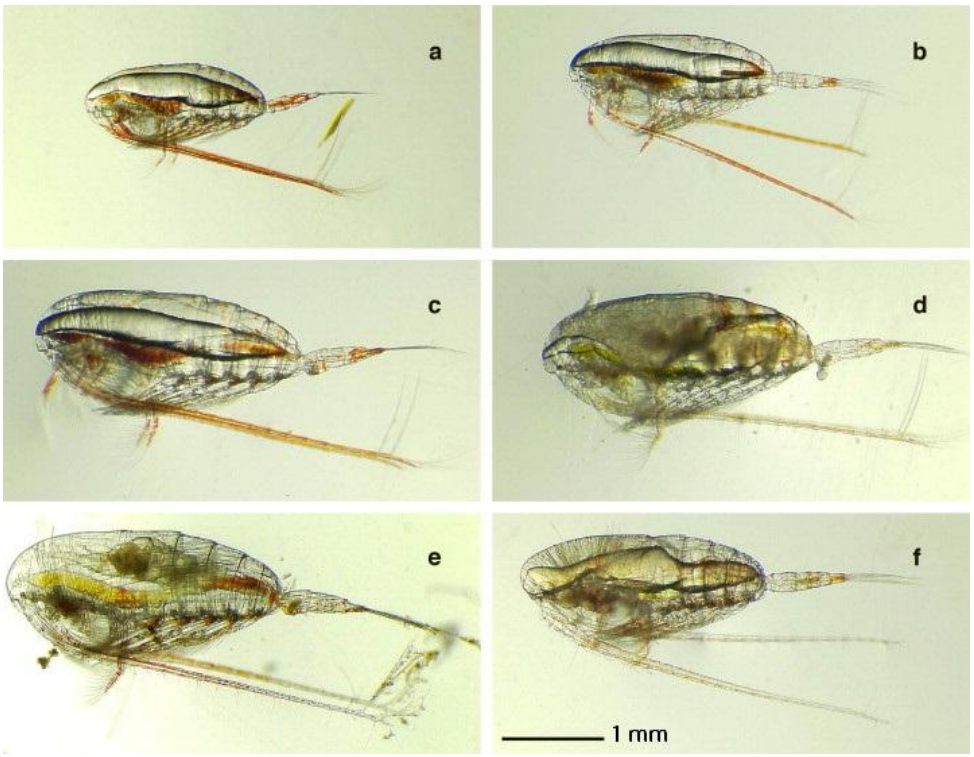

Figure 1.3 *Developmental stages of Calanus finmarchicus. (a) Copepodite IV. (b) Copepodite V. (c) Adult female with developing gonads. (d) Adult female with fully developed gonads. (e) Post-spawning female with few visible eggs. (f.) Adult male copepod. (Hansen et al., 2008a)*

1.6 EFFECTS OF OIL RELATED POLLUTION ON ZOOPLANKTON

Zooplankton has a key part in the marine pelagic food web. The food web is complex and rapidly responds to climate variability, shifts in species distribution and abundance, timing of life history events and trophic relationships (Lenz et al., 2012). Krill and copepods life history can have imperative implications for community structure and function. Several studies have been conducted to discover the effects of oil related pollution on different species of zooplankton, however, the majority of species investigated are copepods and the effects on krill are unknown.

Varela et al. (2006) studied the effects of the *Prestige* oil spill on the plankton community on the N-NW Spanish coast, where calanoid copepods were by far the most dominant groups in the community. This group remained the most dominant even after the spill, and statistical test revealed that no significant changes in abundance were detected. However, the lack of evidence of the effects after the spill was most likely a result of the great variability of the plankton cycles and the short-term impact of the oil on the pelagic system. Jiang et al. (2012) found the acute toxicities and effects of crude oil water accommodated fractions on 15 different species of copepods in a subtropical bay in East China. The copepods showed restlessness, impaired swimming ability, loss of balance, anoxic coma and even death after exposure.

A substantial amount of the marine research in recent years has been dedicated to the boreal keystone copepod, *Calanus finmarchicus*. Several of these studies have focused on the impact of oil pollutants on the survival (Hansen et al., 2011, Faksness et al., 2012, Hansen et al., 2013), feeding, hatching, egg production (Jensen et al., 2008, Jensen and Carroll, 2010, Hjorth and Nielsen, 2011) as well as genetic transcription and expression (Hansen et al., 2007, Hansen et al., 2008b, Jensen et al., 2012) in *C. finmarchicus.*

Oil dispersants have been developed to reduce the environmental damage caused by accidental oil spills. Surfactants are the main component in dispersants, which consist of non-soap detergents with both lipophilic and hydrophilic active agents that allow the dispersant to work. Numerous studies have shown that surfactants can damage several vital functions in the body of an organism. Some of these are inactivation of essential enzymes taken from fresh water protozoan and rat liver (Allen et al., 1965), alteration of membrane permeability in blue mussels (Braaten et al., 1972), interruption of cellular respiration in rainbow trout (Abel and Skidmore, 1975, Mackie et al., 1975), cause membrane lysis in mouse melanoma and rainbow trout (Partearroyo et al., 1990, Partearroyo et al., 1991) or inhibit and stimulate different ATPase enzymatic systems in brine shrimp (Cotou et al., 2001).

A study of the temperate ecosystem response to crude oil and the dispersant Corexit 9527, concluded that oil dispersants should not be used on oil spills during spring blooms, as it may be more toxic to the zooplankton community than when other petroleum hydrocarbons act alone (Parsons et al., 1984a). Ortmann et al. (2012) and Jung et al. (2012) supports these zooplankton findings, though by using Corexit 9500A and Hi-Clean dispersants instead, respectively, and additionally point toward the inhibitory effects of the dispersant on the phytoplankton communities. Another temperate study with chemically dispersed oil implies that marine copepods may be negatively affected by oil in the combination of dispersants. They found the lowest-observed-adverse-effect (LOAE) concentrations of water accommodated hydrocarbon fraction (WAF), WAF plus Hi-Clean, WAF plus Corexit 9500, Hi-Clean alone and Corexit 9500 alone to be 50%, 10%, 0.1%, 1% and 1%, respectively. Thus revealing Corexit 9500 as the most toxic of all the chemicals studied on the copepod *Tigriopus japonicus* (Lee et al., in press).

An experiment in the North Sea showed that the short term effects of oil treated with dispersant were much more pronounced than untreated oil towards the ecosystems zooplankton community. However, as untreated oil is trapped by the sediment in the model systems, its long term effects were expected to be stronger than to the systems who received treatment (Kuiper, 1985). A study of the ecological effects of oil with and without the dispersant Corexit 9550 on a littoral ecosystem in the Baltic Sea (where copepods where the most abundant organism), is somewhat conflicting with the temperate findings. Several of the results indicated a stronger response to oil alone compared to oil and dispersant However, this may have been because the oil left the oil and dispersant system faster than where only oil was added, thus reducing the exposure time significantly (Lindén et al., 1987).

Some very recent research has also considered the potentially different effects between mechanically dispersed and chemically dispersed oil. Olsvik et al. (2012) found that a chemical dispersant did not add to the magnitude of transcriptional responses of mechanically dispersed oil, but rather appeared to lower or modify the transcriptional effect on Atlantic cod larvae's. The 96 hour exposure of oil treated with the Dasic NS dispersant on *Calanus finmarchicus* slightly increased the specific toxicity of the oil at median and low effect levels, but reduced the toxicity at high effect levels, compared to naturally dispersed oil. However, no differences were found in the endpoints between chemically and naturally dispersed oil (Hansen et al., 2012).

There is an evident need for more information on the impact of both oil and chemically dispersed oil on zooplankton and specifically the northern keystone species *Meganyctiphanes norvegica* and *Calanus* spp. Even less is known of the effects of mechanically dispersed oil on these organisms. Although more research has been dedicated to the consequences of oil pollution on *Calanus finmarchicus*, little information can found on the effect of dispersed oil on these species.

1.7 OBJECTIVES

Very scarce laboratory experiments were found in the literature for the Northern krill. This study aims to design an exposure study that would be useful for estimation of the effects of oil spill on krill as in a blowout or oil spill in an arctic ice scenario. Further, we establish some basic parameters of growth and moulting of the Northern krill from the Stavanger area, under different experimental conditions.

The overall objective of this was to analyse the effects of oil and chemically dispersed oil on the two keystone organisms *Meganyctiphanes norvegica* and *Calanus finmarchicus* and additionally compare this to the effects of mechanically dispersed oil on *M. norvegica*. Effects studied were behaviour, respiration and moulting in the Northern krill, and egg production and mortality in *C. finmarchicus.* The results may in turn provide knowledge for development of tools to prepare for environmental management of future operations in sensitive boreal and sub-arctic environments.

2. MATERIALS AND METHODS

Moulting frequency and growth of *M. norvegica* was assessed in a small pre-study by subjecting adult krill to 3 different feeding regimes (2 with different feeds and 1 starved group). The krill moulting rate was recorded and the moults and surviving animals were measured.

Two different exposure scenario studies were tested to assess the toxicity of crude oil to adult krill, and in one case scenario for adult copepods.

1. The toxicity of mechanically dispersed crude oil from a Continuous Flow System (CFS) (Sanni et al., 1998) was determined for adult krill (*M. norvegica*) by continuous exposure of the krill for 14 days. The rates of mortality and moulting were recorded and growth assessed.
2. The toxicity of oil and oil treated with a chemical dispersant from a CFS was assessed to krill (*M. norvegica*) and copepod (*C. finmarchicus*) adults by exposing the animals for 14 days. The rates of mortality, moult, respiration, behaviour (krill) and egg production (*Calanus*) were recorded.

analyse the effects of oil and chemically dispersed

2.1 MOULTING AND CFS EXPOSURE EXPERIMENT (1)

Length and weight measurements of moults and the corresponding whole frozen animals were taken from two different experiments prior to the main experiment. The first of the experiments looked at the effects of different diets on the growth and mortality of the krill *M. norvegica*. The experiment was based on individual *M.norvegica* in 15 separate aquaria (A1 - A15), on three different feeding regimes.

- Artemia nauplii + EZ Larvae (A1 – A5)
- Thalassiosira (algae paste) + EZ Larvae (A6 – A10)
- Starved (A11 – A15)

The EZ Larvae (Zeigler Bros., Inc. USA, 250–600 μm, PL4 - 10) is commercial microcapsulated larval diet, formulated as a completely balanced diet for marine larval and post larval stages. This feed has been used for adult Atlantic krill which is larger than *M. norvegica.* Post larval stages of shrimp are similar in size to the adult krill used in the experiment. Approximately 0.003ml/l of the EZ Larval feed was used together with newly hatched *Artemia salina* nauplii. The Artemia cysts (±530 μm) had high content of highly unsaturated fatty acids (AF Specialty Artemia cysts, Σω3HUFA>15 mg/g dwt, INVE AquacultureNV). The cysts were cultured at 30 °C in 10 l glass bottles, by mixing 7 l seawater and 3 l water, under high oxygenation and constant light. After hatching (24 h) the nauplii were siphoned out from the un-hatched cysts, filtered through a 40 μm mesh, rinsed in seawater (25 °C) and concentrated. The nauplii were re-suspended in filtered seawater and fed to the adult in excess with over 200 nauplii/day/individual krill twice daily. The EZ larvae was also combined with TW 1200 (*Thalassiosira weissflogii)* (Reed Mariculture Inc.) *algae paste. T. weissflogii* is a large diatom (6-20μm x 8-15μm) that is used in the shrimp and shellfish larviculture industry. The large cell size extends the algae feeding period until the end of the post larval stage where high lipid and carbohydrate levels continue to boost survival and growth rates. The algae paste was diluted with filtered seawater and fed 10 000 cells/ml (40 000 cells/ml is in excess) twice daily. The experiment lasted for 59 days.

Individuals that died during this time were replaced with krill from tanks containing 10 animals, receiving the same treatment. This was done to keep a sufficient number of experimental animals the experiment in case of high mortality in early stages of the treatment.

The second experiment was an exposure experiment with four treatments: Control (no oil), low (0.54 mg/l), medium (1.6 mg/l) and high (4.9 mg/l) nominal concentrations of crude oil. The high concentration was based on studies on the copepod *C.finmarchicus* (Hansen et al., 2011), the shrimp *Pandalus borealis* (Beckman et al., 2010) and the amphipod *Gammarus setosus*. (Børseth et al., 2011) and the high effect concentration found on these animals. Two much lower concentrations that are more likely to be relevant for field situation over a longer period of time, were used for the medium and low concentrations. Adult animals of *M. norvegica* were collected at a local site in order to minimise damage on animals during transport and storage. The animals were acclimatised for 8 days in the laboratory and then transferred to experimental aquaria. Each group held two replicates with 14 individuals (24 in total for each treatment) in separate cylinders. Krill intended for respiration experiments were kept under same conditions but with 5 individuals in each cylinder. The krill were fed Artemia nauplii (in excess with 200 nauplii/day/individual krill.), TW 1200 (15000 cells/ml) and EZ larvae (0.003ml/l in each setup) by hand every morning and afternoon.

2.2 CALIBRATION, LENGHT & WEIGHT MEASUREMENTS

Materials needed for microscope calibration, length and weight measurements of the Northern krill are found in table 2.

Table 2.1 *Equipment used for calibration and length measurements of krill samples.*

Equipment
Stereomicroscope
Graticule
Standardised ruler (10 mm)
Fume hood
Forceps
Petri dish
Weight (0.0001g accuracy)
Weighing boat
Oven

2.2.1. Length

The microscope was calibrated by placing a graticule into one of the stereoscopes eyepieces and a 10mm ruler into the field of vision. By comparing the ruler to the eyepiece units (epu) in the graticule, one could determine how many epu 10mm corresponded to. Equation I showes the conversion of measured epu to mm.

$$\text{Millimeters (mm)} = \frac{\text{mm corresponding to calibrated epu value} \cdot \text{measured epu value}}{\text{calibrated epu value at the used magnification}} \qquad [\text{I}]$$

Moult samples were kept at room temperature in small scintillation glass bottles in a 4% formalin solution. For measurements, the exoskeleton was gently lifted out of the formalin solution with forceps and placed into a small petri dish filled with tap water. All handling of

samples in formalin was done under a fume hood. The moult was then placed under the microscope for length measurements. For completely intact moults, length measurements of the abdomen, telson and uropods were conducted, as seen in figure 2. If any parts of the measurements categories were missing, the length of the remaining pieces was measured.

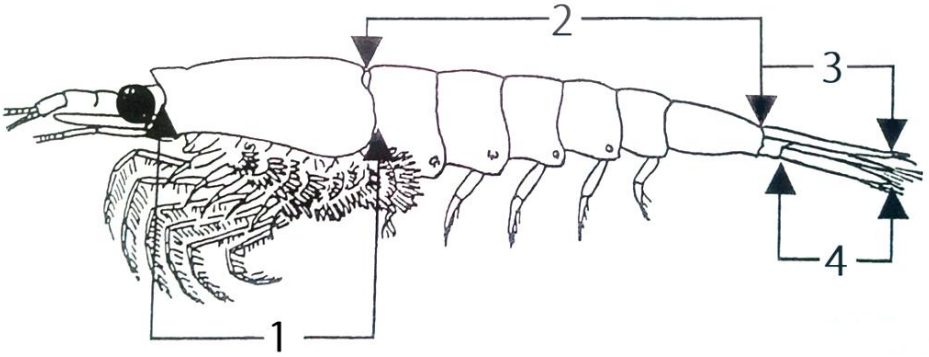

Figure 2.1 *Length measurement categories in the Northern krill: 1) carapace, 2) abdomen, 3) telson and 4) uropods.*

2.2.2. Dry weight

The animals were stored in cryo vials in an ultrafreezer at -80℃ until examination. They were kept on ice between the length and the wet weight measurements. The animals were then dried at 60℃ for 24 to 48 hours and the dry weight recorded.

2.4 CAPTURE AND MAINTENANCE OF ZOOPLANKTON (2)

Krill (*M.* norvegica) was collected in the local fjord off Stavanger between the islands Rennesøy and Åmøy (58˚03.49N, 06.47 E) on the 19th of February 2013 between 3 and 4 am. The temperature at the sampling site was approximately 2°C in the surface layers and 6-7°C at 50-100m depth.

The animals were captured with a modified shrimp trawl fitted with a 2mm macroplankton mesh with a 100 L closed cod end. The trawl was towed at speed of one knot for 20 minutes at 60 meters depth. The krill was gently collected into bowls from the cod end under low light and moved into transfer containers immediately after capture, and kept under low light conditions. The animals were transported to 500 L tanks in the laboratory within 2 hours of capture. The krill were never out of the water. The tanks in the laboratory had a continuous flow of 1 L min^{-1} of temperature controlled filtered seawater. The krill was acclimatised for 2 weeks before the start of the experiment. The initial temperature for the krill was 7°C. Two days after capture the temperature was lowered 0.5°C and every day thereafter until the experimental temperature of 4°C was reached.

The *Calanus* spp. was captured at a local site in Byfjorden off Stavanger (58˚01.87N and 37.70 E) on the 28th of February 2013 using a WP2 net fitted with 335 µm mesh size and closed cod end. The net was lowered to 50 m and towed at 0.6 knots for 10 minutes. The catch was immediately transferred to a 25 L transfer container and divided into three 25 L containers when in the laboratory. The animals were slowly acclimatised to the experimental temperature by lowering the temperature in the holding room 0.5°C daily from 6°C until 4°C was reached.

