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Capital or Income Breeder: The Role of Lipids and Fatty Acid Composition for Successful Reproduction in *Calanus glacialis*

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Marine Coastal Development
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Abstract

This is the first time-series study to investigate the gonad maturation and egg production of *Calanus glacialis* in Svalbard seasonal ice covered fjords, and the first study to investigate the correlation between nutritious status and time of death for *Calanus* spp. males in the Arctic. There is a significant improvement in reproduction success when the females have access to fresh food. Close to 100% of the fed females spawned compared to only half of the starved females, and both egg production rates and hatching success improved.. Females utilized both stored lipids and input of food for reproduction, showing a mixed strategy between capital and income breeding strategy. When food is scarce, the females invest more lipids on fewer eggs as opposed to more eggs, but less lipid per egg when food is abundant. This suggest a life strategy not previously described for *Calanus glacialis* i.e. when food is absent or low females invest in fewer but more lipid-rich eggs to increase the likelihood for this offspring to survive longer and thus increasing the chances to be present when more favourable food conditions finally appear. The fatty acid composition is more important than total lipid content for the hatching success of the eggs, and 16:0, 18:0, 20:5(n-3) and 22:6(n-3) seems to be particularly important. The fatty acid composition of the females change throughout the winter-spring transition, and the changes seems to be related to where in the reproductive cycle they are. Maturation of the gonads appear to rely mostly on fatty acids the female are capable of synthesizing *de novo*. The decrease in stored lipids is also evident in the field data where the total lipid level is significantly lower in late April than in early April**.** Total lipid content of males at the time of death is normally distributed thus, supporting previous suggestions that males die when the stored resources reach a threshold level.

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Maja Karoline Viddal Hatlebakk

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Abbreviations and terms

AF	Adult female
AM	Adult male
BAB	Sampling station in inner basin of Billefjorden
C (I-V)	Copepodite stage (1 to 5)
CF	Condition factor
Chl *a*	Chlorophyll *a*
EPR	Egg production rate
FA	Fatty acid
Falc	Fatty alcohol
MUFA	Monounsaturated fatty acid
N (I-VI)	Nauplii stage (1-6)
OBAB	Sampling station in outer basin of Billefjorden
PCA	Principal component analysis
PUFA	Polyunsaturated fatty acid
R3	Sampling station I Rijpfjorden
SAFA	Saturated fatty acid
TL	Total lipid content
TOD	Time of death
WE	Wax ester

Capital breeder	Reproduction driven by stored resources
Income breeder	Reproduction driven by external food source
Mixed breeder	Reproduction driven by stored and external resources

Introduction

Polar ecosystems are characterized by low temperatures, sea ice, and big variations in solar irradiation, from the complete dark of the polar night, to the constant light from the midnight sun (Varpe 2012). This causes strong seasonal variations in primary production (McNamara and Houston 2008; Varpe 2012).

The primary production of the Arctic ecosystem can be divided into the ice related and the pelagic biota. Under the ice, light conditions vary with snow cover, ice thickness and the amount of sediments in the water (Wassmann et al. 2006; Mundy et al. 2005), but as light increase during the spring, it triggers a short and intensive bloom of ice algae growing on the underside of the ice (Rozanska et al. 2009) until the sea ice melts (Hegseth 1998). When the sea ice starts to melt and break up, the water becomes stratified and the light level increase, triggering the phytoplankton bloom (Wassmann et al. 2006). The bloom lasts for a couple of weeks before it fades out in a smaller summer and autumn production (Falk-Petersen et al. 2009)

The seasonality in the primary production propagates through higher trophic levels (McNamara and Houston 2008), and has resulted in adaptations like diapause (Fiksen 2000), Seasonal vertical migration (McNamara and Houston 2008) long life span, low metabolism (McLaren 1966) and extensive energy storage (Lee et al. 2006; Falk-Petersen et al. 2009).

In the Barents Sea, 70-90% of the mesozooplankton biomass consist of copepods, where the most important herbivorous species in terms of biomass are of the *Calanus* genus (Tande 1991). The *Calanus* species utilize both the ice algal bloom and the phytoplankton bloom, and rapidly convert the fixated carbon into high energy lipids and accumulating essential polyunsaturated fatty acids (PUFAs) (Falk-Petersen et al. 2000; Falk-Petersen et al. 2009; Lee et al. 2006). The lipid level increase from 10-20% of the dry weight in phytoplankton to 50-70% in herbivorous *Calanus* spp. (Falk-Petersen et al. 2007), making these relatively large copepods to key species in effectively transferring essential PUFAs from the primary producers to higher trophic levels of the Arctic ecosystem (Falk-Petersen et al.2009). They are an important food source for plankton-feeders, like the polar cod (*Boreogadus saida* (Lepechin, 1774)), capelin (*Mallotus villosus* (Müller, 1776)) (Wassmann et.al. 2006) and little auk (*Alle alle* (Linnaeus, 1758)) (Mehlum and Gabrielsen, 1993), that again are

important food for marine mammals and seabirds (Falk-Petersen et al. 2007).

In the European Arctic, three *Calanus* species co-exist (Conover 1988; Daase and Eiane 2007). *Calanus finmarchicus* (Gunnerus 1765) is an Atlantic species that is transported by the North Cape Current to the Arctic (Jaschnov 1970; Conover 1988). *Calanus glacialis* Jaschnov 1955 is endemic to the Arctic shelf seas (Conover 1988; Grainger 1961) and *Calanus hyperboreus* Krøyer 1838 is found mainly in the areas of the deeper basins (Jaschnov 1970; Falk-Petersen et al. 2009).

Of the three species, *Calanus glacialis* is the most important in Arctic Shelf seas (Jaschnov 1970; Grainger 1965; Hirche 1994). It has a wide, circumpolar distribution, and specimens also penetrate into the north Atlantic and north Pacific through cold currents such as the East Greenland current and the Labrador Current (Jaschnov 1970).

Calanus glacialis is well adapted to the seasonality and physical environment of the Arctic. Cold temperatures have resulted in a 1-3 year life cycle, consisting of 6 nauplii stages (denoted NI-NVI) and six copepodite stages (denoted CI-CV and AF and AM for adult females and males respectively) (Conover 1988, Tande 1991; Falk-Petersen et al. 2009; Søreide et al. 2010). A schematic presentation of the life cycle of *C. glacialis* is illustrated in Figure 1.

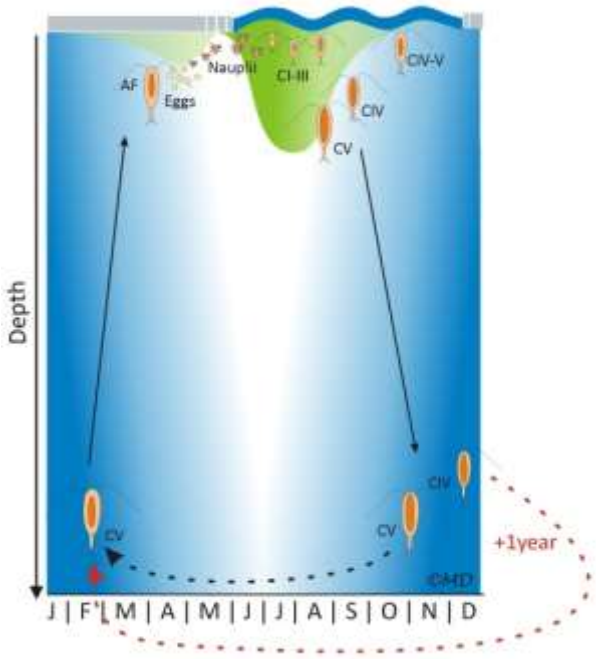

Figure 1: Schematic presentation of the life cycle of *Calanus glacilais.* Figure credit: Malin Daase

For *Calanus glacialis* to be able to complete its lifecycle, it needs to reach a stage where it can store sufficient amounts of lipids before the end of the productive season (Ji et al. 2012). The main overwintering stages are CIV and CV (Falk-Petersen et al. 2009), but they can also spend the winter as CIII (Madsen et al. 2001; Falk-Petersen et al. 2009) or as females (Kosobokova 1999). They descend to depth and enter diapause in autumn (Kosobokova 1999). During the autumn/winter the CV molts to adults. The males appear first, peaking in October, preceding the molting of females at midwinter by 3-4 months (Kosobokova 1999).

When the females ascend to the surface in spring to spawn, it has been shown a close coupling between the bloom of ice algae and the phytoplankton bloom, and the reproductive success of *Calanus glacialis* (Søreide et al. 2010; Leu et al. 2011). Females are able to graze on the ice algae (Runge and Ingram 1988), allowing them to utilize the ice algae for maturation and early spawning (Søreide et al. 2010). From the time of spawning, it takes approximately 3 weeks before the nauplii has developed to the first stage of active feeding, NIII (Søreide et al. 2010; Daase et al. 2011), allowing the nauplii to feed on the phytoplankton bloom, and ensuring a long growth season (Søreide et al. 2010).

Former studies concluded that *Calanus glacialis* was dependent of food for reproduction, i.e. are income breeders (Hirche 1989), but Smith (1990) found spawning *C. glacialis* females in the Fram Strait when the chlorophyll *a* (Chl *a*) concentration in the water was very low, suggesting that *C. glacialis* is capable of reproducing on stored resources only, i.e. capital breeding. However, this and other studies stating *C. glacialis* to be a capital breeder (e.g. Hirche and Kattner 1993) have been done on females collected during spring when ice algae might have been present. There is no doubt that food enhances egg production in *C. glacialis* (Hirche 1989; Tourangeau and Runge 1991; Hirche and Kattner 1993), but whether *C. glacialis* can reproduce in absence of food is still questionable since it cannot be guaranteed that food was not present in the studies above.

For successful reproduction, mating must take place and males must then be mature and present. Males are only present during a rather short time window, mainly during the nonproductive season of the polar night (Kosobokova 1999). Males have reduced feeding appendices and do most likely not feed. Maturation and mating is purely based on stored resources in males, and it is hypothesized that they most likely die when their lipid sac has reached a certain minimum (Kosobokova 1999).

Lipids

There are two major types of storage lipids: Triacylglycerols (TAG) and wax esters (WE). In high-latitude zooplankton, WE is the major storage lipid and it consists of one fatty acid (FA) and one fatty alcohol (Falc) that separate when esterified. TAG consists of three hydrocarbon chains on a glycerol backbone that can be esterified to three FAs (Sargent and Falk-Petersen 1988; Lee et al. 2006). FAs consists of a carboxyl group (-COOH) on a long hydrocarbon chain. They are separated in saturated (SAFA), monounsaturated (MUFA) and

polyunsaturated (PUFA) fatty acids, as they have none, one, or two or more double bonds in the hydrocarbon chain, respectively.

Calanus spp. accumulates lipids from their diet and is also able to biosynthesize *de novo* long chained fatty acids (FA) and fatty alcohols (Falc) (e.g. 20:1(n-9) and 22:1(n-11)) which are stored in a lipid sac which can fill up to 80% of the body cavity of older copepodite stages (Sargent and Henderson 1986; Pascal and Ackman 1976; Vogedes et al. 2010)(Figure 2). In marine ecosystems, Eicosapentaenoic acid (EPA, 20:5(n-3)) and docosahexaenoic acid (DHA, 22:6(n-3)) are essential fatty acids important for healthy development and growth (Kattner and Hagen 2009). These Omega-3 fatty acids and other PUFAs are selectively retained in the copepods and largely reflect their algal diet (Dalsgaard et al. 2003).

In spring, when the females are maturing, half of the lipids are lost in the final stages of maturation, and the WE account for a large portion of this loss (Hirche and Kattner 1993; Jónasdóttir 1999). In the eggs 16:0, 18 FA, and PUFAs, are more abundant than in females (Hirche and Kattner 1993), and especially the PUFAs 20:5(n-3) and 22:6(n-3) have been found to be important for successful hatching of the eggs (Koski et al. 2012).

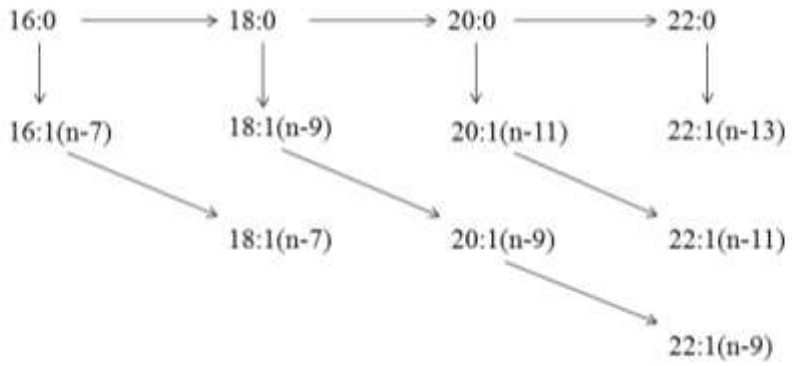

Figure 2: Possible pathways of biosynthesis of fatty acids in calanoid copepods. Figure modified from Sargent and Henderson (1986).

Objectives

In this study I have investigated the reproductive output of feeding versus starved females of *Calanus glacialis*. The females were collected in January, long in advance of the onset of ice algal and phytoplankton production. Females were incubated in filtered sea water in the

laboratory to follow their gonad maturation and reproductive output simultaneously with that in field during the winter-spring transition in 2013. In addition, the total lipid content (TL) have been followed throughout the experiment, and analysis of FA composition of females, nauplii and eggs have been done to investigate the importance of total lipids vs. fatty acid composition for *Calanus glacialis* reproductive success. Males were also collected and incubated in the laboratory to study if there was a correlation between time of death and lipid sac size in males.
The following hypotheses were tested:

H_0) *Calanus glacialis* is a capital breeder
H_1) *Calanus glacialis* need to feed to be able to mature gonads and produce eggs

H_0) Polyunsaturated fatty acids are important in the maturation process of the gonads and the egg production and hatching success of *Calanus glacialis*
H_1) the fatty acid composition is of little importance for the reproduction success of *Calanus glacialis*, it is the total amount of lipids that matter.

H_0) *Calanus glacialis* males die when the lipid storage has reached a minimum threshold value
H_1) the death of *Calanus glacialis* males is controlled by other factors than the amount of total lipids

Material and methods

Sampling

Samples were collected from two fjord in the Svalbard archipelago; Billefjorden and Rijpfjorden (Table 1, Fig3). Billefjorden is a sill fjord on the west coast of Spitsbergen consisting of two basins. The outer basin (max depth ~230 m) is bordered by a sill at ~80 m depth towards Isfjorden and a sill at ~45 m depth towards the inner basin (max depth ~190 m). From December-January to May-June, Billefjorden is usually covered by ice (Arnkværn et al. 2005).

Figure 3: Location of sampling locations; Billefjorden and Rijpfjorden. Figure from Norwegian Polar Institute.

Rijpfjorden, Nordaustlandet, opens northward towards the polar ocean. Rijpfjorden is dominated by cold, Arctic water

masses and is covered by ice up to 9 months a year (Søreide et al. 2010)

Samples were collected once or twice a month from January to May 2013 in Billefjorden, and once in January and once in February in Rijpfjorden (Table 1). In Billefjorden sampling was done at the sampling station BAB, except from February when BAB could not be reached due to difficult sea ice conditions and sampling was done just outside BAB (OBAB), in the first deep basin of Billefjorden. In Rijpfjorden sampling was done at the R3 sampling station.

To follow the environmental conditions, a CTD (Conductivity, Temperature and Depth) profile was sampled with a Saiv CTD probe with a fluorometer attached before each sampling, and water samples were collected at regular depths to analyze Chl *a* concentrations (Gabrielsen et al. unpublished). Ice cores were collected to measure ice algal biomass (Gabrielsen et al. unpublished).

Table 1: Overview of date and position for sampling in Billefjorden and Rijpfjorden 2013. Ice and snow thickness at each sampling is listed as well.

Date	Station	coordinates		Ice thickness	Snow thickness
		N°	E°	(cm)	(cm)
10/01/2013	BAB	78 39.723'	16 44.342'	0	0
12/01/2013	R3	80 17.10’	22 18.6’	0	0
04/02/2013	OBAB	78 34.380’	16 27.480’	0	0
07/02/2013	R3	80 17.10	22 18.6’	0	0
13/03/2013	BAB	78 39.723'	16 44.342'	33	0
09/04/2013	BAB	78 39.723'	16 44.342'	47	0
26/04/2013	BAB	78 39.723'	16 44.342'	50	3
07/05/2013	BAB	78 39.723'	16 44.342'	52	2-5

In January and February sampling were done by ship, FF Helmer Hanssen and KV Svalbard respectively. The samples were taken by multi plankton sampler (MPS;Hydro-Bios,Kiel) consisting of 5 closing nets with 0.25m^2 opening and 200 µm mesh size. Five depth intervals were sampled in Billefjorden (180-150-100-50-20-0 m) and in Rijpfjorden (270-200-100-50-20-0 m). Mass sampling of animals for biochemical analysis and egg incubation were done from bottom to surface with a larger zooplankton net, a WP3 with opening diameter 1 m^2 and mesh size 1mm, equipped with a large cod end for keeping the animals in a better condition.

From March, samples were taken only in Billefjorden due to accessibility. Snowmobiles were used to access the sampling site. Samples were taken through a hole in the ice using a

zooplankton net type WP2 with manual closing mechanism sampling 4 depth intervals (180-100-50-20-0 m).

Community samples for later species identification were fixated in 4% formaldehyde-seawater solution, while samples for egg incubation, image analysis and later lipid analysis were brought back to UNIS alive in large thermoses.

Experimental setup

The copepods were followed individually during the experiment. The males were kept in the dark in labeled 125 mL cups at close to constant temperature (0 to -1°C), and water was changed with filtered seawater at least every 3rd day to assure good water quality. Females were also kept in the dark at the same temperature as the males, but they were kept in egg chambers (Figure 4) made up of two 125 mL cups, where the uppermost cup had a false mesh bottom allowing the eggs to sink down and away from the female to avoid egg predation. Until March, as long as it was safe to assume there were not much algae to be found, sea water was filtered through 1 µm filter (Sartopure PP2 capsule, Sartorius stedim biotech) but from March, Whatmann glass fiber filter, type GF/F (0.7 µm pore size) was used to filter sea water to assure 100% control of no food access.

For feeding, *Thalassiosira nordenskioeldii* P.T. Cleve 1873, grown in the lab on f/2 (Guillard) medium, was added to the filtered seawater at a concentration of approximately 1000 cells mL^{-1}.

Egg chambers and equipment for water exchange were kept separate for fed and starved females to avoid food contamination of the starved females.

Egg production and hatching success

A total of 100 females were incubated for measuring egg production and hatching success; 30 starved females from Billefjorden, 30 starved females from Rijpfjorden, and 40 fed females from Billefjorden. The starved females were sampled mid-January and incubated individually from January 30th. The fed females were sampled and incubated on March 13th, and feeding started on April 3rd. The experiment was terminated on May 5th.

Figure 4: Egg chamber with mesh bottom. Photo: Maja Hatlebakk

The number of eggs was counted every 24±2 hour. The upper part of the egg chamber, containing the female, was moved over in a new cup with filtered sea water. The remaining water was filtered through a 60 µm sieve and the remaining eggs were rinsed into a gridded petri dish. The eggs were counted and kept for seven days at 0 to -1°C for measuring the hatching success. At -1°C *Calanus glacialis* eggs need around 7 days to hatch (Daase et al. 2011). The eggs that had not hatched after one week were assumed to be non-viable. The hatching success was calculated by counting the number of nauplii and unhatched eggs.

In addition to the females incubated for the long time experiment, 30 females where incubated for 24 hours immediately after returning from field in March, early April, late April and May to measure *in situ* egg production rates (EPR). These females were incubated in surface water from the sampling site, screened through 60 mm to remove grazers, to make the conditions as close to *in situ* as possible.

Gonad staging

The level of maturation of the females was decided by visual identification of the developmental stages of the gonads through a stereo microscope. The gonad development stages (GS) were separated into 4 stages of increasing maturity, GS1 to GS4, where GS1 is completely immature and GS4 are ready to spawn (Niehoff and Hirche 1996). When gonads appeared to be between stages, half where counted to the lower stage, and half to the higher stage. Schematic presentation of the four gonad stages can be found in Appendix C.

