

Fate of *Calanus* spp. reproduction and development under different environmental stressors

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Master's Thesis in Biology

Field of study - Arctic Marine Ecology and Resource Biology

BIO-3950 (60 ECT)

December 2016



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Front picture:

top: *Calanus glacialis* adult female

bottom: *Calanus finmarchicus* adult female

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Acknowledgements

First and foremost, I would like to thank my supervisors, Janne E. Søreide and Fredrika Norrbin. For giving me the opportunity to write my thesis at the University Center in Svalbard (UNIS). Especially thanks Janne for letting me join this project and for giving me the freedom to study what I wanted. As well as for good guidance during my time at UNIS.

Thanks to Maja K. V. Hatlebakk for lots of interesting field work both in good and bad weather and thanks to Linn H. Svenndheim, Malin Daase and everyone else who helped me on fieldwork and in the lab with gathering and analysing samples. This would not have been possible without you.

Thanks to the logistic at UNIS and the crew at RV Helmar Hanssen, for helping me with my sampling.

Thanks to Kirstine L. Underbjerg for giving me the opportunity to join the project on consequences of an oil spill in the Arctic.

And thanks to all my friends at UNIS that have these years into a memorable time with jokes, discussions and lots of laughter. Especially thanks to Andreas Alexander for supporting me and for proofreading.

Last but not least, I would like to thank my family for believing in me and encouraging me as well as for always telling me that the world is full of opportunities. I love you for always being just a skype call away when I need someone someone to talk to.

Longyearbyen, 2016
Helene Overaa Eide

Abstract

The large calanoid copepods *Calanus glacialis* and *C. finmarchicus* is a key link between primary producers and higher trophic levels in Arctic marine ecosystems being used as indicator species of respective Arctic and Atlantic water. In this study, the egg production success and population development of *Calanus glacialis* and *C. finmarchicus* were studied in Isfjorden, Svalbard, from February to August 2016. An experimental study, investigating potential effects of different oil spill clean-up methods on *C. glacialis* reproduction was also conducted to study the fate of *Calanus* spp., under different environmental stressors.

The two *Calanus* species had similarly large populations in Isfjorden in winter and entered the surface water simultaneously in late winter-spring. Their reproductive strategy differed although. *C. glacialis* started to produce eggs earlier than *C. finmarchicus* and had a clear egg production maximum at the peak of the spring bloom that dropped to a minimum soon after the bloom. *C. finmarchicus* had a a modest, stable egg production over a relatively long time span from early May to July. The late spawned eggs, however, did not make it, resulting in lower recruitment of *C. finmarchicus* than *C. glacialis* in Isfjorden in 2016. Transformed Atlantic water prevailed in Isfjorden in 2016 and there were no strong evidence of major advective events from February to August. The increase in population numbers in June was thus mainly due to local recruitment, which also the timing of the egg production and the surface temperatures supported. *C. glacialis* timed its reproduction and population development more successfully than *C. finmarchicus* to the prevailing abiotic and biotic environment in Isfjorden. By the end of August, *C. glacialis* had reached its main overwintering stage and was already concentrated at depth. In comparison, *C. finmarchicus* had a bimodal vertical distribution end of August with still high numbers of young developmental stages in the surface.

In the oil exposure experiment dispersant, residue from burnt oil and crude oil were added to the mesocosms placed in seasonal sea ice in Van Mijenfjorden, Svalbard, in February 2015. In May 2015, *C. glacialis* females were incubated in water from these mesocosms. Because of large variation in the data partly due to overall low egg production rates, no significant effect of the different treatments on the reproduction success and development was found. However several of the nauplii from the dispersant treatment showed sign of deformation and reduced survival. In a changing Arctic with less ice, warmer temperatures and more activity, multitude of stressors will together impact the marine ecosystem. In future studies it is therefore important to not study single stressors effect on important biological functional units, but rather a multitude of stressors which not have any impact on its own, but when acting together may have a strong impact.

Keywords

Calanus glacialis · *C. finmarchicus* · Reproduction success · Climate change · Oil spill

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Abbreviations and terms

AF Adult female

AM Adult male

ArW Arctic water

AW Atlantic Water

EP Egg production

EPR Egg production rate

ESC East Spitsbergen Current

CI-CV Copepodite stage 1 to 5

CTD Conductivity, Temperature and Density

Chl a Chlorophyll *a*

IW Intermediate water

LA Lipid sack area

NI-NVI Nauplii stage 1 to 6

NEBA Net environmental benefit analysis

KKD Sample station Karlskronadjupet in Isfjorden

PA Promosome area

PAH Polycyclic aromatic hydrocarbons

PL Promosome length

WSC West Spitsbergen Current

Income breeder Allocates available food to reproduction directly.

Capital breeder Builds up reserves while food resources are plentiful to reproduce at a future date using stored energy only.

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Introduction

The Arctic region is characterized by low temperatures, a fluctuating ice cover and a large seasonality in solar irradiation. The sun stays below the horizon for several months in winter, while the sun is constantly above the horizon in summer. The surface ocean temperature varies little during the year with temperatures usually staying between the freezing point and maximum 8-10°C in summer (Sakshaug et al., 2009).

The Arctic Ocean experiences the largest climate changes (ACIA, 2004), but is the least studied of all the worlds oceans (Blix, 2005). Temperature is increasing rapidly here, particularly in autumn and winter (Kattsov et al., 2005). Some models suggest a sea temperature increase of up to 7°C by the end of the 21st century (ACIA, 2005; IPCC, 2013). As a result of the higher temperature, sea ice concentrations are decreasing. Sea ice is forming later in autumn, breaks up earlier in spring and becomes overall thinner (Wadhams and Davis, 2000; Kwok et al., 2009). In areas, which were earlier dominated by thick multiyear ice, only thin one year ice is left today (Maslanik et al., 2007).

The rising temperatures and the resulting decrease in sea ice will affect the Arctic marine ecosystem, by changing the timing of important biological seasonal events and thus disturb the food-grazer interaction, as well as interactions among organisms (Edwards and Richardson, 2004; Both et al., 2009). Temperate species will be able to survive and reproduce in a warmer Arctic, leading to an Atlantification of the communities in the Arctic Ocean (Wassmann et al., 2006). The change in species composition also introduces stronger competition for resources, especially between Arctic and Atlantic species and new predator-prey relationships is likely to occur with cascading effects on the marine ecosystem (Grebmeier et al., 2006; Aschan et al., 2013).

1.1 Productive season in the Arctic

When the first sunlight reaches through the sea ice, ice algae start to grow inside and at the bottom of the sea ice (Arrigo et al., 2012; Leu et al., 2015). As the ice melts, these algae are released into the water; providing an early food source for the zooplankton (Runge et al., 1991; Søreide et al., 2010). Later, with increased irradiation from the sun and a stratification of the water column, the phytoplankton bloom will start (Leu et al., 2015). These two pulses of ice algae and phytoplankton production are intense, but short. The ice algae may start to grow as early as March in Svalbard, while the phytoplankton bloom commonly starts sometimes between April and June, depending on the sea ice conditions (Hegseth, 1998). Organisms capable to utilize both food sources, however, will have a rather long productive season for growth and reproduction (e.g. Søreide et al., 2010).

1.2 *Calanus* are key species in the Arctic

Mesozooplankton, in particularly the larger Calanoide copepods of the genus *Calanus*, dominate the marine plankton biomass in Arctic and sub-Arctic seas (Wassmann et al.,

2006). The three co-occurring *Calanus* species in Svalbard: *Calanus glacialis*, *C. finmarchicus* and *C. hyperboreus* may account for up to 80% of the zooplankton biomass here (Blachowiak-Samolyk et al., 2008; Søreide et al., 2008).

In Arctic shelf seas, *C. glacialis* is the most important of the three species (Jaschnov, 1970; Grainger, 1965; Hirche and Kwasniewski, 1997; Kosobokova, 1999). *C. finmarchicus* is a boreal species with its main distribution area in the North Atlantic, but it is also numerous in the area south of the Polar Front, in the Barents Sea and along the Norwegian coast where Atlantic water prevails (Conover and Kania, 1988; Melle and Skjoldal, 1998; Wassmann et al., 2006).

C. hyperboreus is an Arctic species with a wide distribution. The main population is found in the deep Greenland Sea and the Nansen Basin of the Arctic Ocean, but it is also found in low abundances in the Nordic Seas and Arctic shelf seas (Conover and Kania, 1988). The three *Calanus* species in the Arctic have similar morphology. This is especially true for *C. glacialis* and *C. finmarchicus*, which can be challenging to distinguish from each other (Gabrielsen et al., 2012).

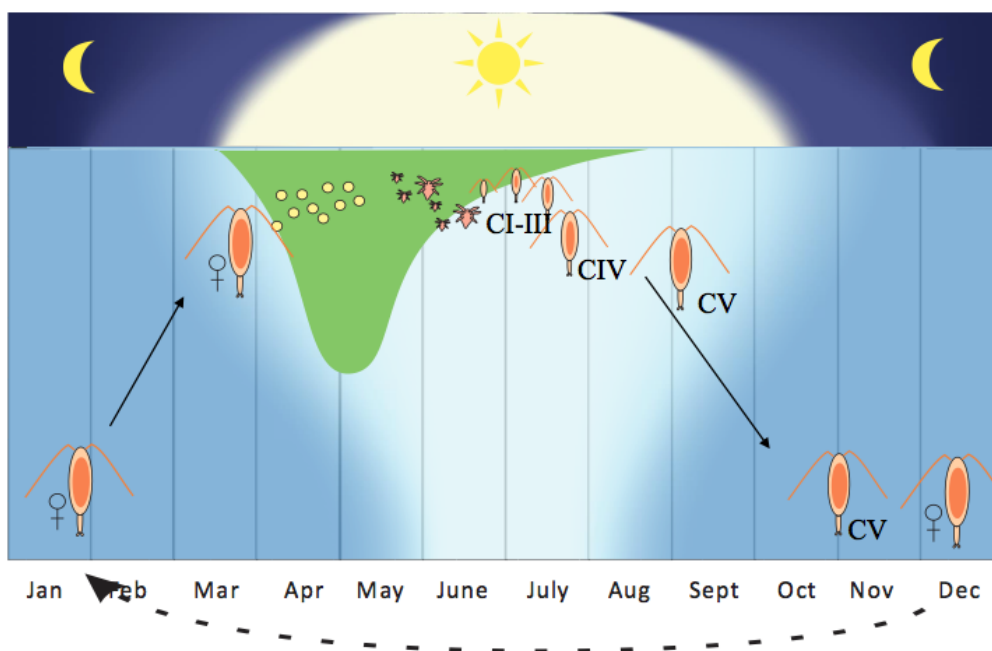


Figure 1: Schematic presentation of a one year life cycle to *C. glacialis* and *C. finmarchicus*. The green field represents phytoplankton bloom (CI-CV: copepodite stages I-V; AF: adult female) (Figure: M.Daase).

Ice cover, timing of the bloom and light conditions influence the life history of *Calanus* spp. (Falk-Petersen et al., 2009). In areas with extensive ice cover, *Calanus* spp. will usually have a multiyear life cycle (Daase et al., 2013). *C. finmarchicus* normally has a one year life cycle, *C. glacialis* a 1 to 2 year life cycle, while *C. hyperboreus* may need up to five years to complete its life cycle (Falk-Petersen et al., 2009).

To cope with long winters with little food, *Calanus* spp. utilizes the spring bloom to accumulate large deposits of lipids (up to 70% of their dry weight) (Falk-Petersen et al., 2009). These consist of *de novo* synthesized wax esters as well as essential polyunsaturated fatty acids (PUFAs) produced by algae (Falk-Petersen et al., 2007). It is important for the *Calanus* species to store enough lipids to survive the winter and invest in early reproduction (Falk-Petersen et al., 2009; Ji et al., 2012). *Calanus* spp. perform distinct seasonal migrations. In autumn, *Calanus* migrate to depth and reduce their metabolism to a minimum, entering diapause (Falk-Petersen et al., 2009; Swalethorp et al., 2011). The main overwintering stages for *C. finmarchicus* is CV, for *C. glacialis* CIV and CV, while *C. hyperboreus* may overwinter as CIII, CIV and CV (Falk-Petersen et al., 2009). During

overwintering, *Calanus* spp. develop from CV to adults (Kosobokova, 1999; Falk-Petersen et al., 2009). In early spring, *Calanus* females will resurface and start spawning, often in synchrony to the spring bloom.