During the adaptation period (14 days), each tank with krill was fed with 7 ml EZ larva 10-50 microns (ZOEA 1 to ZOEA 3) by Zeigler, diluted with seawater to 800 ml, while the *Calanus* was not feed during this period.

2.4 EXPOSURE SYSTEM

The exposure system was designed to examine the biological effects from the water soluble components of oil in water, and oil and dispersant in water, on *M. norvegica* krill and *C. finmarchicus* copepod. Three treatments (control, oil and oil + dispersant (O+D)) were simultaneously tested, using seawater from the same source. A naphthenic-rich crude oil was used in the experiments and Corexit 9500A was selected as the chemical dispersant.

A recirculating continuous flow-through system was established for each of the treatments. The three exposures were tested simultaneously, with animals from the same batch and collection, and seawater from the same source and cooling. To minimise the risk of contamination, the control was kept in a separate controlled temperature room (CT-room) from the oil, and O+D. The oil and O+D treatments each had a 100x100cm header tank were the water level was kept at 18cm to produce 0.180 m^2 header volume. The control treatment had a 40x40m header tank with a 112 cm water level to produce the same header volume. All header tanks were initially filled with cooled (4℃), 2µm sandfiltered seawater taken from 80 m depth outside Mekjarvik in Stavanger. Each header had a continuous low aeration to oxygenate the water, but without disrupting the water surface and providing gentle mixing in the header tank.

The krill were kept in individual cylinders, 24 in total in each treatment, with an 115µm mesh at the bottom. The *Calanus* were placed in the same type of cylinders, 30 animals in each, and three cylinders per treatment (90 in total for each set up) for mortality and one cylinder of animals for egg production. The cylinders were in an exposure tank, standing alongside the header tank (fig. 4). Temperature was kept constant at 4°C by fan cooling. There was illumination on in either of the two CT-rooms. This minimises light induced stress on the animals. All examinations were therefore conducted with headlamps at lowest possible light intensity.

Figure 2.2 *General view of experimental setup; header tank, exposure tank and pump.*

Water was pumped using peristaltic pumps from the header tank to the cylinders and back again, all with intakes and outlets under the water surface. An extractor hood was covered on the oil, and the O+D header tank to provide a good working environment.

In the oil setup, 180 ml crude oil with arctic characteristics was added. The oil was carefully poured onto the surface of the header tank so that it formed a slick on the entire water surface. This was also added to the O+D setup, including 2 % Corexit 9500A dispersant added directly afterwards.

During the experiment, each cylinder in all setups had a flow of approximately 20ml/min. 1ml of a solution with 2 ml EZ larva 10-50 microns, diluted to 164 ml with seawater was given to each cylinder once a day for the first week. For the second week of the experiment, the animals were fed the same feed twice a day. This was due to suspected starving, seen as deaths in the Control group.

A fourth recirculating continuous flow-system was arranged for the effect of mechanical dispersed oil on krill. This system used the same exposure tank as in the three other systems, and the same header tank as in the Control. However, due to a mechanical failure, the system broke down on the first day of exposure, and is therefore not included further in the thesis.

The mortality of the krill was assessed in another part of the experiment and these data will be discussed in context with this project.

The exposure experiment lasted for 14 days. This is a somewhat longer period than expected to be seen for oil in seawater in nature. This was done to asses where mortality would start if animals would be trapped within an area where low mixing would occur. The animals are able to do some dial migration but often maintain their position in the watermass.

2.5 NORTHERN KRILL BEHAVIOUR

The behavioural trends and changes in behaviour of the krill during the two weeks of exposure were observed on day 1, 3, 7 and 13 of the experiment. This was done by characterizing the five most frequent behavioural types made by the krill before the experiment. Observations of behavioural types were recorded over 2 minutes and the length of time the krill spent performing each behaviour was recorded. At the second observation date one extra behaviour was added. This was related to narcosis response of the krill.

2.6 NORTHERN KRILL RESPIRATION MEASUREMENTS

An Oxy-4 mini oxygen meter from PreSens, with a 4-channel fibre optic oxygen transmitter was used for respiration measurements. Glass bottles (115 to 130 ml) were used as respiration chambers. The bottles were cleaned at 90°C, cooled and filled with the same aerated filtered seawater as used in the experiment. The respiration measurements were conducted in complete darkness in a CT-room (at 4°C). Approximately 24 hours before, and up to the start of each experiment, all four electrodes were used to monitor the background oxygen consumption in the seawater. During all experiments, two electrodes were monitoring background oxygen consumption in the seawater and two recorded continuous respiration of single experimental animals. The rest of the respiration chambers were sealed immediately after addition of animal until estimated 20% of the initial oxygen had been consumed by continuous recordings. Then a spot measurement was taken for each chamber. The krill was very active in the chambers and no stirring was needed of the media. However, stirring was needed for the continuous blank measurements.

Individual krill from the oil exposures were gently scooped up from a cylinder with a ladle and transferred into a bowl with clean filtered seawater to rinse off most of the external oil/dispersant chemicals to protect the oxygen electrodes. They were then moved from the bowl into glass beakers, which had been filled with aerated 4°C filtered seawater. To minimise light induced stress to the animals, the beakers were placed on a tray and covered with a thick black plastic cover before being moved to the respiration setup. The beakers content were then gently poured into the respiration bottles through a wide funnel. The krill in the control room were simply moved with a ladle from the cylinders directly into the respiration bottles through a funnel. In total, 8 krill from each treatment were used for the first respiration measurement (2^{nd} day of experiment) and 10 from control and oil on the last (14^{th}) day of the experiment. Because of high mortality, respirations measurements on the 3 remaining krill in O+D were measured at the 13^{th} day, rather than the 14^{th}.

The spot measurements were taken after approximately 5 hours (not below 70% of saturation). All measurements were run for at least 15 minutes or until a clear reading was achieved. Background oxygen consumption was measured on each date and was to be treated as blanks. The oxygen content (mg/l) of the saturated seawater, expressed for particular temperature and salinity, was given by Weiss (1970). The oxygen decrease was monitored in the Oxy –4 mini software and recorded in % O_2 of the saturated O_2 value. The initial oxygen concentration was derived using those levels (eq. II and III).

$$O_2 \text{ consumption (mg/l)} = \frac{\%\ O2\ end - \%\ O2\ start}{(\%\ O2\ start) \cdot (O2\ at\ saturation\ (mg/l))} \qquad [II]$$

O_2 consumption (µmol/g·h) =

$$\frac{\left(O2\ consumed\ \left(\frac{mg}{l}\right) \cdot 1000\ \frac{\mu g}{mg} \cdot (volume\ of\ respiration\ bottle\ (l) - 0.003l)\right) / (32\frac{g}{mol})}{Dw\ krill\ (g) \cdot Conversion\ factor \cdot Time\ used\ (h) \cdot 24} \qquad [III]$$

2.7 NORTHERN KRILL MOULTS

Each cylinder was checked and registered for moults at least once a day throughout the entire experiment.

2.8 *CALANUS* EGG PRODUCTION

For the egg production measurements, 10 *C.finmarchicus* females were transferred from their respective treatment cylinders by gently pipetting each individual from their cylinder and into small glass beakers filled with filtered seawater for examination. Undamaged females were sorted out for egg production using a stereo microscope with cold illumination. Glass beakers were kept cold by placing them on ice during sorting. Individual copepods were then gently pipetted into 1000 ml containers filled with 2µm filtered seawater. Ten replicates were used for each of the three treatments. The containers were placed in a CT-room in the dark for 24 hours. The water was then filtered through a 40µm mesh and the eggs counted under a stereo microscope.

2.9 *CALANUS* MORTALITY

Calanus mortality was registered every second to third day (three days over the weekends). This was done by visual observation on the first days of the experiment with a head light on the lowest light intensity. Later, when mortality could be observed, the animals where gently collected from their respective cylinders with a pipette, and into small glass beakers filled with filtered seawater for examination. The animals were examined under a stereo microscope with cold illumination to separate dead and live *C. finmarchicus*. The live animals were placed back into their respective cylinders.

2.10 WATER CHEMISTRY

Samples for chemical analysis were taken on the day of the experiment was started (one hour after oil and oil plus dispersant were added), day 2, 7 and 14. Results are at the courtesy of IRIS.

2.11 STATISTICAL ANALYSIS

All statistical analyses were conducted using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). To see if the data satisfied the requirements of normal distribution and homogeneous variance, the Kolmogorov-Smirnov and Levene tests were performed, respectively. If these conditions were fulfilled, the experimental groups were compared by One-Way analysis of variance (ANOVA), followed by LSD (Least significant difference), Bonferroni and Scheffe post hoc tests. If the data did not satisfy the normality and homogeneity requirements, the Kuskal-Wallis H test, followed by the Mann-Whitney U test were performed to rank the groups. Values less than, or equal to 0.05 were considered statistically significant.

3. RESULTS

3.1 MOULTING PRE-STUDY

The moulting study started on the 15th of November 2011 and lasted for 55 days to the 9th of January 2012. The animals were divided into 15 aquaria's and given three different feeding regimes;

- Artemia nauplii + EZ Larvae (A1 – A5)
- Thalassiosira (algae paste) + EZ Larvae (A6 – A10)
- Starved (A11 – A15)

Approximately 41 moults were collected during the two months of the experiment. Only two individual had 4 moults, of which one of the animals received the Tha-EZ treatment and the other one was starved. Eight individuals had 3 moults, where four of them came from the Art-EZ treatment and four from the Tha-EZ. The total moulting frequency of the three treatments and the combined moulting in the fed treatments is illustrated in figure 3.1, and total moulting frequency and moults in the starved treatment is illustrated in figure 3.2. From the figures it is clear that the pattern of moulting between the treatments is different. The fed treatments exhibited a synchronised moulting rate of 13-15 days, while the starved group moulted irregularly. The regular feeding appears successful in both reducing mortality and introducing synchronized moulting rhythms.

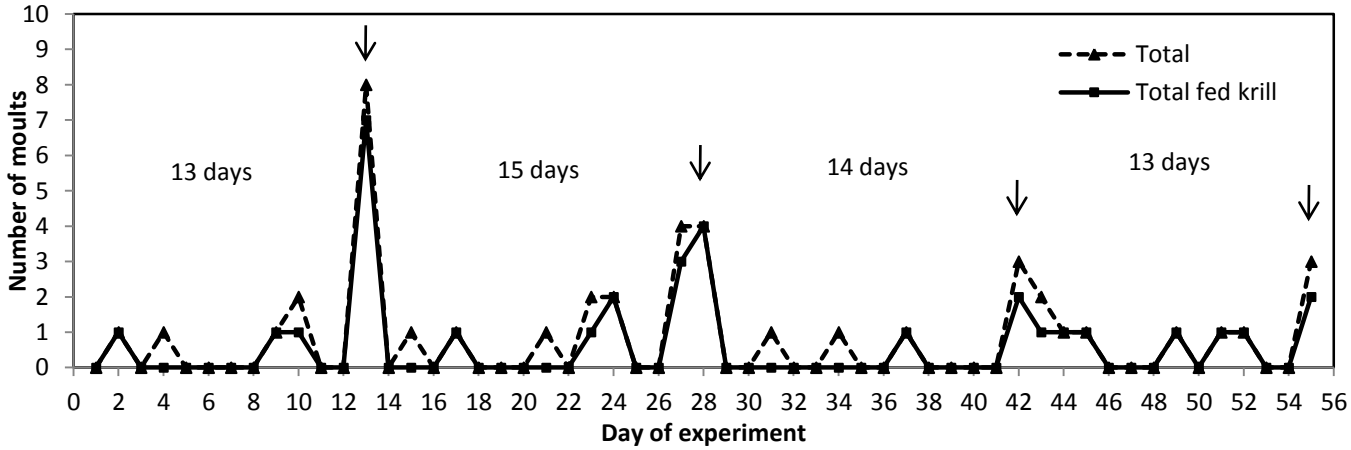

Figure 3.1 *Total moulting frequency and moulting frequency in the fed treatments.*

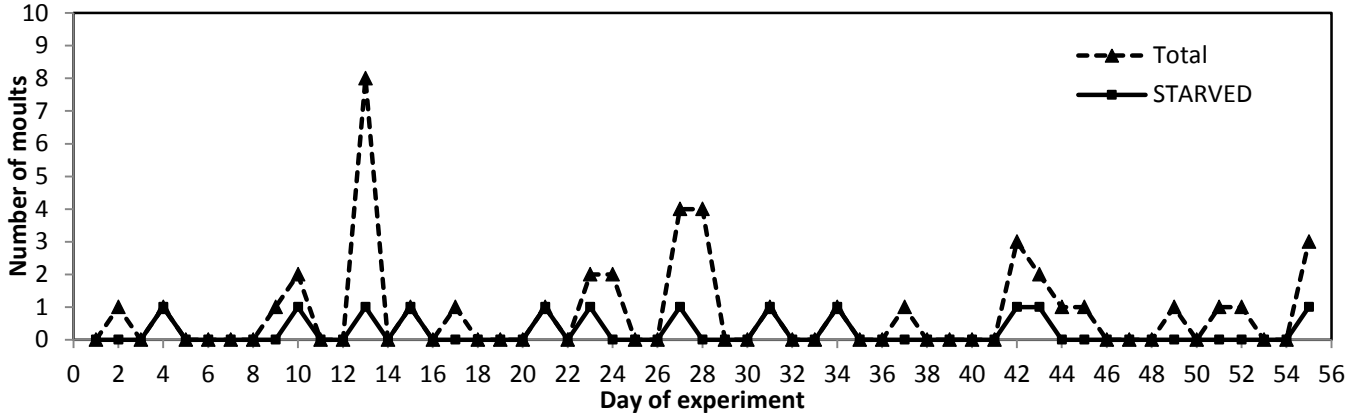

Figure 3.2 *Total moulting frequency and moulting frequency in the starved treatment.*

Table 3.1 summarises the moults collected during the experiment and Table 3.2 lists the corresponding animals tested in the experiment.

Several parameters were measured for the moulting study to obtain numerous data points, and hence ensure that the measurements were reliable. The length of the carapace, abdomen, telson (with and without the split at the end (see fig. 3.3)), uropods, and the wet and dry weight of each animal was measured. For the moults, the length of the abdomen, telson (with and without split) and uropods were measured.

After repeated measurements it became apparent that the two uropods on the same animal occasionally had different lengths and were therefore considered unreliable parameters. The same conclusion was reached for the 'telson with split', as this could get broken and was an unreliable parameter. The abdomen on moults tended to curve, and would not lie in a straight line during measurements. As a result of this, many abdominal lengths on moults were measured with a curve and hence a longer line than the corresponding animals which naturally lay in a straight line. This can to some degree account for the longer abdominal length seen in most of the moults compared to the frozen animal in table 3.2.

The Art-EZ group had an average of 2.5 moults, the Tha-EZ group moulted the most with 2.8 moults, and the starved group moulted the least with 1.5 moults (table 3.1 and 3.2). The seven animals (out of the total seventeen moulting specimens), who only moulted once or twice, did so because their time in the experiment was short and is discounted from the growth/reduction investigation. The two specimens who moulted four times had a telson with split and a uropod, respectively, which were longest in the fourth and final moult (table 3.1). However, the abdomen and the other uropod in both animals were longest in the second moult. The longest segments in the moults (for all but the abdomen of one individual) in all three treatments were in the first or the second moult, and never the third. This indicates that the eight animals which moulted three times (were none where in the starved group) ultimately shrunk throughout the experiment.

The same trend can be seen in the 'mean growth' column in table 3.1, where all but the moults from one animal has a negative mean growth. There was in other words a clear reduction in overall moult length between moults from the same animal during the experiment. The red, bold numbers in the table emphasises the largest segment on moults from the same animal. Each of the segments in the latest (by date) moult from an animal which had moulted more than once was subtracted from the corresponding earliest segment in a moult from the same animal. A mean growth/reduction of the moults was then calculated by calculating an average of the subtracted values. The moults of the starved group shrunk the most (on average -4.6%), while the Art-EZ shrunk the least (on average -3.2%). However, the reduction difference between the groups was small, especially between the Art-EZ and Tha-EZ groups. The Art-EZ group shrunk on average 0.6% less than the Tha-EZ group, and 1.4% less than the starved group.