Image analysis: Estimate of total lipid content and condition factor

The females were photographed once a week, and the males twice a week. The males were photographed more often to have a better resolution around the time of death. The animals were placed individually in water drops in a petri dish. Almost all of the water was removed to make the animals lay on their side to get a lateral view on the picture. Pictures were taken through a stereo microscope using Sony(HDR-HC7 video camera in photo mode) with ocular adapter.

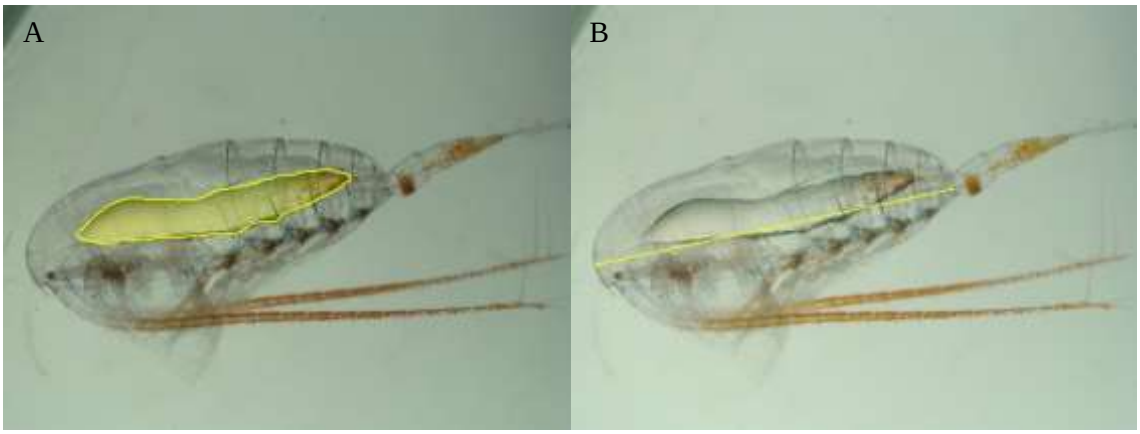

Figure 5: Lateral view of *Calanus glacialis*. The figure shows the outline of the lipid sac (a) and the prozome length (b) photo: Maja Hatlebakk

The pictures were analyzed using imageJ (Rasband, 1997-2013). The number of pixels per 1 mm was given using a picture of calibration slide. For each picture the area of the lipid sac and the prozome length (PL) were measured as illustrated in Figure 5. The area of the lipid sac can be used to estimate total lipid content in mg as described in Vogedes et al. (2010)

$$TL = 0{,}97 * A^{1{,}38} \quad (1)$$

Where A is the area of the lipid sac.

The condition factor (CF) was calculated for each individual based on TL and PL and gives an idea of the nutritional condition of the animal.

$$CF = \frac{TL}{a * PL^b} \quad (2)$$

Where PL is prozome length and a and b are parameters calculated from a linear model.

Parameters a and b are specific for the different copepodite stages. For females $a=3.7\text{x}10^{-4}$ and b=5.1, and for males $a=5{,}87\text{x}10^{-5}$ and b=7.1 (Bailey 2010).

Analysis of fatty acids and fatty alcohols

Fatty acid and fatty alcohol analysis were performed at Alfred Wegener Institute (AWI) in Bremerhaven, Germany. Females from field samples and from different experimental set ups were investigated together with nauplii and unhatched eggs.

Extraction of lipids

Prior to extraction, a known amount, depending on the amount of sample material, of 23:0 fatty acid methyl ester was added as internal standard, for later quantification. The samples were homogenized with a Potter Elvehjem homogenizer in 3x2 mL dichloromethane-methanol (2:1, v:v) (Folch et al.

1957). The supernatant was transferred into a centrifuge vial for separation. Nauplii and algae filters was transferred directly to centrifuge vials and 4 or 8 mL 2:1 dichloromethane-methanol was added respectively. The samples were homogenized in an ultrasonic bath for 10 minutes.

The lipid material was washed with 2 mL (1 mL for nauplii) 0.88% potassium chloride (KCl) in water, and the phases were separated by centrifuging for 5 min. The lower phase, containing the lipids, was transferred to a glass vial using a glass pipette. Another 1 mL dichloromethane was added to the centrifuge vial and it was centrifuged again for 5 minutes. After the second solvent wash, the sample was concentrated and the remaining air was eliminated from the glass vial with gentle stream of nitrogen, and then kept in freezer at -20°C.

Transesterification

Transesterification was done according to Kattner and Fricke (1986). The supernatant was transferred into centrifuge vial with glass pipette and evaporated to dryness under a stream of nitrogen (N_2). The centrifuge vial and its corresponding screw cap was leak checked before usage, to ensure not to lose any solvent during derivatization. Then 250µL hexane and 1mL 3% sulphuric acid (H_2SO_4) in methanol were added. Remaining air in the centrifuge vial was eliminated with a gentle stream of nitrogen and the vial was closed very tight with the screw cap. After heating for 4 hours at 80°C, 4 mL H_2O (milli-q) and 3mL hexane was added and the fatty acid methyl esters and alcohols extracted by shaking of the centrifuge vial for 30 seconds. After phase separation, the upper phase was transferred to a new centrifuge vial using a glass pipette. Extracting was done for another two times. Thereafter the solvent was evaporated to dryness under a stream of nitrogen and a known volume of hexane, depending on the amount of sample material, was added and the sample transferred to a GC vial. Air was eliminated by nitrogen gas and the sample was kept in the freezer at -20°C.

Gas-liquid chromatography

Fatty acid methyl esters (FAME) and fatty alcohols were analyzed in one run. Two similar gas-liquid chromatographs (GCs) Hewlett-Packard 6890N (Agilent technologies Deutschland GmbH & Co. KG) were used depending on the concentration of the sample. The first GC was equipped with a split injector (250°C), a 30m x 0.25 mm i.d. WCOT column (0.25µm film thickness, liquid phase: DB-FFAP) and a flame ionizing detector (280°C). The samples were first injected in split mode and temperature programming from 160°C to 240°C at 4°C/min according to Kattner and Fricke (1986). Less concentrated samples that did not give a satisfactory response, were then injected in the second GC with a splitless injector(250°C), 60m x 0.25 mm i.d. WCOT column (0.25µm film thickness, liquid phase: DB-FFAP), flame ionizing detector (280°C). Temperature programming was from 80°C (2min hold) to 160 at 2°C/min, follow by 2°C/min to 240 (20min hold). The chromatograms were evaluated using the software ChemStation (Agilent, Agilent Technologies Deutschland GmbH & Co.

KG). The fatty acids were identified by comparing relative retention time with known reference standards (37 FAME (Supelco) and a known lab standard).

Statistical analyses

Statistical analyses and graphing were done using Sigmaplot (12.5, Systat Software, San Jose, CA). T-tests were used to compare two groups, and one-way ANOVA were used to compare three or more groups. Significance level was set to $P<0.05$.

To explore patterns in compositional fatty acid composition Principal Component Analysis (PCA) in Canoco 4.5 for Windows was performed (ter Braak and Smilauer 2002). The PCA was performed on untransformed compositional FA data, and FA comprising <2% of total lipids in naupliar and unhatched egg samples were removed prior to analysis to avoid rarely occurring FA influencing the analysis. Ordination techniques and rules of interpretation of PCA ordination plots are summarized by ter Braak and Smilauer (2002). In short, the closer the samples are clustered together, the more similar their FA compositions are. Projection of samples perpendicular to the FA indicates their relative amount of these FA. The FA are standardized and centered and point in the direction of maximum change. Arrow length is proportional to explanatory power. The angle between arrows indicates their correlation, i.e. they are uncorrelated if they are perpendicular to each other and highly correlated (positive or negative) if the angle is small.

Results

Field data

Environmental conditions

In January, the sampling station in Billefjorden was ice free. From February the fjord was starting to freeze, and the sampling was done further out in the fjord (stn. OBAB) in the first deep basin of Billefjorden (Table 1) since the main sampling station (Stn. BAB) could not be reached by ship. Approximate sea ice thickness in February at Stn. BAB was ~10 cm, which increased to ~30 cm in March and reached a maximum thickness of ~50 cm in late April/May (Table 1).

In January and February the water was slightly warmer in the deep compared to March-May, but the water had started to cool in the surface (Fig. 6a). When ice covered, the temperature of

the water column was homogenous at around -1.6 °C. In May, an increased temperature in the surface was observed with a thermocline at 25-50 m where the temperature dropped from approximately -0.9 °C to -1.6 °C (Fig. 6a).

The ice algae growth started in March (0.4 µg chl *a* L^{-1}) with peak chl *a* concentrations (97.4±0.7 µg chl *a* L^{-1}) in the bottom 3 cm in early April (Gabrielsen et al. unpublished data). The onset of sea ice melting had just started in late April and lower ice algal biomass were then observed (14.9 ± 4.1 µg chl *a* L^{-1}).The chl *a* concentration in in the water column was close to zero until early April (Gabrielsen et al. unpublished data). In late April the phytoplankton bloom started, and the chl *a* concentration peaked at 2.2 µg L^{-1} at 7 meters depth. In In May the chl *a* reached even higher concentrations peaking with 7.3 µg Chl *a* L^{-1} at 25 m depth .(Gabrielsen et al. unpublished)

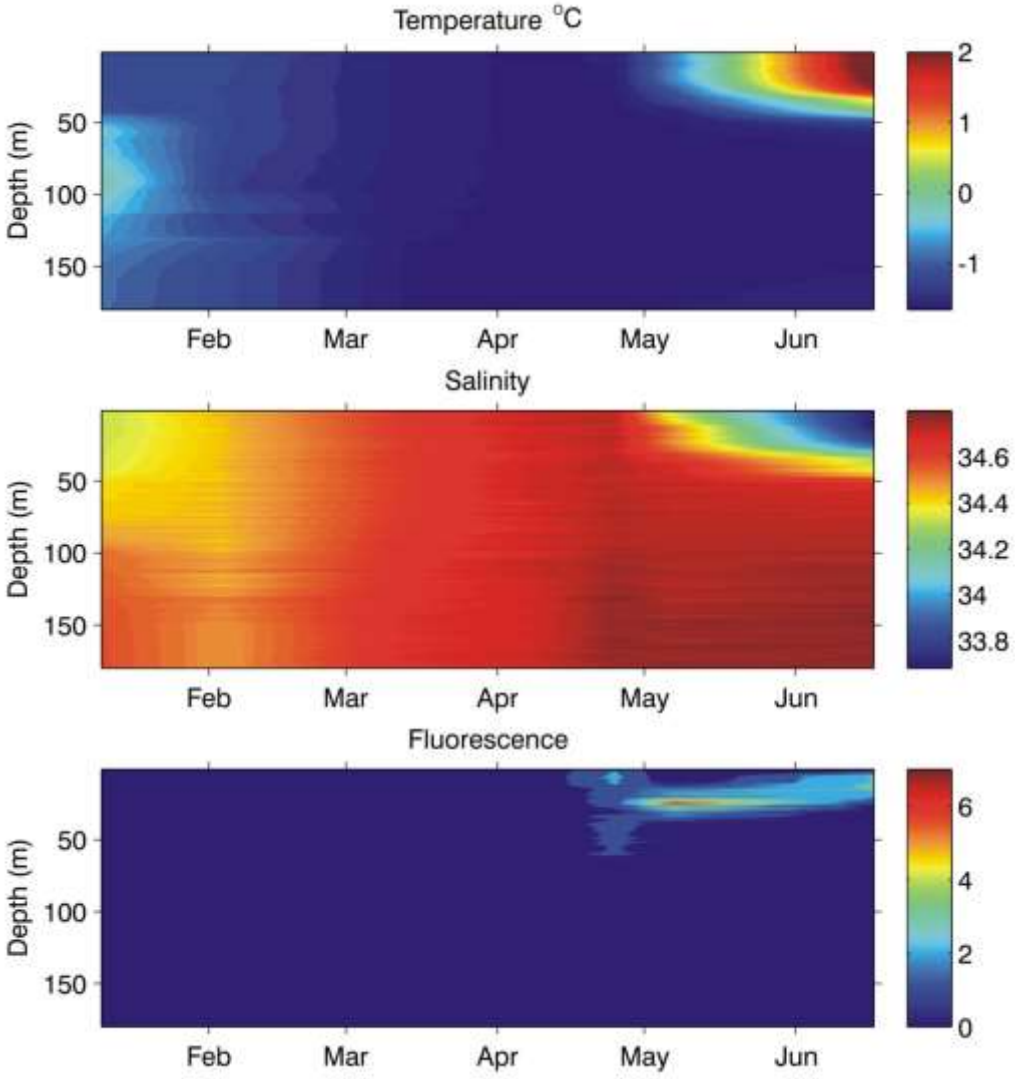

Figure 6: CTD data from Billefjorden, January to June. Top: Temperature profile. Middle: Salinity profile indicating local water masses close to Arctic water. Bottom: Chlorophyll *a* concentration (µg L^{-1}) obtained from *in situ* Chl *a* fluorescence measurements.

Community composition

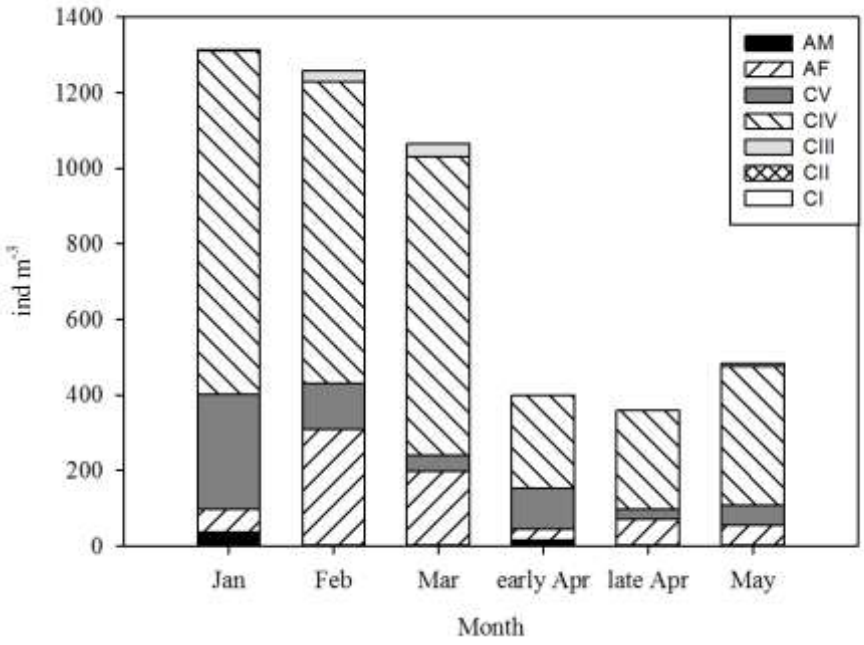

Figure 7: Community composition and densities of *Calanus glacialis* in Billefjorden (mean for the whole water column), winter-spring 2013. Young development stages (CI and CII) were not present.

Community samples showed that *Calanus glacialis* was the most abundant of the three *Calanus* species in Billefjorden, comprising 97% (97.2±1.9) of the *Calanus* community. The total abundance of *C. glacialis* decreased throughout the winter-spring transition, most notably from March to early April (Fig. 7). Males were almost absent except in January and a few found in early April. Females were always present, but were most abundant in February and March.

In situ egg production rates

In March and early April none of the females spawned (Table 2). In late April, 37% of the females spawned, producing 19.4 eggs female^{-1} on average, with a hatching success of 76.7%. In May, 70% of the females spawned with an average of 55.9 eggs female^{-1} and a hatching success of 96.2%.

Table 2: Egg production data for 24 hour incubation of females from Billefjorden.

	March	Early April	Late April	May
Total Females	30	30	30	30
Producing eggs	0	0	11	21
Total eggs	0	0	581	1678
Hatching (%)	-	-	79.69	96.19
egg/fem	0	0	19.4	55.9
SD	0	0	32.7	45.2
CV	-	-	168.8	80.9

Gonad stages

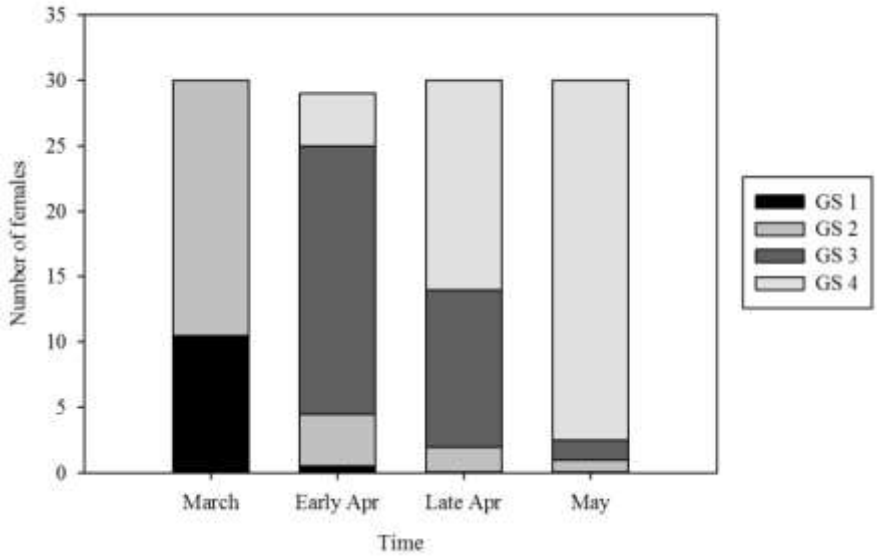

Figure 8: Gonad stage (GS) composition of females from Billefjorden. GS 1 is completely immature, GS 2 immature, GS3 almost mature and GS4 is ready to spawn (Niehoff and Hirche 1996)

The gonad status was determined for the females used in the 24 hour *in situ* egg production measurements (Figure 8). In early April the gonad status could not be determined on one of the females due to internal parasites, most likely a parasitic ciliate which infected the whole body cavity. In March 2/3 of the females had started the maturation process. In early April the first few mature females where found, and most were close to mature. By late April, no immature females were found, and about half of the females were mature. In May almost all the females were mature.

Capital or income breeder

The fatty acid composition of the *Thalassiosira nordenskioeldii* culture used for feeding the copepods in the laboratory, were dominated by 14:0, 16:0 and 16:1(n-7) (totally ~ 75%), and were low in PUFAs (maximum 13.3 (±1.1)% (Table 3). The Chl *a* concentration varied between 20.5 (±0.2) and 31.9 (±0.7) µg L^{-1}, averaging at 27.1 µg L^{-1} (Boissonnot 2013).