C. glacialis is known to produce eggs in synchrony with the early ice bloom (Søreide et al., 2010). From the time of spawning it takes approximately three weeks for the nauplii to reach the first feeding stage, NIII (Søreide et al., 2010; Daase et al., 2011). Thus, by utilizing the ice bloom for spawning, the *C. glacialis* offspring will be able to fully utilize the pelagic bloom (Søreide et al., 2010). The life cycle of *C. glacialis* and *C. finmarchicus* is illustrated in Figure 1.

The three *Calanus* species in Svalbard waters have developed different reproduction strategies (Falk-Petersen et al., 2009). *C. hyperboreus* is a capital breeder. It will start spawning in deep water during the winter by utilizing lipid reserves accumulated during the previous spring bloom. In contrast, *C. finmarchicus* is primarily an income breeder, which means that it depends on recent feeding in order to initiate spawning. *C. glacialis* is a mixed breeder, using both strategies. It is capable of spawning prior to the spring bloom, but will normally need food to reach maximum egg production (EP) rates (Melle and Skjoldal, 1998).

1.3 Environmental challenges

1.3.1 Global warming

The zooplankton in the Arctic must be adapted to survive in an area with a highly seasonal light regime and an area with large fluctuations in ice cover. The timing of ice melt, thickness of ice and the formation of new ice are all critical for the survival of zooplankton (Scott et al., 2000).

Reduced thickness and extent of sea ice will change the underwater light climate radically, which again will affect the timing of the spring bloom of both ice algae and phytoplankton. This may lead to cascading effects on secondary producers like *C. glacialis*, especially if it doesn't manage to adapt its life strategy to the changes in the physical and biological environment (Søreide et al., 2010). A potential mismatch with the spring bloom might lead to a strong decline in the population of *C. glacialis*. In contrast, an earlier phytoplankton bloom and a longer productive season will likely be beneficial for *C. finmarchicus* (Wassmann et al., 2011). However, a warmer climate also favor the microbial loop and may particularly favor the small specimens, rather than the larger copepods in general (Iversen and Seuthe, 2011).

Svalbard is situated in the dynamic transition zone between Arctic and Atlantic waters (Nilsen et al., 2008). The West Spitsbergen Current (WSC) brings warm and saline Atlantic water (AW; >35 PSU, >3°C; (Nilsen et al., 2008)) into the Arctic Ocean, up along the west coast of Spitsbergen and through the Barents Sea (Loeng, 1991). The East Spitsbergen Current (ESC), which runs first southwards along the eastern coast of Spitsbergen and then northwards along the west coast of Spitsbergen (Cottier et al., 2005) fills the shelf region with cold and less saline Arctic Water (ArW; 34.4-34.8 PSU and <0°C; Loeng, 1991) that again mixes with freshwater run-off from land and sea ice melting (i.e. surface waters) and Atlantic water, resulting in an intermediate water mass (IW; 34.4-34.7 PSU, >0°C; (Nilsen et al., 2008)).

C. glacialis and *C. finmarchicus* are often used as indicator species of Arctic and Atlantic water, respectively (Kwasniewski et al., 2003; Wassmann et al., 2006; Daase and Eiane, 2007). As the climate becomes warmer it has been suggested that *C. finmarchicus* will move northwards and into waters that traditionally have been dominated by *C. glacialis* and *C.*

hyperboreus (Wassmann et al., 2011). A change in *Calanus* distribution in the Arctic may lead to cascading trophic effects in the entire Arctic food web (Falk-Petersen et al., 2007).

1.3.2 Oil spill in the Arctic

With increased temperatures and decreasing sea ice concentrations in the Arctic, new areas become accessible for ship traffic and oil exploiting (ACIA, 2004). Up to 13% of the worlds undiscovered oil resources and 30% of the worlds undiscovered gas reserves are located in the Arctic (Gautier et al., 2009). As the global demand of energy increases, there will also be an increased attention to the Arctic (AMAP, 2010). With increasing human activity, there is also an increased risk of pollution from drilling waste, discharge of produced water and accidental oil spills (Balk et al., 2011).

There has to days date not been any large oil spill in the Arctic, the closest is the Exxon Valdez spill in 1989 and the oil spill in the Gulf of St. Lawrence in 1969 (AMAP, 2010). The oil spill had large consequences on the marine life in the area (AMAP, 2010). With warmer climate and a decrease of multi year sea ice the activity level in the Arctic is increasing, with that the potential risk of an oil spill will also increase. The consequence of an oil spill is highly dependent on type of oil, region and the time of the year (Jiang et al., 2010; Brandvik and Faksness, 2009). In open water, winds and waves will usually break the oil down, in the Arctic the oil is at risk of being trapped by the sea ice (Brandvik and Faksness, 2009; Board et al., 2014)

The Arctic is one of the most challenging areas for oil spill response in the world, with extreme weather, drifting ice, polar night, remoteness and poor infrastructure (AMAP, 2010). When accidents happen, knowledge of what actions to take in the Arctic is important. Therefore, the International Association of Oil and Gas Producers and the Arctic Response Technology Joint Industry Program initiated a project to develop a net environmental benefit analysis (NEBA) tool to assess the environmental effects of Arctic oil spills and specific treatment technologies that may be applied during an oil spill response in this region.

In order to develop this NEBA tool, it is important to increase the knowledge about the biological response to an oil spill. Oil toxicity of weathered oil in the Arctic is not thoroughly understood, especially in relation to sea ice and represents an important data gap. A full evaluation of oil spill response alternatives will require toxicity data for targeted valuable ecosystem components (Word, 2013).

Polycyclic aromatic hydrocarbons (PAH) include those compounds that have the most serious environmental effects of the compounds in crude oil (National Research Council, 2003). PAHs in ocean water enter copepods passively by diffusion and actively during feeding, either through the ingestion of oil droplets in the water or by ingesting oil-coated algae (Conover, 1971; Gyllenberg, 1981; National Research Council, 2003). Because of their lipophilic nature, these compounds may be stored in the copepods lipid reserves and may pose a significant risk of bioaccumulation, when these toxic compounds are transferred to higher trophic levels. When metabolized, the hydrocarbon compounds can cause both lethal and sub-lethal effects. Sub-lethal toxic effects of contaminants in marine organisms include impairment of physiological processes that alter the energy available for growth and reproduction and other effects on reproductive and developmental processes, including direct genetic damage (Capuzzo et al., 1988).

Several studies have been conducted on *C. glacialis* to study the effect of oil on *Calanus* reproduction (Jensen and Carroll, 2010; Gardiner et al., 2013), but not the effect of the different treatment methods to clean up after an oil spill. This is also essential to know when choosing between different clean up methods.

1.4 Aim and goals for this study

The overall aim of this study was to increase our knowledge on *Calanus glacialis* and *C. finmarchicus* reproduction and development under different environmental stressors. This to determine these two key species fate in a future warmer Arctic. The following research questions were focused upon:

1. Are the population abundance and stage composition of *C. glacialis* and *C. finmarchicus* comparable in locations resembling more sub-Arctic conditions were they co-occur?
2. Do *C. glacialis* and *C. finmarchicus* exhibit different timing and duration of egg production when living under the same environmental conditions?

In addition an oil exposure experiment was conducted to study the effect different oil treatments on the *C. glacialis* reproduction The following hypotheses were tested:

- H0: The three different clean up methods natural attenuation, dispersant and *in-situ* burning of the oil, will have no effect on the *C. glacialis* reproduction
- H1: There will be be an treatment effect on *C. glacialis* reproduction
- H2: The three oil spill treatment methods: natural attenuation, dispersant and *in-situ* burning will have different effects on the *C. glacialis* reproduction.

Materials and Methods

2.1 Study area

Samples were collected in Isfjorden, the largest fjord in Svalbard, situated in western Spitsbergen (Figure 2). Isfjorden is an open fjord and due to its geographic location, it is strongly influenced by Atlantic water, but may also be influenced by Arctic water in the coastal current on the shelf moving northwards along West Spitsbergen (Nilsen et al., 2008). Several glaciers are discharging into the fjord, either directly or in one of its four side arms. The mouth of the fjord is about ten kilometers wide and 455 m deep, but most of the fjord (55%) is shallower than 100 m (Nilsen et al., 2008). In recent years only the side arms of Isfjorden with a distinct sill have been ice covered during winter (<http://polarview.met.no/>). In this study, monthly sampling in Karlskronadjupet (KKD) was conducted (Fig. 2b) KKD is located approximately 40 km inwards from the entrance of Isfjorden in the deepest innermost basin at approximately 270 m depth.

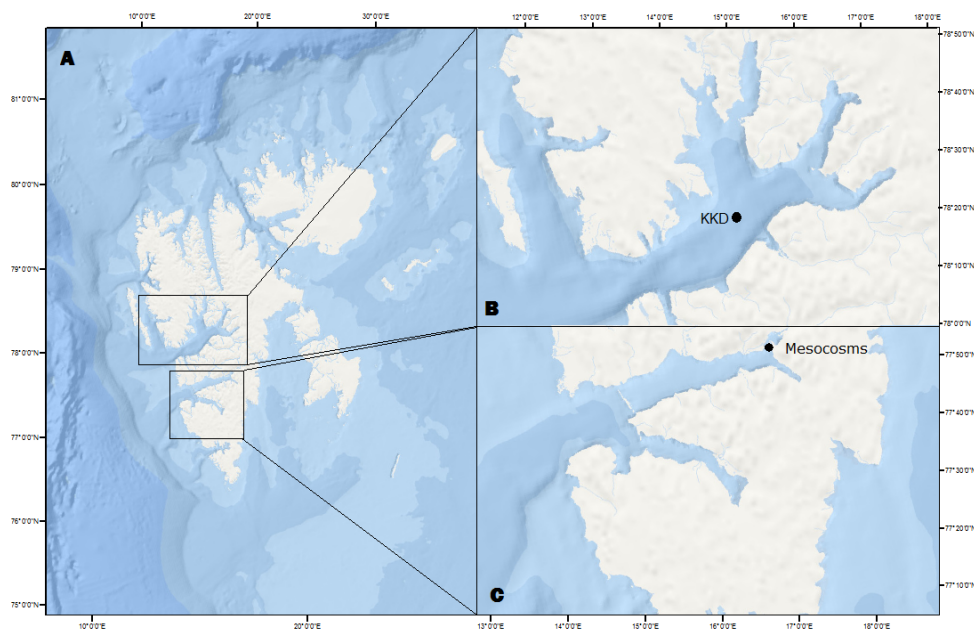


Figure 2: Map showing the sampling location. The sampling station KKD (Karlskronadjupet) and the location for the mesocosm deployment is marked

2.2 Sampling

Sampling took place approximately once a month between February and August 2016 (Table 1). At the start of every sampling a CTD (Conductivity, Temperature and Density) profile

was taken with a Saiv CTD (CTD/STD model SD204) with a fluorometer attached.

For the analysis of chlorophyll *a* (Chl *a*) concentrations, additional water samples were collected at the surface (1m), 15 m, Chl *a* max, and close to the bottom (250 m) with a Niskin bottle. Zooplankton was collected with a Multinet Midi (Multi Plankton Sampler [MPS], Hydrobios) or a WP2 manual closing net (Hydrobios). Both nets had a 0.25 m² net opening and 200 µm mesh size. The MPS consisted of five closing nets, whereas the WP2 closing net had only one net. Five standard sampling depths were sampled 0-20 m; 20-50 m; 50-100 m, 100-200 m and 200-bottom. Community samples for species identification were preserved in 4% formaldehyde-seawater solution buffered with either hexamine or borax within 1 hour after sampling. Additional surface water samples and live zooplankton samples from the upper 50 m were brought back to UNIS for egg production measurements (see 2.4).

Table 1: Overview of samples collected at KKD (78.316667 N, 15.133331 E) from February to August 2016

Date	Community samples	Net	Egg experiment	Boat
17/02/2016	260-100-50-20-0	WP2	✓	UNIS Polaris
21/03/2016	260-100-50-20-0	WP2	✓	UNIS Polaris
04/04/2016	250-100-50-20-0	WP2	✓	UNIS Polaris
29/04/2016	250-50-0	WP2	-	KV Svalbard
02/05/2016	-	WP2	✓	KV Svalbard
09/05/2016	-	WP2	✓	RV Helmar Hansen
15/05/2016	260-200-100-50-20-0	MPS	✓	RV Helmar Hanssen
05/06/2016	260-200-100-50-20-0	MPS	✓	Viking explorer
05/07/2016	260-200-100-50-20-0	MPS	✓	Viking explorer
18/08/2016	-	WP2	✓	RV Helmar Hanssen
31/08/2016	260-200-100-50-20-0	MPS	✓	RV Helmar Hanssen

2.3 Sample analyses

Water samples were analysed for Chl *a* biomass by filtering triplicates of 300 to 500 ml seawater onto GF/F glass microfiber filters (Whatman, England). The filters were either frozen at -80°C or put in liquid nitrogen for later analyses or immediately extracted in 10 ml

of methanol. Chlorophyll was extracted for 24h following the method in Holm-Hansen and Riemann (1978). The Chl *a* biomass was then measured with a 10-AU- 005-CE fluorometer (Turner, USA).