Table 3.1 *Moults from the moulting experiment. The 'Mean growth' value stands for mean growth between moults from the same animal. The 'G/R of telson' value is the growth/ reduction between the moult and the post-moult specimen. All moults within two horizontal lines come from the same individual. The red bold numbers highlight the largest moult from the same individual. Blank lines/open places represent un-measurable moults or moult segments.*

Date	Group	Abdomen (mm)	Telson (mm)	Telson + split (mm)	Uropod (mm)	Uropod (mm)	Mean growth (%)	G/R of telson (%)
28.11.11	A1	17.5	**4.3**	6.0	5.5	5.3		0
12.12.11	A1	**18.0**	4.2	**6.2**	5.5	5.3	0.7	2.4
27.12.11	A1		4.2	6.1	5.3	5.2		2.4
28.11.11	A2	18.2	4.2	5.8	4.8	4.6		-2.5
13.12.11	A2	17.4	**4.2**	**5.8**	4.8	4.6	-0.1	-7.9
29.12.11	A2	**17.5**	4.1	5.6	4.8	**4.8**		-5.3
28.11.11	A3	17.1	4.3	**6.0**	**5.4**	**5.2**		-10.5
12.12.11	A3	16.9	**4.4**	5.9	5.2	4.8	-8.4	-13.2
09.01.12	A3	17.1	3.9	5.6	4.5	4.6		0
24.11.11	A4	**18.1**	4.1	5.9	**5.2**	**5.2**		0
09.12.11	A4	17.4	4.2	**6.1**	4.8		-2.7	-2.5
27.12.11	A4	16.9	4.2	5.7	5.1	4.9		-2.5
28.11.11	A5	16.4	**4.0**	**5.3**	**4.6**	**4.6**		-11.4
12.12.11	A5						-5.4	
09.01.12	A5		3.9	4.8	4.3	4.4		-8.6
28.11.11	A6	**17.8**	3.9	**5.5**	**4.8**	**4.8**		2.6
13.12.11	A6	17.1	3.9	5.4	4.7	4.7	-3.9	2.6
28.12.11	A6		3.7	5.4	4.6	4.6		7.7
25.11.11	A7							
08.12.11	A7	**16.9**	3.9	5.2	**4.7**	4.6	-1.0	-5.6
22.11.11	A7							
03.01.12	A7	16.5	3.9	**5.3**	4.5	4.6		-5.6
28.11.11	A8		**4.2**	5.5	4.8	4.9		-10.8
09.12.11	A8	**16.8**	4.1	**5.7**	4.8	4.9	-7.6	-8.1
05.01.12	A8	16.6	3.8	4.9	4.5	4.4		0
28.11.11	A9	17.7	**4.2**	**6.2**	4.4	5.2		-5.1
13.12.11	A9	17.7	4.1	5.9	4.1	5.2	-2.4	-2.6
30.12.11	A9	17.7	4.0	6.0	4.4	4.9		0
17.11.11	A10	17.4	4.0	**6.0**	**5.3**	**5.2**		2.5
02.12.11	A10		4.0	5.6	5.2	5.1	-4.1	2.5
13.12.11	A10	**18.1**	3.9	5.3	5.1	4.8		5.0
06.01.12	A10	17.7	3.8	5.7	4.3	4.3		2.6
19.12.12	A11	15.9	3.8	5.3		4.5		
28.12.11	A11							
30.11.11	A12	**21.2**	4.4	**6.8**	5.8	5.8	-7.9	2.3
16.12.11	A12	19.0	4.4	6.3				2.3
19.11.11	A13	16.6	4.2	5.9	**5.2**	5.1	-0.4	2.4
06.12.11	A13	16.6	4.2	5.9	5.1	5.1		2.4
25.11.11	A14							
08.12.11	A14	**16.8**	4.1	5.7	**5.2**	4.4	-1.6	
27.12.11	A14		4.0	5.2				
09.01.12	A14	16.1	4.1	5.7	4.8	**4.5**		
28.11.11	A15	16.9	**6.2**	**6.2**	**5.4**	**5.4**	-8.5	-39.5
12.12.11	A15	16.9	4.3	5.8	5.3	5.2		2.3

All the sampled moults were clear and unpolluted and few showed signs of broken telson or uropods. No clear difference could be seen between the treatments by visual observation of the moults or the frozen animals. Figure 3.3 show the end part of a typical moult from the experiment, illustrating the telson and telson with split measurements.

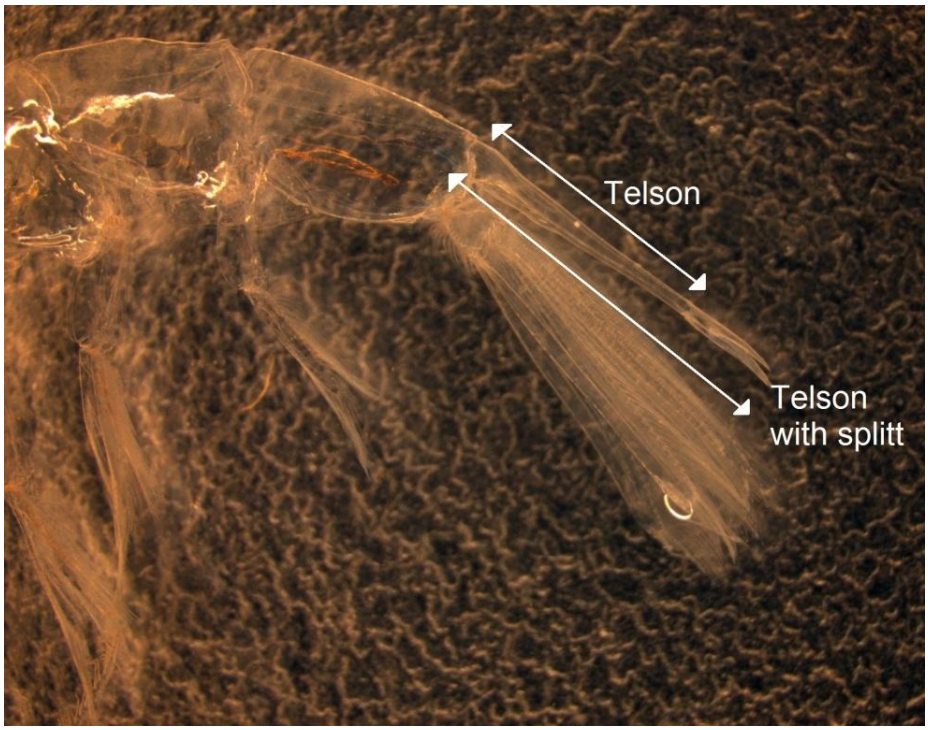

Figure 3.3 *The end part of a moult from the moulting experiment, illustrating the telson and telson with split length measurements.*

To verify growth or reduction of the krill during the course of experiment, the animals and the corresponding moults were compared. This however, proved more difficult than expected. When comparing the length of the segments measured on the animals with the length of the corresponding segments in the animal's last moult, the segments on the animals were in general smaller than on the moult. From the measurement results, it may therefore appear like the krill expands its moult before shredding it, resulting in a moult that is generally larger than the animal. Still, there are exceptions to this generalization, emphasised as bold, red numbers in table 3.2. The krill could also have shrunk as there are examples of shrinking of the Northern krill in literature.

When looking at table 3.2, there was only one, of the total six krill in the Art-EZ group which had a segment (uropod) that was longer than the corresponding segment in the animal's last moult. Because the telson length is covered in the 'G/R of telson' value, it is excluded from the comparisons here. In the Tha-EZ group, four of the total six animals had a one segment (telson with split or uropod) which were longer than in the moult. In the starved group, four of the total eight animals had one or two segments (abdomen or uropod) which were longer than in the moult. Only within the starved group was the abdomen among one of the segments which had grown. The starved group was also the group were the animals had most increasing segments, indicating growth. From these observations, the Art-EZ group had fewest animals which showed growth to some degree and the starved group had the most animals which showed growth. However, this is conflicting with the reduction seen between the last and the first moult in each group. Art-EZ was the group with the least reduction, and the starved group was the one with the most reduction. The moult results indicate an overall

reduction in length during the time the animals were in the experiment, while the animal lengths are the final length the krill had at the time of dead or the end of the experiment. The starved group was also the group with the least animals surviving to the end of the experiment, making the growth observations somewhat invalid. Two of the four animals which indicated growth in the starved group, died two and a half and three weeks before the end of the experiment, and might therefore still have shrunk had they lived till the end. All of the animals in the Art-EZ group, which showed the least growth among the animals, lived till the experiment ended.

Because the telson length remained the most reliable of the parameters measured, this length was used to measure the growth increment at moult INC value. The 'G/R of telson' column in table 3.1 compares moults with the post-moult animal to give an idea of the INC. Positive values indicate that the telson on the final animal was longer than in the moult. Values within the Art-EZ treatment between the first moult and the final animal varied between -11.4% and 0%, within Tha-EZ they were from -10.8% to 2.5%, and within the starved treatment they were between -39.5% and 2.3%. The largest negative value was in other word found in the starved treatment, indicating that the telson in the first moult was much longer than the final length of the telson in the animal and that the animal ultimately had shrunk. The INC value in the last moult and the post-moult specimen in Art-EZ varied between -8.6% and 2.4%, in Tha-EZ it was between -5.6% and 7.7% and in the starved treatment it was between 2.3% and 2.4%. The two highest values (5.0 and 7.7%) were found in the Tha-EZ, indicating that krill in this treatment had grown the most. The starved group did not have any negative INC values when comparing the krill with its last moult, however, there were no telson lengths in this treatment within the last three weeks of the experiment.

A significant statistical difference between the telson length of the moults of the Art-EZ and the Tha-EZ group ($p = 0.002$), and the Tha-EZ and the starved group ($p = 0.005$) were found by the Mann-Whitney test. This may be because of the different feed the krill received, but it may also be because the krill selected for the different treatments by chance were of a larger or smaller size. The remaining moult and animal data in each of the groups did not reveal a significant difference from each other.

Table 3.2 *Animals from the moulting experiment. The bold red numbers represents the parts of the animal that were longer than the corresponding parts in the animal's last moult. The horizontal lines separate the three different treatments. Open places represent segments of the animals that were not measurable.*

Date	Group	Abdomen (mm)	Telson (mm)	Telson + split (mm)	Uropod (mm)	Uropod (mm)	Carapace (mm)	Dry wt. (mg)	No. of moults
09.01.12	A1		**4.3**	6.0	5.2	4.8	8.5	60.4	3
03.01.12	A2	18.2	4.1		4.6	4.5	8.7	44.0	1
09.01.12	A2	16.1	3.9	5.4	4.3	4.4	7.7	43.8	2
09.01.12	A3	16.4	3.9	**5.7**	4.6	4.6	8.2	52.2	3
09.01.12	A4	16.5	4.1		3.4	3.4	8.5	54.0	3
09.01.12	A5	14.9	3.6	4.4	3.9	4.0	7.3	28.2	3
09.01.12	A6	16.4	**4.0**	**5.6**	4.6	4.5	7.7	50.1	3
09.01.12	A7	15.9	3.7	5.1	4.2	4.3	7.6	38.7	4
09.01.12	A8	15.9	3.8	**5.1**	4.4	4.3	8.1	41.5	3
04.01.12	A9	16.1	4.0	5.4	**4.5**	4.6	7.7	41.1	3
20.12.11	A10	16.8	**4.1**	**5.6**	4.6	4.6	8.1	55.8	3
06.01.12	A10	15.9	3.9	5.5	4.3	4.3	7.7	36.8	1
19.11.11	A11	17.1			4.7		8.5	51.1	0
28.11.11	A11	15.6	4.0	5.4	3.9	4.3		48.3	0
29.11.11	A11	14.9			3.5		6.7	29.0	2
22.12.11	A12	**19.1**	**4.5**	6.2		4.3	9.5	72.3	2
04.01.12	A13	**17.8**	**4.3**	5.7	5.1	**5.2**	8.8	63.4	2
22.11.11	A14	15.3	4.0	5.6	4.1	4.8	8.0	43.9	0
09.01.12	A14	**19.7**					9.8	57.2	4
19.12.11	A15	**17.4**	**4.4**	6.1	4.9	4.7	8.8	53.5	2

3.2 CFS EXPOSURE EXPERIMENT ON *M. NORVEGICA* (1)

The CFS oil exposure experiment on *Meganyctiphanes norvegica* was conducted in early February 2012 and lasted for fourteen days. Adult Northern krill was exposed to three difference oil concentration, High (4.9 mg/L), Medium (1.6 ml/L) and Low 1(0.54 mg/L). Because of extremely high mortality at the highest nominal oil concentration with almost all the individual dead after day one, the experimental moults belong to the Medium, Low and Control treatments. Sampling for experimental parameters was done on day seven (week 1) and day fourteen (week 2) but moulting and mortality was registered on a daily basis. The Medium exposuregroup was sampled only at the seventh day due to mortality.

In contrast to the previous preliminary moulting study, a difference between the three treatments in the exposure experiment could be seen by visual observation of the moults. All of the seven moults from the Medium exposure group had telson and uropods which were brown at the end and were cut to some degree (fig. 3.4 and 3.5). Many of the moults with brown and cut uropods also had swimming feet with a fury appearance. Of the thirteen moults in the Low exposure group, six of them looked similar to the moults in the Medium treatment, with brown, cut uropods and "fury" swimming feet. Four other moults in the Low group had uropods which were cut to some extent, but not brown. Of the remaining three moults, one of them was polluted with yellow particles, slightly cut and brown, while the last two were fine (fig. 3.6). The Control group moulted the most during the experiment, and ended with a total of nineteen moults. Eight of the moults had brown and to some slight degree, cut telson and uropods. Another seven were cut, but not brown, while the last four moults were fine (fig. 3.7-3.8). Table 3.3 summarises the visual observations of the moults.

Table 3.3 *Summary of the visual observations of the telson and uropods on the moults in the Control, Low and Medium exposure groups.*

	Control	Low	Medium
Brown & cut	8 (42%)	6 (46%)	6 (86%)
Only cut	7 (37%)	4 (31%)	1 (14%)
Fine	4 (21%)	2 (15%)	
Other		1 (8%)	
Total moults	19	13	7

Figure 3.4 *Telson and uropods on a moult from the Medium exposure treatment. The telson and uropods appears brown at the end and the uropods are ragged and cut.*

Figure 3.5 *Telson and uropods on a moult from the Medium exposure treatment. The telson and uropods appears brown at the end and the uropods are ragged and cut.*

Figure 3.6 *Hindquarter on a moult from the Low exposure treatment. All swimming feet appear fury. Some slight degree of brown marks on the telson and uropods.*

Figure 3.7 *Telson and uropods on a moult from the Control treatment. The uropods appear slightly brown and cut, while the telson is fine.*

Figure 3.8 *Telson and uropods on a moult from the Control treatment that appears to be neither cut nor brown.*

The parameters measured were length of carapace, abdomen, telson (with and without the split at the end), uropods, and the wet and dry weight of the animals. Using the One-Way ANOVA test, no significant statistical difference could be seen between the Control, low or medium treatments at the first or the second week of exposure in any of the parameters listed. Some of the most reliable parameters when examining krill are the length of the animal's carapace, telson, and the wet and dry weight, and are therefore the elements examined closer here. Although no significant difference were seen between the treatments, variations can still be observed in the carapace and telson length, and wet and dry weights of the animals (fig. 3.9-3.12) .

When examining the mean carapace (fig. 3.9) and telson length (fig.3.10), the Medium exposure group appeared to grow the most, and the Low grew the least. The reason for the apparently high growth in the Medium treatment is most likely that the smallest animals died first, hence increasing the groups mean carapace length without truly growing.

The same trend can be seen in the wet (fig. 3.11) and dry weight (fig. 3.12) of the animals, were the mean weight of the Medium group is higher than that of the Low group. However, the Control was the heaviest group of the three treatments. Thus even though the animals in the Medium treatment were the longest in respect to carapace and telson length after the smaller organism had died, the Control treatment was the group with the heaviest krill. This may indicate that the animal's length does not necessarily correspond with its weight, or that this was a result of the random selection of the animals when dividing the krill into the three treatments. Another hypothesis is that the surviving krill in the Medium exposure group was not able to digest their food because it was coated in oil and hence gained little weight.

Still, the error bars with a 95 % confidentiality interval for the mean values for each treatment are overlapping, making it hard to state any clear difference in length or weight between any of the treatments. It might be that the fourteen days of the experiment is not long enough to separate between length or weight of the krill, or that the animals still have sufficient lipid stores to obscure possible effects in growth.