Table 3: Fatty acid composition of *Thalassiosira nordenskioeldii*

	1st week				2nd week				3rd week			
	%		µg/mL		%		µg/mL		%		µg/mL	
Fatty acid												
14:0	15.04	±0.38	0.0624	±0.001	16.79	±0.15	0.059	±0.0021	16.98	±0.35	0.046	±0.0008
15:0	0.28	±0.4	0.0011	±0.0016	0.21	±0.15	0.001	±0.0005	0.00	±0	0.000	±0
15:1(n-5)	1.62	±0.04	0.0067	±0.0001	1.79	±0.02	0.006	±0.0002	1.74	±0.03	0.005	±0.0001
16:0	26.99	±0.9	0.1120	±0.0009	33.55	±0.27	0.118	±0.0043	31.32	±0.72	0.084	±0.0012
16:1(n-7)	32.32	±0.55	0.1342	±0.004	25.84	±0.73	0.091	±0.0023	27.26	±1.37	0.073	±0.001
16:1(n-5)	0.84	±0.03	0.0035	±0	1.24	±0.02	0.004	±0.0002	1.50	±0.03	0.004	±0.0001
16:2(n-4)	0.35	±0.02	0.0014	±0	0.34	±0.03	0.001	±0.0001	0.00	±0	0.000	±0
16:3(n-4)	0.00	±0	0.0000	±0	0.00	±0	0.000	±0	0.00	±0	0.000	±0
16:4(n-1)	0.59	±0.19	0.0024	±0.0008	0.77	±0.55	0.003	±0.002	1.14	±0.08	0.003	±0.0001
18:0	2.29	±0.11	0.0095	±0.0002	3.59	±0.41	0.013	±0.0019	5.46	±1.02	0.015	±0.0033
18:1(n-9)	3.20	±0.02	0.0133	±0.0005	1.96	±0.16	0.007	±0.0009	2.24	±0.41	0.006	±0.0013
18:1(n-7)	1.00	±0.02	0.0042	±0.0003	1.06	±0.02	0.004	±0.0002	1.29	±0.08	0.003	±0.0001
18:2(n-6)	2.00	±0.01	0.0083	±0.0003	2.83	±0.02	0.010	±0.0005	2.37	±0.05	0.006	±0.0001
18:3(n-3)	0.09	±0.12	0.0004	±0.0005	0.00	±0	0.000	±0	0.00	±0	0.000	±0
18:4(n-3)	1.08	±0.06	0.0045	±0.0004	1.00	±0.04	0.004	±0.0002	1.12	±0.01	0.003	±0.0001
20:0	0.14	±0.2	0.0006	±0.0009	0.00	±0	0.000	±0	0.00	±0	0.000	±0
20:1(n-11)	0.00	±0	0.0000	±0	0.00	±0	0.000	±0	0.00	±0	0.000	±0
20:1(n-9)	0.00	±0	0.0000	±0	0.00	±0	0.000	±0	0.00	±0	0.000	±0
20:1(n-7)	0.00	±0	0.0000	±0	0.00	±0	0.000	±0	0.00	±0	0.000	±0
20:3(n-6)	1.93	±0.35	0.0081	±0.0018	0.00	±0	0.000	±0	0.00	±0	0.000	±0
20:4(n-3)	0.10	±0.13	0.0004	±0.0006	0.00	±0	0.000	±0	0.00	±0	0.000	±0
20:5(n-3)	5.09	±0.43	0.0212	±0.0027	4.78	±0.17	0.017	±0.001	3.98	±0.06	0.011	±0.0006
22:1(n-11)	1.99	±0.48	0.0084	±0.0024	1.35	±0.24	0.005	±0.0009	1.06	±1.06	0.003	±0.003
22:1(n-9)	0.12	±0.17	0.0005	±0.0008	0.00	±0	0.000	±0	0.00	±0	0.000	±0
22:1(n-7)	0.14	±0.2	0.0006	±0.0009	0.00	±0	0.000	±0	0.00	±0	0.000	±0
22:5(n-3)	0.00	±0	0.0000	±0	0.00	±0	0.000	±0	0.00	±0	0.000	±0
22:6(n-3)	2.13	±0.05	0.0088	±0.0006	2.07	±0.02	0.007	±0.0002	2.16	±0.56	0.006	±0.0017
24:1(n-9)	0.68	±0.07	0.0028	±0.0002	0.82	±0.04	0.003	±0	0.38	±0.38	0.001	±0.001
Sum												
Total	100.00	±0	0.4155	±0.0175	100.00	±0	0.353	±0.0146	100.00	±0	0.270	±0.01
MUFA	41.92	±0.44	0.1742	±0.0086	34.06	±0.46	0.120	±0.0036	35.47	±0.42	0.096	±0.0024
PUFA	13.33	±1.1	0.0556	±0.007	11.80	±0.41	0.042	±0.0029	10.77	±0.48	0.029	±0.0024
SFA	44.75	±1.44	0.1857	±0.0027	54.14	±0.14	0.191	±0.0083	53.76	±0.05	0.145	±0.0053

Egg production and hatching success

For the starved females, 17 (56.67%) and 14 (46.67%) females, from Billefjorden and Rijpfjorden produced eggs, respectively. The total number of eggs and over all hatching success were 585 eggs with a 62.22% hatching success for Billefjorden and 622 eggs with a 63.83% hatching success for Rijpfjorden, giving an average of 19.5 eggs per female from Billefjorden, and 20.73 eggs per female from Rijpfjorden. The average time between batches was 4.94 days (±2.49) for Billefjorden and 4.26 days (±2.45) for Rijpfjorden.

For the fed females 37 of 38 (97%) produced eggs. In total, 6029 eggs were produced, with a hatching success of 90.12%. This resulted in an average of 163 eggs per female. The average time between batches was 2.9 (±1.22) days. From the first day of egg production to termination of the experiment, the average number of eggs per female per day was 0.43 (±0.63) for starved females from Billefjorden and 0.58 (±1.03) for starved females from Rijpfjorden. EPR of the fed females were significantly higher (P<0.001, t-test of means) at 6.23(±3.67) for fed females. The fed females started to feed immediately when they were offered food, and based on faecal pellets production, they kept a steady average production of approximately 30 pellets female^{-1} day^{-1}.

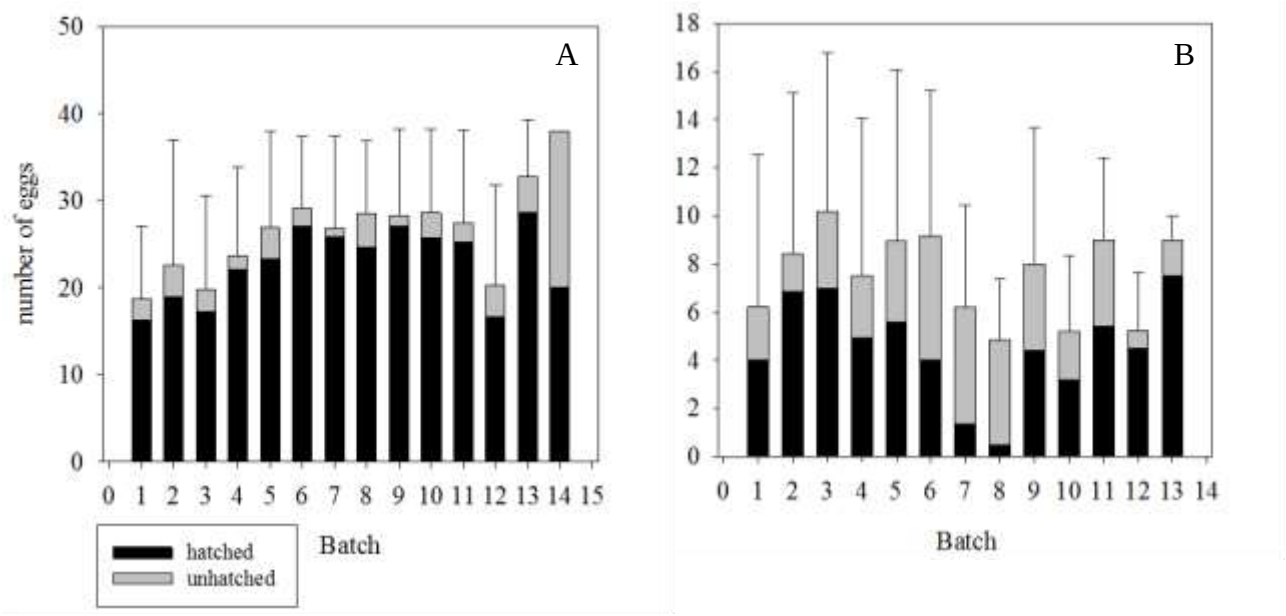

Figure 9: Average number of hatched and unhatched eggs per female per batch for fed females (A) and starved females (B). Calculations were done only for spawning females and performed on each batch, from the first to the las batch during the incubation experiments.

For the fed females, 50% were producing eggs after one week, and after two weeks all except three females produced eggs. The number of eggs was increasing throughout the experiment, from 18.7 (± 8.4) eggs ind^{-1} in the first batch, to 32.8(± 6.6) eggs ind^{-1} in batch 13 (Figure 9a). A scatter plot of the egg production data was made, and a linear regression fitted, showing a significant increase of egg production with time (R^2=0.49; *P*=0.005) (figure not shown). The hatching success did not change significantly over time for the fed females (*P* = 0.241, one-way ANOVA), and was on average 88.0 (±17.5)%.

For the starved females (Figure 9b) there were no significant changes in average number of eggs produced with time (p = 0.415, one-way ANOVA). The overall average was 7.5 (± 5.1) eggs $female^{-1}$ $batch^{-1}$. The hatching success decreased rather steep from batch 1 to a minimum in batch 8(13.3 ± 26.7%), for so to increase again (Figure 9b)

Detailed egg production data is presented in Tables A-1, A-2 and A-3 in appendix A

Mortality

The starved females from Billefjorden decreased linearly (R^2=0.97) with a slope of -0.311 females day^{-1}, or 2.18 females $week^{-1}$. At the end of the experiment, only four females were still alive. The starved females from Rijpfjorden also decreased linearly (R^2=0.96) with a slope of -0.352 females day^{-1}, or 2.46 females $week^{-1}$. Only three females were still alive at the end of the experiment. Only one of the feeding females died from natural causes before May 1st. By the end of the experiment on May 5th, four more females had died. Females taken out for FA analysis or that obviously died from handling is not included in calculations of the mortality rate. The decline of living females throughout the experiment is shown in appendix B.

Lipid consumption and egg production

The lipid level decreased throughout the experiment for both starved and fed females. The starved females that produced eggs had 0.241 (± 0.079) mg ind^{-1} at the beginning of the experiment. Based on pictures taken before and after the first egg batch, 41.5(±12.9)% and 56.2 (±15.9)% of the TL was used for maturation. Egg production continued to 77.9 (± 25.4)% of the lipids were used, and at the time of death, 82.5 (± 14.8)% of the TL had been consumed.At the time of death, the average value of TL was 0.060 (±0.058)mg ind^{-1} for the females from Billefjorden and 0.09 (±0.081)mg ind^{-1} for females from Rijpfjorden. The average value of the condition factor was 0.26 (±0.22) for females from Billefjorden and 0.38 (± 0.24) for the females from Rijpfjorden.

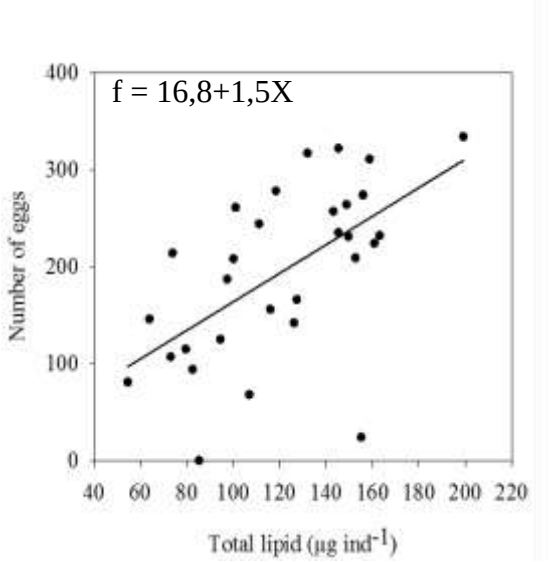

Figure 10: Total number of eggs against total lipid content at start of the experiment for fed females

The fed females started out at 0.121 (± 0.035) mg ind^{-1}. Based on estimates of TL for first picture taken after egg production started, 52.4 (± 22.5)% of the lipids had been used for maturation and first egg production. At the end of the experiments 83.6 (± 13.9)% had been consumed. The lipid levels of the fed females at the end of the experiment were variable, with an average of 0.027 (± 0.024)mg ind^{-1} and a CF of 0.12 (±0.16), implying that internal lipids were utilized even when the females were actively feeding.

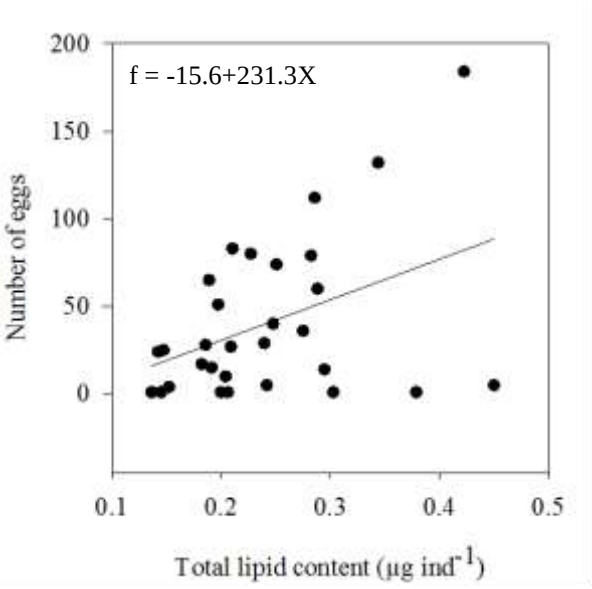

Figure 11: Total number of eggs against total lipid content at start of the experiment for starved females

For the fed females, total number of eggs increased when the female had higher TL prior to egg production, as shown in Figure10. The increase in EPR with TL was significant (Figure 10; *P*=0.0007) but the fit of the linear regression was not very high (R^2=0.34), implying that TL have some effect on the egg production, but is not the main factor explaining differences in EPR.

Also for the starved females the total number of eggs showed a significant increase with increasing lipid content prior to maturation (P=0.02), but the fit of the regression line (R^2=0.17) was even poorer than for the fed females (Figure 11).

Investment in offspring

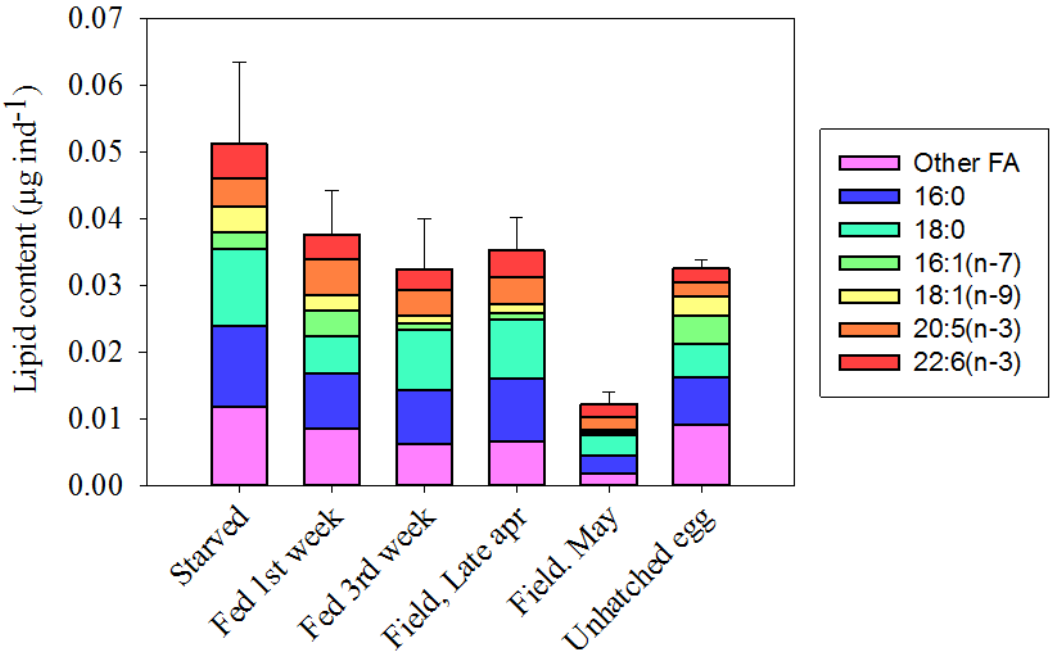

Figure 12: Total lipid content and fatty acid composition of nauplii and unhatched eggs of *Calanus glacialis*.

The TL of nauplii and unhatched eggs was estimated from the analysis of FA composition, and the results are presented in Figure 12. The highest lipid levels were found in nauplii produced by starved females (0.052 ±0.012 µg ind^{-1}), and the lowest in nauplii from 24 hour incubation in May (0.012 ±0.001 µg ind^{-1}). The nauplii from fed females in the laboratory for 1 and 3 weeks had a total lipid content of 0.038 ±0.007 µg ind^{-1} and 0.034 ±0.009 µg ind^{-1} respectively, the nauplii from late April had 0.036 ±0.005 µg ind^{-1}, and the unhatched eggs 0.033 ±0.001 µg ind^{-1}, making them very even in total lipid content and placing them between the starved and May nauplii. Significant differences in TL were only found between the nauplii from field in May and the other groups (P<0.05, one-way ANOVA).

Four fatty acids were abundant in all nauplii and unhatched eggs: 16:0, 18:0, 20:5(n-3) and 22:6(n-3), but also 16:1(n-7) and 18:1(n-9) showed relatively high amounts (Figure12). All

samples showed the same pattern, but the absolute amount varied between the different samples.

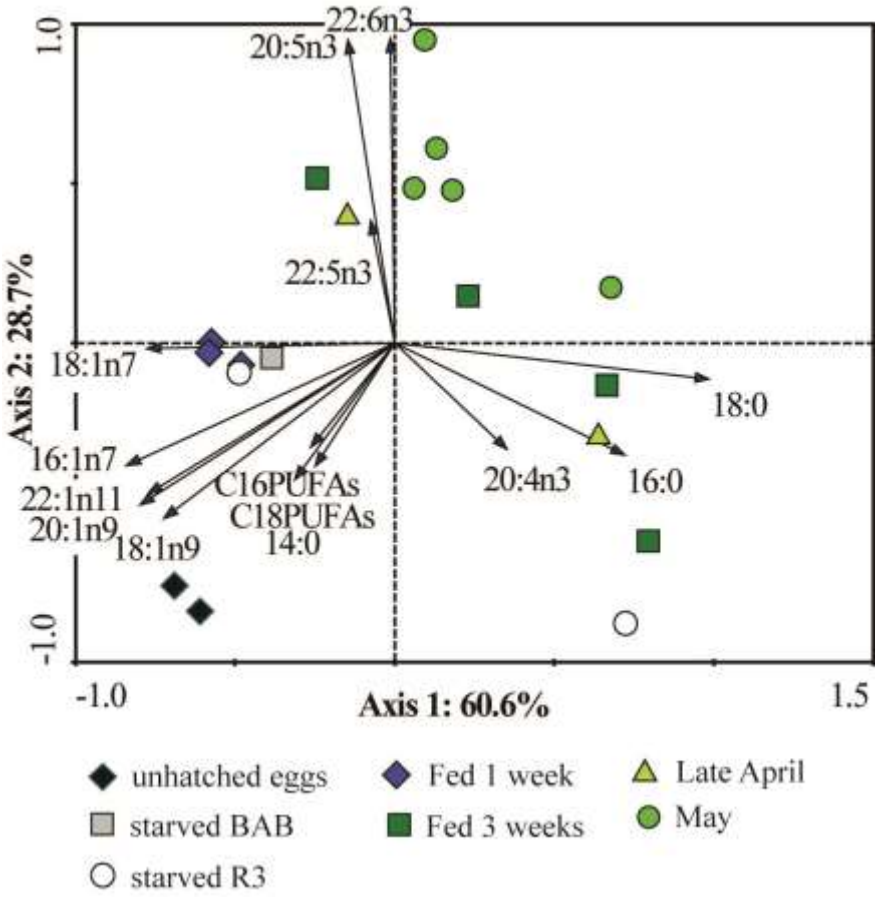

Figure 13: Principal component Analysis (PCA) plot of the relative fatty acid composition of nauplii from the long-term laboratory incubations and the 24 hrs egg production incubation on females in field. The 2-dimensional plot show >90% of the total fatty acid variability and there are three main groups: The unhatched eggs down to the left with low proportions of 20:5n3 and 22:6n3 and high of for instance 16:1n7, the nauplii from starved females and from fed females for only 1 week to the left in the middle with intermedium levels of 20:5n3 and 22:6n3 and 16:1n7, and nauplii from fed females for three weeks and from *in situ* EPR measurements to the right with medium to high proportions of 16:0 and 18:0, and 20:5n3 and 22:6n3.

The 2-dimensional PCA plot of the relative fatty acid composition of the nauplii from the lab and field and the unhatched eggs (figure 13)displayed almost 90 % of the total fatty acid variability. The most important fatty acids for separation on axis 1 were 16:0 and 18:0, and on axis 2 the PUFAs 20:5(n-3) and 22:6(n-3). The nauplii from field in May and from females fed for three weeks in the lab are all from well fed females and are grouped together in an area reflecting relatively high levels of the main PUFAs 20:5n3 and 22:5n3, and the saturated fatty acids 16:0 and 18:0. The unhatched eggs are grouped together in the opposite direction, reflecting relatively low levels of the PUFAs and and higher levels of MUFAs, particularly

16:1n7. The nauplii from starved females show some variation along axis 1, but not so much on Axis two. They are all low in relative amounts of the PUFAs 20:5n3 and 22:6n3. The nauplii from females fed for 1 week in the lab appear similar to the nauplii from starved females.