Prior to quantitative and qualitative analysis of *Calanus* spp. population structures, large macro-zooplankton was removed. Samples were thereafter diluted to 50-200 ml volume, depending on the density and 5 ml subsamples were taken using a Henson-Stempel pipette. Analysis was done under the stereomicroscope at 10-40x magnification. All *Calanus* spp. were enumerated and identified from subsamples until a minimum of 200 individuals was reached. The species of the *Calanus* could be identified by determining them according to their developmental stage and measuring their prosome length under a stereo microscope. To distinguish between *C. finmarchicus*, *C. glacialis* and younger stages of *C. hyperboreus* the size classes, obtained from Daase and Eiane (2007) were followed (Table 2). *Calanus* spp. adult males (AM) were not identified to species, since their size ranges are not known. *Calanus* eggs and nauplii were not identified to species level and were counted from sub samples until a minimum of 50 individuals was reached.



Figure 3: The chambers used to incubate to females. The uppermost cup had a mesh bottom

Table 2: Length classes used to distinguish the three *Calanus* species copepodite stages from each other. Table from Daase and Eiane (2007), length is corrected for CV and AF (Daase unpublished)

Stage	Prosome length (μm)		
	<i>C. finmarchicus</i>	<i>C. glacialis</i>	<i>C. hyperboreus</i>
CI	< 810	810-900	> 900
CII	< 1170	1170-1350	> 1351
CIII	< 1470	1470-1950	> 1950
CIV	< 2010	2010-2910	^a
CV	< 2900	> 2900	^a
AF	< 2950	> 2950	^a

^a Distinguished by the presence of a spike on the 5th thoracic segment

2.4 Egg production

Thirty adult females (AF) of *C. glacialis* and thirty *C. finmarchicus* AF were incubated in order to measure egg production and egg hatching success. Those sixty females were picked randomly from a surface sample (50-0 m). As the necessary number of sixty females could not be found every month, less AF were incubated in some months.

Only females from the upper 50 m were incubated as it was assumed that only those were actively producing eggs. The incubation experiment was always started immediately after return to the lab within 4 hrs of sampling. Females were incubated individually in a

chamber with a false bottom of mesh size of 300-500 μm mesh size (Fig. 3) to allow eggs to fall to the bottom, but prevent the females to predate on them (Basedow and Tande, 2006). The females were incubated for 24 hours in order to estimate the daily egg production.

After 24 hours the eggs were transferred to a petri dish and counted under a stereo microscope in a cold lab (6°C). The eggs were incubated in the petri dish at close to *in-situ* temperatures in the dark. After eight days the eggs were counted for hatching success. Eggs, which had not hatched after eight days, were assumed to be non-viable (Daase et al., 2011). Hatching success was calculated by comparing the number of nauplii to the original number of eggs.

The females from the egg incubation experiments were placed in a droplet of water in a petri dish. Most of the water was removed to make the animal lie on its side. This was done to get a lateral image of the individual. Pictures were taken through a stereo microscope using a Sony video camera (HDR-HC7) in photo mode with an ocular adapter. The pictures were analyzed using the program ImageJ (Rasband et al., 1997). Pictures of a calibration slide were taken to calculate the number of pixels per 1 mm. For each picture of the AF, the prosome length (PL), prosome area (PA) and area of the lipid sack (LA) were measured (Fig. 2.4). The lipid sack area was used to estimate the total lipid content (TL) in mg as described in Vogedes et al. (2010):

$$TL = 0.97 \cdot A^{1.38}.$$

A is here the lipid sack area. Since there is a difference in size between females of the two species, the percentage of lipids in the body was also calculated by using the proportion of LA to PA (LA/PA).

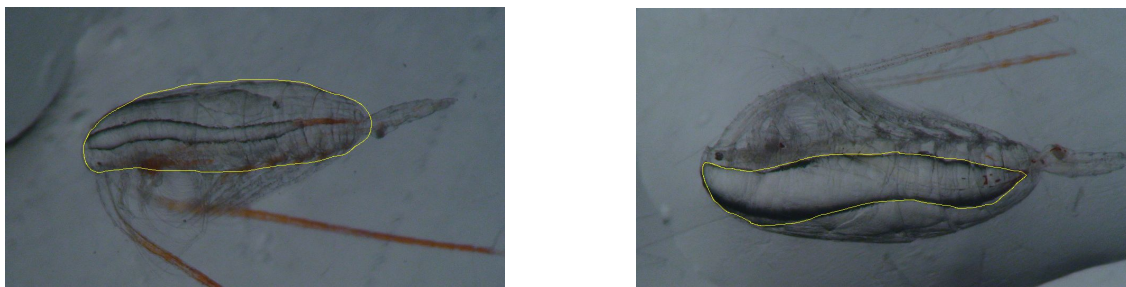


Figure 4: (Left) Picture of an adult female with a small lipid sack area (LA) with the prosome area (PA) marked. (Right) Picture of an adult female with a large lipid sack area (LA), LA is marked.

2.5 Exposure experiment

A larger mesocosm experiment was conducted in Van Mijenfjorden (77.8684°N 16.7540°E), on the west coast of Spitsbergen (Fig. 2 C). The experiment was performed to investigate the effect of the oil spill response methods on natural ecosystems: Natural attenuation, chemical dispersion and burning of oil were investigated by exposing the natural ecosystems for these three treatments within a closed mesocosm. The oil exposure experiments were part of a larger experiment run by Arctic Response Technology (Joint Industry Programme, 2012). More information about the experiment set-up can be found in Appendix D.1. In this specific study the focus was on the egg hatching success of eggs produced by *C. glacialis* females exposed to the different oil spill response methods. Investigations of the daily egg production were carried out by a project partner (Appendix E.1).



Figure 5: (Left) Mesocosms in Svea. (Right) Experimental set-up (©Kirstine Larsen Underbjerg).

Females used in this experiment were collected on the 7th of May 2015 in Isfjorden (KKD) and were stored cold and dark until the experiment started on the 16th of May. For each treatment 15 replicates of three females were incubated in 1 L glass bottles (Fig. 5), containing 200 μm filtered mesocosm water (Table 5). The incubation bottles were stored in a climate regulated room at 0°C under dim light.

Every second day the diatom *Thalassiosira weissflogii* ($\sim 400 \mu\text{g C L}^{-1}$) was added as food to all the bottles. This was done to avoid food limitation affecting the reproduction.

To investigate the egg production, the content from each bottle was gently rinsed through a 50 μm sieve every 24 h to collect eggs and females. The females were incubated in the same water during the entire experiment, so therefore the filtered water was returned to the bottle. The filtered copepods and eggs were put into a petri dish with chilled 0.2 μm filtered seawater. The females were immediately returned to the experimental bottles using a plastic pipette.

Eggs were kept on ice-chilled petri dishes and counted under a stereo microscope in a temperature controlled room (5°C). To investigate effects on egg hatching success, eggs were collected from a control sample before the beginning of the experiment (Day 0) and after 11, 12 and 13 days of incubation. The eggs were incubated in the dark at 0°C. To investigate how many days it took for the eggs to hatch, the eggs and nauplii were counted every 24 h. Eggs, which had not hatched after eight days were assumed to be non-viable. Hatching success was estimated as the proportion of the initial number of eggs, which hatched into nauplii. Nauplii were counted again 21 days after incubation to estimate survival. Nauplii survival was estimated as the proportion of initial number, which had developed to NII. During the calculation of hatching success, only egg batches with more than ten eggs were included. For nauplii survival only replicates with five or more hatched egg were included in the calculations.

2.6 Statistical analyses

Statistical analyses were performed with R (R Development Core Team, 2008). The different treatments in the oil exposure study were compared using Students t-test or 1-way Analysis of variance (ANOVA). If the p values were less than 0.05 Tukeys-HSD (Honesty Significant Difference) post hos test was used to determine which treatments were different. Values were considered significantly different at $p < 0.05$

The Akima package (Akima et al., 2013) was used to make the interpolation diagram in Figure 6. All other graphs were made with the ggplot2 package (Wickham, 2009).

Results

3.1 Field data

3.1.1 Environmental conditions

The year 2016 has been one of the warmest years in Svalbard, resulting in record-low ice cover in Svalbard fjords (<http://polarview.met.no/>). In the winter season 2015-2016 Isfjorden was never ice covered, except from the innermost bays of the few sill fjords. In 2016, Isfjorden was mainly characterized by Transformed Atlantic waters (TAW) and some intermediate water masses (IW) (Fig. 6, Appendix A). Minimum temperatures (1-2 °C) were measured during April and May (Fig. 6). In June, a freshwater layer on top started to appear which were quickly warmed up by solar radiation. Maximum surface temperatures were measured in July (5°C). It was local processes such as winter cooling and convection and meltwater river run-off combined with solar radiation that mainly modified the hydrography from February through August (Fig. 6a, b). Single, stronger, advective events were not detected. The spring bloom started the second week of May (Fig. 6c). Elevated Chl *a* values were still detectable in the upper 50 m in June and July, but by the end of August low winter Chl *a* values were again prevailing (Fig. 6c).

3.1.2 *Calanus* population structure

In winter and early spring *C. glacialis* and *C. finmarchicus* occurred in similar high numbers. However the two species had different population structures. In February, *C. finmarchicus* was mainly dominated by CV, while *C. glacialis* was dominated by CIV (Fig.7). Both populations were mainly concentrated below 100 m depth in February, but especially *C. finmarchicus* started to be more evenly distributed in the water column in March (Fig. 8, 9).

In late April-May, both species, and in particularly the females, concentrated in the upper 50 m. More than 90% of the *C. glacialis* females and 89% of the *C. finmarchicus* females were captured in this surface layer (Fig. 8, 9). A strong reduction in population numbers were observed during the winter-spring transition. The total abundance of *C. finmarchicus* decreased by 96% and *C. glacialis* decreased by 87% from February to May (Fig. 7).

In May the smaller copepodite stages CI and CII started to appear, and in June these smaller stages totally dominated the populations of both species. The abundance of *C. glacialis* and *C. finmarchicus* increased by two orders of magnitude from May to June, but only *C. glacialis* reached similar high population numbers as found in February by the end of August (Fig. 7). In August, *C. glacialis* had reached its main overwintering stages CIV (58%) and CV (35%) and 90% of its population was found below 200 m depth (Fig. 9). *C. finmarchicus* was also in an advanced population developmental state by the end of August, mainly comprised of CIV (30%) and CV (60%). The vertical distribution of *C. finmarchicus*, however, was bimodal with still a relatively large proportion of young developmental stages present in the upper 20 m (Fig. 8).