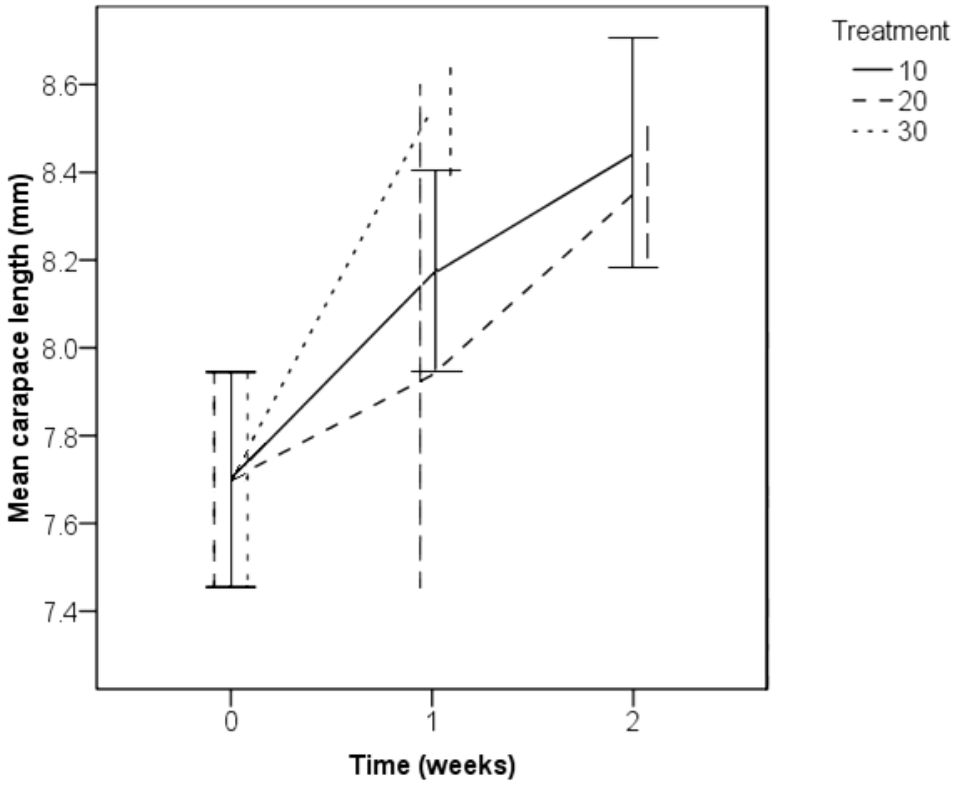

Figure 3.9 *Mean carapace length over time in the three exposure treatments, where 10 = Control, 20 = Low and 30 = Medium oil exposure. The vertical lines represent error bars with a 95 % confidence interval.*

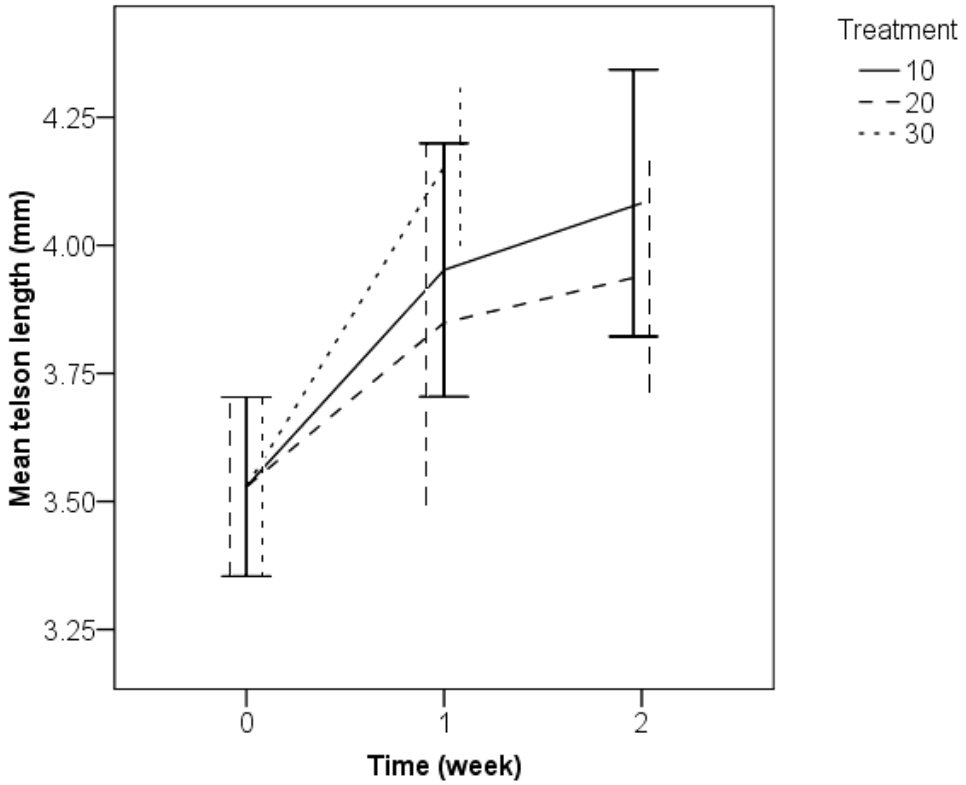

Figure 3.10 *Mean telson length over time in the three exposure treatments, where 10 = Control, 20 = Low and 30 = Medium oil exposure. The vertical lines represent error bars with a 95 % confidence interval.*

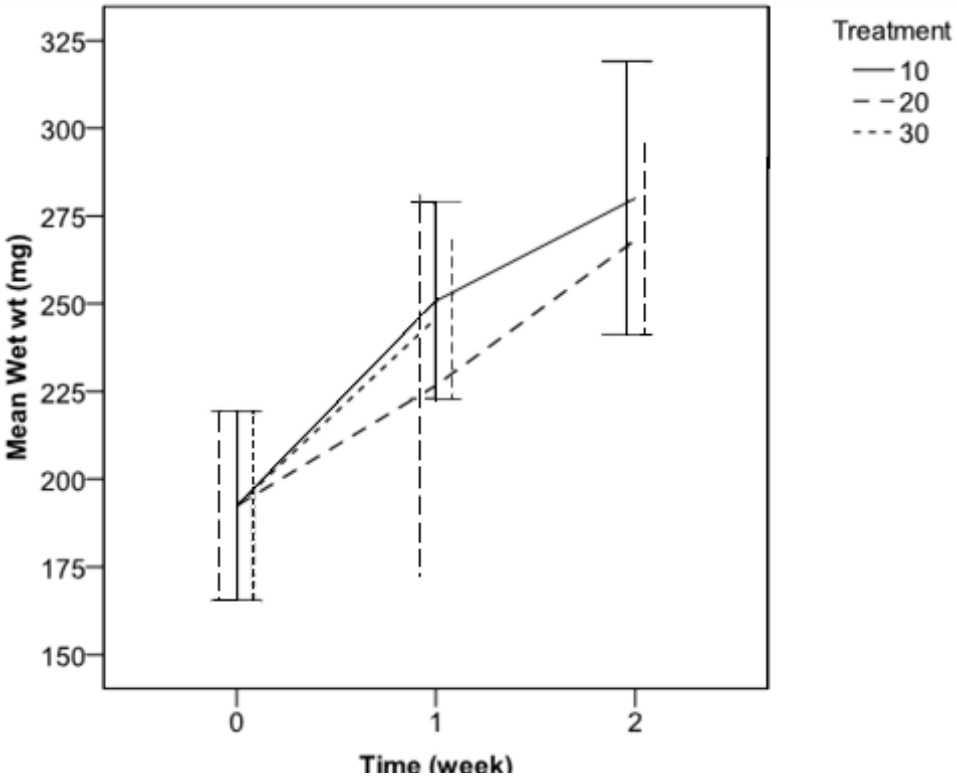

Figure3.11 *Mean wet weight over time in the three exposure treatments, where 10 = Control, 20 = Low and 30 = Medium oil exposure. The vertical lines represent error bars with a 95 % confidence interval.*

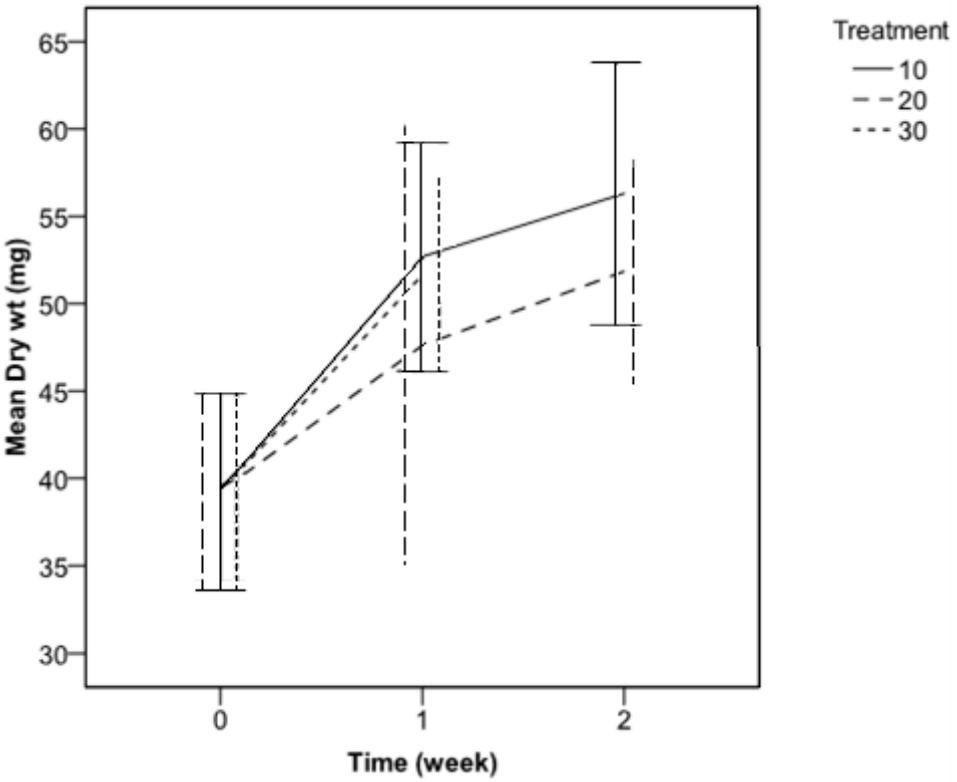

Figure 3.12 *Mean dry weight over time in the three exposure treatments, where 10 = Control, 20 = Low and 30 = Medium oil exposure. The vertical lines represent error bars with a 95 % confidence interval.*

3.3 EXPOSURE EXPERIMENT ON *M. NORVEGICA* AND *C. FINMARCHICUS* (2)

The objective of this thesis was to establish an exposure study that would be useful for estimation of the effects of oil in ice or arctic blowout scenarios on keystone northern marine organisms. The experimental setup was therefore not designed to add mixing to the treatments, but to simulate calm waters as in arctic seas with ice covers. Nonetheless, a third and separate experimental setup was designed with mechanical mixing to look at the effects of oil in waters with natural dispersion caused by wave action. However, water flow in and out of the experiment and aeration of the header tank allowed some gentle mixing of the water.

The laboratory simulation of calm waters had no wave action and little mixing in any of the treatments, when in reality some wave action and underwater currents would have caused slight mixing even in ice covered waters. More movement of the water would therefore have been more accurate to simulate potential field conditions. Little mixing in water masses is not unheard of from the field, though it is most likely unusual for such conditions to be continuous for fourteen days. However, the setup with oil and mechanical mixing would have covered the mixing effects to some extent. Unfortunately, the mechanical treatment collapsed after the first day of exposure due to a problem with the outlet and was consequently not completed.

The length of the exposure experiment of fourteen days was perhaps not realistic when considering oil spills or blowout scenarios, as the oil would have been more diluted and degraded in the field, hence reducing exposure times compared to than we expected to see in the laboratory. The reason for the extended time span in the experiment was to gather information on how long it would take until the effects of oil/oil plus dispersant were seen on animals under controlled conditions, rather than simulating accurate environmental conditions. This way, when creating a model approach for ecological impact assessment systems, a suitable time period can be set. Simulating the effects of mixing and loss of volatiles in oil under different environmental conditions is a complex matter and more realistic for a field study than a controlled laboratory study.

The closed CFS worked excellently for the purpose of the exposure system. External contamination was eliminated and contaminants, organisms and food were kept in a closed circuit. However, because of the closed system, living organisms like Artemia nauplii was not given as feed seeing that this would cause extra contamination in the system. Appropriate food concentrations to closed systems with krill were at this point unknown, and an adapted concentration of 1ml of solution with 2 ml EZ larva 10-50 microns, diluted to 164 ml with seawater was given to each cylinder once a day. This concentration appeared to be a modest portion. Mortality seen among the krill in the Control treatment after the first week of exposure confirmed this, and the rations were consequently doubled.

The closed exposure system worked excellently for the purpose of the experiment. However, because of the closed system, living organisms like Artemia nauplii or algae were not given as feed seeing that this could cause extra contamination in the system. Appropriate food concentrations to a closed systems with krill were not known for a static system, and an adapted concentration of 1ml of solution with 2 ml EZ larva 10-50 microns, diluted to 164 ml with seawater was given to each cylinder once a day. This concentration appeared to be a modest portion. Mortality seen among the krill in the Control treatment after the first week of exposure confirmed this, and the rations were consequently doubled with improvement in the mortality data.

Though the recirculating system worked well for the three setups, the water supply to the cylinders, using peristaltic pumps, could have functioned better. The pressure in the tubes

into the exposure tanks was not as high as initially predicted and should optimally have circulated back to the header tank. Instead they were secured higher up where gravity maintained a more or less constant water level and pressure and the tubes outlets were taken into the tank where the cylinders were placed. The excess water then flowed back to the header through the main outlet of the tank.

In earlier experiments with the Northern krill, more than one animal was placed in the same cylinder, resulting in increased mortality among the krill (A. Ingvarsdóttir, pers. comm.). To avoid this outcome, individual krill were placed in separate cylinders for this experiment.

The cold air ventilation and extractor hoods placed over the Oil and O+D header tanks in the exposed CT-room did not ensure a good enough working environment. Fume masks was therefore worn as an extra precaution. Working light was kept at a minimum, though the animals still showed some reaction to the low light from the head lamps used. Optimally, an even weaker, possibly red light would have caused less reaction in the light-sensitive test specimens.

The exposure study consisted of four different treatments, Control (zero oil), Oil (0.1%), O+D (oil plus 2% chemical dispersant, Corexit 9500A) and MDO (0.1% mechanically dispersed oil). The total concentration of polycyclic aromatic hydrocarbons (TPAH) in the seawater of the different treatments over the course of the experiment is shown in figure 3.13. The MDO treatment collapsed after the first day of exposure, although a chemical analysis was conducted on the second day on the water that was still left standing. The results show that the MDO treatment clearly exhibited the highest PAH concentrations at both the first (one hour after exposure) and the second sampling (day two). The low TPAH content in the Control at the fourteenth and last day of exposure is due to a small amount of naphthalene (0.052µg/L) which slowly increased in the treatment over the experimental period. The reason for this is unknown, yet previous studies with krill have detected naphthalene at the end of the experimental time in tissue samples that have never been exposed to oil (A. Ingvarsdóttir, pers. comm.). Further investigation on the subject is therefore highly needed.

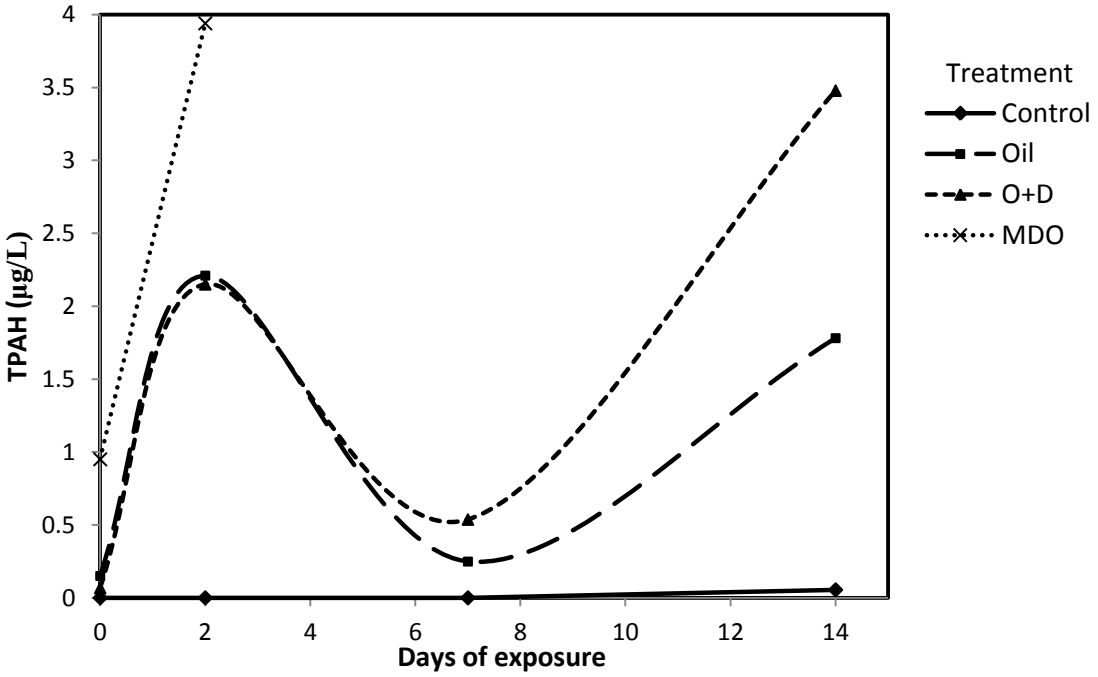

Figure 3.13 *Total concentration of polycyclic aromatic hydrocarbons in the different treatments*

3.3.1 Northern krill behaviour

Northern Krill behavioural recordings were conducted at day one, three, seven and thirteen of the exposure experiment. Observations of behavioural types were recorded over 2 minutes and the length of time the krill spent performing each behaviour was recorded. The results are given in seconds of krill behaviour per minute. The krill's most common behavioural patterns were arranged into six different categories, ranked from the lowest to the most active behaviour.

1. Lies still on the bottom
2. Very slow venting/shaking of feet on the bottom
3. Venting with feet while lying on the bottom
4. Venting with feet while standing still in the water column
5. Turning while swimming vertically
6. Swimming round along the wall

Behavioural observation of the Northern krill illustrated a clear difference between the treatments over the course of the experiment. The difference was most obvious between the exposed treatments and the Control treatment. At the start of the exposure, all treatments showed a comparable mixture of both active and inactive behaviour (fig. 3.14). This trend more or less remained in the Control treatment throughout the experiment.

The Oil and O+D treatments resembled each other in behavioural trend, although the behaviours in the O+D treatment were more pronounced and appeared earlier than in the Oil treatment. The krill appeared restless and highly active up to the first week of exposure in the Oil treatment, and up to the third day in the O+D treatment, compared to the Control. This corresponds with other published behavioural research on marine organisms, reporting of hyperactivity and increased swimming speeds after short-time exposure of pollutants. The induced hyperactivity resembled an escape reaction permitting exposed animals to evade stressful conditions.

On the seventh day of exposure in the Oil treatment, and the third day in O+D, the behaviour of the exposed specimens decreased from the high activity behaviours to an increasing time spent in the low activity behavioural type number 2. This behaviour was added in the course of the experiment as it had not been performed before by untreated animals. Behaviour number 2 eventually took over in the O+D treatment, and a similar trend was spotted in the Oil group as well. Behaviour number 2 could be described as narcosis, a condition of stupor or unconsciousness produced by a drug or other chemical substance (Anstie, 1865), in this case oil and oil plus dispersant. All krill which started performing behaviour number 2 spent most of their time in this state, and eventually died within one to two days after first performing the behaviour. This behavioural type never appeared in the Control group, although a low degree of mortality was observed in this treatment.