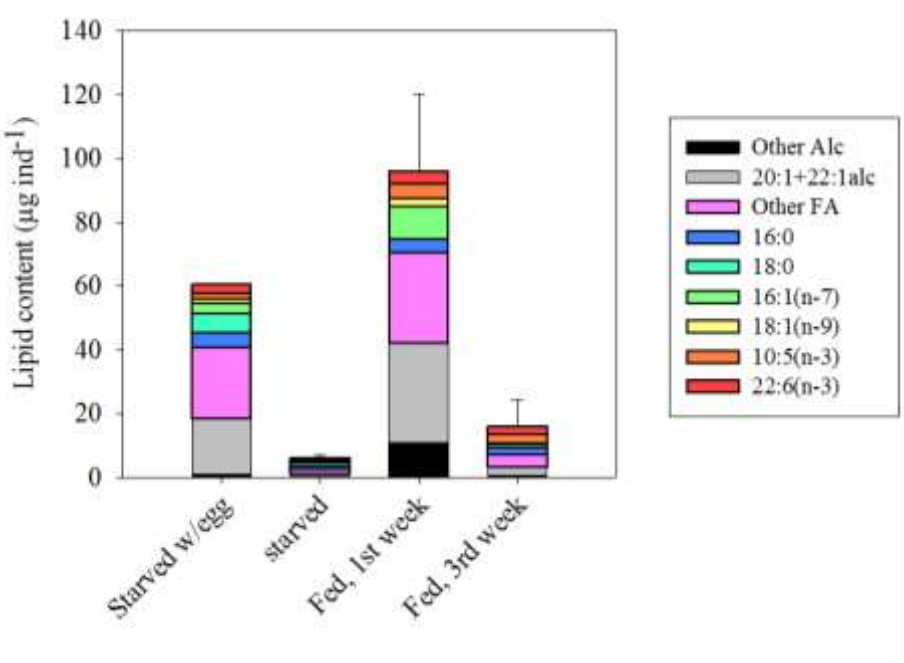

Figure 14: Fatty acid composition of fed and starved females at the end of the laboratory incubation experiments.

The FA composition of females from the lab at the end of the experiments indicates that the starved females stopped producing eggs when the lipids were depleted and that the stored lipids were utilized even when the female had access to food (Figure 14). The females that stopped producing eggs reached very low lipid levels(6.0 ± 1.1 µg ind^{-1}) compared to the female that still produced eggs at the end of the experiment (60.3 µg ind^{-1}). Of all the females at the end of the experiment, the lipid level is highest in females still producing eggs, except for the PUFAs 20:5(n-3) and 22:6(n-3) where the fed females appear to have a higher absolute content. Except from one female from Rijpfjorden, all the starved females had stopped producing eggs when the experiment was terminated. The lipid content of the fed females decreased significantly from the first week to the 3rd ($P<0.05$) indicating that the internal lipids were utilized despite food being abundant.

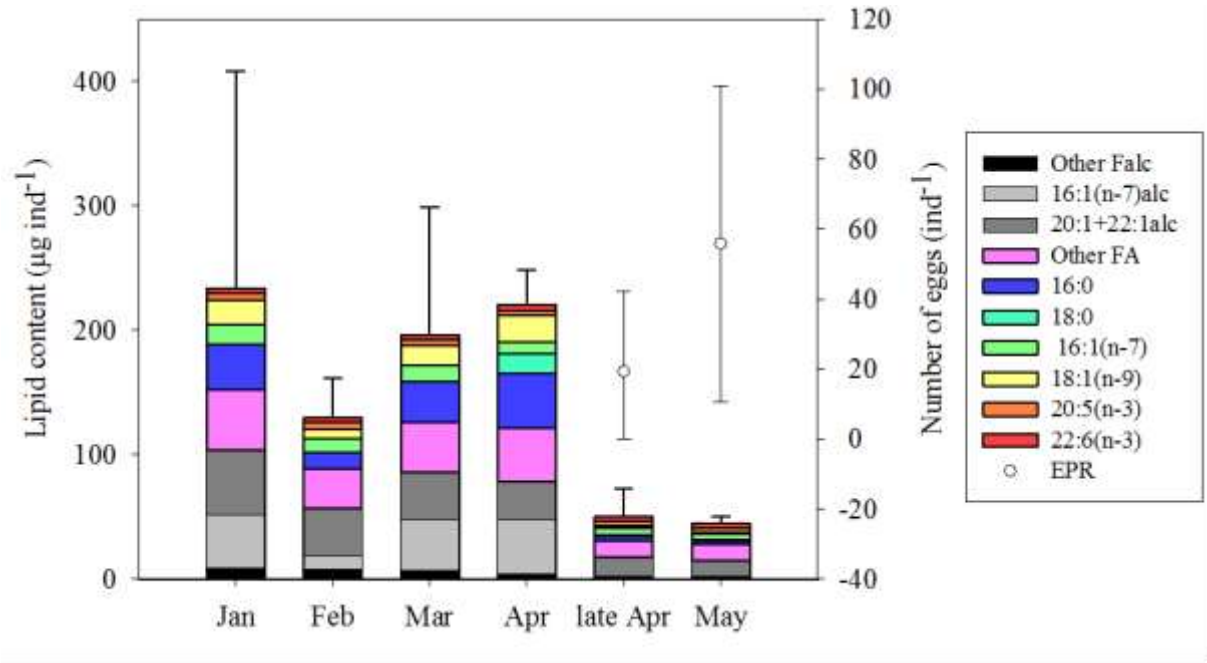

Figure 15: Total lipid content and the fatty acid composition and the egg production rates throughout the winter/spring transition for females from Billefjorden. In March, no egg production. *In situ* egg production rates not measured in January and February since no females were mature.

The total lipid content and relative fatty acid and fatty alcohol composition, of females from field throughout the winter and spring are shown in Figure 15. The fatty acid composition was stable from late April to May, but varied earlier in the year. The total lipid content was stable until April, but dropped significantly from early to late April. The main part of thei s drop was a decrease in fatty alcohols, and particularly 16:1(n-7)alc. The actual amount of the PUFAs 20:5(n-3) and 22:6(n-3) did not change (both $p>0.05$, t-test of means), but the relative amount increased significantly (both $p<0.001$, t-test of means) from April to late April. Both actual and relative amount of 18:1(n-9) increased until April and dropped to late April. The actual amount of 16:1(n-7) was stable until March before it started dropping steadily till May, but increased in relative amounts. 18:0 was almost absent except from in April. 16:0 decreased both in actual and relative amounts from January to February, but then increased significantly to April (P <0.05, t-test of means), before dropping to late April (P <0.001, t-test of means).

Males and time of death

The avearage TL decreased by 0.0074 ± 0.0016 mg day^{-1} throughout the experiment. The data is presented in Figure 16 where 0 is TOD, and the x-axis is days prior to death. There was a significant decrease (P <0.01), but the fit of the linear regression was moderate (R^2=0.54.).

Individual plots were made of the four males that lived longer than two weeks (minimum five points of estimated TL), and all of these fit very well ($0.70 < R^2 < 0.85$, figure not shown), indicating that the reduction in stored lipids were linearly, but the reduction rate varied between individuals. One male was not included in the calculations due to punctured lipid sac, and one estimate of TL was removed due to clearly too high estimate.

Figure 17 show the death rate of the males throughout the experiment. The mortality is highest during the first two weeks of the experiment when it peaked at -4 ind day^{-1}. After 11 days, the 28th of February, only 4 of 34 males were still alive. Average life span from start of incubation on 15th of February was 9.5±6.0 days.

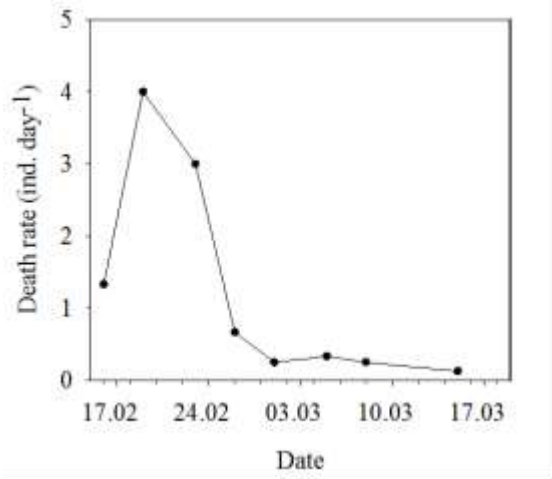

Figure 16: Average total lipid content throughout the experiment

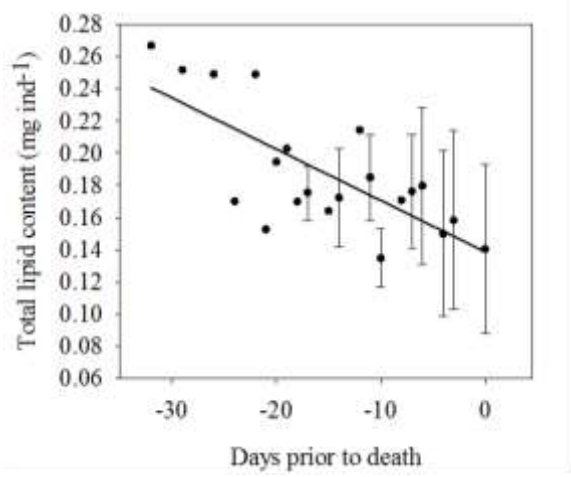

Figure 17: Average death rate throughout the experiment

Discussion

This is the first study to investigate the gonad maturation and egg production of *Calanus glacialis* in seasonal ice covered fjords in Svalbard, and the first study to investigate the correlation between nutritious status and time of death for *Calanus* spp. males in the Arctic. The main fjord studied was Billefjorden, a seasonal ice covered sill fjord with restricted water exchange, which makes it an ideal fjord to study population dynamics. In closed fjord systems special local population adaptations may evolve. However, parallel studies of *C. glacialis* from another seasonal ice covered fjord in this study, Rijpfjorden, without a sill, largely agreed with the findings from Billefjorden suggesting Billefjorden to be a representative location to study important reproductive processes for *C. glacialis* in general.

Calanus glacialis dominate the *Calanus* community in Billefjorden (this study; Arnkværn et al. 2005). Its high abundance in this sill fjord suggests it to successfully reproduce here despite this fjords rather harsh environment with subzero temperatures and long lasting sea ice cover with sea ice break-up normally occurring in June.

Capital or income breeder

The total lipid content of both the fed and the starved females decreased during the incubation experiments. For both fed and starved females, around half of the lipids were used for maturation which corresponds to previous findings for *Calanus glacialis* (Hirche and Kattner 1993) and for *Calanus finmarchicus* (Jónasdóttir 1999). When the incubation experiment was terminated, the lipid content of the fed females was reduced to 84% of the initial lipid content, indicating that the stored lipids were close to depletion despite access to fresh food. This is further supported by the positive relationship between lipid content prior to egg production and total amount of eggs produced (Figures 10 and 11) , indicating that the total egg production is a function of both female nutritious status and the food access. Despite that fed females were feeding well (the incubated females produced on average 30 faecal pellets per day), the fed females did not reach maximum EPR of around 80-100 eggs per female (Melle and Skjoldal 1998). This could be due to stress of being kept in the laboratory in small chambers or that the incubation was ended before maximum EPR were reached. Another cause could be poor algal food quality, since the diatoms they were given were rather low in the PUFAs 20:5(n-3) and 22:6(n-3) that are essential omega-3 fatty acids for copepod egg production, egg hatching and growth (Pond et al. 1996; Jónasdóttir et al. 2005; 2009)

Nevertheless, this study shows that *Calanus glacialis* is fully capable of reproducing without food. However, females produce one order of magnitude more eggs when food is available. These findings are also consistent with the results from Hirche and Kattner (1993) who also found a clear potential of capital breeding, but that daily egg production increased six-fold when algal food was available. Both EPR and hatching success were higher for fed vs starved females in this study (Figure 9). Close to 100% of the fed females spawned compared to only half of the starved females. Later sampling in the year (March vs. January) of females for the feeding experiment versus the starved experiment may have led to a selection of females with higher fitness/ condition being picked for the fed incubation experiment in March since females in poor condition by then may have been lost from the population. However, the algal food concentrations in sea ice and water were still negligible in March(Figure 6), females were still not fully matured (Figure 8) and the abundance of females was still high (Figure 7). A significant decline in female abundance (and overall population abundances) were first seen in April, while a strong decline in female nutritious status (lipid content) was first seen in late April (Figures 7 and 15) The number of eggs per batch for starved females produced varied between 1 and 30 without any clear trend in increase or decrease in numbers of eggs per batch with time. The fed females increased their egg production with time (Figure 9), starting at an average of 18.7 (± 8.4) and ending at 32.8 (± 6.6) (ignoring the last batch where data is based on only one female). This suggests that females need some time to fully mature and reach maximum egg production after onset of favourable food concentrations. This is also consistent with observations from the parallel 24 hour *in situ* egg production incubations with females collected from field (Table 2, Figure 15). In late April, the first eggs started to appear but the EPR were low even if the algal biomass was high in the upper 5 m (2-3 µg Chl *a* L^{-1}), probably due to sea ice melting and ice algae being released into the water column (Gabrielsen *et al*. unpublished). First in May when high concentrations of algal food had been available for a while, females started to reach max EPR of around 80 eggs $female^{-1}$ day^{-1} (Melle and Skjoldal 1998).

Eggs from females in the *in situ* egg production measurements, incubated in water from the sampling site had high hatching success (Table 2), comparable or slightly higher than the hatching success observed for eggs from fed females in the laboratory. For the fed and starved females in the long-term incuabtions experiments In contrast, a strong decline in egg hatching success were observed for the starved females as the stored fat resources got depleted,

reaching as low as 13% (Fig. 9). However, the hatching success increased again towards the end of the starved incubation experiment reaching > 80% hatching success for the last two batches comparable high as for eggs from fed females. Only five females produce more than eight batches, so the increase could be caused by chance, but when looking at the individuals, they also showed this pattern of decrease and increase individually. The routines of handling the females in the lab were the same during the whole experiment, so it seems unlikely that the change in hatching success were caused by difference in the handling.

When food is scarce, the females seem to invest more lipids per egg and fewer eggs as opposed to more eggs, but less lipids per egg when food is abundant (see below). The Chl *a* concentration in the surface water of Billefjorden was quite high in May (Figure 6), the phytoplankton bloom had started, and it can be assumed that the females thus had access to food of high quality in terms of high PUFA content (Søreide et al. 2010). The females fed in the laboratory were offered a monoculture of the diatom *Thalassiosira nordenskioeldii*. These fed females invested less lipids per egg than the starved females, but still significantly more lipids per egg than the females in the field in May did. The females from Billefjorden in late April that produced eggs invested about the same amount as the females fed in the laboratory. When these females were collected, the Chl *a* concentration in the surface water was just starting to increase, indicating that feeding conditions had just started to be favourable. The starved females invested the highest lipid amount per egg, but had the lowest EPR, while females captured in May had the highest EPR, but invested the least amount of lipid per egg (Figure 12). This suggest a life strategy not previously described for *Calanus glacialis* i.e. when food is absent or low females invest in fewer but more lipid-rich eggs most likely to increase the likelihood for this offspring to survive longer and thus increases the chance to be present when more favourable food conditions finally appear. So not only the timing of spawning and number of eggs, but also the nutritious status of the individual egg is important strategic moves by the females to secure high reproductive success (e.g. Varpe et al. 2007)

Different diatom species have been used as food in *Calanus* egg production studies, and the EPR have varied. Hirche (1989) used *Thalassiosira antarctica* Comber 1896, and got an EPR of 41.8 ± 38.1 eggs female^{-1} day^{-1}. Tourangeau and Runge (1991) used *Thalassiosira weisflogii* (Grunow) Fryxell and Hasle 1977, and got an EPR of 18.6 eggs female^{-1} day^{-1}. Both of which had a higher EPR than the present study (6.2 ±3.7). The relative amount of PUFAs in the algal food offered to females incubated in the laboratory in this study was rather

low (mean ~12%, Table 3), which may explain the relatively low EPR for the surplus fed females in the laboratory versus females in field (e.g. Koski et al. 2012).

Relative and absolute fatty acid composition

A series of plots and regressions were conducted to find some relationships between the total lipid content and the egg production and hatching success (Appendix E) but no significant relationships were found, suggesting that not only the quantity but also the quality or more precise the fatty acid and fatty alcohol composition play an important role.

The FA composition of the females change throughout the winter spring transition (Figure 15), and the changes seems to be related to where in the reproductive cycle they are. Maturation of the gonads appear to rely mostly on fatty acids and fatty alcohols the females are capable of synthesizing *de novo*. Maturation is very costly in terms of lipids, both as seen in this study, where around half of the stored lipids of the incubated females were used prior to egg production, and as noted in other studies (Hirche and Kattner 1993; Jónasdóttir 1999). From January to March the relative and absolute FA composition was rather stable. In early April, however, when females in the field were close to reach final maturation a distinct change in FA composition appeared with a significant increase in absolute values of 16:0, 18:1(n-9) and particularly 18:0, that was less than 0.1 µg ind^{-1} prior to April, increasing to >15 µg ind^{-1} in early April for so to decrease again to less than 0.1 µg ind^{-1} in late April and May. This increase is coupled with a decrease in Falc (Appendix D, Table D-1), indicating that these are cleaved and mobilized for use. Both relative and actual amount of the mentioned FAs decrease again to late April when the females have reached full maturty, indicating that these FA may be particularly are important for the final maturation stage.

This can indicate that in the final maturation process involve some internal timing of lipid catabolism of for instance the long chained FA and alcohols 20:1n9 and 22:1n11, as well as the Falc 16:1n7 (Sargent and Henderson 1986). The variability in the above mention FA and Falc were exceptionally high in April when females were about to fully mature. This high variability at this particularly time of the year, and not in the other months, may be due to big changes in FA and Falc composition between immature and females in their final maturation process since females in different maturation stages was found in April. However, further studies of FA and Falc composition of females in different gonad maturity stage are needed to draw conclusions.

A drop in total lipid content was seen from beginning to late April (Figure 12). Despite this drop though, some FAs do not drop as much, but appear to be kept. The PUFAs 20:5(n-3) and 22:6(n-3) do not decrease much in absolute values and thus increase in relative values with the drop in total lipids from early to late April. These two PUFAs have already been found to be very important for reproduction and egg hatching success (Koski et al. 2012). These PUFAs are essential and need to be kept on a certain level as they are principal membrane constituents (Kattner and Hagen 2009) and thus can be a limiting factor for reproduction.

Fatty acid composition of the nauplii

Unhatched eggs had the lowest relative PUFA content, while naturally fed females (May) produced nauplii with the highest relative content of 20:5n3 and 22:6n3 (Fig. 13). In absolute values, however, the amount of 20:5n3 and 22:6n3 did not differ much among the nauplii, but the absolute values of the SAFAs 16:0 and 18:0 and partly the MUFA 16:1n7 did (Appendix D). This suggests that the eggs need a certain minimum amount of the essential omega-3 fatty acids 20:5n3 and 22:6n3 to make a viable egg. The FA composition rather than the total lipid content is therefore determinable for the egg hatching success, and a minimum amount of the 20:5(n-3) and 22:6(n-3) seem to be required Since the nauplii can't eat before reaching stage NIII (Søreide et al. 2010, Daase et al. 2011), the newly hatched nauplii are assumed to be representative of the FAs transferred from the female to the egg. All the samples of nauplii and the unhatched eggs show similar patterns of FA composition with relative high amounts of 16:0, 18:0, 20:5(n-3) and 22:6(n-3) (Figure 12). We also here see the same pattern as in the TL of the egg that more is invested in each egg when the availability and quality of the food decreases. The nauplii from the 24 hour incubation in May are clearly among the lowest measurements in all FAs, but are among the highest in relative amount of the four most abundant FA, indicating the importance of these FAs for successful reproduction. The PCA (Figure 13) further supports the importance of these FAs. The nauplii vary more in the relative amount of 16:0 and 18:0 than of the PUFAs, 20:5(n-3) and 22:6(n-3), but the relative amount of the PUFAs are what separated the nauplii from the unhatched eggs. This indicates that the hatching success is more sensitive to variations in PUFAs than in SAFAs
This corresponds with the findings of Koski et al. (2012) who also found 20:5(n-3) and 22:6(n-3) to be the most important FA for hatching success, but in *Calanus finmarchicus*. Koski et al. (2012) stated that the FA composition of the egg was not altered by the female,

but that it reflected the FA composition of the diet of the female. This is not in accordance with this study, where the algae were relatively low in some lipids the fed nauplii had relatively high content of, most notably 18:0.