3. Results

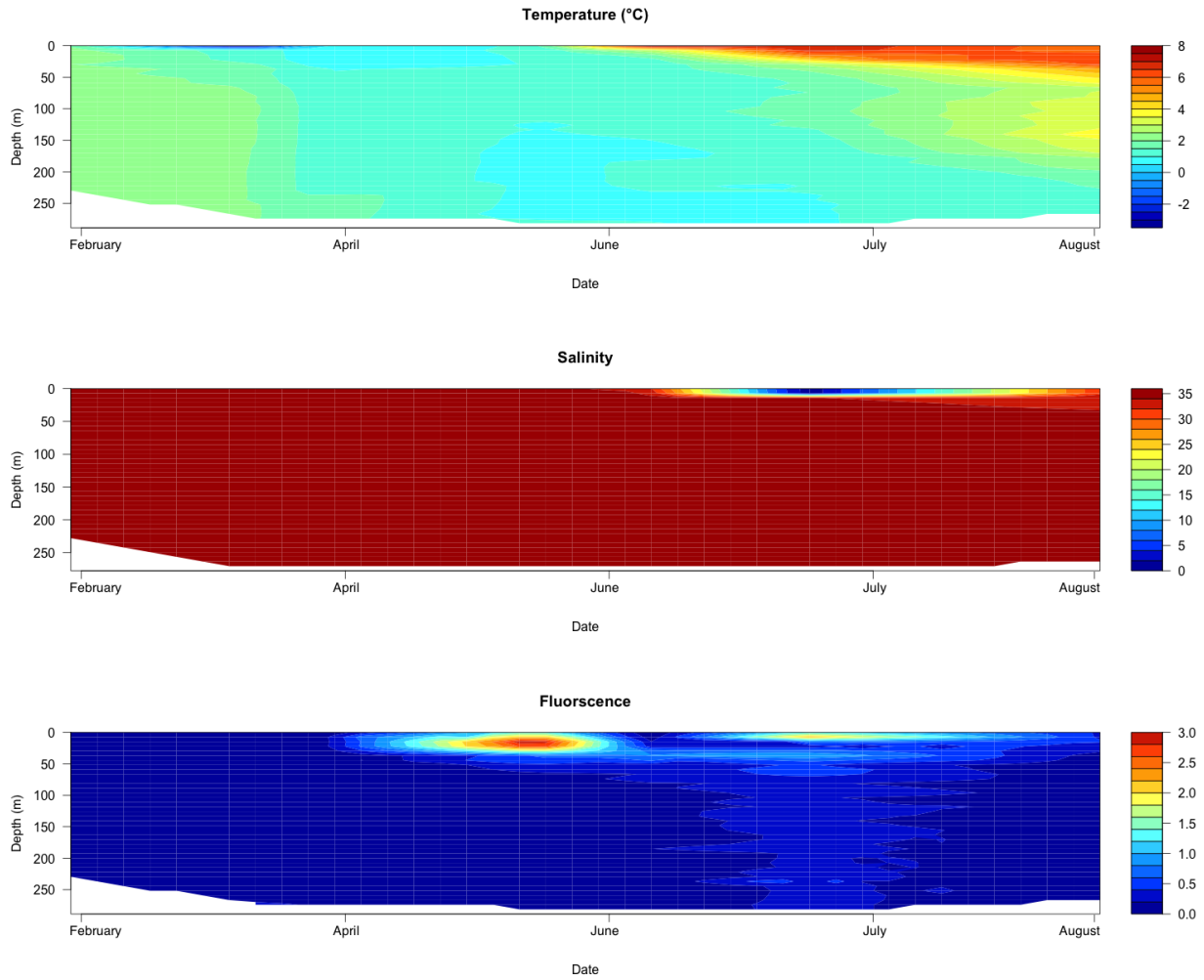


Figure 6: CTD data from Isfjorden, January to August 2016. Top: Temperature profile, middle: Salinity profile and bottom: Chlorophyll *a* concentration ($\mu\text{g L}^{-1}$) from Chl *a* fluorescence measurements.

From February to April very few *Calanus* spp. eggs and nauplii were present in the water column (Fig. 10). In May, the number of *Calanus* nauplii increased from close to zero to 228 ind. m^{-3} , but these nauplii mainly comprised of the older nauplii stages NIV and NV. From July to the end of the sampling period in late August very few nauplii were found (Fig. 10)

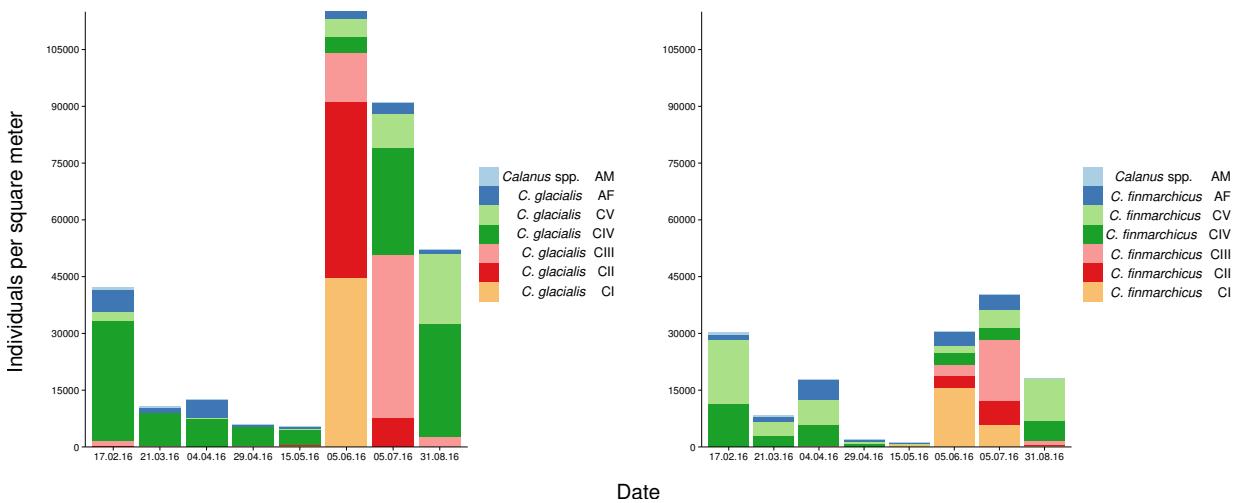


Figure 7: Total abundance and copepodite stage composition *C. glacialis* (left panel) and *C. finmarchicus* (right panel) in Isfjorden from February to August 2016

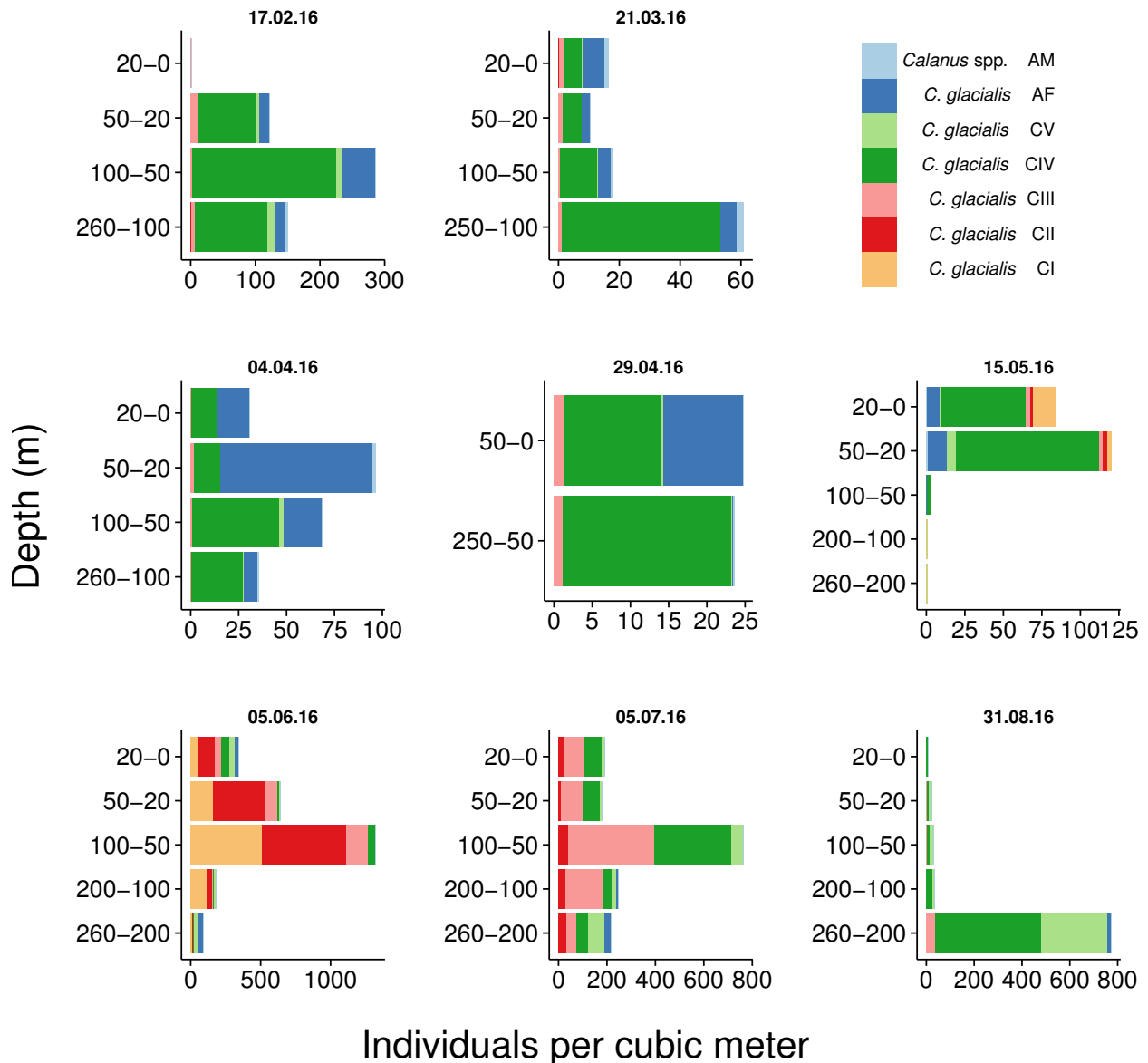


Figure 8: Vertical distribution of *Calanus glacialis*

3.1.3 *In-Situ* egg production and hatching success

During the first sampling date in February, both *C. glacialis* and *C. finmarchicus* were present in the surface, but neither of them produced eggs (Tables 3, 4). In March, *C. glacialis* started to produce a few eggs, while *C. finmarchicus* did not start to spawn before the beginning of April. *C. glacialis* had clearly highest egg production rates during the spring bloom in mid-May (Table 3). In comparison, *C. finmarchicus* never showed a defined peak in its egg production but had a modest egg production (~ 20 EPR F^{-1} day $^{-1}$) over an extended period from mid-May to July (Table 4).

When calculating the number of females with the daily EPR, *C. glacialis* had a population egg production peak in mid-May and *C. finmarchicus* in early June (Fig. 11). Since *C. finmarchicus* had a higher number of females present in the surface and a rather long time period of relatively high EPR, the total number of eggs produced by *C. finmarchicus* was higher than *C. glacialis*.

The hatching success for *C. glacialis* was high (73-93%) early in the reproduction. The 9th of May there was a sudden drop (36%), but a week later, when the spring bloom was