The narcosis behaviour seemed to be reflected in the krill mortality (fig. 3.14). As behaviour number 2 appeared on the third day in the O+D treatment, mortality started to rapidly increase between the fourth and fifth day. Similarly, behaviour number 2 appeared on the seventh day in the Oil exposure and the mortality started to increase between ninth and tenth day. Behavioural observations in other words preceded the increase in mortality among the krill, as the observations seemed to be linked to the lethal effects of oil exposure.

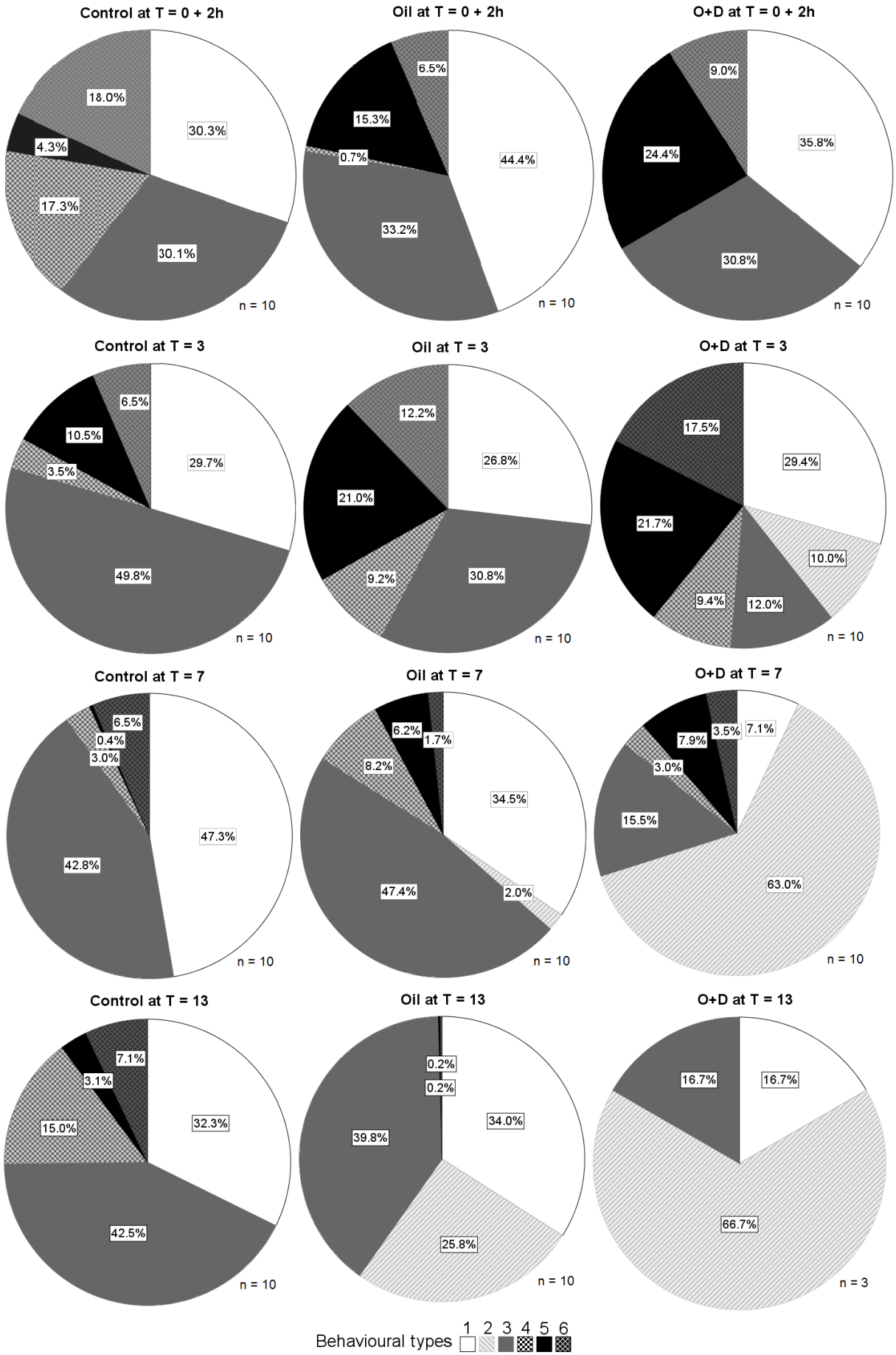

Figure 3.14 *Graphical summary of the behaviours in the different treatments over the course of the experiment. T = day of the experiment.*

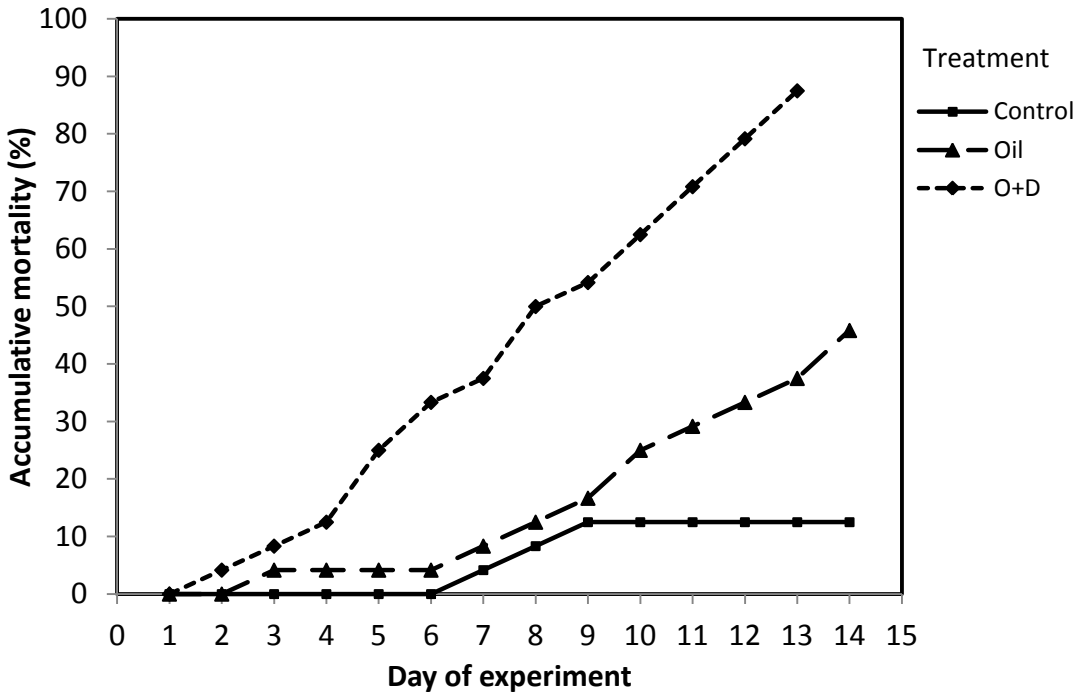

Figure 3.15 *Mortality of krill in exposure experiment. (Courtesy of Anna Ingvarsdóttir, unpublished data).*

According to the One-Way ANOVA test none of the behavioural groups were found to be significantly different at the first day of exposure. On the third day of exposure, behavioural group 3 showed a statistically significant difference between the Control and O+D treatments, according to the LSD, Scheffe and Bonferroni post hoc tests. All other groups were not considered significantly different. On the seventh day of exposure, behavioural group number 1 and 2 showed a significant difference between Control and O+D treatment, and the Oil and O+D treatment, according to the Kruskal-Wallis and Mann-Whitney tests. Group number 3 also showed a significant difference between the Oil and O+D treatments according to the previously listed post hoc tests. On the thirteenth day of exposure behavioural group number 2 and 6 showed a statistically significant difference between the Control and O+D treatment, and the Control and Oil treatment, according to the Kruskal-Wallis and Mann-Whitney non-parametric tests. Table 3.4 summarises these statistical findings.

The Mann-Whitney test also revealed a development in behavioural types within the separate treatments over the course of the experiment. When looking at the development within the Control treatment, a significant statistical difference in behaviour 5 between the third and seventh day of exposure were observed. The oil treatment developed a difference in two behavioural groups during the two weeks of the experiment. The low activity behavioural group number 2 exhibited a difference between day zero and thirteen and the third and thirteenth day of exposure. The second difference in the oil treatment was observed in the high activity behaviour number 6, between the third and seventh day, and the third and thirteenth day of exposure. In the O+D treatment, a similar trend was seen in behaviour. The low activity behaviour number 2 developed a difference between day zero and seven, day zero and thirteen, the third and seventh day and the third and thirteenth day of exposure. And the high activity behaviour number 5 was statistically different between the third and seventh day, and the third and thirteenth day of exposure. Table 3.5 summarises the statistic findings within each individual treatment.

Table 3.4 S*tatistical findings of the differences between the treatments in Northern Krill behavioural recordings. NSD = Not significantly different, SD = significantly different,* * = $P \leq 0.05$, ** = $P \leq 0.01$ and *** = $P \leq 0.001$.

FIRST DAY OF EXOPSURE

Behaviour 1				**Behaviour 2**				**Behaviour 3**				**Behaviour 4**				**Behaviour 5**				**Behaviour 6**			
	O+D	Oil	Cont.		O+D	Oil	Cont.		O+D	Oil	Cont.		O+D	Oil	Cont.		O+D	Oil	Cont.		O+D	Oil	Cont.
Cont.	NSD	NSD		Cont.	NSD	NSD		Cont.	NSD	NSD		Cont.	NSD	NSD		Cont.	NSD	NSD		Cont.	NSD	NSD	
Oil	NSD			Oil	NSD			Oil	NSD			Oil	NSD			Oil	NSD			Oil	NSD	-	
O+D				O+D				O+D				O+D				O+D				O+D			

THIRD DAY OF EXOPSURE

Behaviour 1				**Behaviour 2**				**Behaviour 3**				**Behaviour 4**				**Behaviour 5**				**Behaviour 6**			
	O+D	Oil	Cont.		O+D	Oil	Cont.		O+D	Oil	Cont.		O+D	Oil	Cont.		O+D	Oil	Cont.		O+D	Oil	Cont.
Cont.	NSD	NSD	-	Cont.	NSD	NSD		Cont.	SD**	NSD		Cont.	NSD	NSD		Cont.	NSD	NSD		Cont.	NSD	NSD	
Oil	NSD	-	-	Oil	NSD			Oil	NSD			Oil	NSD			Oil	NSD			Oil	NSD		
O+D	-	-	-	O+D				O+D				O+D				O+D				O+D			

SEVENTH DAY OF EXOPSURE

Behaviour 1				**Behaviour 2**				**Behaviour 3**				**Behaviour 4**				**Behaviour 5**				**Behaviour 6**			
	O+D	Oil	Cont.		O+D	Oil	Cont.		O+D	Oil	Cont.		O+D	Oil	Cont.		O+D	Oil	Cont.		O+D	Oil	Cont.
Cont.	SD***	NSD	-	Cont.	SD**	NSD		Cont.	NSD	NSD		Cont.	NSD	NSD		Cont.	NSD	NSD		Cont.	NSD	NSD	
Oil	SD**	-	-	Oil	SD**			Oil	SD*			Oil	NSD			Oil	NSD			Oil	NSD		
O+D	-	-	-	O+D				O+D				O+D				O+D				O+D			

THIRTEENTH DAY OF EXPOSURE

Behaviour 1				**Behaviour 2**				**Behaviour 3**				**Behaviour 4**				**Behaviour 5**				**Behaviour 6**			
	O+D	Oil	Cont.		O+D	Oil	Cont.		O+D	Oil	Cont.		O+D	Oil	Cont.		O+D	Oil	Cont.		O+D	Oil	Cont.
Cont.	NSD	NSD	-	Cont.	SD**	SD*		Cont.	NSD	NSD		Cont.	NSD	NSD		Cont.	NSD	NSD		Cont.	SD*	SD***	
Oil	NSD	-	-	Oil	NSD			Oil	NSD			Oil	NSD			Oil	NSD			Oil	NSD		
O+D	-	-	-	O+D				O+D				O+D				O+D				O+D			

Table 3.5 *Statistical findings of the behavioural development within the individual treatments. NSD = Not significantly different, SD = significantly different,* * = $P \leq 0.05$ and ** = $P \leq 0.01$.

CONTROL

Behaviour	0^{th} and 3^{rd}	0^{th} and 7^{th}	0^{th} and 13^{th}	3^{rd} and 7^{th}	3^{rd} and 13^{th}	7^{th} and 13^{th}
1	NSD	NSD	NSD	NSD	NSD	NSD
2	NSD	NSD	NSD	NSD	NSD	NSD
3	NSD	NSD	NSD	NSD	NSD	NSD
4	NSD	NSD	NSD	NSD	NSD	NSD
5	NSD	NSD	NSD	SD**	NSD	NSD
6	NSD	NSD	NSD	NSD	NSD	NSD

OIL

Behaviour	0^{th} and 3^{rd}	0^{th} and 7^{th}	0^{th} and 13^{th}	3^{rd} and 7^{th}	3^{rd} and 13^{th}	7^{th} and 13^{th}
1	NSD	NSD	NSD	NSD	NSD	NSD
2	NSD	NSD	SD*	NSD	SD*	NSD
3	NSD	NSD	NSD	NSD	NSD	NSD
4	NSD	NSD	NSD	NSD	NSD	NSD
5	NSD	NSD	NSD	NSD	NSD	NSD
6	NSD	NSD	NSD	SD*	SD**	NSD

O+D

Behaviour	0^{th} and 3^{rd}	0^{th} and 7^{th}	0^{th} and 13^{th}	3^{rd} and 7^{th}	3^{rd} and 13^{th}	7^{th} and 13^{th}
1	NSD	NSD	NSD	NSD	NSD	NSD
2	NSD	SD*	SD*	SD**	SD*	NSD
3	NSD	NSD	NSD	NSD	NSD	NSD
4	NSD	NSD	NSD	NSD	NSD	NSD
5	NSD	NSD	NSD	SD*	SD*	NSD
6	NSD	NSD	NSD	NSD	NSD	NSD

3.3.2 Northern krill respiration

Northern krill respiration was measured twice during the experiment, at the second and fourteenth day of exposure. At the second day, eight animals were tested from each treatment, while at the fourteenth and final day, ten animals were tested from the Control and Oil treatments. Because of high mortality the O+D respiration was measured on the thirteenth, rather than the fourteenth day, with only three animals.

As the seawater used in the experiment was filtered, a very low background oxygen consumption was expected, however, this value was not subtracted from the final respiration results. The two first background oxygen measurements were suspiciously high (close to 40% of the oxygen consumed by the krill). This was due to no mixing of the water in the bottles at the end of the measurements. Without mixing, the electrodes will not measure the oxygen content sufficiently. These data were therefore not usable. The two final background measurements were either close to, or below zero, indicating no, or extremely low oxygen consumption. The water used had been filtered with 2µm filter and at such low temperature we are confident that the background respiration is negligible and will not affect the respiration data. Because of the activity of the krill in the respiration chambers during respiration measurements the water was naturally mixed, creating an even oxygen level within the chamber, and no stirring was needed. However, the last respiration measurements on some of the Oil and all the three O+D animals required a metal stirrer at the end of the measurements as these animals were extremely inactive or in a state of narcosis.

According to the one-way ANOVA test, the respiration rates at the different treatments were not statistically different at the second day of exposure. However, there was observed a significant difference between the Control and the Oil and O+D treatments at experimental day thirteen and fourteen, according to the LSD, Scheffe and Bonferroni post hoc tests. No significant difference was seen between the Oil and O+D treatments, although the variation in oxygen consumption between the two on the last day of exposure was considerable. The reason for this is the low number of krill which were left in the O+D group, compared to the Oil treatment were not enough to cause a statistical difference. Table 3.6 summarises the statistical findings.

Table 3.6 *Statistical findings of the respiration rates between the exposure treatments. NSD = Not statistically different, SD = Statistically different, * = $P \leq 0.05$.*

2nd day of exposure				**13th and 14th day of exposure**			
	Control	Oil	O+D		Control	Oil	O+D
O+D	NSD	NSD		O+D	SD *	NSD	
Oil	NSD			Oil	SD *		
Control				Control			

Although no statistical difference was seen between the treatments at the second day of exposure, a visual difference in the groups median respiration rates were observed. The median respiration rate in the Control treatment was 42.5 μmol O_2/g·h, the krill in the Oil treatment used 50.0 μmol O_2/g·h and the O+D treatment used 52.5 μmol O_2/g·h (fig. 3.14). The higher oxygen consumption of the exposed krill corresponds with previous studies on zooplankton exposed to oil pollution. The higher respiration rate is most likely connected with the induced hyperactivity exposed animals suffer as an escape reaction to avoid stressful conditions. This is confirmed in the behavioural analysis where increased activity level was seen in Oil and O+D treatments at the third day of exposure, compared to the Control (fig. 3.14).

At the fourteenth and final day of exposure, there was an obvious difference in the oxygen consumption between the treatments. This is confirmed by the statistical findings, however, the Oil and O+D treatments were not considered significantly different. Yet, as seen in the measurements conducted at the second day of exposure, a visual difference between the treatments median respiration rates could still be seen. The median respiration rate in the Control was 32 μmol O_2/g·h, the Oil used 24 μmol O_2/g·h and the O+D used 10.5 μmol O_2/g·h (fig. 3.16). Here the oxygen consumption in the exposed animals is distinctly lower than in the Control. This might be because of stress on vital organs and cell repair, reserving energy for maintenance and repair of other systems rather on respiration and/or narcosis.