Males at TOD

Total lipid content at TOD is normally distributed (appendix E), thus the data support the hypothesis that males die when the stored resources reach a certain minimum, which in this study was found to be (0.14 ±0.05 mg ind^{-1}). This is a higher lipid content than observed in females at TOD.

The lipid content of the males decrease linearly towards TOD (Fig. 16), but the average life span from incubation was only 9.5 (±6.0) days. The death rate was highest during the first two weeks (Fig. 17), after which most of the males were dead. The time of death in the lab is consistent with the community samples from Billefjorden, where the males are absent or occur in very low abundances in February.

It is previously suggested that the *Calanus glacialis* males die due to lipid storages reaching a certain minimum value (Kosobokova 1999). The results of the present study support this suggestion, but other potential factors can't be ruled out. A study on males should be conducted earlier in the winter when they are more abundant (Kosobokova 1999), and with a more thorough investigation of the potential impact of other factor such as FA composition, protein and external cues on TOD before a conclusion can be drawn.

Concluding remarks

Calanus glacialis was found to be capable of maturing and producing viable offspring without access to food, but the reproduction output was an order of magnitude lower compared to actively feeding females. However, fed females also used internal lipid storages to produce eggs, and almost depleted them, indicating that C. glacialis has a mixed strategy being dependent on both internal stoarges and access to food of high quality (i.e. high PUFA content) to successfully reproduce. . Interestingly, the amount of lipids invested per individual egg also varied with food availability,suggesting a reproduction strategy not previously described for *C. glacialis*: when food is absent or low, females invest in fewer but more lipid-rich eggs and when food is favourable females produce many, but lipid-poor eggs. This strategy increase the likelihood for offspring to survive until more favourable food

conditions appear, and to maximize offspring production when the food conditions are favourable.
Thus the hypothesis of *Calanus glacialis* being a capital breeder is neither rejected nor confirmed, as the females are able to alter their reproductive strategy according to the food availability.

Four fatty acids were found to be especially abundant in all nauplii: 16:0, 18:0, 20:5(n-3) and 22:6(n-3), and the relative content of the PUFAs separated the unhatched eggs from the nauplii. From the fatty acid composition of the females from field, it appears that different fatty acids are used for maturation of the gonads than what is important for the nauplii, and so the females might be able to supply eggs with fatty acids not found in the food. A positive relationship was found between total lipid content and the amount of eggs produced, but it explained very little of the variation in egg production. The highest hatching success was found both for eggs/nauplii of both high and low lipid content.
Thus the hypothesis of fatty acid composition being more important than total lipid content is kept.

The lipid content of the males decrease steadily until the time of death, and is normally distributed when the males die. However, the lipid content at time of death is relatively high. Thus the hypothesis of male dying when the lipid content reach a minimum threshold value is not rejected or confirmed. More studies, including a closer look at males fatty acid composition, are needed before a final conclusion can be drawn.

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Appendix A: Egg production and Hatching success

Table A-1: Number of hatched (H) and unhatched (U) eggs per batch, with sum of H, U and total number of eggs and overall hatching success for specimen and batch of starved females from Billefjorden

	1			2			3			4			5			6		
Specimen	date	H	U	date	H	U	date	H	U	date	H	U	date	H	U	date	H	U
B1																		
B2																		
B3	28.feb	1	0	03.mar	14	0	10.mar	15	0	17.mar	10	0						
B4	27.feb	4	12	03.mar	4	0	10.mar	2	2	15.mar	3	0	22.mar	8	1			
B5																		
B6	27.feb	4	5	03.mar	11	3	08.mar	13	0	11.mar	7	2	14.mar	8	7			
B7	20.mar	1	2	28.mar	1	3	08.apr	1	7	14.apr	3	3	22.apr	0	1	29.apr	1	0
B8																		
B9	28.feb	13	0	11.mar	3	0	18.mar	12	0									
B10																		
B11	01.mar	0	1															
B12	20.mar	1	2	27.mar	5	0	01.apr	7	0									
B13	01.mar	3	1	07.mar	1	3	11.mar	9	0	28.mar	0	3	03.apr	0	7			
B14	27.feb	12	14	03.mar	11	0	08.mar	4	0	15.mar	0	2	20.mar	1	0	25.mar	0	5
B15	13.mar	17	0	21.mar	9	8	26.mar	0	17	29.mar	0	3	01.apr	0	10	06.apr	0	11
B16																		
B17	30.mar	1	0															
B18	27.feb	0	1	02.mar	6	0	07.mar	8	1	11.mar	7	4						
B19																		
B20																		
B21																		
B22	28.feb	4	0	07.mar	1	0												
B23																		
B24																		
B25	01.mar	3	4	07.mar	6	1	11.mar	2	3	14.mar	0	4	22.apr	0	6	24.apr	0	1
B26	01.mar	7	1	04.mar	1	0	06.mar	4	0	08.mar	4	0	12.mar	1	3	15.mar	0	4
B27	04.mar	7	1	06.mar	13	1	10.mar	6	0	14.mar	8	2	19.mar	4	1	23.mar	7	1
B28	011.mar	4	1	14.mar	0	1	17.mar	2	3	23.mar	0	1	27.mar	1	1			
B29																		
B30																		
Sum		82	44		86	20		85	33		42	24		23	37		8	22
Total		126			106			118			66			60			30	
Hatch. Succ. (%)		65.08			81.13			72.03			63.64			38.33			26.67	

Specimen	7			8			9			10			11			12						
	date	H	U	date	H	U	date	H	U	date	H	U	date	H	U	date	H	U	Sum H	Sum U	Sum egg	Hatch.s.
B1																			0	0	0	
B2																			0	0	0	
B3																			40	0	40	100.00
B4																			21	15	36	58.33
B5																			0	0	0	
B6																			43	17	60	71.67
B7																			7	16	23	30.43
B8																			0	0	0	
B9																			28	0	28	100.00
B10																			0	0	0	
B11																			0	1	1	0.00
B12																			13	2	15	86.67
B13																			13	14	27	48.15
B14	31.mar	0	2																28	23	51	54.90
B15	16.apr	0	5																26	54	80	32.50
B16																			0	0	0	
B17																			1	0	1	100.00
B18																			21	6	27	77.78
B19																			0	0	0	
B20																			0	0	0	
B21																			0	0	0	
B22																			5	0	5	100.00
B23																			0	0	0	
B24																			0	0	0	
B25	26.apr	0	7	30.mar	0	8	03.apr	0	7	06.apr	4	1	08.apr	10	3	14.apr	6	2	31	47	78	39.74
B26																			17	8	25	68.00
B27	27.mar	1	2	30.mar	2	1	04.apr	5	0	06.apr	1	1	12.apr	5	1	19.apr	4	0	63	11	74	85.14
B28																			7	7	14	50.00
B29																			0	0	0	
B30																			0	0	0	
Sum		1	16		2	9		5	7		5	2		15	4		10	2				
Total		17			11			12			7			19			12					
Hatch.succ. (%)		5.88			18.18			41.67			71.43			78.95			83.33					

Table A-2: Number of hatched (H) and unhatched (U) eggs per batch, with sum of H, U and total number of eggs and overall hatching success for both specimen and batch of starved females from Rijpfjorden

	1			2			3			4			5			6			7		
Specimen	date	H	U	date	H	U	date	H	U	date	H	U	date	H	U	date	H	U	date	H	U
R1																					
R2																					
R3	07.mar	5	1	17.mar	0	4	21.mar	6	1												
R4																					
R5																					
R6																					
R7	18.mar	0	1																		
R8																					
R9	20.mar	1	13	24.mar	8	4	29.mar	1	0	01.apr	6	3	03.apr	1	0	08.apr	12	3	14.apr	1	11
R10	17.mar	11	1	20.mar	19	2	24.mar	11	0	28.mar	8	2	01.apr	10	7	06.apr	0	11	19.apr	0	1
R11	11.mar	1	0	23.mar	1	2															
R12																					
R13																					
R14																					
R15	28.feb	9	6	03.mar	16	3	06.mar	1	23	08.mar	3	0	11.mar	23	0	15.mar	10	3	20.mar	10	4
R16	15.mar	1	0	17.mar	12	0	20.mar	18	5	24.mar	10	0	27.mar	11	0	30.mar	10	0	03.apr	0	8
R17	18.mar	1	0	20.mar	0	1															
R18	20.mar	1	0																		
R19	11.mar	10	0	15.mar	21	1	19.mar	21	2	22.mar	15	15	25.mar	16	6	28.mar	4	18	02.apr	0	4
R20																					
R21																					
R22	08.apr	1	0																		
R23																					
R24	16.mar	0	1																		
R25																					
R26	04.mar	1	0	11.mar	2	0	15.mar	4	3												
R27																					
R28	09.apr	0	1																		
R29																					
R30																					
sum		42	24		79	17		62	34		42	20		61	13		36	35		11	28
Total		66			96			96			62			74			71			39	
Hatch.s.		63.64			82.29			64.58			67.74			82.43			50.70			28.21	

Table A-2 continued

	8			9			10			11			12			13					sum	Hatch.s.
Specimen	date	H	U	date	H	U	date	H	U	date	H	U	date	H	U	date	H	U	sum H	sum U	egg	(%)
R1																			0	0	0	
R2																			0	0	0	
R3																			11	6	17	64.71
R4																			0	0	0	
R5																			0	0	0	
R6																			0	0	0	
R7																			0	1	1	0.00
R8																			0	0	0	
R9	19.apr	1	0																31	34	65	47.69
R10																			59	24	83	71.08
R11																			2	2	4	50.00
R12																			0	0	0	
R13																			0	0	0	
R14																			0	0	0	
R15	23.mar	0	5	25.mar	1	2	27.mar	0	3	01.apr	2	6	02.apr	2	0	07.apr	5	3	82	58	140	58.57
R16	06.apr	0	8	07.apr	13	6	10.apr	0	5	15.apr	0	5							75	37	112	66.96
R17																			1	1	2	50.00
R18																			1	0	1	100.00
R19	04.apr	0	4	10.apr	3	3	15.apr	11	0	21.apr	10	3	24.apr	6	1	05.mai	10	0	127	57	184	69.02
R20																			0	0	0	
R21																			0	0	0	
R22																			1	0	1	100.00
R23																			0	0	0	
R24																			0	1	1	0.00
R25																			0	0	0	
R26																			7	3	10	70.00
R27																			0	0	0	
R28																			0	1	1	0.00
R29																			0	0	0	
R30																			0	0	0	
sum		1	17		17	11		11	8		12	14		8	1		15	3				
Total		18			28			19			26			9			18					
hatch.s.(%)		5.56			60.71			57.89			46.15			88.89			83.33					

Table A-3: Number of hatched (H) and unhatched (U) eggs per batch, with sum of H, U and total number of eggs and overall hatching success for both specimen and batch of fed females

	1			2			3			4			5			6			7			8		
Specimen	date	H	U	date	H	U	date	H	U	date	H	U	date	H	U	date	H	U	date	H	U	date	H	U
F1	08.apr	1	0																					
F2	10.apr	23	1																					
F3	05.apr	9	2	07.apr	22	5	09.apr	16	0															
F4	08.apr	33	1	10.apr	26	3																		
F5	09.apr	11	5																					
F6	10.apr	15	0																					
F7	12.apr	25	0	15.apr	14	17	18.apr	25	2	21.apr	29	0	22.apr	19	2	26.apr	24	3	29.apr	28	1	01.mai	26	1
F8	25.apr	19	1	28.apr	26	2	02.mai	16	3	05.mai	13	1												
F10	06.apr	15	0	09.apr	13	0	12.apr	16	18	14.apr	25	0	16.apr			18.apr	40	2	21.apr	33	0	24.apr	37	0
F11	12.apr	27	1																					
F12	06.apr	16	0	10.apr	21	0	12.apr	9	2	14.apr	14	0	16.apr			17.apr	18	7	19.apr	37	1	21.apr	32	0
F13	08.apr	13	3	11.apr	12	1	13.apr	20	2		-	-	18.apr	20	0	21.apr	19	5	24.apr	26	4	27.apr	17	0
F14	05.apr	0	1	13.apr	24	8	15.apr	26	3	18.apr	37	1	25.apr	46	0	28.apr	40	2	01.mai	32	1	04.mai	38	3
F15	04.apr	0	19	08.apr	13	1	10.apr	10	0	13.apr	16	3		-	-	18.apr	16	2	21.apr	22	0	24.apr	25	4
F17	08.apr	5	0	12.apr	13	0	18.apr	-	-	25.apr	2	12	29.apr	6	7	03.mai	21	1	05.mai	0	1			
F20	04.apr	3	9	06.apr	15	0	08.apr	8	1	11.apr	19	0	14.apr	26	8	17.apr	21	3	19.apr	24	0	21.apr	28	15
F21	06.apr	12	6	08.apr	14	0	10.apr	20	2	14.apr	35	5	16.apr	-	-	17.apr	26	5	19.apr	32	4	25.apr	19	4
F22	06.apr	13	4	08.apr	8	0	10.apr	14	3	13.apr	12	1	17.apr	20	1	19.apr	37	1	21.apr	19	0	24.apr	20	10
F23	06.apr	0	1	10.apr	1	0		-	-	13.apr	18	1	15.apr	33	0	18.apr	37	0	21.apr	36	0	25.apr	16	11
F24	06.apr	14	1	10.apr	16	1	13.apr	14	0	14.apr	23	1	18.apr	23	1	20.apr	28	0	22.apr	39	0	24.apr	19	6
F25	06.apr	16	7	10.apr	34	0	13.apr	2	0	14.apr	27	1	17.apr	17	2	19.apr	32	0	22.apr	32	0	25.apr	43	0
F26	06.apr	29	1	08.apr	17	4	12.apr	0	10	14.apr	15	0	17.apr	13	23	19.apr	46	2	26.apr	5	1			
F27	23.apr	23	0	03.mai	1	0																		
F28	10.apr	15	1	13.apr	19	2	15.apr	23	1	18.apr	29	0	20.apr	33	1	22.apr	28	1	26.apr	27	0	28.apr	12	0
F29	04.apr	11	6	07.apr	16	0	09.apr	22	0	11.apr	30	0	14.apr	21	0		-	-	19.apr	17	-	25.apr	37	13
F30	25.apr	15	12	28.apr	14	34	29.apr	14	4	04.mai	1	0												
F32	15.apr	-	-	17.apr	18	0	19.apr	17	2	24.apr	27	0	01.mai	26	0	04.mai	23	2						
F33	17.apr	33	0	19.apr	65	5	23.apr	21	0	26.apr	41	0	02.mai	41	3									
F34	17.apr	29	0	19.apr	10	0	23.apr	35	3	25.apr	35	0	29.apr	34	2	01.mai	28	0	04.mai	28	4			
F35	17.apr	20	0	20.apr	23	1	22.apr	3	0	26.apr	40	3	29.apr	35	0	02.mai	23	0	05.mai	39	0			
F36	15.apr	9	-	17.apr	6	1	19.apr	14	1	21.apr	8	1	23.apr	29	1	25.apr	13	1	29.apr	6	2	01.mai	25	2
F37	13.apr	21	0	16.apr	-	-	18.apr	1	0	20.apr	33	0	22.apr	2	0	26.apr	29	0	30.apr	23	0	03.mai	26	0
F38	18.apr	29	1	21.apr	21	4	23.apr	48	4															
F39	15.apr	26	-	17.apr	29	0	19.apr	26	1	23.apr	28	3	25.apr	23	2	28.apr	36	3	30.apr	30	1	03.mai	27	-
F40	15.apr	22	0	17.apr	30	0	20.apr	14	6	25.apr	25	0	28.apr	6	2	01.mai	18	2						
F41	14.apr	19	0	16.apr	-	-	18.apr	23	0															
F42	13.apr	20	0	15.apr	27	22	18.apr	25	4	21.apr	8	8	24.apr	3	19	26.apr	21	4	28.apr	25	1	01.mai	31	0
Sum		591	83		568	111		482	72		590	41		476	74		624	46		560	21		478	69
total		674			679			554			631			550			670			581			547	
Hatch.s. (%)		87.01			83.65			87.00			93.50			86.55			93.13			96.39			86.28	

Table A-3 continued

	9			10			11			12			13			14					hatch.s.
Specimen	date	H	U	date	H	U	date	H	U	date	H	U	date	H	U	date	H	U	sum H	Sum U	sum egg	(%)
F1																			1	0	1	100.00
F2																			23	1	24	95.83
F3																			47	7	54	87.04
F4																			59	4	63	93.65
F5																			11	5	16	68.75
F6																			15	0	15	100.00
F7	04.mai	16	3																206	29	235	87.66
F8																			74	7	81	91.36
F10	27.apr	33	1	30.apr	26	2													238	23	261	91.19
F11																			27	1	28	96.43
F12	24.apr	30	0	27.apr	31	0	30.apr	19	0	03.mai	11	2	05.mai	27	1				265	13	278	95.32
F13																			127	15	142	89.44
F14																			206	18	224	91.96
F15	26.apr	29	3	29.apr	22	2	01.mai	23	2	04.mai	18	2							194	38	232	83.62
F17																			47	21	68	69.12
F20	24.apr	11	0	26.apr	34	9	28.apr	10	0	30.apr	0	7	02.mai	22	6	05.mai	20	18	241	76	317	76.03
F21	27.apr	28	1	29.apr	40	3	02.mai	27	4	04.mai	26	9							281	43	324	86.73
F22	26.apr	38	1	28.apr	16	1	30.apr	30	3	02.mai	25	3	05.mai	27	4				279	32	311	89.71
F23	01.mai	29	0	05.mai	28	3													198	16	214	92.52
F24	27.apr	23	2	29.apr	23	8	03.mai	25	2	05.mai	4	1							251	23	274	91.61
F25	28.apr	43	1																246	11	257	95.72
F26																			125	41	166	75.30
F27																			24	0	24	100.00
F28	30.apr	16	3	03.mai	20	0													222	9	231	96.10
F29	27.apr	36	0	30.apr	44	3	03.mai	31	3	05.mai	41	3							306	28	334	91.62
F30																			44	50	94	35.06
F32																			111	4	115	96.52
F33																			201	8	209	96.17
F34																			199	9	208	95.67
F35																			183	4	187	97.86
F36	03.mai	14	2	05.mai	9	2													133	13	146	90.51
F37	05.mai	21	0																156	0	156	100.00
F38																			98	9	107	91.59
F39	05.mai	29	0																254	10	264	95.78
F40																			115	10	125	92.00
F41																			42	0	42	100.00
F42	05.mai	24	2																184	60	244	75.41
Sum		420	19		293	33		165	14		125	27		76	11		20	18				
total		439			326			179			152			87			38					
Hatch.s. (%)		95.67			89.88			92.18			82.24			87.36			52.63					