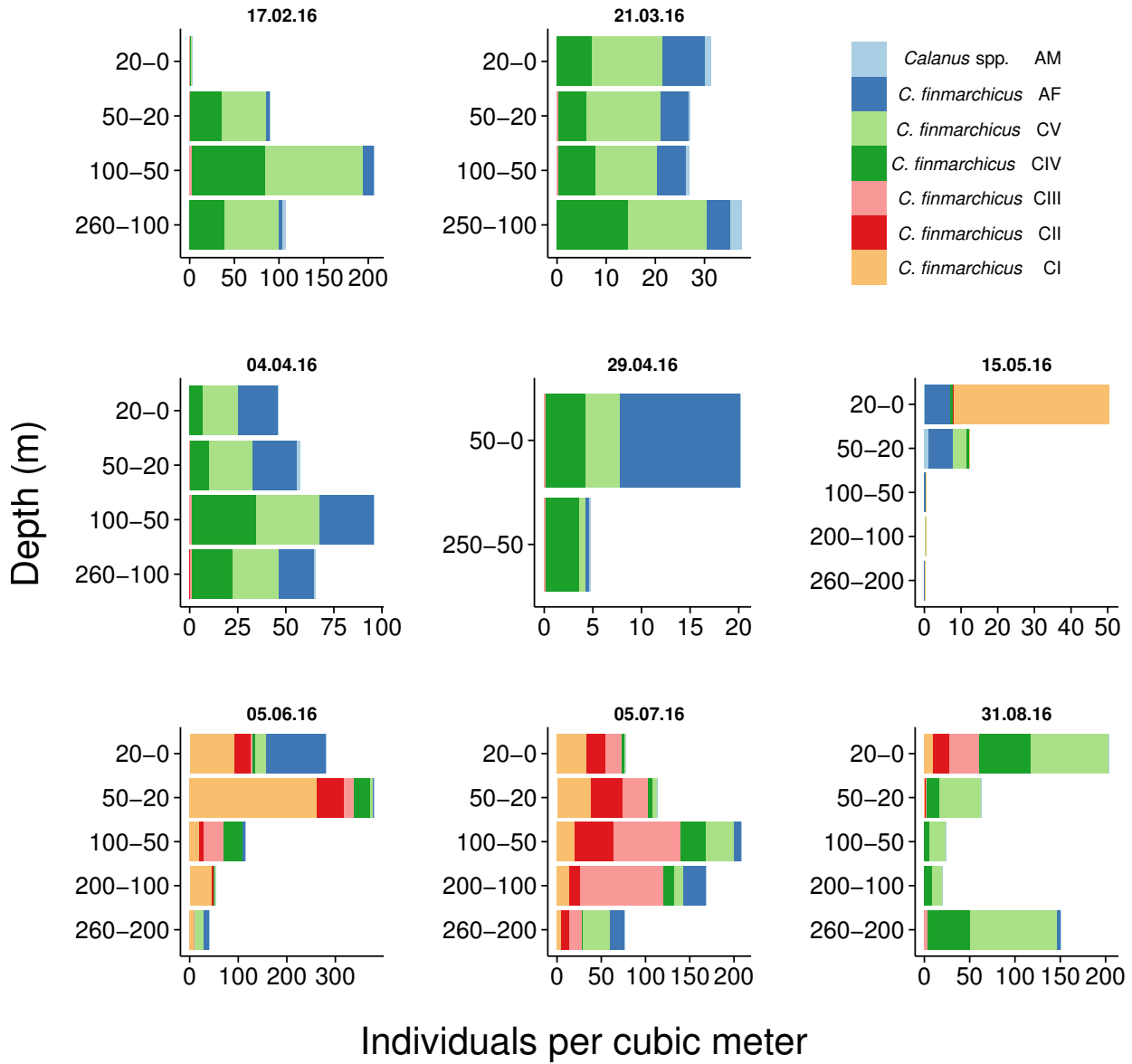


Figure 9: Vertical distribution of *Calanus finmarchicus*

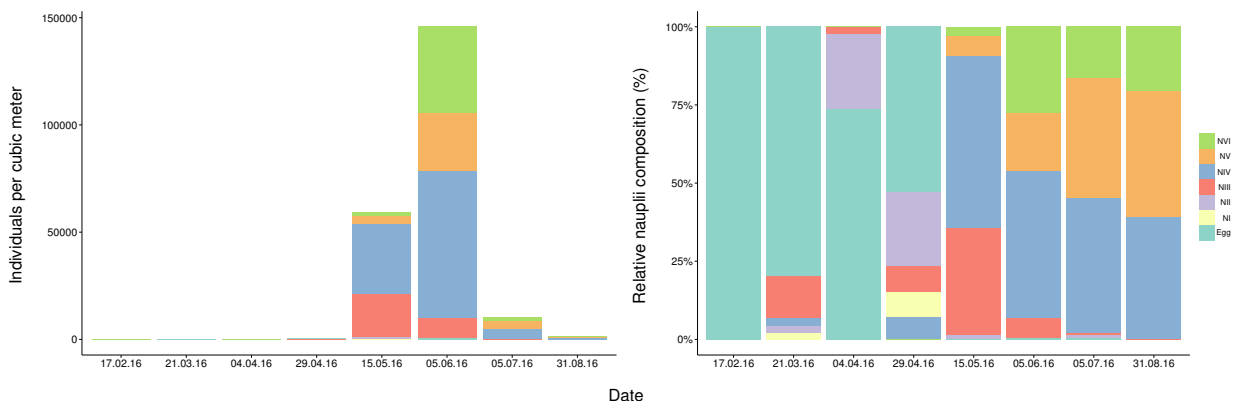


Figure 10: (left) Abundance of egg and nauplii stages per square meter and (right) relative abundance (%) of egg and nauplii stages at KKD during the sampling period

at its most intensive the egg hatching success was again high and it continued to be high in June. In July and August, the number of *C. glacialis* females were close to zero, but those

found in the surface waters produced eggs, but at a very low rate and with poor hatching success

The egg hatching success to *C. finmarchicus* was low in the beginning (30%), but similarly high to that found for *C. glacialis* in May (Tables 3, 4). Highest egg hatching success for *C. finmarchicus* was found in early June (> 89%; Table 4).

The percentage of deformed nauplii was zero, except during the spring bloom when a small percentage of naupliar deformation (<2.8%) was found for both species (Tables 3, 4).

Table 3: *Calanus glacialis* egg production (EP) measurements from Isfjorden 2016. Temp. is the incubation. Chl *a* is from the Chl *a* max. (AF=adult females, PA=Prosom area, LA=Lipid sack area).

Date sampled	Chl <i>a</i> GF/F 0.7µm	Temp. (°C)	N. of AF	PL*	LA*	% Egg laying AF	EP day ⁻¹ *	Max egg clutch	Hatching success (%)	Deformed nauplii
17.02.16	<0.1 ^a	3.2	30	3.25±0.24	0.80±0.36	0	-	-	-	-
21.03.16	<0.1 ^a	3.0	30	3.26±0.25	0.58±0.32	16.7	1.4± 4,4	20	-	-
04.04.16	~0.7 ^a	3.3	30	3.23±0.18	0.41±0.16	23.3	1.4±3.9	18	73.2	0
02.05.16	0.13	3.3	27	3.08±0.30	0.26±0.13	24	8.9±17.6	55	93.3	0
09.05.16	2.55	2.0	30	3.48±0.28	0.36±0.55	53.3	18.7±31.7	128	36.1	0
15.05.16	11.18	2.0	29	3.11±0.30	0.37±0.36	82.7	58.9±42.4	129	87.7	0.34
05.06.16	0.16	3.4	10	3.02±0.36	0.78±0.47	70	5.2±7.1	22	74.0	0
05.07.16	~1.75 ^a	3.5	1	-	-	100	38	38	44.7	0
18.08.16	~1.18 ^a	2.0	5	3.06±0.13	0.05±0.05	100	5.8±9.6	23	8.7	0
31.08.16	<0.1 ^a	2.0	0	-	-	0	-	-	-	-

*(mean±SD)

^a Chl *a* values obtained from fluorescence values (Fig. 6)

Table 4: *Calanus finmarchicus* egg production (EP) measurements from Isfjorden 2016. Temp. is the incubation. Chl *a* is from the Chl *a* max. (AF=adult females, PA=Prosom area, LA=Lipid sack area).

Date sampled	Chl <i>a</i> GF/F 0.7 μ m	Temp. ($^{\circ}$ C)	N. of AF	PL*	LA*	% Egg laying AF	EP day $^{-1}$ *	Max egg clutch	Hatching success (%)	Deformed nauplii
17.02.16	<0.1 ^a	3.2	30	2.79 \pm 0.09	0.56 \pm 0.15	0	-	-	-	-
21.03.16	<0.1 ^a	3.0	30	2.71 \pm 0.20	0.47 \pm 0.18	0	-	-	-	-
04.04.16	\sim 0.7 ^a	3.3	31	2.68 \pm 0.20	0.38 \pm 0.15	12.9	0.3 \pm 1.1	6	30.0	0
02.05.16	0.13	3.3	29	2.59 \pm 0.13	0.08 \pm 0.07	37.9	8.3 \pm 14.6	50	79.1	0
09.05.16	2.55	2.0	30	2.61 \pm 0.23	0.16 \pm 0.17	70	21.4 \pm 28.1	96	60.3	0
15.05.16	11.18	2.0	30	2.66 \pm 0.20	0.09 \pm 0.09	70	19.8 \pm 25.9	89	67.3	2.75
05.06.16	0.16	3.4	34	2.81 \pm 0.59	0.35 \pm 0.27	52.9	17.8 \pm 27.4	90	89.3	1.5
05.07.16	\sim 1.75 ^a	3.5	5	-	-	60	18.8 \pm 26.9	65	50.0	0
18.08.16	\sim 1.18 ^a	2.0	16	2.66 \pm 0.27	0.14 \pm 0.18	50.0	7.5 \pm 13.5	46	14.6	0
31.08.16	<0.1 ^a	2.0	6	2.64 \pm 0.10	0.09 \pm 0.13	0	-	-	-	0

*(mean \pm SD)

^a Chl *a* values obtained from fluorescence values (Fig. 6)

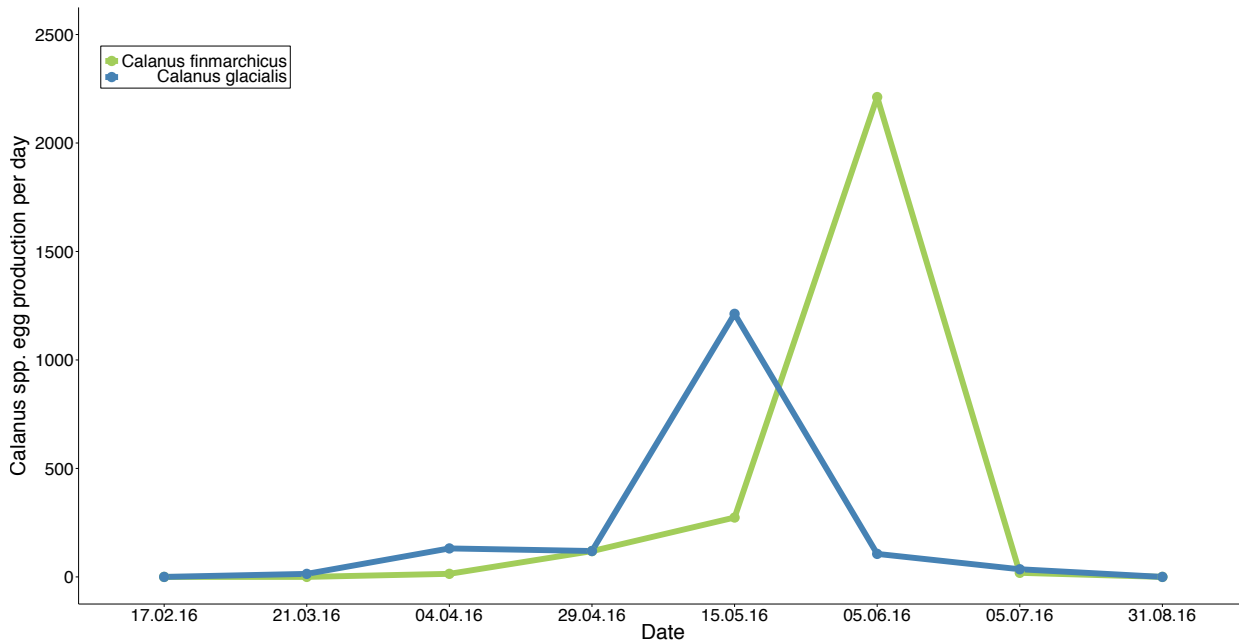


Figure 11: Estimate of the total egg production female⁻¹ day⁻¹. Egg production day⁻¹ was multiplied with female abundance in the upper 50 m

3.2 Oil experiment

3.2.1 Chemical analysis

In the oil exposure experiment the PAH concentration in the experimental incubation bottles was significant higher in the dispersant treatment (16.936 $\mu\text{g PAH L}^{-1}$) than in the other treatments, and the control (Table 5). More than 90% of the PAHs detected were naphthalens. In addition, there were smaller amounts of fluornes and phenanthrenes and some pyrenes (<1%)

Table 5: Mean concentration of total PAH in the mesocosm water samples. Data are mean \pm SD

Treatment	Mean PAH ($\mu\text{g/L}$) \pm SD
Control	0.090 \pm 0.070
Burnt oil	0.261 \pm 0.62
Dispersant and oil	16.935 \pm 9.772
Natural attenuation	2.190 \pm 1.152

3.2.2 The effect of oil spill treatments on egg production and egg hatching success of exposed *C. glacialis*

In general, the daily egg production rates was comparable low (mean 4 EPR d⁻¹) in all treatments and in the control (mean 4-5 EPR d1) (Appendix C. 1.). However, there was a higher cumulative egg production in the dispersed oil treatment compared to the other treatments and the control (Fig. 12).

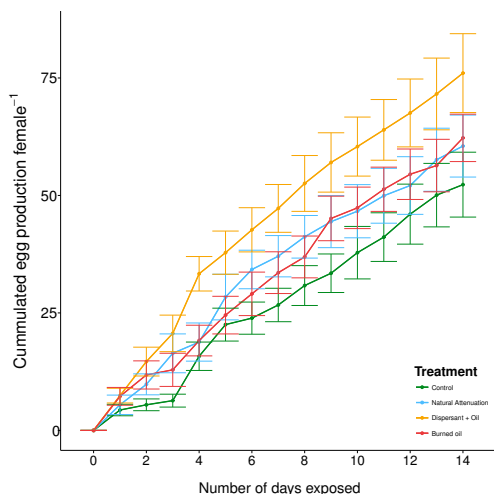


Figure 12: Cumulated egg production per female *Calanus glacialis* in the oil exposure experiment (\pm SE)

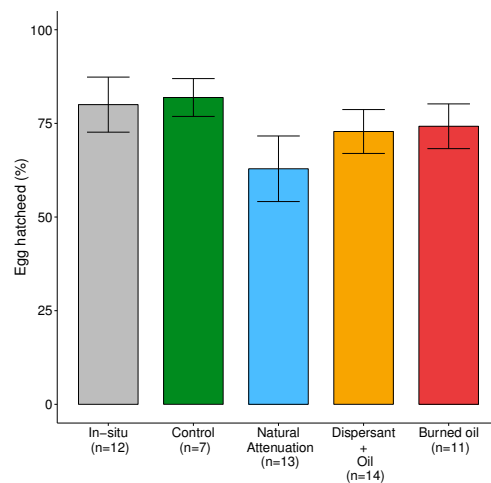


Figure 13: Average egg hatching success (%) per incubated female (\pm SE). Eggs were incubated for eight days. (n=number of replicates)

The egg hatching success had a tendency to be somewhat lower for those eggs produced by *C. glacialis* incubated in the Natural attenuation treatment compared to the other treatments (Fig. 13). There was, however, no significant difference between the four treatments (ANOVA $P=0.26$), although a t-test showed that there was a small difference in the hatching success between the control and natural attenuation treatment (t-test; $p=0.049$).