It is worth noting that several of the krill in the Oil and all of the krill in the O+D treatment showed a very low activity level and one of the total three surviving organisms in the O+D treatment died at the end of the respiration recordings. Again, these respiration rates correspond with the animal's behaviour, recorded in the behavioural measurements at the thirteenth day of exposure. Higher activity naturally requires higher oxygen levels than lower activity behaviours. Low activity animals are reserving energy on repair of the toxic effects caused by the oil exposure. The lower respiration rates in the Oil and O+D treatments are also associated with increased mortality, clearly seen in figure 3.13.

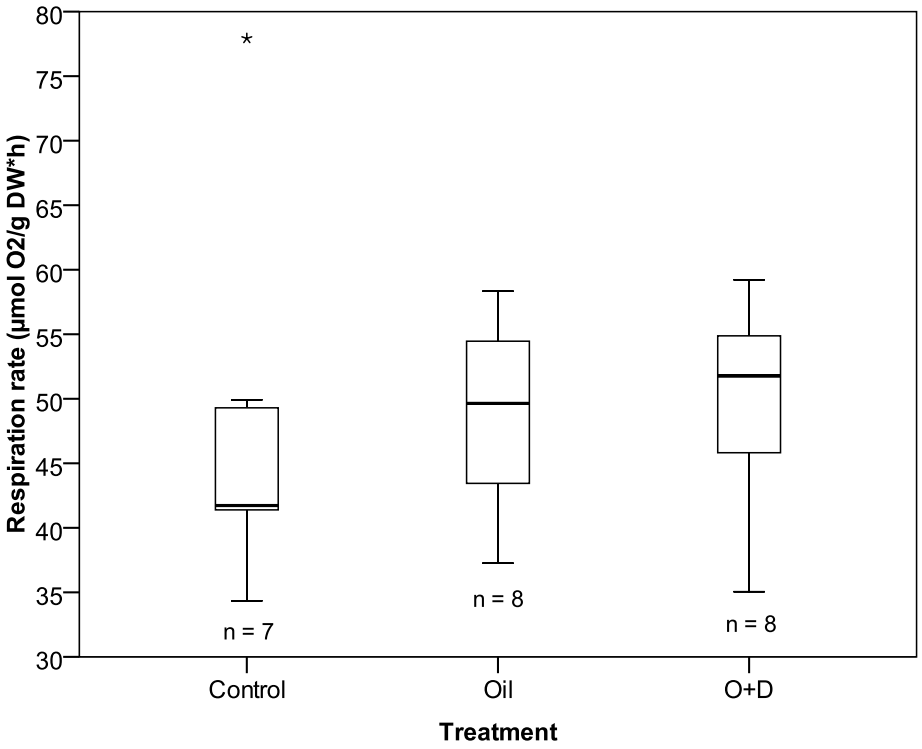

Figure 3.16 *Respiration rates of M. norvegica after two days of exposure. The whiskers show the 95% probability range of respiration rates recorded and the boxes lineate 50% of the respiration data. The horizontal bar represents the median respiration rate and the star represents an extreme outliner.*

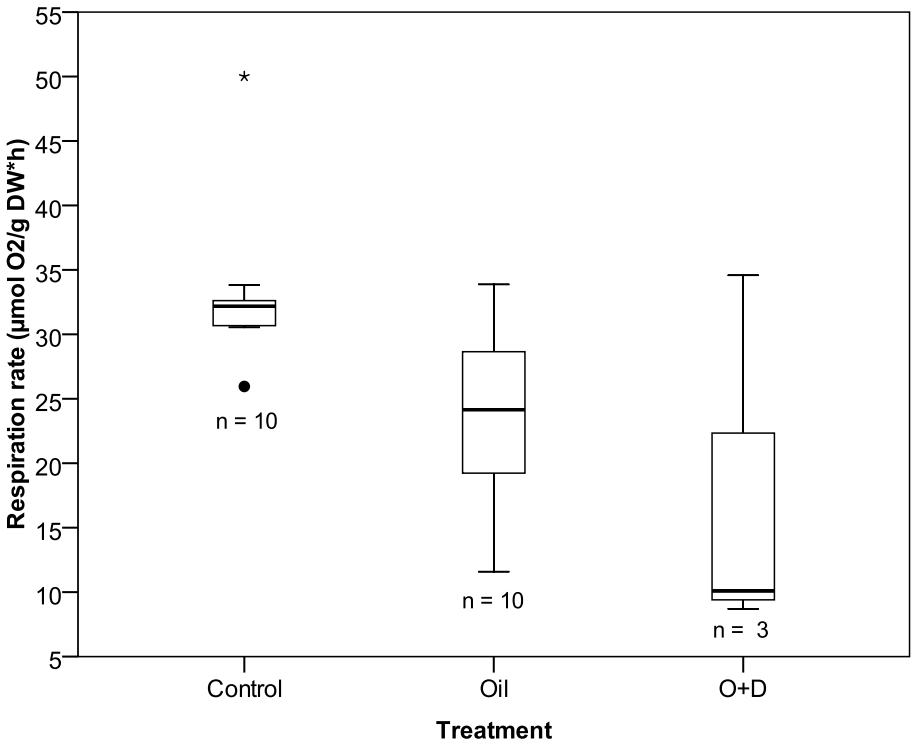

Figure 3.17 *Respiration rates of M. norvegica after 14 days (O+D only received 13 days) of exposure. The whiskers show the 95% probability range of respiration rates recorded and the boxes lineate 50% of the respiration data. The horizontal bar represents the median respiration rate, the circle represents a mild outliner, while the star is an extreme outliner.*

3.3.3. Northern krill moulting

The moulting rate of the twenty-four *M. norvegica* in each of the three treatments was recorded daily throughout the exposure experiment. After the first day of exposure, the largest increment in moulting rate was seen in the Control treatment. The krill in this treatment moulted five of the groups' total eight moults within the first day of the experiment. After this, the animals kept a more or less steady moulting rate all through the experiment, with one moult every third to sixth day. The Oil and O+D treatments illustrated a second and different moulting pattern from that of the Control treatment. All of their moults came within the fifth (O+D treatment) and sixth (Oil treatment) day of exposure. The animals in the Oil group additionally appeared to lag approximately a day behind the initial moulting of the Control group which appeared at the first day of exposure and the O+D treatment lay three days behind. The oil and oil plus dispersant seemed to postponed the moulting response in the krill.

The total and final moult count was however, very similar in all three treatments. The Control and Oil groups ended up with eight moults, and the O+D group ended with seven moults. Table 3.7 contains the accumulated moults data, while figure 3.18 illustrates this graphically. Overlap of the error bars between the three treatments in fig. 3.18 made it hard to separate the different lines and where therefore removed.

No statistical tests were found to be suitable for the accumulated moults data, and were therefore not conducted. There does not seem to be any difference in moulting rates between the 3 different treatments although there seems to be a slight difference in the timing of the moulting.

Table 3.7 *Accumulated moults in the three treatments during the exposure period. The bold, red numbers emphasises the different groups moulting rate.*

Time (days)	Control	Oil	O+D
1	**5**	**3**	0
2	5	**5**	**2**
3	5	**7**	**5**
4	**6**	7	**6**
5	6	7	**7**
6	6	**8**	7
7	6	8	7
8	6	8	7
9	6	8	7
10	**7**	8	7
11	7	8	7
12	7	8	7
13	7	8	7
14	**8**	8	7

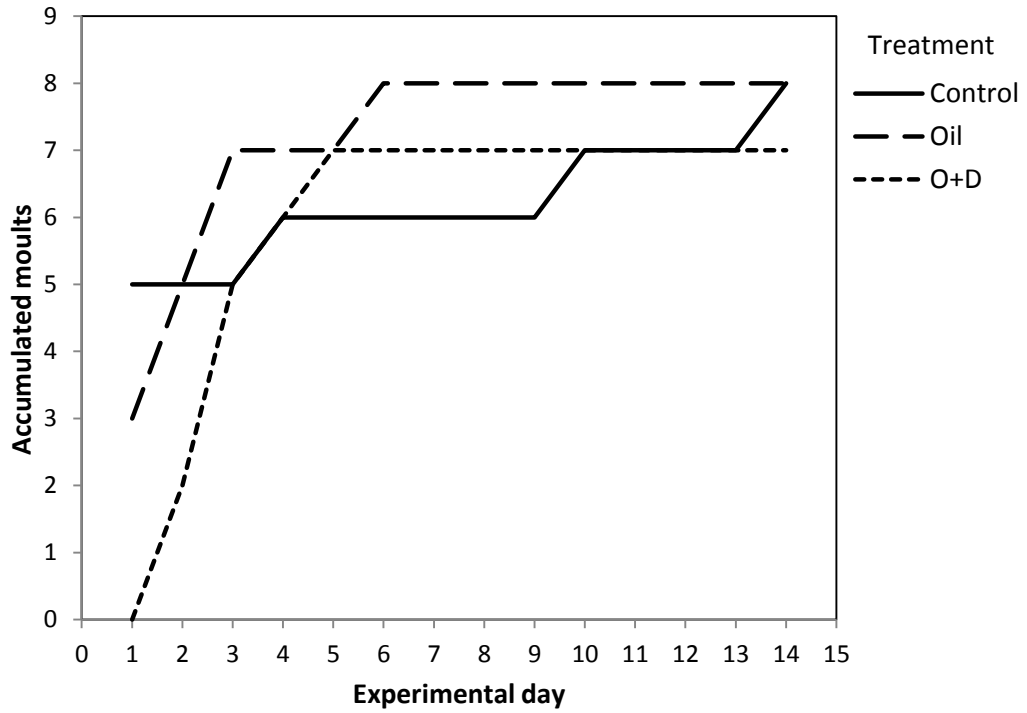

Figure 3.18 *The moulting rate in each of the three treatments during the exposure period.*

3.3.4 *Calanus* egg production

Calanus finmarchicus egg production was measured twice during the experiment, at the second and tenth day of exposure. Egg production of ten *Calanus* females was examined from each treatment.

The egg production data was not normally distributed according to the Kolmogorov-Smirnov test, so a Mann-Whitney test was performed to look for statistical difference between the treatments. This test revealed no significant statistical difference between the treatments at neither the second nor the tenth day of experiment. However, some differences between the treatments can still be seen in figure 3.19 and 3.20. The mean egg production rate in the Control treatment at the second day of exposure was 9.0 eggs/female·day, the mean in the Oil was 6.1 eggs/female·day, and the mean in the O+D treatment was 1.3 eggs/female·day. On the tenth day of exposure the variance between the treatments was lower, though the Control still produced more eggs than the exposed *Calanus.* The mean egg production rate in Control was 3.1 eggs/female·day, the mean in the Oil and O+D treatment was 0.1 eggs/female·day The variance within each treatment is very high and due to the fact that some females did not produce any eggs at all. This can possibly be explained by them not having received a sperm sac from a male, that their eggs were not mature for release, their reserves were not enough for egg production or because of their feeding history.

The graphs show that there appears to be considerably less egg production seen for the oil exposed treatments although they were not statistically different. The overall egg production decreased with time, but that was to be expected as the feeding for the *Calanus* was not optimal and to continue higher production they would have needed the right mixture of live algae. The same trend is seen at both sampling times with the control *Calanus* consistently producing more eggs than the exposed animals.

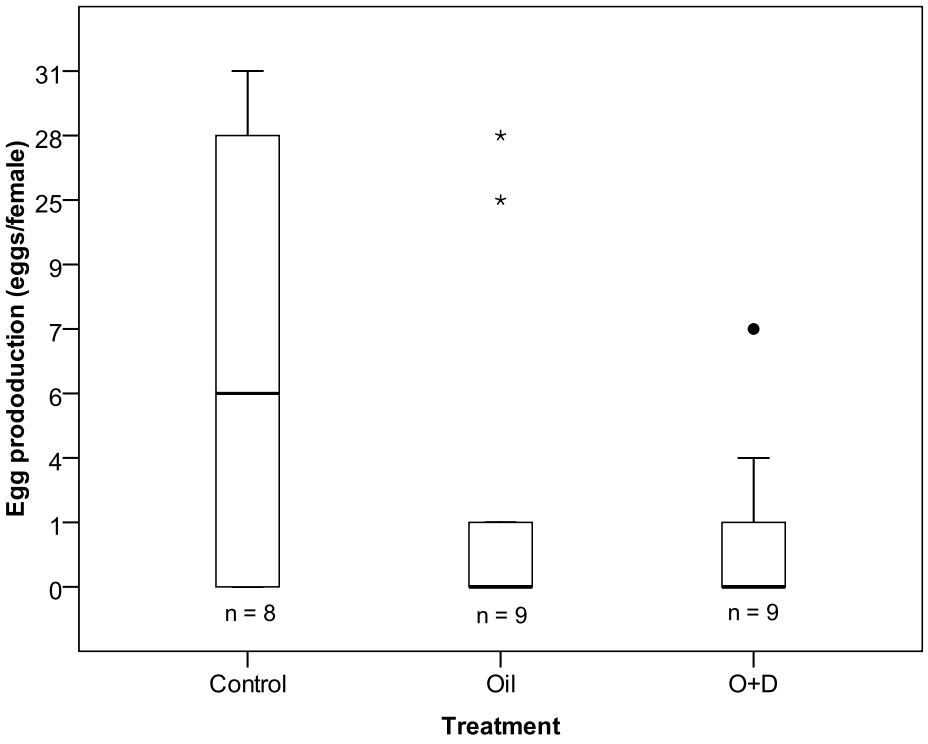

Figure 3.19 *Egg production of C. finmarchicus after 2 days of exposure. The boxes lineate 50% of the egg production data and the horizontal bar represents the median egg production rate. The circle represents a mild outliner, while the stars are extreme outliners.*

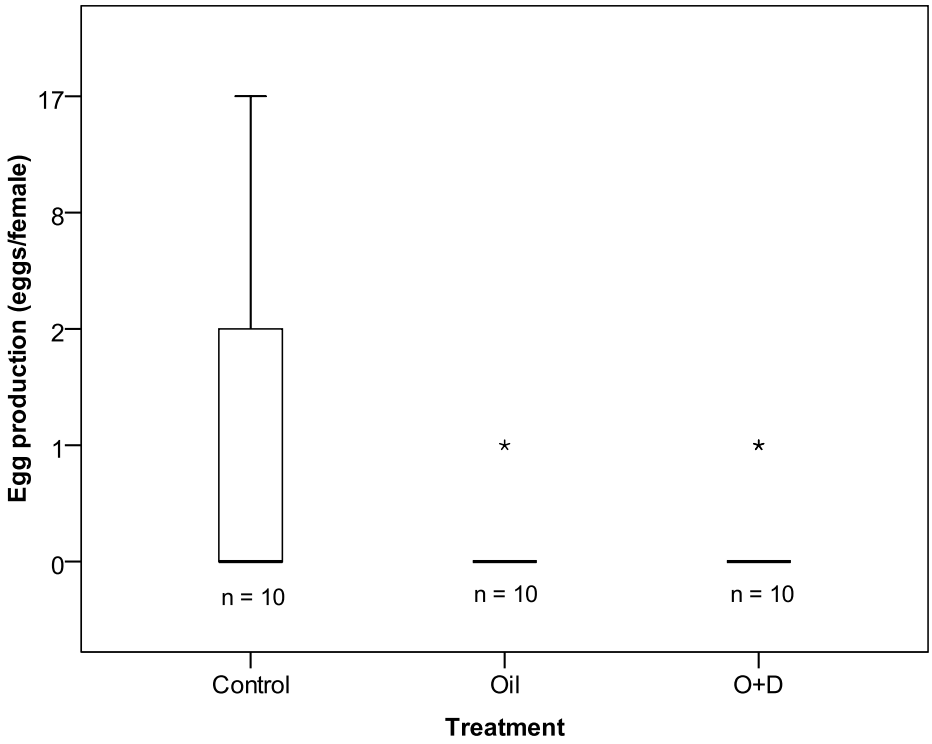

Figure 3.20 *Egg production of C. finmarchicus after 10 days of exposure. The boxes lineate 50% of the egg production data and the horizontal bar represents the median egg production rate. The stars represent extreme outliners.*

3.3.5. *Calanus* mortality

The mortality of *Calanus finmarchicus* was recorded throughout the exposure experiment in the Control, Oil and O+D treatments. Each treatment had three cylinders containing 30 animals, resulting in a total of 90 animals per set up. Mortality was registered every second to third day (three days over the weekends). Animals which were suspected dead were examined under the microscope to check for movement (fig. 3.21).

Figure 3.21 *Mortality registration of Calanus finmarchicus at 25x magnification.*

The first and largest spike in mortality occurred sometime over the first weekend, and was recorded on the sixth day of exposure. We registered 56% and 5 % dead in the O+D and Oil treatment, respectively (fig. 3.22). A pronounced difference in mortality between the three groups could clearly be seen at the end of the experiment (the error bars of the different treatments at the same date did not overlap). The O+D treatment ended up with 96.1 % of the *Calanus* dead, the Oil treatment ended with 38.1 % and the Control ended with 17.8 %. The mortality at the end of the experiment was in other word approximately 2 times higher in the Oil treatment compared to the Control, and 5 times higher in the O+D group than the Control. The mortality in the Control treatment was most likely related to low or incorrect feeding of the animals. The final mortality count in the O+D treatment exceeded the initial amount of animals placed in the cylinders (table 3.8). This can be due to a mistake when counting the animals at the beginning of the experiment or a misplacing of a couple of individual when sorting the egg production animals that got the same treatment. This will however not affect the mortality results as those have been corrected for the final amount of animals in the cylinders.