Appendix B: Mortality of females

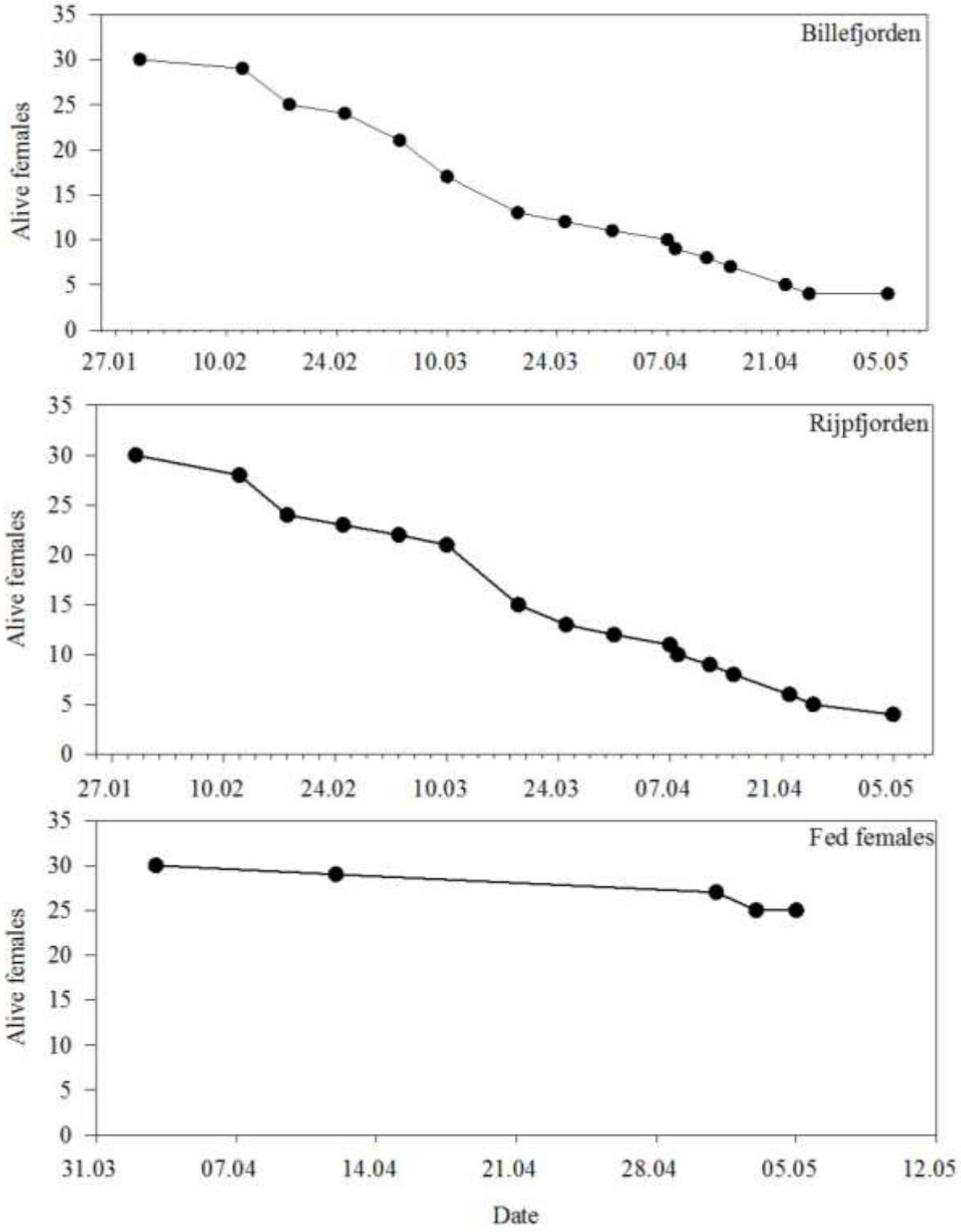

Figure B-1: Decrease in number of living females over time in the long time incubation experiments. Top: Starved females from Billefjorden. The number of females decreases steadily, and a well fitted linear regression (R^2=0.973) gave a slope of -0.311 females day^{-1}, or 2.18 females $week^{-1}$. Only four females were still alive at the end of the experiment. Middle: Starved females from Rijpfjorden. The number of females decreases steadily, and a well fitted linear regression (R^2=0.956) gave a slope of -0.352 females day^{-1}, or 2.46 females $week^{-1}$. Only three females were still alive at the end of the experiment. Bottom: Fed females. Only one of the feeding females died from natural causes before May 1st. By the end of the experiment on May 5th, four more females had died.

Appendix C: Gonad stages

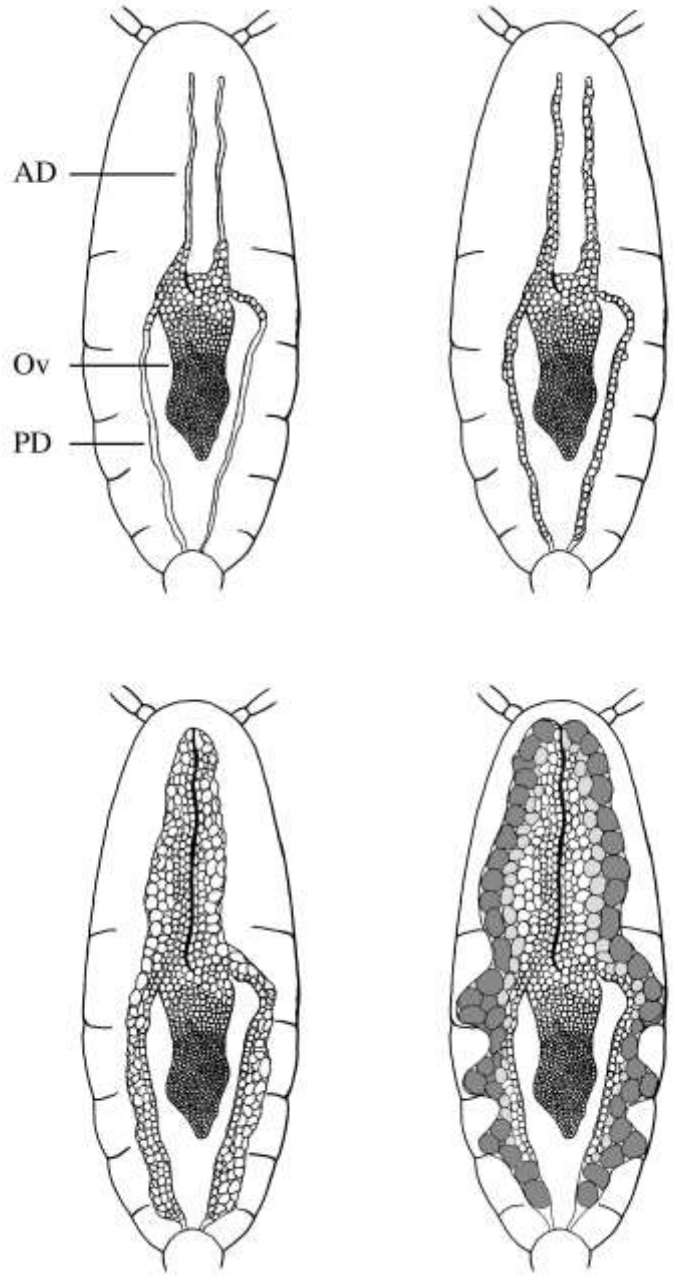

Figure C-1: Gonad stages of *Calanus* spp. Top left: GS1, recognized on no cells in the diverticula. Top right: GS2, one row of oocytes in diverticula. Bottom left: GS3, multiple rows of small, immature oocytes in diverticula. Bottom right: GS4, at least one row of mature oocytes, pouch formations on posterior diverticula. Figure credit: Barbara Niehoff, from Niehoff and Hirche (1996)

Appendix D: Fatty acid composition

Table D-1: Fatty acid composition of females from Billefjorden through the winter and spring

	January				February				March			
	%		µg/ind		%		µg/ind		%		µg/ind	
Fatty acid												
14:0	5.51	±1.47	11.64	±5.22	6.40	±0.86	8.72	±0.92	5.03	±1.21	9.87	±3.41
15:0	0.26	±0.12	0.81	±0.62	0.50	±0.05	0.69	±0.1	0.31	±0.07	0.74	±0.42
15:1(n-5)	0.27	±0.29	0.26	±0.19	0.09	±0.06	0.11	±0.08	0.15	±0.22	0.12	±0.16
16:0	10.28	±6.07	35.93	±39.65	8.78	±4.18	13.39	±9.17	12.19	±5.27	32.33	±20.45
16:1(n-7)	7.57	±2.66	15.47	±6.91	8.20	±1.55	11.06	±1.12	7.43	±2.79	13.56	±3.57
16:1(n-5)	0.36	±0.05	0.85	±0.54	0.40	±0.07	0.54	±0.04	0.20	±0.15	0.31	±0.26
16:2(n-4)	0.56	±0.12	1.61	±1.39	0.40	±0.1	0.54	±0.11	0.47	±0.16	0.98	±0.6
16:3(n-4)	0.13	±0.09	0.17	±0.16	0.29	±0.08	0.39	±0.11	0.00	±0	0.00	±0
16:4(n-1)	0.80	±0.55	2.11	±1.39	1.10	±0.41	1.43	±0.38	0.59	±0.18	1.49	±0.88
18:0	0.92	±1.31	0.61	±0.86	0.09	±0.13	0.10	±0.14	0.11	±0.16	0.09	±0.12
18:1(n-9)	6.14	±2.7	19.83	±20.24	5.25	±1.52	7.72	±3.88	6.39	±1.82	15.93	±9.3
18:1(n-7)	0.69	±0.24	1.37	±0.54	0.78	±0.11	1.12	±0.39	0.89	±0.15	2.11	±1.14
18:2(n-6)	2.32	±1.34	8.15	±8.83	1.91	±0.88	2.91	±1.95	2.79	±1.29	7.46	±4.82
18:3(n-3)	0.58	±0.19	1.25	±0.73	0.70	±0.06	0.97	±0.15	0.48	±0.05	1.01	±0.43
18:4(n-3)	0.58	±0.49	0.66	±0.49	1.16	±0.21	1.58	±0.27	0.41	±0.36	0.96	±1.17
20:0	0.55	±0.34	2.00	±2.07	0.57	±0.12	0.83	±0.35	1.19	±0.74	3.15	±2.54
20:1(n-11)	0.11	±0.15	0.07	±0.1	0.00	±0	0.00	±0	0.12	±0.17	0.09	±0.13
20:1(n-9)	8.07	±3.67	14.62	±6.23	5.03	±3.68	7.76	±5.42	6.26	±2.92	10.81	±2.15
20:1(n-7)	0.35	±0.25	0.45	±0.39	0.16	±0.02	0.22	±0.03	0.20	±0.29	0.15	±0.22
20:3(n-6)	0.85	±1.03	3.91	±5.22	0.24	±0.03	0.34	±0.12	2.69	±1.99	7.80	±6.03
20:4(n-3)	0.45	±0.08	1.06	±0.67	0.46	±0.11	0.61	±0.04	0.27	±0.08	0.59	±0.36
20:5(n-3)	2.73	±1.1	5.52	±2.66	3.89	±0.84	5.22	±0.65	2.16	±0.83	3.91	±1.01
22:1(n-11)	3.90	±1.61	7.48	±3.3	4.67	±1.09	6.25	±0.92	3.33	±1.83	5.47	±0.87
22:1(n-9)	0.71	±0.25	1.40	±0.53	0.74	±0.1	1.01	±0.1	0.55	±0.22	0.99	±0.24
22:1(n-7)	0.68	±0.52	2.58	±3.07	0.58	±0.59	0.97	±1.11	0.84	±0.46	2.34	±1.55
22:5(n-3)	0.51	±0.22	1.68	±1.68	0.59	±0.31	0.91	±0.67	0.52	±0.12	1.20	±0.69
22:6(n-3)	1.85	±0.53	3.91	±1.74	2.58	±0.44	3.49	±0.22	2.04	±0.66	3.82	±1.13
24:1(n-9)	2.10	±1.79	8.46	±9.99	2.63	±2.39	4.36	±4.6	2.84	±1.47	7.81	±5.11
Fatty alcohol												
14:0	0.30	±0.26	0.87	±0.62	0.61	±0.18	0.81	±0.11	0.46	±0.2	0.81	±0.19
16:0	2.81	±1.17	5.22	±2.03	3.30	±0.85	4.38	±0.55	2.65	±1.55	4.27	±0.63
16:1(n-7)	9.77	±11.18	43.70	±57.35	6.57	±7.03	11.12	±13.18	14.13	±9.19	40.65	±28.33
18:1(n-9)	0.61	±0.3	1.12	±0.4	0.94	±0.27	1.24	±0.19	0.58	±0.24	1.03	±0.29
18:1(n-7)	0.64	±0.31	1.18	±0.45	0.82	±0.25	1.08	±0.18	0.59	±0.21	1.09	±0.31
20:1(n-9)	16.59	±6.88	31.34	±13.11	17.08	±5.7	22.36	±5.23	13.02	±5.46	23.19	±5.69
22:1(n-11)	9.44	±3.94	19.79	±10.14	12.49	±4.38	16.29	±4.1	8.13	±2.83	15.10	±4.51
Sum												
Total	100.00		257.10	±174.57	100.00		140.51	±31.98	100.00		221.23	±103.51
MUFA	30.96	±3.96	72.85	±41.36	28.52	±3.54	41.11	±13.53	29.21	±4.79	59.68	±23.19
PUFA	11.37	±0.51	30.04	±21.09	13.32	±1.02	18.39	±2.8	12.40	±1.88	29.22	±15.57
SFA	17.52	±4.65	50.99	±45.89	16.35	±3.5	23.73	±10.07	18.82	±4.39	46.18	±25.98
Falc	40.16	±2.08	103.21	±67.91	41.82	±4.75	57.28	±6.97	39.56	±1.33	86.14	±38.99
WE	80.28	±4.18			83.53	±9.63			78.95	±2.77		

Table D-1 continue

	April				Late April				May			
	%		µg/ind		%		µg/ind		%		µg/ind	
Fatty acid												
14:0	3.86	±0.48	9.37	±0.25	7.11	±0.21	3.62	±1.49	6.74	±0.79	3.10	±0.61
15:0	0.27	±0.1	0.69	±0.28	0.28	±0.01	0.14	±0.06	0.26	±0.03	0.12	±0.01
15:1(n-5)	0.12	±0.17	0.26	±0.36	0.57	±0.02	0.29	±0.12	0.39	±0.03	0.18	±0.03
16:0	17.95	±0.99	44.21	±5.91	7.15	±0.41	3.61	±1.41	7.03	±1.13	3.22	±0.68
16:1(n-7)	3.75	±0.87	9.24	±2.54	10.44	±1.14	5.63	±3.13	9.99	±2.4	4.60	±1.44
16:1(n-5)	0.19	±0.02	0.47	±0.03	0.35	±0.02	0.18	±0.08	0.33	±0.03	0.15	±0.03
16:2(n-4)	0.42	±0.26	1.03	±0.66	0.35	±0.25	0.13	±0.09	0.61	±0.14	0.28	±0.09
16:3(n-4)	0.20	±0.29	0.42	±0.6	0.16	±0.02	0.08	±0.03	0.23	±0.02	0.10	±0.01
16:4(n-1)	0.16	±0.18	0.44	±0.51	0.17	±0.01	0.09	±0.05	0.10	±0.07	0.05	±0.04
18:0	7.49	±10.6	15.64	±22.12	0.98	±0.39	0.59	±0.48	1.57	±0.85	0.72	±0.42
18:1(n-9)	8.85	±0.58	21.83	±3.14	3.77	±0.09	1.94	±0.87	2.63	±0.38	1.21	±0.24
18:1(n-7)	1.59	±0.74	3.73	±1.28	1.09	±0.03	0.56	±0.25	1.19	±0.04	0.55	±0.07
18:2(n-6)	4.43	±0.48	10.97	±2	0.90	±0.04	0.47	±0.23	0.76	±0.29	0.35	±0.15
18:3(n-3)	0.36	±0.2	0.93	±0.54	0.00	±0	0.00	±0	0.00	±0	0.00	±0
18:4(n-3)	0.72	±0.11	1.80	±0.41	0.25	±0.01	0.13	±0.05	0.26	±0.02	0.12	±0.02
20:0	1.05	±0.11	2.54	±0.09	0.22	±0.04	0.10	±0.03	0.17	±0.08	0.08	±0.04
20:1(n-11)	0.08	±0.11	0.16	±0.23	0.35	±0.02	0.18	±0.07	0.34	±0.04	0.16	±0.03
20:1(n-9)	3.96	±1.01	9.60	±2.19	8.81	±0.72	4.67	±2.39	9.54	±1.27	4.30	±0.31
20:1(n-7)	0.14	±0.12	0.33	±0.26	0.52	±0.07	0.26	±0.09	0.45	±0.03	0.21	±0.02
20:3(n-6)	2.08	±1.45	5.45	±3.76	0.13	±0.02	0.06	±0.02	0.07	±0.05	0.03	±0.02
20:4(n-3)	0.30	±0.13	0.77	±0.4	0.23	±0.02	0.12	±0.06	0.27	±0.01	0.13	±0.02
20:5(n-3)	1.55	±0.17	3.77	±0.43	7.57	±1.11	3.66	±1.01	7.17	±0.35	3.26	±0.25
22:1(n-11)	2.21	±0.6	5.34	±1.11	4.78	±0.31	2.47	±1.1	5.47	±0.4	2.52	±0.45
22:1(n-9)	0.47	±0.1	1.13	±0.22	1.00	±0.09	0.51	±0.23	1.25	±0.14	0.57	±0.06
22:1(n-7)	1.15	±0.32	2.90	±1.02	0.23	±0.05	0.13	±0.08	0.33	±0.05	0.15	±0.03
22:5(n-3)	0.34	±0.17	0.87	±0.46	0.19	±0.14	0.11	±0.1	0.25	±0.18	0.12	±0.09
22:6(n-3)	1.81	±0.11	4.43	±0.27	7.20	±0.92	3.51	±1.06	7.88	±0.51	3.58	±0.24
24:1(n-9)	3.59	±1.1	9.08	±3.37	1.33	±0.15	0.65	±0.2	1.40	±0.07	0.64	±0.06
Fatty alcohol												
14:0	0.24	±0.08	0.58	±0.17	0.57	±0.04	0.29	±0.12	0.47	±0.01	0.21	±0.03
16:0	0.92	±0.19	2.24	±0.35	2.25	±0.11	1.17	±0.56	1.62	±0.15	0.74	±0.11
16:1(n-7)	16.55	±11.52	43.59	±30.33	0.90	±0.08	0.48	±0.25	0.92	±0.17	0.42	±0.08
18:1(n-9)	0.26	±0.04	0.63	±0.07	0.76	±0.03	0.39	±0.16	0.56	±0.02	0.26	±0.04
18:1(n-7)	0.28	±0.04	0.68	±0.15	0.65	±0.03	0.34	±0.17	0.59	±0.08	0.27	±0.05
20:1(n-9)	7.58	±1.42	18.49	±3.31	17.76	±1.23	9.40	±4.79	16.83	±1.21	7.68	±1
22:1(n-11)	5.05	±0.58	12.40	±1.93	10.96	±0.53	5.53	±2.15	12.34	±1.32	5.70	±1.14
Sum												
Total	100.00		245.99	±28.78	100.00		51.49	±22.74	100.00	±0	45.76	±5.6
MUFA	26.11	±1.58	64.05	±7.33	33.25	±1.61	17.47	±8.59	33.31	±2.36	15.23	±2.15
PUFA	12.39	±1.96	30.89	±7.44	17.16	±2.22	8.36	±2.5	17.59	±0.68	8.01	±0.7
SFA	30.62	±10.56	72.45	±15.93	15.73	±0.29	8.06	±3.47	15.78	±2.83	7.24	±1.67
Falc	30.88	±9.8	78.60	±30.94	33.85	±0.88	17.60	±8.19	33.32	±0.76	15.27	±2.11
WE	61.56	±19.49			67.55	±1.64			66.57	±1.54		