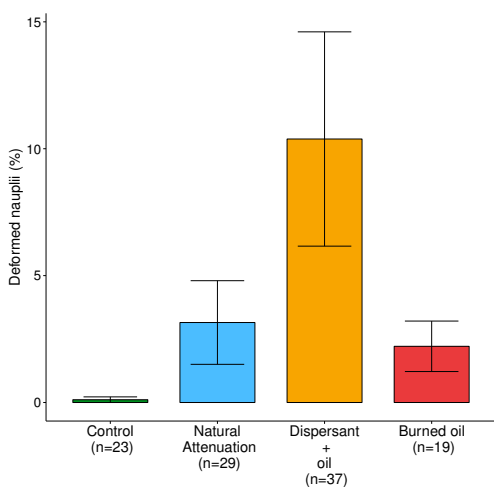


Figure 14: Average egg hatching (%) per incubated female *C. glacialis* (\pm SE). Each bar represent one treatment (n=number of replicates).

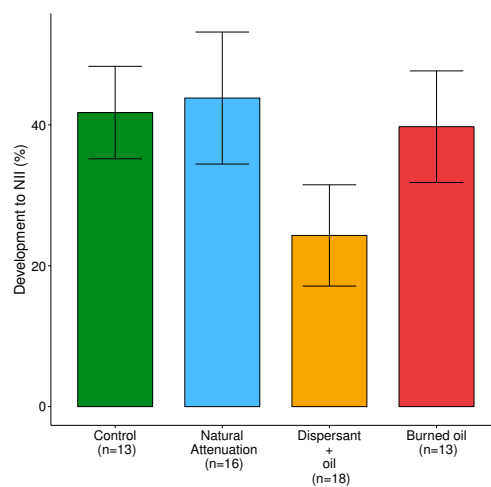


Figure 15: Average number off nauplii from the oil exposure experiment that survived and developed to NII (\pm SE). Each bar represent one treatment (n=number of replicates).

During the incubation experiment it was found that several of the nauplii was born with different degree of deformations (Fig.14, Appendix E.2). The highest numbers of deformed nauplii was found in the dispersant treatment (ANOVA $p=0.004$). In this study, the nauplii were followed to the next naupliar stage to investigate if the deformed nauplii where able to survive and develop to the next naupliar stage NII. There was a tendency that less of the nauplii from the dispersant treatment survived, but this trend was not significant due to high variability (ANOVA, $p=0.25$).

Discussion

C. finmarchicus and *C. glacialis* have different environmental optima and thus different core distribution areas (Jaschnov, 1970; Conover and Kania, 1988; Melle and Skjoldal, 1998). In Isfjorden, both *C. finmarchicus* and *C. glacialis* co-occurred in similar high abundances, comparable to maximum numbers found for *C. finmarchicus* and *C. glacialis* in Svalbard waters (Basedow et al., 2004; Blachowiak-Samolyk et al., 2008; Gluchowska et al., 2016; Daase and Eiane, 2007).

C. finmarchicus may reach five times higher abundances ($\sim 150.000 \text{ ind m}^{-2}$) in its core distribution area in the North Atlantic (Melle et al., 2014). In the core of the West Spitsbergen Current and in fjords strongly influenced by Atlantic or transformed Atlantic water e.g. Kongsfjorden and Isfjorden, *C. finmarchicus* is also numerous (up to $30.000 \text{ ind m}^{-2}$) (Blachowiak-Samolyk et al., 2008; Weydmann et al., 2014; Gluchowska et al., 2016, this study). In fjords with restricted water mass exchange in West Spitsbergen, e.g. sill fjords, the abundance of *C. finmarchicus* is generally poor (Arnkværn et al., 2005). In comparison, *C. glacialis* can be especially abundant in such sill fjords which often are glacial fjord bays with extensive cold water formation and seasonal sea ice cover (Nilsen et al., 2008; Gluchowska et al., 2016). For instance, in the sill fjord Billefjorden a similar large population of *C. glacialis* exists as found for the Isfjorden proper in this study (Arnkværn et al., 2005) which equals the high numbers of *C. glacialis* found in Hinlopen and Rijpfjorden in Eastern Svalbard, which are among the highest population numbers reported in Arctic shelf seas (Søreide et al., 2008).

The strong decline in population numbers of both *C. glacialis* and *C. finmarchicus* during the winter-spring transition in Isfjorden may indicate unfavorable leading to starvation or potential parasitic or viral infection (Daase et al., 2014). However, such high mortality doesn't need to mean that the populations are not sustainable. For instance in Loch Eteve, Scotland, which has a large local population of *C. finmarchicus* experience similar decline in population numbers from winter to spring as found in Isfjorden (Clark et al., 2012). Similar declines in population numbers during winter-spring are also observed for *C. glacialis* and *C. finmarchicus* in Svalbard fjords with otherwise relatively large *Calanus* populations (Arnkværn et al., 2005; Søreide et al., 2010).

4.1 *Calanus* spp. egg production - capital or income breeder?

C. glacialis is known to be capable of capital breeding, but it commonly produce much more eggs when actively feeding, while *C. finmarchicus* is known to be a primarily income breeder (Melle and Skjoldal, 1998). *C. glacialis* is known to start spawning at low rates prior to the spring bloom, while spawning in *C. finmarchicus* is more closely related to the onset of the spring bloom (Nielsen and Hansen, 1995; Melle and Skjoldal, 1998; Niehoff et al., 2002; Madsen et al., 2001, 2008). Although both species reproduced prior to the spring bloom in this study, which also were supported by the reduction in their lipid sac sizes over

this period, *C. glacialis* started to produce eggs earlier than *C. finmarchicus*. Even though the lipid sacks constituted the same percentage of the body in the two species, *C. glacialis* was overall much larger in size than *C. finmarchicus* and could thus afford to start the egg production earlier than *C. finmarchicus* (Melle and Skjoldal, 1998; Miller et al., 2000). Interestingly, *C. glacialis* had a distinct peak in egg production simultaneously with the spring bloom, while *C. finmarchicus* had a more modest EPR stretched over a longer time period from May through July/early August.

The egg production of *C. glacialis* in this study was similar to the one observed in previous studies where the maximum EPR lies between 55-88 eggs female⁻¹ day⁻¹ during peak egg production (Ashjian et al., 1995; Melle and Skjoldal, 1998). This supports that *C. glacialis* in Isfjorden is capable of producing eggs in the higher, maximum range.

However, daily EPR of up to 70 eggs f⁻¹d⁻¹ for *C. finmarchicus* has been found (Diel and Tande, 1992). Temperature is very important for *C. finmarchicus* egg production. It has been shown that the egg production in *C. finmarchicus* is drastically reduced at temperatures below 6°C (Melle et al., 2014). Isfjorden was most likely too cold for the *C. finmarchicus* females to reach their maximum egg production capacity.

The females of both *C. glacialis* and *C. finmarchicus* were utilizing the peak of the bloom to fuel egg production, which was also evident from the non-increasing lipid sac areas during May. After the bloom, the number of *C. glacialis* females as well as the daily EPR was low, while *C. finmarchicus* continued to produce eggs at similar modest rates until August.

Other studies from the Arctic also show that *C. glacialis* is capable to fine-tune its reproduction to the short, but intensive spring bloom that characterizes high latitude seas (Søreide et al., 2008; Kjellerup et al., 2012). In contrast, the egg production strategy of *C. finmarchicus* fits the prolonged primary production at temperate latitudes better (Niehoff et al., 2002; Kjellerup et al., 2012). The earlier the better was the conclusion for *Calanus* reproduction at high latitudes (Varpe, 2012). When studying the copepod recruitment of the two species in summer, it is clearly seen that the late spawned *C. finmarchicus* eggs did not make it to copepodite stage I. The very low naupliar abundance in July supports that eggs produced in summer did not even make it to the older naupliar stages. This could be a result of poorer food availability combined with increased predation pressure (McLaren et al., 1988). Primary production usually becomes strongly nutrient depleted in stratified waters in summer and by the end of August also strongly light limited due to river and particle run-off from land and shorter day lengths (Marquardt et al., 2016). Further south at more temperate latitudes the productive season is longer and also normally includes a small autumn bloom (Leu et al., 2011).

Females of *C. glacialis* remaining in June, stopped to reproduce and rather invested in building up their lipid storages, potentially preparing for a new overwintering. Some studies have suggested that adult females of *C. glacialis* may be iteroparous, i.e. that they are able to survive and reproduce a second year (Kosobokova, 1999).

4.2 Egg hatching success

In the seasonal study in Isfjorden, the hatching success for both species varied greatly during the season. For *C. glacialis* the hatching success was high early in the season for so to decrease prior to the spring bloom. This can be explained by the use of internal resources with sufficient amounts of essential polyunsaturated fatty acids (PUFAs) in the early phase, for so to experience PUFA limitations later if no input of fresh food is possible. It is uncertain why the egg hatching success for *C. glacialis* was low on the 9th of May, especially as the same was not observed in *C. finmarchicus*. However, it may take some time to assimilate the algal food after a long starvation period (Freese et al., 2016). In general, the hatching success

to *C. finmarchicus* was overall low prior to the spring bloom when the temperature was on its lowest. In an experimental study, testing temperature effects on *Calanus* egg hatching success, the hatching success remained similarly high (>90%) within the temperature range from 0 to 10°C for both *C. glacialis* and *C. finmarchicus* (Weydmann et al., 2015). However, studies of egg hatching success in the field revealed that *C. finmarchicus* had significantly lower egg hatching success at temperatures below 6°C (Melle et al., 2014)

4.3 Potential effects of oil spill on *C. glacialis* reproduction

The risk of an oil spill in the Arctic increases with increased human oil and shipping activities there. The results of the oil exposure study doesn't make it possible to give clear advises. How to treat an oil spill will be dependent on the location and the time of the year. The mesocosm was placed out in the fjord in February and in May, when the mesocosm water was sampled, a thick layer of oil under the ice could still be found. Due to the sampling method, mesocosm water was sampled below the thickest part of the oil layer. The oil got trapped in the sea ice. If an oil layer is present in spring, the females will migrate up and into the oil, resulting in too much exposure, leading to higher concentrations of PAHs at the sea ice-water boundary than used in this study. If the oil gets trapped in the ice it could as well lead to a reduction in ice algae since the dark oil prevents lights to penetrate. Ice algae is utilized by the *C. glacialis* to mature and produce eggs (Søreide et al., 2010). The combination of decrease in ice algae biomass and high PAH, will likely have an even larger negative effect on the *C. glacialis* reproduction. One small stressor may not potentially have a measurable negative effect, but several small stressors combined, may be deadly.

In the oil exposure experiment a very low EPR was observed. During the 14 days experiment an average production of 4-5 eggs female⁻¹ day⁻¹ for the three treatments and the control were observed. This was very low compared to the maximum EPR of *C. glacialis* in the seasonal study in Isfjorden in 2016. In 2015, the spring bloom started around the 7th of May, approx. 1 week earlier than in 2016 in Isfjorden. When the oil exposure experiment started on the 16th of May, the *C. glacialis* females may have been already past its reproduction peak, especially since they were starved for almost a week prior to the experiment. Despite that *C. glacialis* was fed ad libitum during the experiment, *C. glacialis* was not able to increase its reproduction to maximum levels again. Its EPR remained low, comparable to the EPR measured for *C. glacialis* in early June in 2016. The differences in EPR between the control and the treatments were non-significant, but the treatments showed a trend of having a higher cumulative EPR than the control.