Statistical analysis of the data was found to be very complicated. The data had neither normal distribution nor homogeneous variance and a parametric test like the Two-Way ANOVA was therefore not suitable. A Two-Way non-parametric tests alternative is the Scheirer-Ray-Hare test. This test is not adapted for low n-replicate numbers. Attempts to run the data with the Scheirer-Ray-Hare test did not provide with sensible outputs and the danger of getting erroneous results with this test was found to be too high. It was therefore decided to run a Two-Way ANOVA (to show difference for day, treatment and combination of the two) even though the conditions ANOVA require were not satisfied. This was done only to see if

there were found to be any differences developing. This test should not be performed with so few data (three replicates for each factor combination) as the ANOVA requires normal distribution and homogeneity of variances that cannot be tested reliably.

According to the Two-Way-ANOVA test there was found to be a significant difference in mortality between all factors ($p<0.001$.). This means that the mortality in both the treatments and the timing within the experiment are significantly different from each other. This would suggest that there is a difference between all three treatments and that also the day of experiment is an effect factor. We can see that in fig. 3.22 the mortality at the end is twice as high in the Oil group than seen in Control and five times higher in the O+D group when compared with Control and that the error bars (±SE) of the different treatments at the same date does not overlap. Mortality increases sharply in the O+D group but is less pronounced in the other two groups. On day eleven the mortality of the Oil exposed animals increases.

Both the percentage of mortality and the timing of the mortality spikes are comparable to the mortality observed among the krill in the corresponding treatments (fig. 3.13). In both cases a mortality spike appeared around the fourth to fifth day of exposure in the O+D treatment. Similarly, an increase was seen in the Oil treatment around the ninth to eleventh day of exposure in both species. However, *C. finmarchicus* appear slightly more sensitive to the oil plus chemical dispersant combination and slightly less sensitive to the crude oil alone, than the Northern krill.

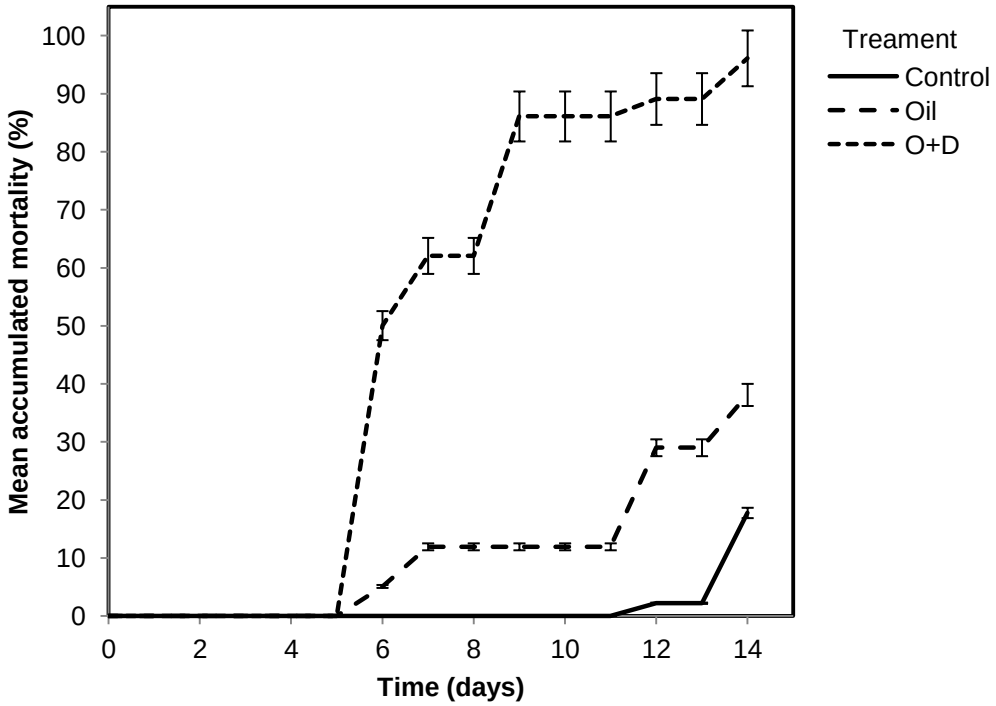

Figure 3.22 *Mean accumulated mortality of Calanus finmarchicus in the three different treatments during the exposure experiment. The vertical lines represent error bars with a 95 % confidentiality interval.*

Table 3.8 *Summary of the mortality results.*

	Cylinder 1	Cylinder 2	Cylinder 3	Mean	Mean cumulated mortality (%)
Dead Control	5	8	3	5.3	17.8
Live Control	26	21	27	24.7	
Dead + live	31	29	30	30.0	
Dead Oil	7	13	12	10.7	38.1
Live Oil	20	20	16	18.7	
Dead + live	27	33	28	29.3	
Dead O+D	36	28	32	32.0	96.1
Live O+D	1	3	0	1.3	
Dead + live	37	31	32	33.3	

4. DISCUSSION

Expanding petroleum activities into northern marine areas have resulted in need for tools that specify Arctic specific characteristics when assessing environmental risks for these regions. Such activities will increase the possibility of shipping accidents and accidental oil discharges together with produced water discharges from drilling rigs in those areas. Sometimes quickly diluted and degraded to lower concentrations, such emissions may impose a longer term threat to plankton fauna through sub-lethal effects from bioactive components either at low levels, or the potential for bioaccumulation to other levels through the food chain. Zooplanktons are widely distributed in the marine environment and because of their limited mobility, have little ability to escape the stress of an oil spill.

Although some studies have indicated that Arctic species do not differ from non-arctic species on the individual level (De Hoop et al., 2011, Olsen et al., 2011), Arctic species have characteristics that might influence severity and duration of potential impacts in a different manner than non-arctic species would. Such characteristics are the strong seasonality of feeding, reproductive abilities and timing, food availability and longevity and developmental times (Chapman and Riddle, 2005). As *Meganyctiphanes norvegica* and *Calanus finmarchicus* are considered keystone planktonic species in these areas and source of food for several commercially important fish species, concern is raised that these, and hence the associated ecosystem, can be seriously affected in the event of an oil spill.

The objective of this thesis was to establish some basic parameters from a pre study and use the information to conduct an exposure study that would be useful for estimation of the effects of oil in ice or blowout scenarios on northern marine keystone organisms. These results may in turn provide knowledge for development of tools to prepare for environmental management of future operations in sensitive boreal and sub-arctic environments.

4.1 MOULTING PRE-STUDY

Moult and growth are essential parameters for the construction of energy budgets for crustaceans. Measurements of moult lengths inform us of the growth and possible shrinkage in the animal, and hence the life history and condition of the test species. Buchholz (1991) has summarized laboratory experiments on moult and growth of krill. The study showed that the first growth increment at moult (INC) under laboratory conditions was always the largest, most likely still reflecting the field situation, and the value would decrease thereafter. This corresponds with our results for moult measurements, were the longest segments generally were measured for the first moult. The moult lengths usually decreased from that point on, seen as an overall negative mean growth of the moults. The Tha-EZ treatment moulted the most (on average 2.8 moults), and the starved group moulted the least (on average 1.5 moults (table 3.1 and 3.2)). The moults in the Art-EZ treatment shrunk the least (-3.2%), while the starved group shrunk the most (-4.4%). This was the only measurement where the Art-EZ treatment appeared more favourable than the Tha-EZ treatment, shrinking on average 0.6% less than the Tha-EZ.

The INC value has been reported to vary as much as -15% to +21%, when typically comparing the uropod, carapace or antennal scale length between the moult and post-moult animal in Antarctic krill (Buchholz, 1991). While in the Northern krill, INC have been showed to vary between -6% to +6% (Buchholz and Buchholz, 2010) and -0.1% to +0.1% (Cuzin-Roudy et al., 2004). Because the telson length remained the most reliable of the parameters measured, this length was used to measure the INC value, seen as the 'G/R of telson' column in table 3.1. All of the treatments exceeded the lower end of the reported *M.*

norvegica INC values (-11.4% to 0% in Art-EZ and -10.8% to 2.5% in Tha-EZ), while only the starved group also exceeded the lower range of the Antarctic krill (-39.5% to 2.3%). When comparing the length of the telson in the first moult with the final telson length of the krill, and when comparing the last moult with the post-moult krill, the Tha-EZ treatment exhibited the highest INC values. The Tha-EZ treatment also held more krill with segments (the telson length excluded) which were longer than in the animal's last moult, than in the Art-EZ treatment. Although the starved treatment was the group with krill that had the most segments which were longer than in the animal's last moult, most of them were from two and a half to three weeks before the experiment ended, and are therefore not valid as true indications of growth.

In this study, a clear difference in moulting patterns was visible between the fed and starved treatments. The two feeding treatment exhibited regular moulting periods with a intermoult period (IMP) of 13-15 days while the unfed animals did not show any clear rhythm to their moulting (Fig. 3.1 and 3.2). The interrelationship between IMP and INC depends on the exact timing of the moult phases (Tarling et al., 2010). These may in turn be affected by environmental conditions. The IMP has been found to be around 13 days in an adult krill of ca 30 mm at 10°C (Cuzin-Roudy and Buchholz, 1999, Buchholz et al., 2006). However at temperatures of 8°C, longer IMPs were observed. This is in accordance with the results seen for the krill measured here, where intermoult time was found to be between 13.5 and 15 days for krill size approximately 28mm. Longer IMPs were seen for the starved animals as well as irregular moulting times. This could indicate that stressful conditions potentially affect hormonal regulation of moult timing. Synchronized moults may also be absent in the starved groups due to the lack of regular feeding regimes. Regular feeding in the laboratory may trigger fast ingestion and subsequent accumulation of reserves. As a result, moult preparatory processes and finally actual ecdysis are accelerated and at the same time synchronize between individuals (Buchholz, 2003). The regular feeding appeared successful in both reducing mortality and introducing synchronized moulting rhythms.

Summarizing the results, little difference could be seen between the two feeding treatments (Art-EZ and Tha-EZ). The moults in the Art-EZ group shrunk 0.6% less than in the Tha-EZ treatment, while the Tha-EZ treatment moulted the most and had the most animals with segments that were longer than in the animal's last moult. However, the starved group was clearly the most unfavourable treatment, with the fewest moults and the most shrinking krill. Because both of the fed treatments ended with a mean negative growth, long term conditions and feed were most likely not optimal throughout the experiment. A possible reason for the negative growth might come from maintenance procedures, as the growth increment at moult has been reported to be sensitive to such disturbances (Buchholz, 1991) or that the krill were not in the appropriate age group for growth measurements. Growth studies on krill should optimally focus on sub-adult animals, thus avoiding complications generated by sexual maturation and production of sperm and eggs during the experiment (Buchholz, 2003). The krill in our study was a mixture both adult males and females. Adult Northern krill with high age will also, as most other living creatures, grow more slowly over time (Boysen and Buchholz, 1984). In addition should experimental times be kept short (from days to a few weeks) in order to produce reliable growth data. This has been fully acknowledge in the establishment of the instantaneous growth rate technique (overview in Nicol, 2000).

4.2 CFS EXPOSURE EXPERIMENT ON *M. NORVEGICA* (1)

The most important means of entry of oil in zooplankton is through the particulate dietary pathway (Corner et al., 1976, Harris et al., 1977). Oil droplets in the particulate form

can aggregate with, or absorb, naturally occurring particles. Consequently, food particles are trapped by oil. Krill is capable of shifting their feeding pressure to correspond with the most abundant particles in seawater (Parsons et al., 1967). As the oil droplets are similar in size to naturally occurring particles they can be ingested by the krill.

In contrast to the moulting study, a clear difference between the different treatments in the exposure experiment could be seen (fig. 3.4-3-8). There were pronounced differences between the Medium moults and the other groups and a difference was also found between the Low and Control treatments. The Control group had moults in the best condition. This observation does not appear to be sited in literature per date as oil exposure studies on krill are not available. A possible reason for the brown, cut externals might be because of bacterial infections. Oil works as a carbon source for several bacterial strains, and many microbial communities have been shown to flourish when exposed to oil (Harayama et al., 2004, Bao et al., 2012, Ortmann et al., 2012, Viggor et al., 2013).

Although no significant statistical difference was found between the treatments, variations were still observed in the carapace and telson length, and wet and dry weights of the animals (fig. 3.9-3.12). When examining the mean carapace and telson length, the Medium exposure group appeared to grow the most, and the Low grew the least. The reason for this is possibly that the smallest animals in the Medium group most likely died first, hence increasing the mean carapace length without truly growing. Reports of size-dependent mortality show that smaller organisms are worse affected than individuals with larger size when confronted with oil pollution (e.i. Jiang et al., 2012). Additionally, the data in the Medium treatment was only from the first week, and the evidently high growth trend in this treatment might still have decreased drastically had the animals survived until the end of the experiment.

A similar trend can be seen in the wet (fig. 3.11) and dry weight (fig. 3.12) of the animals, were the mean weight of the Medium group is higher than that of the Low group. However, the Control was the heaviest group of the three treatments. Thus even though the animals in the Medium treatment were the longest in respect to carapace and telson length after the smaller organism had died, the Control treatment was the group with the heaviest krill. This may indicate that the animal's length does not necessarily correspond with its weight, or that this was a result of the random selection of the animals when dividing the krill into the three treatments. Another hypothesis is that the surviving krill in the Medium exposure group was not able to digest their food because it was coated in oil, as stated by Parsons et al. (1967), and hence gained little weight.

4.3 EXPOSURE EXPERIMENT ON *M. NORVEGICA* AND *C. FINMARCHICUS* (2)

4.3.1 Northern krill behaviour

Behavioural observations conducted on organisms exposed to oil related compounds often involve feeding (Barata et al., 2002, Calbet et al., 2007) and hatching rates (Barata et al., 2002, Bellas and Thor, 2007) but swimming speeds or activity levels are rarely recorded. However, changes in behaviour are becoming an increasingly popular approach to analyse effects of anthropogenic pollution (Clotfelter et al., 2004, Zala and Penn, 2004). Long-term exposure at low concentration of pollutants will not necessarily result in lethal toxicity, making traditional mortality tests less relevant. Behaviour observations capture differences occurring at sub-lethal concentrations and transmit important information about impairment

and subtle ecological processes, making behaviour an appropriate parameter (Krang, 2007, Ward et al., 2008).

Swimming behaviour is a primary part of krill ecology and appears to be highly sensitive to a variety of natural factors (Buskey et al., 1987, Tiselius, 1992, van Duren and Videler, 1995, Michalec et al., 2012). To the authors' knowledge, no behavioural research on the effects of oil pollution on krill has yet been conducted. Still, Michalec et al. (2013) observed the behavioural responses in the copepod *Eurytemora affinis* to sub-lethal concentrations of polycyclic aromatic hydrocarbons. The effects were observable as increased swimming speed and activity, mostly within 30 min of exposure and persisted or faded during a period of depuration in uncontaminated water of similar duration. Similar, hyperactivity was observed in the amphipod *Corophium valuator* exposed to 25 and 50% water accommodated fractions (WAFs) of weathered crude oil, while exposure to 100% led to narcosis (Kienle and Gerhardt, 2008). The induced hyperactivity resembled an escape reaction permitting animals to evade stressful conditions.

Behavioural observation of the Northern krill illustrated a clear difference between the treatments over the course of the experiment (fig. 3.14). The Oil and O+D treatments illustrated the same behavioural trend, although the behaviours in the O+D treatment were more pronounced and appeared earlier than in the Oil treatment. The krill appeared restless and highly active up to the first week of exposure in the Oil treatment, and up to the third day in the O+D treatment, compared to the Control. This corresponds with the reports of hyperactivity in marine organisms by Michalec et al. (2013) and Kienle and Gerhardt (2008) after short-time exposure of pollutants.

After this point, the behaviour of the exposed specimens decreased from the high activity behaviours to the low activity behavioural type number 2, believed to be narcosis. All krill that started performing behaviour number 2 spent most of their time in this state, and eventually died within one to two days of first performing the behaviour. This behavioural type never appeared in the Control treatment, although a low degree of mortality was observed in this treatment. Again, the results match the findings in Kienle and Gerhardt (2008) where animals exposure to 100% WAFs of weathered crude oil appeared to go into a state of narcosis. The overall results also correspond with the observations Jiang et al. (2012) found in 15 different species of copepods. Specimens exposed to crude oil WAFs showed restlessness, impaired swimming ability, loss of balance, anoxic coma and even death.

Behavioural observations are highly informative, and in this study preceded the increase in mortality among the krill. Behaviour represents an endpoint of particular interest in ecotoxicological studies. A reasonable number of studies have examined the effects of contaminants on the behaviour of aquatic organisms. However, test specimens, experimental conditions, contaminants and incubation times vary significantly, making comparison of the results difficult. A model approach which can be used in ecological impact assessment models is therefore highly needed.