Table D-2: Fatty acid composition of females from Rijpfjorden

	January				February			
	%		µg/ind		%		µg/ind	
Fatty acid								
14:0	7.82	±0.71	9.62	±2.13	5.95	±1.4	9.87	±2.02
15:0	0.37	±0.14	0.48	±0.21	0.30	±0.08	0.59	±0.31
15:1(n-5)	0.29	±0.23	0.35	±0.25	0.18	±0.26	0.16	±0.23
16:0	6.03	±0.71	7.36	±1.43	12.05	±4.76	24.96	±14.55
16:1(n-7)	6.88	±2.34	9.63	±6.39	4.45	±1.27	7.28	±1.68
16:1(n-5)	0.40	±0.08	0.48	±0.06	0.24	±0.2	0.31	±0.22
16:2(n-4)	0.44	±0.17	0.63	±0.44	0.39	±0.11	0.69	±0.34
16:3(n-4)	0.10	±0.08	0.14	±0.1	0.06	±0.08	0.05	±0.07
16:4(n-1)	0.43	±0.18	0.52	±0.18	0.27	±0.2	0.45	±0.45
18:0	1.12	±0.79	1.53	±1.25	0.18	±0.25	0.16	±0.22
18:1(n-9)	3.98	±0.59	4.85	±0.98	6.94	±2.13	13.94	±7.6
18:1(n-7)	0.87	±0.19	1.12	±0.5	0.48	±0.37	0.82	±0.83
18:2(n-6)	1.66	±0.36	1.97	±0.25	3.10	±1.26	6.45	±3.75
18:3(n-3)	0.58	±0.41	0.57	±0.41	0.66	±0.09	1.16	±0.39
18:4(n-3)	0.99	±0.38	1.13	±0.29	0.58	±0.17	1.00	±0.42
20:0	0.25	±0.11	0.30	±0.11	0.64	±0.36	1.39	±0.96
20:1(n-11)	0.26	±0.19	0.35	±0.27	0.14	±0.2	0.13	±0.18
20:1(n-9)	10.10	±0.84	13.07	±5.27	7.18	±2.81	11.17	±0.95
20:1(n-7)	0.33	±0.26	0.40	±0.28	0.25	±0.35	0.22	±0.31
20:3(n-6)	0.22	±0.06	0.25	±0.01	0.76	±0.88	1.54	±2.01
20:4(n-3)	0.39	±0.14	0.45	±0.12	0.16	±0.11	0.25	±0.22
20:5(n-3)	3.70	±0.47	4.72	±1.64	2.40	±0.62	3.94	±0.74
22:1(n-11)	6.36	±0.99	8.22	±3.46	4.57	±1.51	7.30	±1.04
22:1(n-9)	0.97	±0.1	1.23	±0.44	0.73	±0.15	1.23	±0.28
22:1(n-7)	0.40	±0.01	0.51	±0.17	1.07	±0.68	2.36	±1.72
22:5(n-3)	0.41	±0.06	0.54	±0.25	0.49	±0.14	0.93	±0.51
22:6(n-3)	2.65	±0.74	3.19	±0.75	2.11	±0.34	3.61	±0.99
24:1(n-9)	0.88	±0.42	1.04	±0.41	3.80	±2.66	8.41	±6.68
Fatty alcohol								
14:0	0.70	±0.07	0.91	±0.37	0.34	±0.28	0.45	±0.33
16:0	3.13	±0.52	3.94	±1.23	2.03	±0.71	3.22	±0.38
16:1(n-7)	1.41	±0.62	1.79	±0.81	12.59	±8.48	28.39	±20.1
18:1(n-9)	0.85	±0.13	1.06	±0.26	0.65	±0.32	0.98	±0.02
18:1(n-7)	0.80	±0.12	1.04	±0.42	0.41	±0.34	0.53	±0.38
20:1(n-9)	20.90	±0.17	26.46	±8.53	14.18	±6.09	21.74	±2.81
22:1(n-11)	13.35	±0.2	16.87	±5.3	9.68	±2.99	15.66	±3.28
Sum								
Total	100.00		126.69	±40.86	100.00		181.34	±65.83
MUFA	31.71	±3.52	41.25	±17.5	30.03	±2.08	53.33	±17.74
PUFA	11.57	±2.15	14.09	±3.04	10.97	±0.71	20.07	±7.63
SFA	15.59	±1.2	19.28	±4.69	19.12	±3.57	36.97	±17.57
Falc	41.14	±1.07	52.06	±16.5	39.87	±2.36	70.96	±23.49
WE	82.23	±2.16			79.59	±4.68		

Table D-3: Fatty acid composition of fed females

	1st week				3rd week			
	%		µg/ind		%		µg/ind	
Fatty acid								
14:0	6.85	±0.99	6.34	±0.73	4.57	±0.89	0.80	±0.49
15:0	0.41	±0.1	0.37	±0.07	0.36	±0.09	0.06	±0.04
15:1(n-5)	0.20	±0.1	0.17	±0.04	0.14	±0.19	0.01	±0.01
16:0	4.57	±0.78	4.23	±0.68	13.16	±1.96	1.95	±0.8
16:1(n-7)	10.75	±1.69	10.26	±2.61	6.66	±1.42	1.14	±0.63
16:1(n-5)	0.26	±0.02	0.25	±0.05	0.06	±0.09	0.00	±0
16:2(n-4)	0.64	±0.06	0.60	±0.11	0.66	±0.06	0.10	±0.05
16:3(n-4)	0.14	±0.1	0.12	±0.09	0.25	±0.18	0.05	±0.04
16:4(n-1)	0.91	±0.32	0.88	±0.34	0.40	±0.21	0.08	±0.06
18:0	0.00	±0	0.00	±0	1.15	±1.62	0.06	±0.09
18:1(n-9)	2.47	±0.46	2.28	±0.32	2.26	±0.47	0.35	±0.17
18:1(n-7)	0.74	±0.1	0.68	±0.08	2.37	±0.34	0.35	±0.15
18:2(n-6)	0.60	±0.1	0.56	±0.1	0.70	±0.12	0.11	±0.06
18:3(n-3)	0.15	±0.13	0.15	±0.12	0.26	±0.03	0.04	±0.02
18:4(n-3)	0.08	±0.11	0.10	±0.14	0.28	±0.07	0.04	±0.01
20:0	0.34	±0.07	0.31	±0.02	0.06	±0.08	0.00	±0
20:1(n-11)	0.00	±0	0.00	±0	0.00	±0	0.00	±0
20:1(n-9)	10.77	±1.51	10.40	±3.39	5.84	±2.08	1.10	±0.82
20:1(n-7)	0.24	±0.02	0.22	±0.04	0.04	±0.05	0.00	±0
20:3(n-6)	0.00	±0	0.00	±0	0.02	±0.03	0.00	±0
20:4(n-3)	0.27	±0.07	0.25	±0.04	0.06	±0.08	0.00	±0
20:5(n-3)	5.36	±1.22	4.85	±0.25	18.43	±3.87	2.64	±1
22:1(n-11)	5.09	±1.47	5.21	±2.75	2.30	±1.06	0.45	±0.38
22:1(n-9)	1.09	±0.15	1.08	±0.41	0.97	±0.29	0.17	±0.13
22:1(n-7)	0.04	±0.06	0.05	±0.07	0.05	±0.07	0.00	±0
22:5(n-3)	0.00	±0	0.00	±0	0.20	±0.15	0.04	±0.03
22:6(n-3)	4.28	±0.9	3.89	±0.21	18.80	±3.44	2.73	±1.06
24:1(n-9)	0.95	±0.24	0.86	±0.01	3.39	±1.15	0.46	±0.15
Fatty alcohol								
14:0	0.65	±0.17	0.59	±0.13	0.09	±0.13	0.01	±0.01
16:0	3.80	±1.06	3.72	±1.38	0.30	±0.1	0.06	±0.04
16:1(n-7)	4.46	±3.41	5.04	±4.72	2.68	±1.51	0.53	±0.37
18:1(n-9)	0.69	±0.18	0.63	±0.14	0.14	±0.11	0.02	±0.03
18:1(n-7)	0.82	±0.18	0.75	±0.11	0.23	±0.13	0.04	±0.03
20:1(n-9)	19.87	±0.47	19.01	±4.46	7.11	±2.91	1.38	±1.01
22:1(n-11)	12.51	±1.36	12.22	±4.17	6.03	±3.02	1.21	±0.99
Sum								
Total	100.00		96.05	±24.21	100.00		16.02	±8.18
MUFA	32.60	±0.69	31.44	±8.51	24.08	±2.23	4.04	±2.3
PUFA	12.42	±2.32	11.39	±1.18	40.04	±6.98	5.84	±2.31
SFA	12.17	±1.89	11.26	±1.43	19.29	±2.5	2.89	±1.23
Falc	42.81	±3.56	41.96	±13.94	16.58	±7.24	3.25	±2.42
WE	85.36	±7.17			33.00	±14.39		

Table D-4: Fatty acid composition of starved females at the end of the experiment

	Billefjorden		Rijpfjorden		Rijpfj. w/egg	
	%	µg/ind	%	µg/ind	%	µg/ind
Fatty acid	S_BAB	S_BAB	S_R4	S_R3	FE_R4	FE_R3
14:0	4.3328	0.2098	4.8644	0.3445	8.4515	5.1005
15:0	0.4101	0.0199	0.3470	0.0246	0.2145	0.1295
15:1(n-5)	0.4061	0.0197	0.4755	0.0337	0.3331	0.2010
16:0	11.7353	0.5683	16.6206	1.1772	7.2944	4.4021
16:1(n-7)	3.5628	0.1725	1.5389	0.1090	5.1866	3.1301
16:1(n-5)	0.2056	0.0100	0.1782	0.0126	0.2064	0.1245
16:2(n-4)	0.2245	0.0109	2.1537	0.1525	0.1366	0.0824
16:3(n-4)	0.3661	0.0177	0.0893	0.0063	0.0851	0.0514
16:4(n-1)	0.4603	0.0223	0.0969	0.0069	0.1990	0.1201
18:0	5.0502	0.2446	23.5142	1.6655	10.1271	6.1116
18:1(n-9)	3.9036	0.1890	3.3858	0.2398	2.1315	1.2864
18:1(n-7)	2.0203	0.0978	1.0610	0.0752	0.6093	0.3677
18:2(n-6)	0.8719	0.0422	1.9543	0.1384	0.9889	0.5968
18:3(n-3)	0.0000	0.0000	0.4199	0.0297	0.0590	0.0356
18:4(n-3)	0.4419	0.0214	0.5367	0.0380	0.1314	0.0793
20:0	1.0857	0.0526	2.1663	0.1534	0.5139	0.3101
20:1(n-11)	0.1880	0.0091	0.0966	0.0068	0.0000	0.0000
20:1(n-9)	5.9628	0.2888	7.7062	0.5458	12.9364	7.8070
20:1(n-7)	0.1614	0.0078	0.1887	0.0134	0.3192	0.1926
20:3(n-6)	0.2549	0.0123	0.3119	0.0221	0.0700	0.0422
20:4(n-3)	0.2640	0.0128	0.2228	0.0158	0.2382	0.1437
20:5(n-3)	11.3651	0.5504	8.2671	0.5856	3.4717	2.0951
22:1(n-11)	3.0914	0.1497	4.0208	0.2848	6.5619	3.9600
22:1(n-9)	1.5942	0.0772	1.3836	0.0980	2.6390	1.5926
22:1(n-7)	0.2178	0.0105	0.1705	0.0121	0.2004	0.1209
22:5(n-3)	1.0239	0.0496	0.0000	0.0000	0.3699	0.2232
22:6(n-3)	19.5160	0.9451	1.4584	0.1033	4.1526	2.5061
24:1(n-9)	6.2717	0.3037	0.3482	0.0247	1.9858	1.1984
Fatty alcohol						
14:0	0.4386	0.0212	0.2825	0.0200	0.1003	0.0606
16:0	0.4603	0.0223	0.2005	0.0142	0.7912	0.4775
16:1(n-7)	1.2620	0.0611	0.2809	0.0199	0.1085	0.0655
18:1(n-9)	0.1627	0.0079	0.1745	0.0124	0.3093	0.1866
18:1(n-7)	0.1744	0.0084	0.1562	0.0111	0.3510	0.2119
20:1(n-9)	6.8226	0.3304	9.1784	0.6501	17.7300	10.6999
22:1(n-11)	5.6912	0.2756	6.1493	0.4356	10.9963	6.6362
Sum						
Total	100.0000	4.8428	100.0000	7.0830	100.0000	60.3494
MUFA	27.5855	1.3359	20.5541	1.4558	33.1096	19.9814
PUFA	34.7887	1.6848	15.5111	1.0986	9.9023	5.9760
SFA	22.6141	1.0952	47.5125	3.3653	26.6015	16.0538
Falc	15.0117	0.7270	16.4223	1.1632	30.3867	18.3382
WE	29.9718		32.6285		60.2985	

Table D-5: Fatty acid composition of nauplii from feeding experiment in lab

	1st week				3rd week			
	%		µg/ind		%		µg/ind	
Fatty acid								
14:0	4.23	±0.59	0.0016	±0.0002	3.89	±0.19	0.0013	±0.0003
15:0	0.15	±0.11	0.0001	±0	0.34	±0.16	0.0001	±0.0001
15:1(n-5)	0.81	±0.04	0.0003	±0	0.93	±0.33	0.0003	±0.0002
16:0	22.37	±1.9	0.0083	±0.001	24.77	±2.39	0.0083	±0.0026
16:1(n-7)	10.06	±1.43	0.0039	±0.0012	3.25	±1.79	0.0010	±0.0005
16:1(n-5)	0.41	±0.12	0.0002	±0	0.34	±0.07	0.0001	±0
16:2(n-4)	1.05	±0.12	0.0004	±0.0001	1.05	±0.35	0.0003	±0.0001
16:3(n-4)	0.09	±0.13	0.0000	±0.0001	0.04	±0.07	0.0000	±0
16:4(n-1)	0.37	±0.38	0.0001	±0.0001	0.03	±0.05	0.0000	±0
18:0	14.81	±0.62	0.0056	±0.0012	26.84	±6.25	0.0090	±0.0035
18:1(n-9)	6.10	±0.1	0.0023	±0.0004	3.18	±0.4	0.0010	±0.0002
18:1(n-7)	2.73	±0.44	0.0010	±0.0003	1.94	±0.33	0.0006	±0.0001
18:2(n-6)	2.33	±0.45	0.0009	±0.0002	1.90	±0.82	0.0006	±0.0002
18:3(n-3)	0.23	±0.17	0.0001	±0.0001	0.17	±0.11	0.0000	±0
18:4(n-3)	0.54	±0.57	0.0002	±0.0002	0.71	±0.5	0.0003	±0.0002
20:0	0.58	±0.09	0.0002	±0.0001	0.95	±0.26	0.0003	±0.0002
20:1(n-11)	0.18	±0.13	0.0001	±0	0.03	±0.05	0.0000	±0
20:1(n-9)	4.24	±0.58	0.0016	±0.0005	1.13	±0.93	0.0004	±0.0003
20:1(n-7)	0.48	±0.53	0.0002	±0.0002	0.11	±0.19	0.0001	±0.0001
20:3(n-6)	0.06	±0.08	0.0000	±0	0.00	±0	0.0000	±0
20:4(n-3)	0.28	±0.2	0.0001	±0.0001	1.72	±1.43	0.0006	±0.0006
20:5(n-3)	14.00	±1.24	0.0053	±0.0014	12.02	±3.55	0.0038	±0.0009
22:1(n-11)	0.94	±0.39	0.0004	±0.0002	0.19	±0.23	0.0001	±0.0001
22:1(n-9)	0.21	±0.16	0.0001	±0.0001	0.05	±0.08	0.0000	±0
22:1(n-7)	0.24	±0.34	0.0001	±0.0001	0.12	±0.16	0.0001	±0.0001
22:5(n-3)	0.70	±0.11	0.0003	±0	0.68	±1.03	0.0002	±0.0003
22:6(n-3)	9.63	±0.85	0.0036	±0.0006	10.23	±3.18	0.0032	±0.0008
24:1(n-9)	1.91	±0.21	0.0007	±0.0001	2.07	±0.66	0.0007	±0.0004
Fatty alcohol								
14:0	0.16	±0.23	4.70E-05	±0.0001	0.45	±0.29	1.50E-04	±0.0001
16:0	0.11	±0.15	0	±0	0.87	±0.77	3.20E-04	±0.0003
16:1(n-7)	0.00	±0	0	±0	0.00	±0	0.00	±0
18:1(n-9)	0.00	±0	0	±0	0.00	±0	0.00	±0
18:1(n-7)	0.00	±0	0	±0	0.00	±0	0.00	±0
20:1(n-9)	0.00	±0	0	±0	0.00	±0	0.00	±0
22:1(n-11)	0.00	±0	0	±0	0.00	±0	0.00	±0
Sum								
Total	100.00	±0	0.0376	±0.0067	100.00	±0	0.0329	±0.0075
MUFA	28.31	±1.9	0.0108	±0.0026	13.35	±3.25	0.0044	±0.0014
PUFA	29.29	±1.42	0.0110	±0.0021	28.54	±6.53	0.0091	±0.0017
SFA	42.13	±2.11	0.0157	±0.0022	56.79	±8.43	0.0190	±0.0065
Falc	0.27	±0.38	0.0001	±0.0001	1.32	±0.98	0.0005	±0.0004
WE	0.50	±0.71			2.51	±1.9		

Table D-6: Fatty acid composition of nauplii from starvation experiment in lab

	Billefjorden		Rijpfjorden				Unhatched eggs			
	%	µg/ind	%		µg/ind		%		µg/ind	
Fatty acid	Snaup_BAB	Snaup_BAB	SnaupR7		SnaupR6		SunhatE		SunhatE	
14:0	4.3812	0.0028	3.9605	±0.34	0.0018	±0.0003	3.7173	±0.01	0.0012	±0
15:0	1.0970	0.0007	0.0000	±0	0.0000	±0	0.0000	±0	0.0000	±0
15:1(n-5)	0.3638	0.0002	0.7612	±0.11	0.0004	±0.0001	0.8460	±0.03	0.0003	±0
16:0	21.8898	0.0142	23.9192	±2.71	0.0112	±0.0042	22.1489	±0.76	0.0072	±0
16:1(n-7)	4.1846	0.0027	5.1731	±1.06	0.0022	±0.0002	13.0370	±0.19	0.0043	±0.0002
16:1(n-5)	0.4251	0.0003	0.3807	±0.17	0.0002	±0	0.5165	±0.02	0.0002	±0
16:2(n-4)	1.0698	0.0007	1.1734	±0.02	0.0005	±0.0001	1.5294	±0.12	0.0005	±0
16:3(n-4)	0.0000	0.0000	0.0000	±0	0.0000	±0	0.0000	±0	0.0000	±0
16:4(n-1)	1.3788	0.0009	0.0756	±0.08	0.0000	±0	0.0000	±0	0.0000	±0
18:0	15.3056	0.0099	24.9261	±9.08	0.0124	±0.0072	15.2233	±0.39	0.0050	±0.0001
18:1(n-9)	8.2712	0.0054	7.4315	±1.57	0.0032	±0.0002	8.6325	±0.4	0.0028	±0.0002
18:1(n-7)	1.9846	0.0013	2.0772	±0.49	0.0009	±0	3.4459	±0.03	0.0011	±0
18:2(n-6)	3.9106	0.0025	2.3229	±0.09	0.0011	±0.0003	2.5450	±0.11	0.0008	±0.0001
18:3(n-3)	0.9938	0.0006	0.5619	±0.05	0.0002	±0	0.6345	±0.07	0.0002	±0
18:4(n-3)	0.0000	0.0000	0.1848	±0.18	0.0001	±0.0001	0.0000	±0	0.0000	±0
20:0	0.6444	0.0004	0.7775	±0.27	0.0004	±0.0002	0.7446	±0.26	0.0002	±0.0001
20:1(n-11)	0.5370	0.0003	0.2370	±0.03	0.0001	±0	0.0000	±0	0.0000	±0
20:1(n-9)	4.1580	0.0027	4.5668	±2.69	0.0017	±0.0007	7.6411	±0.31	0.0025	±0.0002
20:1(n-7)	0.0000	0.0000	0.0000	±0	0.0000	±0	1.3103	±1.31	0.0004	±0.0004
20:3(n-6)	0.0000	0.0000	0.1483	±0.15	0.0000	±0	0.0000	±0	0.0000	±0
20:4(n-3)	1.8635	0.0012	0.8475	±0.33	0.0004	±0.0003	0.5586	±0	0.0002	±0
20:5(n-3)	9.9426	0.0065	7.9776	±3.24	0.0032	±0.0005	6.4193	±0.23	0.0021	±0
22:1(n-11)	1.0545	0.0007	0.9434	±0.49	0.0004	±0.0001	2.1472	±0.02	0.0007	±0
22:1(n-9)	0.2800	0.0002	0.3167	±0.07	0.0001	±0	0.0000	±0	0.0000	±0
22:1(n-7)	0.3976	0.0003	0.0000	±0	0.0000	±0	0.0000	±0	0.0000	±0
22:5(n-3)	0.2854	0.0002	0.1587	±0.16	0.0001	±0.0001	0.0000	±0	0.0000	±0
22:6(n-3)	12.2853	0.0080	8.8674	±3.17	0.0036	±0.0003	6.7888	±1.02	0.0022	±0.0004
24:1(n-9)	1.5694	0.0010	1.3600	±0.31	0.0006	±0	2.1137	±0.48	0.0007	±0.0002
Fatty alcohol										
14:0	0.9522	0.0006	0.5128	±0.51	0.0003	±0.0003	0.0000	±0	0.0000	±0
16:0	0.7742	0.0005	0.3383	±0.34	0.0002	±0.0002	0.0000	±0	0.0000	±0
16:1(n-7)	0.0000	0.0000	0.0000	±0	0.0000	±0	0.0000	±0	0.0000	±0
18:1(n-9)	0.0000	0.0000	0.0000	±0	0.0000	±0	0.0000	±0	0.0000	±0
18:1(n-7)	0.0000	0.0000	0.0000	±0	0.0000	±0	0.0000	±0	0.0000	±0
20:1(n-9)	0.0000	0.0000	0.0000	±0	0.0000	±0	0.0000	±0	0.0000	±0
22:1(n-11)	0.0000	0.0000	0.0000	±0	0.0000	±0	0.0000	±0	0.0000	±0
Sum										
Total	100.0000	0.0649	100.0000	±0	0.0454	±0.0124	100.0000	±0	0.0326	±0.0013
MUFA	23.2258	0.0151	23.2475	±6.77	0.0097	±0.0002	39.6903	±0.05	0.0129	±0.0005
PUFA	31.7297	0.0206	22.3182	±5.78	0.0094	±0.0001	18.4756	±0.85	0.0060	±0.0005
SFA	43.3180	0.0281	53.5832	±11.71	0.0258	±0.012	41.8341	±0.9	0.0136	±0.0002
Falc	1.7264	0.0011	0.8512	±0.85	0.0005	±0.0005	0.0000	±0	0.0000	±0
WE	3.3724		1.6023	±1.6			0.0000	±0		