Highest PAH concentrations was found in the dispersant treatment, the treatment that had the highest cumulative EPR. In a study by Jensen and Carroll (2010), where *C. glacialis* was exposed to crude oil, it was shown that crude oil decreased the EPR. However in other studies (Jensen et al., 2008) no large effects on EPR was found when *Calanus* was exposed to oil (Jensen et al. 2008). If the exposure experiment had been conducted earlier during the peak of EPR and not after, a larger effect of oil on the reproduction might have been observed. 11% of the nauplii from the dispersant treatment showed rather large deformations (Appendix. E.2). The nauplii from the three treatments (burned, dispersant and natural attenuation) showed, in comparison to the control and the seasonal study in 2016, a significantly higher occurrence of deformed *C. glacialis* nauplii. Some deformed nauplii during the spring bloom in May 2016 was observed for both *C. glacialis* and *C. finmarchicus*. Some diatoms have anti-predator responses, producing toxic aldehydes, which are known to increase the number of non-viable eggs and cause nauplii deformation (Poulet et al., 1995; Starr et al., 1999; Uye, 1996). A presence of toxic diatoms in May,

could potentially explain the low egg hatching success of *C. glacialis* in May as well as the few occurrences of nauplii deformations. The likelihood for toxic diatoms in Isfjorden is not known since no one has yet studied toxic algae in the Arctic.

The dispersant treatment showed the lowest survival to NII. The differences were, however, not significant due to the large variability among treatments. Eggs were removed from the mesocosm water every 24h and incubated in filtered sea water. Since eggs are produced at different times, some eggs might have been incubated in the mesocosm water for 24h, while other eggs were removed from the mesocosm water soon after spawning. This could be the reason for the large variability seen in nauplii deformation. If the eggs had been continued to be incubated in the treatment water, the difference between treatments might have been more clear-cut.

A positive effect of sea ice trapping the oil is, that the window of opportunity for using dispersant and burning of oil is much higher (Dickins et al., 2008; Brandvik and Faksnes, 2009; Buist et al., 2011). To burn oil or use dispersant, the oil needs to be in a certain condition and film thickness. In open water the oil spreads out to a thin layer quite fast, making it hard to ignite. The dispersant treatment was the treatment, which indicated some negative effects on the *C. glacialis* reproduction. The dispersant divides the oil into smaller droplets, which then might be easier taken up by organisms like *C. glacialis*. If possible, burning of oil should be used rather than the use of dispersants.

The dispersant treatment was also the lowest percentage of individuals surviving to NII. There are however no significant differences between the different treatments. This was caused by the large variance within the groups. Eggs were removed from the mesocosm water every 24h and incubated in filtered sea water. Since eggs are produced at different times, some eggs might have been incubated in the mesocosm water for 24h, while other eggs were removed from the mesocosm water soon after. This could be the reason for the large variance of the results. If the eggs had been incubated in the mesocosm water, the difference between the different treatments might have been more clear.

4.4 Successful population recruitment?

Isfjorden is situated at 78°N, but resembles sub-Arctic more than Arctic environmental conditions (Wiedemann et al. 2016, this study). Relatively warm sea temperatures, no sea ice formation and a rather long primary productive season characterized the year 2016 and previous years (Marquardt et al., 2016, e.g.). Both *C. glacialis* and *C. finmarchicus* were abundant in Isfjorden, suggesting extensive water mass mixing between Atlantic water and colder and less saline Arctic shelf water before the water entered Isfjorden. No major advective events were possible to detect in the hydrography data, but exchange of surface water masses due to wind cannot be ruled out (Nilsen et al., 2008). The distinct increase in young *Calanus* copepodites from May to June may partly be explained by wind driven advection (Nilsen et al., 2008). However, *C. glacialis* exhibited high egg production rates in May which can support the massive local copepod recruitment in June. A similar increase in recruitment was not found for *C. finmarchicus*. If extensive water mass exchange with the shelf outside had occurred, it could be expected that also a similar high increase in *C. finmarchicus* population would have been likely (Basedow et al., 2004).

The smaller copepodite stages CI and CII can be challenging to identify to species and may therefore have been misidentified (Gabrielsen et al., 2012). The time it takes from hatching to CI and CII and the high CI and CII abundances in June, however, fits well with the timing of the *C. glacialis* peak in egg reproduction in mid-May (McLaren et al., 1988). The CI found in July was identified to be *C. finmarchicus*. If a large part of the CI in June were to be *C. finmarchicus*, too, a large increase in *C. finmarchicus* CIII should have

been observed in July, but this was not the case. Therefore it can be concluded, that most the CI and CII *C. glacialis* in June were likely to be correctly identified and that local production rather than advective processes took place spring and summer 2016 in Isfjorden. The lower recruitment of *C. finmarchicus* compared to *C. glacialis* was likely due to low egg production and hatching success prior to the bloom, only modest egg production during the spring bloom and late spawned eggs not being able to develop due to poorer food condition and higher predation in summer (see above).

By the end of August, *C. glacialis* had similar or even higher population numbers than found in February, while *C. finmarchicus* had lower. *C. glacialis* had reached its main overwintering stages CIV and CV by the end of August and was concentrated at depth. *C. finmarchicus* was not in the similar late state by the end of August. A large part of the population was still in the surface.

C. finmarchicus is able to feed efficiently on smaller algal cells than *C. glacialis*, which makes it possible for *C. finmarchicus* to still grow and develop under post bloom conditions (Hansen et al., 1990). However, even if the young stages present in end of August would make it to the overwintering stage, a decrease in the *C. finmarchicus* population is likely to occur. *C. finmarchicus* is most likely dependent on advection of individuals in order to have a sustainable population in Isfjorden over time. In a future warmer climate, however, *C. finmarchicus* will probably experience more successful recruitment.

4.5 Conclusion

In Isfjorden, *C. glacialis* seems to have a strong, sustainable population under the current primary production and temperature regime. *C. finmarchicus*, however, may to a lesser degree be sustainable due to a higher degree of mismatch between reproduction, development and growth to the abiotic and biotic environment in Isfjorden. Particularly, an increase in the spring and early summer temperatures will have a strong positive effect on the *C. finmarchicus* reproduction and growth. A large and sustainable population of *C. finmarchicus* in Isfjorden is therefore likely in the future if the warming continues as predicted. Whether this will be on the expense of *C. glacialis* is not possible to conclude from this study. However, if *C. glacialis* continues to spawn earlier than *C. finmarchicus* and to match its reproduction and growth mainly to the spring bloom and *C. finmarchicus* continues to reproduce somewhat later the competition for resources will likely be low and ensuring co-existence of both species also in the future.

Increased human activities in the Arctic increase the likelihood for accidental oil spills. Based on the results from the experimental study in 2015 and the seasonal field study in 2016, the conclusion is that negative effects are more likely to be found when using dispersant to clean up oil spills. No significant negative effects could however be proven in this study. Nevertheless, the overall low egg production rates during the experiment, resembling post-blooming egg production rates in field, and the short exposure of the eggs in the treatments likely led to the high variability seen and thus non-significant effects despite that some clear trends were observed.

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Appendices

A. Water masses in Isfjorden

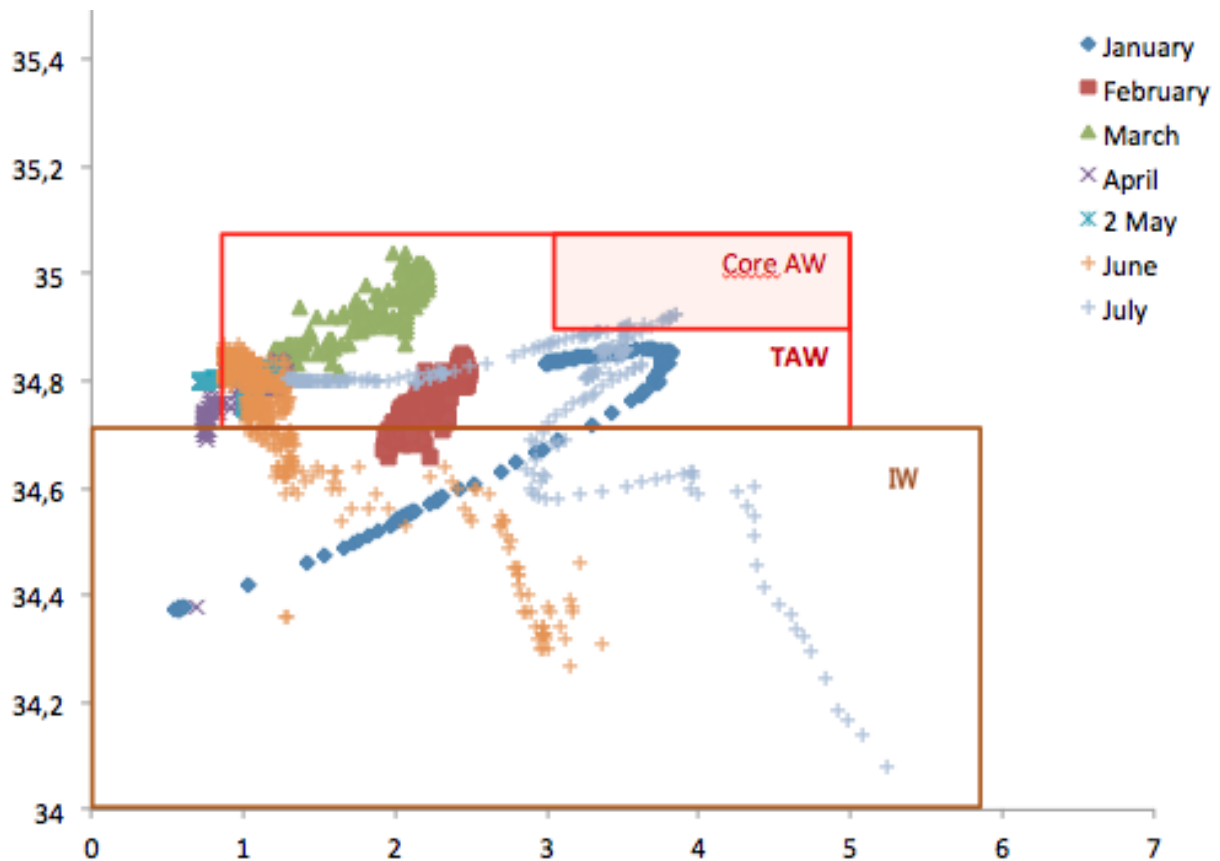


Figure A.16: Temperature/Salinity plot of the different water masses obtained from the CTD data. Water masses is defined according to (Nilsen et al., 2008) (IW:Intermediate water, TAW: Transformed Atlantic water)

B. *Calanus* spp. community composition

B.1 Copepodite composition

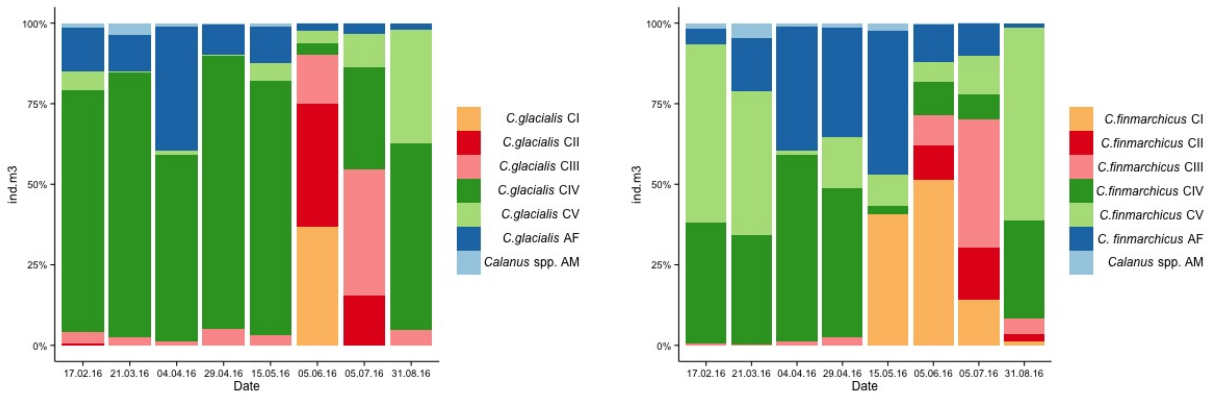


Figure B.17: Relative abundance (%) of *Calanus glacialis* (left) and *Calanus finmarchicus* (right) during the study period from February 2016 to August 2016