4.3.2 Northern krill respiration

Respiration rates are a good indicator of animal's metabolic and adaptive abilities, and are therefore an excellent tool when examining the environmental effects of pollution on exposed organisms. The keystone organism *Meganyctiphanes norvegica* is known for its high aerobic metabolism, but poor anaerobic capacity (Spicer et al., 1999). Saborowski at al. (2002) found that the respiration rates of three different populations of *M. norvegica* displayed near identical rates of oxygen uptake (30-35 μmol O_2/g DW·h) when tested at the environmental temperatures they were each experiencing. However, comparison of oxygen

consumption rates between studies is particularly difficult because of methodological and ecological differences and the thermal history of the animal (Spicer and Saborowski, 2010). Consequently, there is a range of oxygen uptake values reported for the Northern krill. Respiration rates in the Gulf of St. Lawrence in Feb-Aug (2-10℃) ranged from 60.3–92.9 μmol O_2/g DW·h, and in Kosterfjorden (Sweden) in Dec-Sep (5-10℃) they ranged from 21.9–40.6 μmol O_2/g DW·h (Saborowski et al., 2002). While recent work has focused on physiological effects, the ecophysiological effects of pollution are generally are widely understudied in *M. norvegica.*

In an earlier study of oil toxicity in copepods it is described how the physical obstacle of oil fractions acts as a barrier to oxygen transfer between air and water, hence reducing the amount of oxygen animals are able to get a hold of (Kontogiannis and Barnett, 1973). Exposure of naphthalene, a major component in crude oil, to mud crabs resulted in increased oxygen consumption and decreased activity of respiratory enzymes (Ahmad et al., 2003). The same effect were observed in copepods exposed to low concentrations of oil WAFs, which showed an increased oxygen consumption (Kontogiannis and Barnett, 1973, Smith and Hargreaves, 1984) by the influence of oil toxicity.

Respiration rates from the experiment corresponded with the behavioural trends seen between the treatments at both the measured early and late exposure days. Although no statistical significant differences were seen between the treatments at the second day of exposure, the exposed treatments median respiration rates were visually higher than those in the Control treatment (fig. 3.16). The Control used 10 μmol O_2/g·h less than the O+D treatment, and 7.5 μmol O_2/g·h less than the Oil treatment. This corresponds to the higher activity observed in the behavioural recordings in the Oil and O+D treatments at the third day of exposure, compared to the slightly lower activities seen in Control, and the results of Ahmad at al. (2003), Smith and Hargreaves (1984) and Kontogiannis and Barnett (1973). The median respiration rate of 42.5 μmolO_2/gh in the Control were not significantly different from the ones found in Saborowski at al. (2002), while the rates of 50.0 μmolO_2/gh and 52.5 μmolO_2/gh in the oil and O+D treatments, respectively, were distinctly higher.

At the fourteenth and final day of exposure, there was a clear difference in oxygen consumption between the treatments. This was confirmed by statistical findings, however, the Oil and O+D treatments were not considered significantly different. Yet, as seen in the measurements conducted at the second day of exposure, a visual difference between the treatments median respiration rates could still be seen (fig. 3.17). The Control used 21.5 μmol O_2/g·h more than the O+D and 8 μmol O_2/g·h more than the Oil treatment. These findings did not correlate to the increased oxygen consumption normally seen in organisms exposed to oil. However, tests conducted by Ahmad at al. (2003) and Smith and Hargreaves (1984) were only after relatively short-time exposure (up to three days), and are therefore not completely comparable with the findings presented here where the exposure was considerably longer (fourteen days) and the animals had started displaying narcosis.

It is worth noting that several of the krill in the Oil and all of the krill in the O+D treatment showed a very low activity and one of the total three surviving organisms in the O+D treatment died at the end of the respiration recordings. Again, these respiration rates correspond with the animal's behaviour, recorded in the behavioural measurements at the thirteenth day of exposure. Higher activity naturally requires higher oxygen levels than lower activity behaviours.

4.3.3 Northern krill moults

Synchronized moults of krill have been reported under both laboratory (Mackintosh, 1967, Clarke, 1976) and field conditions (Buchholz et al., 1996). And as mentioned under 4.1, the initiation of regular feeding regimes in the laboratory may trigger synchronize moulting between individuals (Buchholz, 2003). Krill exposed to high acute oil pollution have been observed to have the highest moult counts shortly after exposure unlike krill kept under control conditions, and adult krill in low oil concentration (0.05 mg/L nominal concentration of oil) moulted less than krill under control conditions (A. Ingvarsdóttir, pers. comm.).

This was however not the case in this study, where the total and final moult count was very similar in all three treatments (table 3.7 and fig. 3.18). The krill in the Control treatment moulted five of the groups' total eight moults within the first day of the experiment. After this, the group kept a more or less steady moulting rate all through the experiment, with one moult every third to sixth day. The Oil and O+D treatments illustrated a second and different moulting pattern from that of the Control treatment. All of their moults came within the fifth (O+D treatment) and sixth (Oil treatment) day of exposure. The animals in the Oil group additionally appeared to lag approximately a day behind the stress moulting which appeared at the first day of exposure in the Control treatment and the O+D treatment lay three days behind. The oil and oil plus dispersant seemed to show a postponed moulting response. It seems that moulting is a parameter that is difficult to interpret. Moulting is under hormonal control but factors like feeding, feeding history and temperature will also affect it and may additionally be influenced by environment, seasonality and photoperiod (Teschke et al., 2008, Seear et al., 2009). It is possible that under experimental conditions stress could induce and increase moulting occurrences, and lower level of stress (starvation and low oil exposure) increased the IMP. No papers have to this day been published on the subject, and the results can therefore not be compared with other studies. Because of the lag in the first moult peek varies between treatments, the phenomena should be further investigated to find if this is a trend, and might be used as an indicator of stress, or if it is a coincidence.

4.3.4 *Calanus* egg production

The egg production rate in copepods is a sub-lethal response parameter which has been shown to be 2-8 times more sensitive to pollutants such as polycyclic aromatic hydrocarbons (PAH), than the lethal response (Bellas and Thor, 2007). This study suggests that short-term exposure of pollutants might have long-term effects on copepods that would otherwise go unnoticed in mortality measurements. Egg production is an important aspect of the adult biology of copepods because it integrates a number of metabolic processes and therefore has a high ecological value (Berdugo et al., 1977). Furthermore, the egg production rate has been reported with a lower degree of variability than the mortality rate, and is for that reason a more suitable impact parameter (Bellas and Thor, 2007).

Two different studies have tested the effect of the PAH pyrene, a major component in oil, on the egg production in the copepod *Calanus finmarchicus*. Both studies show that the egg production rate was significantly reduced after exposure (Jensen et al., 2008, Hjorth and Nielsen, 2011). However, Jensen and Carroll (2010) did not find any significant difference in cumulative egg production when *C.finmarchicus* was exposed to crude oil.

No significant statistical difference between the treatments was found at neither the second nor the tenth day of the experiment, corresponding with the results of Jensen and Carroll (2010). However, variations in the mean egg production rates between the treatments were still seen. The mean egg production rate in the Control group at the second day of

exposure was 2.9 eggs/female·day higher than in the Oil treatment and 7.7 eggs/female·day higher than in the O+D treatment with an average of 9.0 eggs per female in the Control group. The reduced egg productions in the exposed treatments correlates to the findings by Jensen et al. (2008) and Hjorth and Nielsen (2011). Here there was observed a stronger reduction, and hence impact in the O+D treatment, than in the Oil treatment. The reduced egg production can be explained by either a direct or indirect effect caused by the oil or the oil plus dispersant. The direct effect may have caused an impairment in the maturation of the gonads or/and steps in the oogenesis by the chemicals or its toxic metabolites, as proposed by Cowles and Remillard (1983). The indirect effect on the egg production involves a reduced grazing activity by the effects of the chemicals, as suggested by Ott et al. (1978) and Jensen et al. (2008). Additionally, a lag period of an extra day before the start of egg production has been observed for *C.finmarchicus* when exposed to pyrene (Hjorth and Nielsen, 2011) such that the eggs produced in the present study in the exposed treatments might not have been counted because the incubation period was not long enough. The mean control value of 9.0 eggs/female·day corresponds with the *C.finmarchicus* pre-bloom mean egg production rate in the Norwegian Sea of 10 eggs/female·day (Stenevik et al., 2007).

On the tenth day of exposure the variance between the treatments was lower, though the Control still produced more eggs than the exposed *Calanus.* The mean egg production rate in the Control treatment was 3.0 eggs/female·day higher than in the Oil and the O+D treatment. The overall egg production decreased with time (9.0 eggs/female·day in the Control at the second day of the experiment, compared to 3.1 eggs/female·day at the tenth day), but that was to be expected as the feeding for the *Calanus* was not optimal and to continue higher production they would have needed the right mixture of live algae. The food portions were also suspected to be too low during the first week of the experiment, due to suspected starving seen as deaths in the krill Control group. Egg production in *C.finmarchicus* has been found to rapidly change in response to food availability (Runge, 1985, Niehoff, 2004, Hjorth and Nielsen, 2011)) and studies on the effect of food abundance on the copepods biology have shown that growth and egg production rates decline with the food level and food quality (Hirche et al., 1997, Campbell et al., 2001, Madsen et al., 2008).

In both the early and late egg production recordings conducted during the experiment, the variance within each treatment was high due to the fact that some females did not produce any eggs at all. The reason for this can possibly be explained by them not having received a sperm sac from a male, that their eggs were not mature for release, their reserves were not enough for egg production or because of their feeding history.

4.3.5. *Calanus* mortality

Survival remains the one of the most applied response parameter when evaluating the impact of toxic substances, and copepods have been used comprehensively for toxicity testing and establishment of tolerance levels in exposure scenarios. Some of these studies have focused on the impact of oil pollutants on the survival of the boreal keystone copepod, *Calanus finmarchicus* (table 4.1).

Table 4.1 *LC_{50} values of oil related compounds on Calanus finmarchicus.*

Compound	LC_{50} (mg/L)	Study time (h)	Reference
Naphthalene	7.02	96	(Neverdal et al., 2006)
WAF's of marine diesel	1.60	96	(Hansen et al., 2013)
WAF's of artificially weathered crude oil	0.50	144	(Hansen et al., 2011)
Dispersed produced water (as pentane)	0.91	48	(Broch et al., 2013)
Naturally dispersed oil	0.80	96	(Hansen et al., 2012)
Chemically (Dasic NS) dispersed oil	0.49	96	(Hansen et al., 2012)
Dispersant Corexit 9500A	30.4	96	(Hansen et al., 2010)

Recent studies show that resorting to chemical dispersants to manage oil spills is a controversial matter, owing to the dispersant enhanced bioavailability of oil in the water column and the potential for increased toxicity caused by the added dispersant (Gulec et al., 1997, Gulec and Holdway, 2000, Ramachandran et al., 2004, Couillard et al., 2005, Schein et al., 2009, Milinkovitch et al., 2011) . Likewise have Lee et al. (in press) found that when testing the acute toxicity of WAFs of crude oil, chemically dispersed WAFs and the dispersant on the copepod *Tigriopus japonicus*, the dispersant Corexit 9500 was the most toxic (LC_{50}-96h = 34.9%) of all the chemicals tested. The LC_{50} of the chemically dispersed WAFs after 96h was found to be 45.6%.

A pronounced difference was found in mortality both between the days of exposure, the treatments and the combination of the two (days-treatment). The O+D treatment ended up with 96.1% of the *Calanus* dead, the Oil treatment ended with 38.1 % and the Control ended with 17.8%. The mortality in the Control treatment was most likely related to low or incorrect feeding of the animals, as mortality normally is not observed in *Calanus* Controls (Hansen et al., 2011). Nevertheless, there was in other words close to five times higher mortality in the O+D treatment than in the Control, and approximately two times higher mortality in the Oil than in the Control. The highest spike in mortality was recorded on the sixth day of exposure and could be seen as 56% mortality in the O+D treatment. According to this, the median lethal concentration in the O+D treatment was reached within approximately 144h 0.1% chemically dispersed oil. Because the time span of the results matches few of the published data on *C. finmarchicus*, comparison is difficult. However, the O+D treatment close to 50% more lethal than the Oil treatment at the fourteenth day of exposure, corresponding to all cited literature on toxicity effects of oil and oil plus dispersant.

The high mortality observed in the O+D treatment compared to both the Control and Oil groups advice against application of chemical dispersants in environments with high concentrations *C. finmarchicus.* However, the exposure period in the present study does not reflect ordinary environmental conditions, but was chosen to observe the time span likely to be relevant for an experimental model study on oil pollution in Arctic environments. Chemical dispersants dilute and hence remove oil from the water faster than natural dispersion, thus reducing the exposure time marine organisms may be subjected to, significantly. Because of this, the overall environmental impact might still be lower with addition of toxic chemical dispersants, than without (Lindén et al., 1987).

Both the percentage of mortality and the timing of the mortality spikes are comparable to the mortality observed among the Northern krill in the corresponding treatments (fig. 3.13), making the two species comparable in oil spill scenarios. However, *C. finmarchicus* appear slightly more sensitive to the oil plus chemical dispersant combination and slightly less sensitive to the crude oil alone, than the Northern krill. Still, the two species comparable ability to survive periods with non-live food is not know, and *M. norvegica* is known for its sensitivity to maintenance procedures (Buchholz, 1991), making comparisons difficult.

5. CONCLUSION

The objectives of the study were completed successfully. Basic parameters of two different feeds and a starving treatment have been recorded. The main emphasis was on recording "growth" (negative or positive) as INC, but both IMP and moulting rhythm has been established for the local Stavanger population of *M. norvegica*. The information gathered has been used to select parameters and develop experimental conditions for the Northern krill.

From these parameters an exposure system that can be used for blowout or "oil in ice" situations has been designed and tested. The other objective reached was to run a longer term exposure (for fourteen days) to test the designed system for maintaining animals for longer periods within experimental conditions. The system would be suitable for both short and longer term exposures. It is however implausible, that the animals would be subjected for so long to such high oil or dispersant concentrations, as more mixing and potential influx of fresh seawater by currents would result in a shorter period of exposure and most likely lower initial mortality rates would be observed.

To reach more realistic situations, a short acute oil exposure experiment (two to five days), with and without dispersant and with dispersant only would be an option. Additionally, it is recommended to look at survival after exposure with a longer term recovery period to be able to estimate further sub-lethal conditions. It has been reported that sub-lethal effects from oil derived toxicity can be seen during such recovery periods after oil exposures (Goksøyr et al., 1991, Middaugh et al., 1998, Ingvarsdóttir et al., 2012). Mixing of the oil and water for more turbulent conditions as was planned within the experiment. This would give further information on potential impacts on the animals in the field as the volatiles in the oil would be released earlier and potentially be more lethal than the oil slick, but would also disperse and dissolute faster, resulting in a shorter exposure time. Unfortunately this setup failed and had to be discontinued from the experiment.

A behavioural bioassay for krill under control and exposure conditions was successfully established. This assay showed effects from the oil and oil plus dispersant three to five days before mortality rates started to increase substantially, and turned out to be a valuable parameter to compare our results to other known endpoints used for toxicological studies. The majority of the krill in the Oil and O+D treatments appeared hyperactive at the start of exposure, though later the activity levels in a large fraction of the animals in these treatments decreased to what appeared to be narcosis. Respiration rates at the early and late experimental days correlated to the krill behavioural observations at the corresponding dates, with high oxygen consumption in the exposed treatments at first, then lower consumption towards the end.

Moulting frequency was found to be a parameter that was difficult to interpret, as there are indicators to krill moulting due to stress and some conflicting results were seen in this and a previous exposure experiment. The egg production rates in *C. finmarchicus* showed no significant difference between the treatments and very high variance within each sample. However, a slight reduction in the Oil treatment compared to the Control, and an even higher reduction in the O+D treatment was observed. This was due to highly variable egg production outputs from the females. If counts is made only from the females that produce eggs, the Control has much higher values of eggs/producing female. In order to be able to look only at producing females, much higher amount of individuals would have been used for the egg production experiment. The logistics for such an increase might not be feasible, both in terms of keeping the animals while producing and the increased effort needed to filter and count the eggs. Observed mortality for both krill and *Calanus* indicated that dispersant would increase mortality substantially.

As a result, caution should be taken when considering the direct application of dispersant in natural environments, even though it has the advantage of rapidly removing crude oil. Time of year, environmental conditions, weather and currents will also have to be considered. These results may provide knowledge for development of tool to prepare for environmental management of future operations in sensitive boreal and sub-arctic conditions.

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APPENDIX

Table A. *Mean temperature and oxygen levels in the Control treatment over the course of the experiment.*

Day of exp.	Oxygen (ml)	Oxygen (%)	Temp. (˚C)
1	10.40	99.55	3.90
2	10.62	100.95	3.80
3	10.51	100.70	3.80
6	10.64	101.23	3.67
7	10.39	99.93	4.10
8	10.39	100.37	4.30
9	10.40	100.27	4.20
10	10.51	100.33	3.80
13	10.41	100.33	4.20
14	10.40	100.07	4.13

Table B. *Mean temperature and oxygen levels in the Oil treatment over the course of the experiment.*

Day of exp.	Oxygen (ml)	Oxygen (%)	Temp. (˚C)
1	10.14	97.70	4.20
2	10.10	97.45	4.25
3	10.12	97.45	4.20
6	10.36	98.75	3.80
7	10.19	97.57	3.93
8	10.48	96.70	2.40
9	10.20	97.60	3.90
10	10.05	96.47	4.00
13	10.06	96.43	4.00
14	10.07	96.40	3.90

Table C. *Mean temperature and oxygen levels in the O+D treatment over the course of the experiment.*

Day of exp.	Oxygen (ml)	Oxygen (%)	Temp. (˚C)
1	9.95	96.00	4.20
2	9.85	95.20	4.30
3	9.93	95.65	4.20
6	10.18	97.05	3.70
7	10.18	97.27	3.80
8	10.38	95.70	2.40
9	10.23	97.70	3.80
10	10.15	97.37	3.97
13	10.05	96.37	4.00
14	10.17	97.60	3.87