Table D-7: Fatty acid composition of nauplii from 24 hour incubation of females from field

	Late April				May			
	%		µg/ind		%		µg/ind	
Fatty acid								
14:0	3.7914	±0.15	0.0014	±0.0001	2.8848	±0.71	0.0004	±0.0001
15:0	0.5190	±0.52	0.0002	±0.0002	0.0000	±0	0.0000	±0
15:1(n-5)	0.7333	±0.27	0.0003	±0.0001	0.5996	±0.09	0.0001	±0
16:0	26.0696	±1.03	0.0095	±0.0016	22.9224	±1.34	0.0028	±0.0004
16:1(n-7)	2.9040	±1.69	0.0010	±0.0005	3.1296	±1.28	0.0004	±0.0002
16:1(n-5)	0.3663	±0.05	0.0001	±0	0.0000	±0	0.0000	±0
16:2(n-4)	0.6233	±0.1	0.0002	±0.0001	1.1422	±0.59	0.0001	±0.0001
16:3(n-4)	0.0000	±0	0.0000	±0	0.0000	±0	0.0000	±0
16:4(n-1)	0.0000	±0	0.0000	±0	0.0000	±0	0.0000	±0
18:0	23.7820	±5.98	0.0089	±0.0033	25.1864	±3.75	0.0031	±0.0006
18:1(n-9)	3.6282	±0.06	0.0013	±0.0002	3.1779	±0.2	0.0004	±0.0001
18:1(n-7)	1.7491	±0.4	0.0006	±0.0001	2.3881	±0.29	0.0003	±0
18:2(n-6)	2.3962	±0.44	0.0009	±0.0003	1.8343	±0.25	0.0002	±0
18:3(n-3)	0.2979	±0.02	0.0001	±0	0.1407	±0.17	0.0000	±0
18:4(n-3)	0.1393	±0.14	0.0000	±0	0.2027	±0.25	0.0000	±0
20:0	1.0948	±0.08	0.0004	±0.0001	0.9349	±0.17	0.0001	±0
20:1(n-11)	0.0000	±0	0.0000	±0	0.0000	±0	0.0000	±0
20:1(n-9)	0.9268	±0.29	0.0003	±0.0001	0.5627	±0.12	0.0001	±0
20:1(n-7)	0.0000	±0	0.0000	±0	0.3668	±0.48	0.0000	±0.0001
20:3(n-6)	0.0000	±0	0.0000	±0	0.0000	±0	0.0000	±0
20:4(n-3)	2.5223	±0.46	0.0009	±0.0003	0.0000	±0	0.0000	±0
20:5(n-3)	11.8925	±3.27	0.0041	±0.0006	15.6576	±1.9	0.0019	±0.0003
22:1(n-11)	0.2765	±0.02	0.0001	±0	0.1761	±0.22	0.0000	±0
22:1(n-9)	0.1400	±0.14	0.0000	±0	0.0000	±0	0.0000	±0
22:1(n-7)	0.0742	±0.07	0.0000	±0	0.0000	±0	0.0000	±0
22:5(n-3)	0.5245	±0.52	0.0002	±0.0002	0.4785	±0.4	0.0001	±0
22:6(n-3)	11.3307	±2.11	0.0040	±0.0002	15.6019	±1.99	0.0019	±0.0003
24:1(n-9)	2.0275	±0.15	0.0007	±0	2.3003	±0.23	0.0003	±0
Fatty alcohol								
14:0	0.8390	±0.17	0.0003	±0.0001	0.1609	±0.2	0.0000	±0
16:0	1.3516	±0.45	0.0005	±0.0002	0.1518	±0.2	0.0000	±0
16:1(n-7)	0.0000	±0	0.0000	±0	0.0000	±0	0.0000	±0
18:1(n-9)	0.0000	±0	0.0000	±0	0.0000	±0	0.0000	±0
18:1(n-7)	0.0000	±0	0.0000	±0	0.0000	±0	0.0000	±0
20:1(n-9)	0.0000	±0	0.0000	±0	0.0000	±0	0.0000	±0
22:1(n-11)	0.0000	±0	0.0000	±0	0.0000	±0	0.0000	±0
Sum								
Total	100.0000	±0	0.0361	±0.0048	100.0000	±0	0.0123	±0.0017
MUFA	12.8260	±3.01	0.0045	±0.0005	12.7009	±1.81	0.0016	±0.0003
PUFA	29.7267	±5.08	0.0105	±0.0004	35.0579	±3.23	0.0043	±0.0006
SFA	55.2567	±7.46	0.0203	±0.0053	51.9285	±4.78	0.0064	±0.0011
Falc	2.1906	±0.62	0.0008	±0.0003	0.3126	±0.39	0.0000	±0.0001
WE	4.2012	±1.17			0.5760	±0.72		

Appendix E: TL and KF plots for starved females

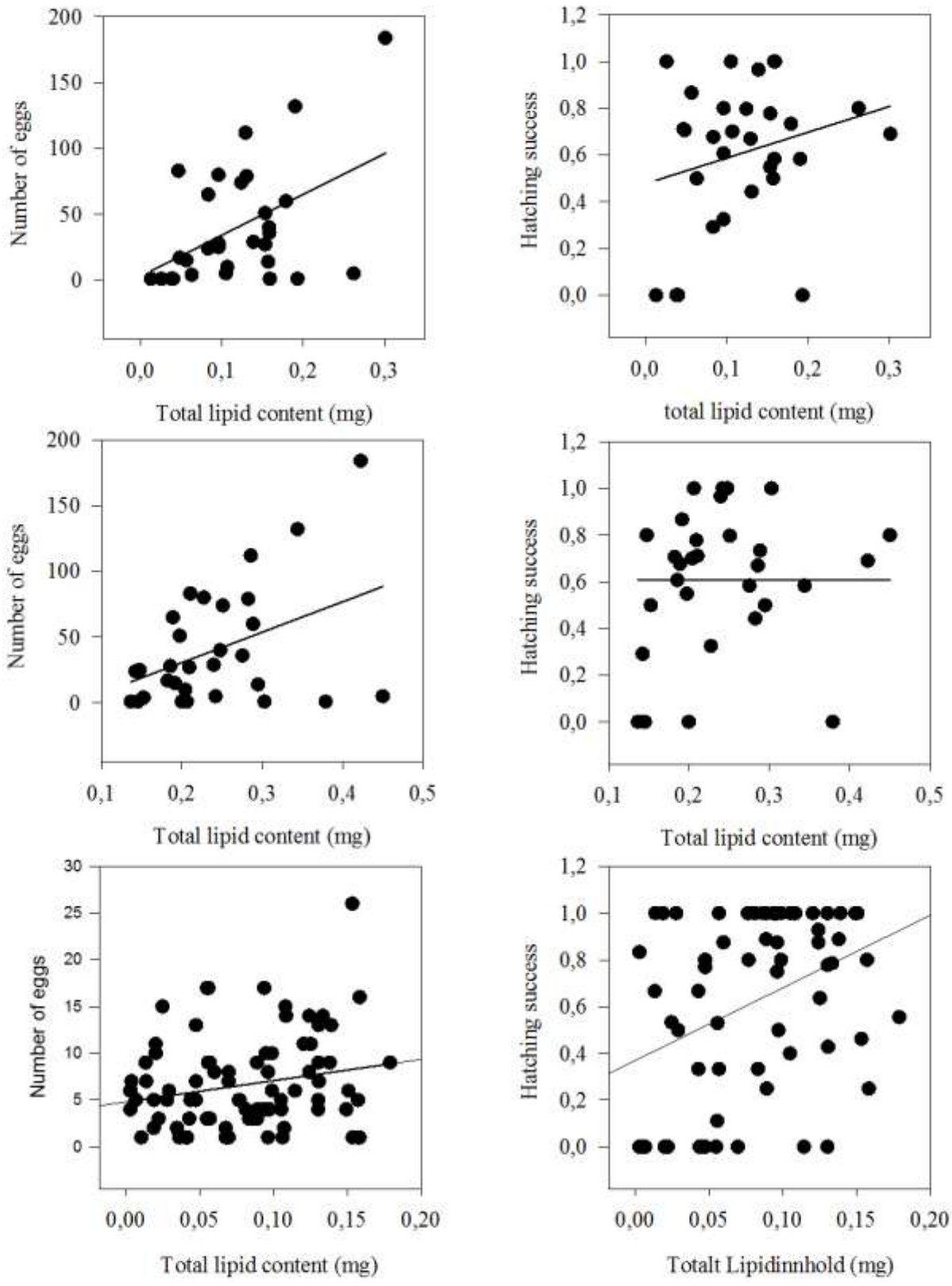

Figure E-1: Total lipid content vs. egg production and hatching success. Top: Total number of eggs and overall hatching success of individual vs total lipid content at first egg batch, Middle: Total number of eggs and over all hatching success of individual vs total lipid content at beginning of experiment, bottom: number of eggs and hatching success of each egg batch vs total lipid content at approximate time of batch

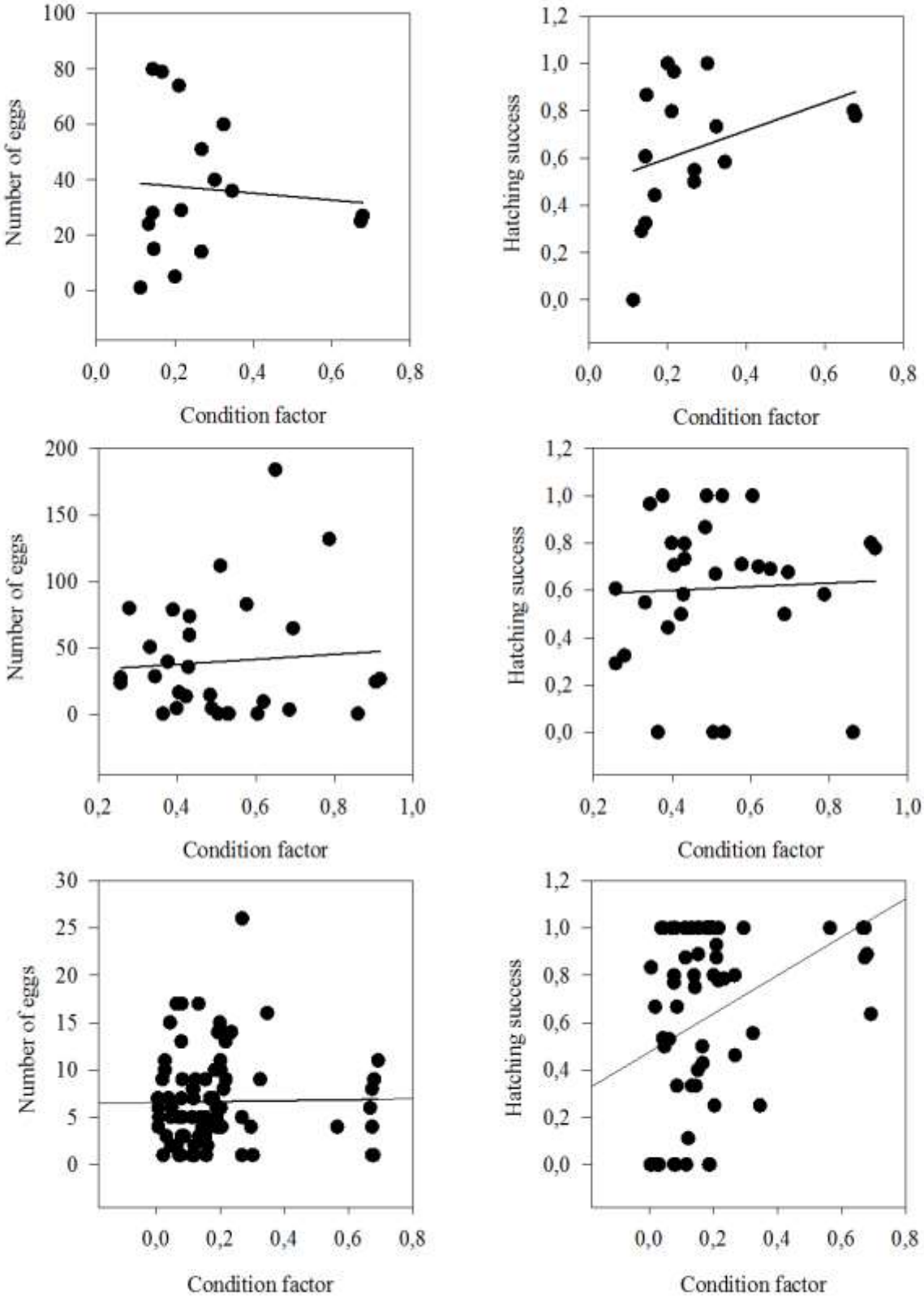

Figure E-2: Condition factor vs. egg production and hatching success. Top: Total number of eggs and overall hatching success of individual vs condition factor at first egg batch. Middle: Total number of eggs and over all hatching success of individual vs condition factor at beginning of experiment, bottom: number of eggs and hatching success of each egg batch vs condition factor at approximate time of batch

Appendix F: Total lipid content of males at time of death

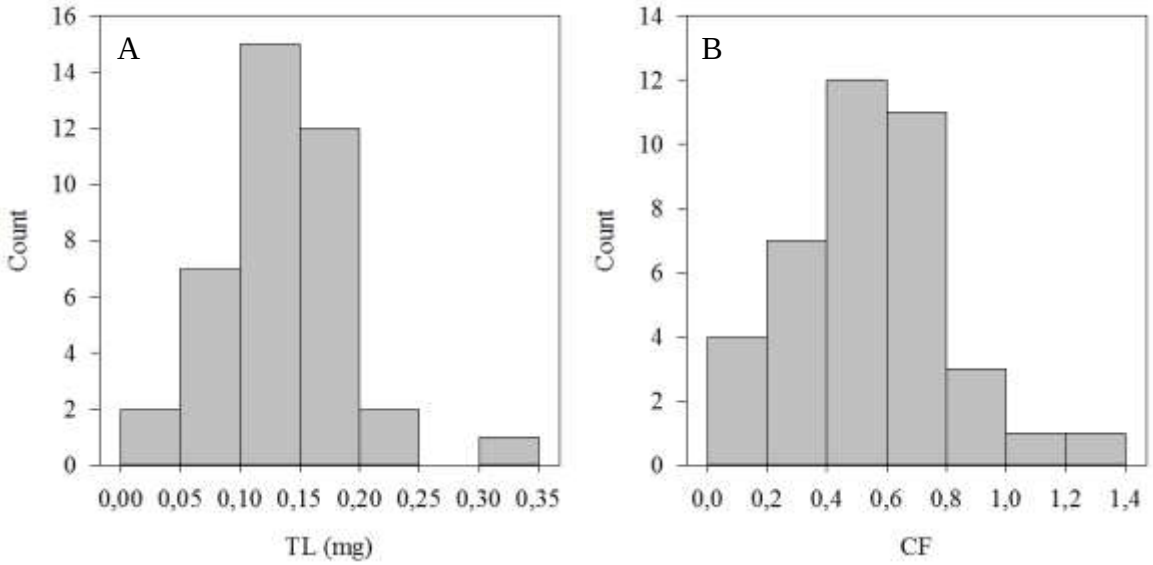

Figure F-1: frequency histograms of estimated total lipid content (a) and condition factor (b) for males at time of death.

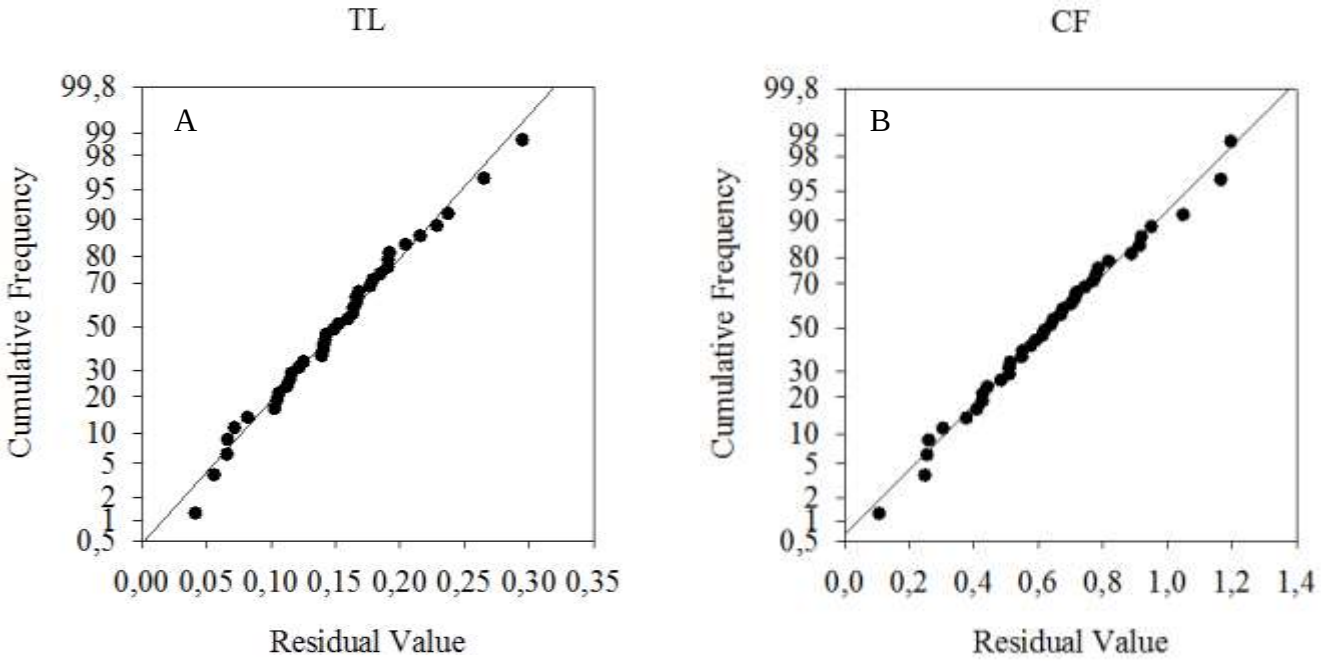

Figure F-2: Normal distribution plots for total lipid content (a) and condition factor (b) at time of death of male *C. glacialis*