B.2 Egg and nauplii composition

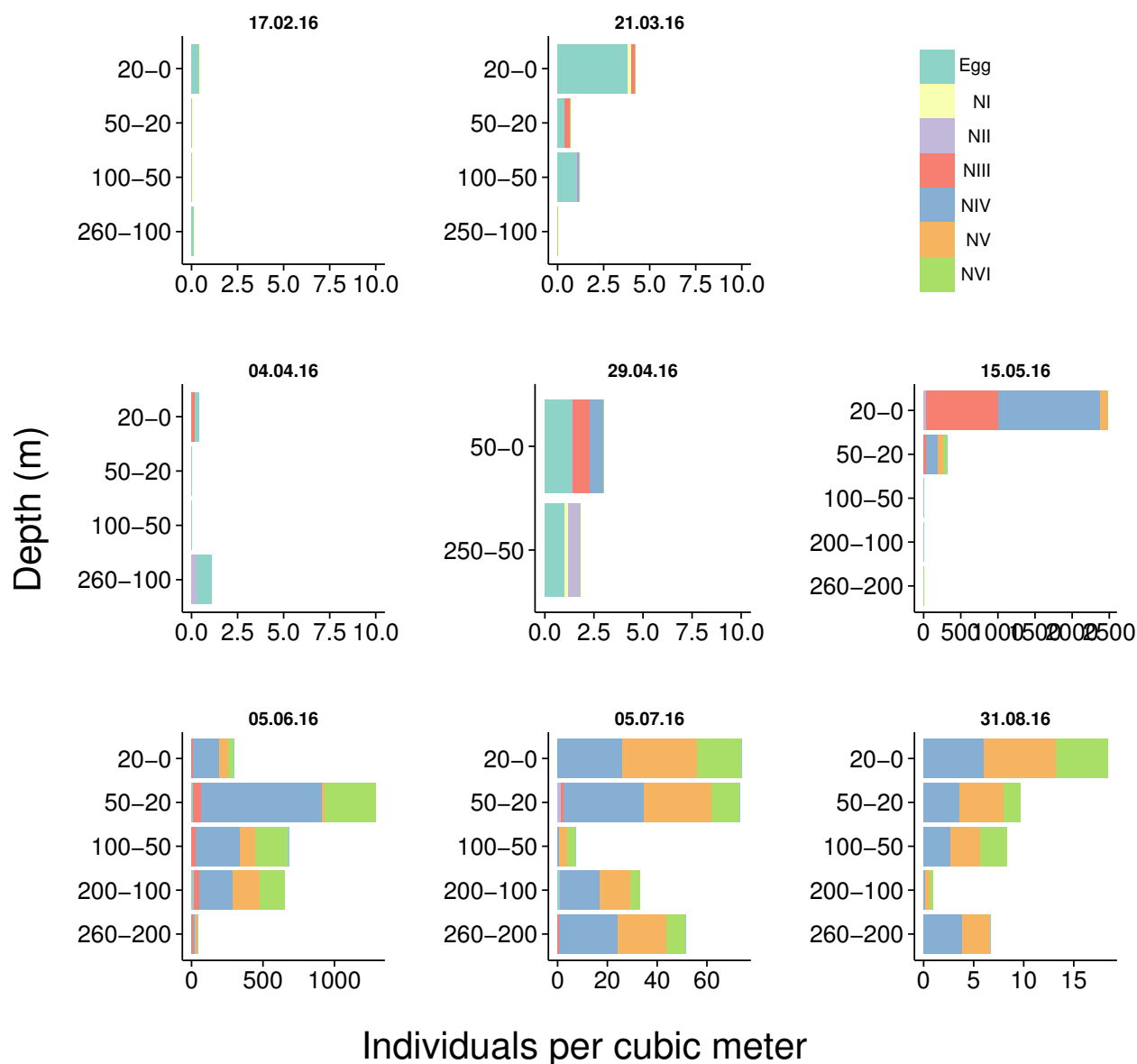


Figure B.18: Abundance and vertical distribution of *Calanus* egg and nauplii in Isfjorden from February to August 2016.

C. Female condition

Table C.1: Length and lipid content of *Calanus glacialis* female.*(mean±SD)

Date	N of females	Prosome length (PL)*	Prosome area (PA)*	Lipid sack area (LA)*	LA/PA*	Total lipid (mg)
17.02.16	30	3.25±0.24	2.70±0.51	0.82±0.36	0.30±0.10	0.15
21.03.16	30	3.26±0.25	2.55±0.49	0.58±0.32	0.22±0.08	0.09
04.04.16	30	3.23±0.18	2.53±0.33	0.41±0.16	0.16±0.05	0.06
02.05.16	27	3.08±0.30	2.35±0.47	0.26±0.13	0.12±0.07	0.03
09.05.16	30	3.48±0.28	3.00±0.79	0.36±0.55	0.08±0.08	0.05
15.05.16	29	3.11±0.30	2.47±0.56	0.37±0.36	0.13±0.11	0.05
05.06.16	10	3.02±0.36	2.40±0.58	0.78±0.47	0.31±0.14	0.14
18.08.16	5	3.06±0.13	2.42±0.30	0.05±0.05	0.06±0.07	0.00
31.08.16	0	-	-	-	-	

Table C.2: Length and lipid content of *Calanus finmarchicus* females.*(mean±SD)

Date	N of females	Prosome length (PL)*	Prosome area (PA)*	Lipid sack area (LA)*	LA/PA*	Total lipid (mg)
17.02.16	30	2.79±0.09	1.99±0.17	0.56±0.15	0.29±0.09	0.09
21.03.16	30	2.71±0.20	1.74±0.28	0.47±0.18	0.27±0.09	0.07
04.04.16	31	2.68±0.20	1.70±0.30	0.38±0.15	0.22±0.07	0.05
02.05.16	29	2.59±0.13	1.57±0.38	0.08±0.07	0.05±0.05	0.01
09.05.16	30	2.61±0.23	1.67±0.30	0.16±0.17	0.09±0.06	0.02
15.05.16	30	2.66±0.20	1.74±0.29	0.09±0.09	0.09±0.08	0.01
05.06.16	34	2.81±0.59	1.82±0.24	0.35±0.27	0.18±0.14	0.05
18.08.16	16	2.66±0.27	1.75±0.43	0.14±0.18	0.08±0.09	0.01
31.08.16	6	2.64±0.10	1.90±0.82	0.09±0.13	0.05±0.07	0.01

D. Oil exposure experiment

D.1 Experimental set-up

In February 2015 eight cylindrical enclosures with an open top and bottom (3 m deep, 1.5 m in diameter) were anchored in the sea ice. The mesocosms were placed 800 m from shore and at a water depth of 15-25.

Three different chemical treatments (two replicates) were added to the mesocosms; crude oil, crude oil mixed with chemical dispersant and residue from burnt crude oil. In addition, the set-up included a control treatment (two replicates). Untreated "KOBBE" crude oil from the Goliat oil field (provided by ENI Norge A/S), and was used to represent crude oil composition of an Arctic oil spill. For the natural attenuation treatment 20 L crude oil was gently added to the mesocosm. This volume was used as it was considered to represent initial crude oil concentration during an actual offshore oil spill in the Arctic. In the chemical dispersant treatment, an additional x L of OSR52 Finasol (Total Special Fluids) was added. In the burning treatment, 4 L of residue from 20 L burnt crude oil (INERIS laboratories), was added to the mesocosm. After addition, sea ice was allowed to form naturally.

Water from the mesocosm was sampled on 14th of May 2015 during the beginning of the ice algal bloom and six weeks before sea ice break up. Mesocosm water for the exposure experiment was collected through boreholes in the sea ice. Water was pumped from 10 cm above the lower ice edge using a hand-operated vacuum pump (Biltema hevertpump, 4,6 l/min, -2.4 °C) with a 200 µm filter, and stored dark at 1 °C in 23 L glass bottles until returning to the laboratory.

E. Oil exposure results

E.1 Daily egg production

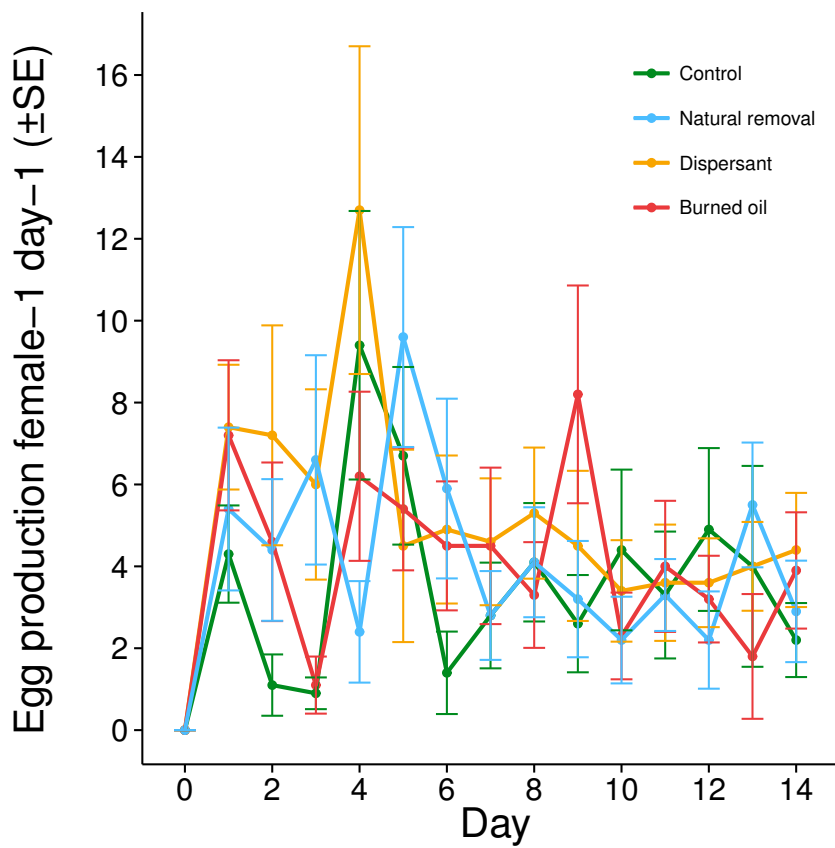


Figure E.19: Daily egg production per female

E.2 Nauplii deformation

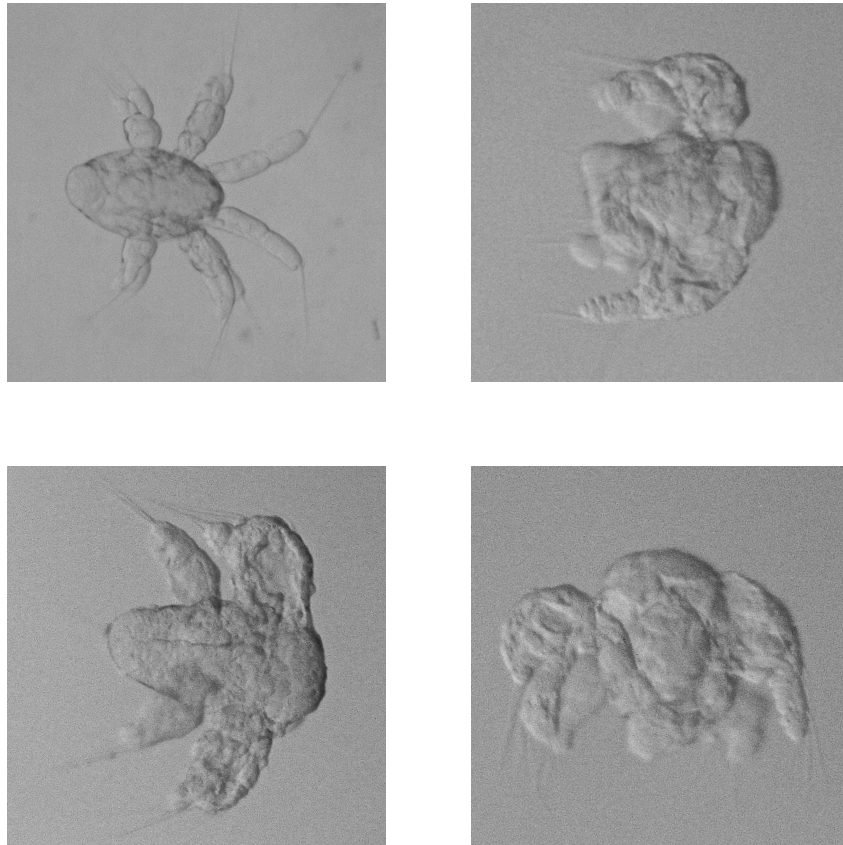


Figure E.20: Pictures of *Calanus glacialis* nauplii (A) A normal nauplii from the control (B-D) Various pictures of deformed nauplii.