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Effects of temperature and food availability on feeding and egg production of *Calanus hyperboreus* from Disko Bay, western Greenland

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Abstract:

The effects of temperature and food availability on feeding and egg production of the Arctic copepod *Calanus hyperboreus* were investigated in Disko Bay, western Greenland, from winter to spring 2009. The abundance of females in the near bottom layer and the egg production of *C. hyperboreus* prior to the spring bloom document that reproduction relies on lipid stores. The maximum *in situ* egg production (± SE) of 54 ± 8 eggs female^{-1} d^{-1} was recorded in mid-February at chlorophyll *a* concentrations below 0.1 µg l^{-1}, whereas no egg production was observed in mid-April when the spring bloom developed. After reproduction, the females migrated to the surface layer to exploit the bloom and refill their lipid stores. In 2 laboratory experiments, initiated before and during the spring bloom, mature females were kept with and without food at 5 different temperatures ranging from 0 to 10°C and the fecal pellet and egg production were monitored. Food had a clear effect on fecal pellet production but no effect on egg production, while temperature did not have an effect on egg or fecal pellet production in any of the experiments. Analyses of carbon and lipid content of the females before and after the experiments did not reflect any effect of food or temperature in the pre-bloom experiment, whereas in the bloom experiment a clear positive effect of food was detected in female biochemical profiles. The lack of a temperature response suggests a future warmer ocean could be unfavorable for *C. hyperboreus* compared to smaller *Calanus* spp. which are reported to exploit minor temperature elevations for increased egg production.

Keywords: *Calanus hyperboreus* ; Egg production ; Fecal pellet production ; Effect of temperature

INTRODUCTION

The annual productivity cycle in arctic ecosystems is greatly influenced by inter annual variations in sea ice cover and solar irradiance as the breakup of the sea ice increases available light to the surface water in the spring. In Disko Bay the breakup of the sea ice varies greatly between years (Nielsen and Hansen 1995; Madsen et al. 2001; Hansen et al. 2006; Madsen et al. 2008a; Madsen et al. 2008b; Dünweber et al. 2010). However, a general increase in mean air temperature of 0.4˚C pr. year and a reduction in sea ice cover of 50% have been observed from 1991 to 2004 (Hansen et al. 2006). This makes Disko Bay an ideal site for investigating the impact of climate change mediated variation in the ice cover on succession pattern in the pelagic food webs.

The three *Calanus* species *C. hyperboreus, C. glacialis* and *C. finmarchicus* are key species in arctic marine ecosystem. With their ability to convert phytoplankton to high energy wax esters they provide an energy rich food source for fish, seabirds and marine mammals (Falk-Petersen et al. 2009; Heide-Jørgensen and Acquarone 2002; Karnovsky et al. 2003). All three *Calanus* species are adapted to arctic conditions by having multiple year lifecycles with seasonal ontogenetic migration and accumulation of lipids during spring and summer, as well as hibernation and arrested development in winter (Conover 1988; Madsen et al. 2001; Melle and Skjoldal 1998; Nielsen and Hansen 1995). *C. glacialis* and *C. hyperboreus* are true arctic species while *C. finmarchicus* have their main distribution in the Atlantic. However, in Disko Bay all three co-exist (Conover 1988; Hirche 1987; Madsen et al. 2001).

In early spring, when the breakup of the sea ice triggers the formation of the spring bloom, the *Calanus* species ascend from the deep waters (Madsen et al. 2001) and start feeding to support egg production and refuel lipid reserves (Nielsen and Hansen 1995). When the bloom has ceased and

66 the *Calanus*-species have refilled their lipid stores, they stop eating and descend to the near-bottom
67 layers where they slow down their metabolism and over-winter in a stage of diapauses (Lee et al.
68 2006).
69
70 *Calanus hyperboreus* differs from *C. glacialis* and *C. finmarchicus* in a number of traits including
71 lifecycle, feeding and reproductive strategies. *C. hyperboreus* has the longest lifecycle of the three,
72 lasting typically between two and five years (Madsen et al. 2001; Scott et al. 2000). In contrast to
73 the two others, *C. hyperboreus* does not produce eggs after their ascent. They complete spawning
74 during winter in the deep waters using their internal lipid stores to fuel egg production and their
75 eggs ascend freely to the photic zone (Hirche and Niehoff 1996, Melle and Skjoldal 1998). Winter
76 spawning gives *C. hyperboreus* an advantage since the eggs have developed to the first feeding
77 nauplii-stage at the onset of the bloom. This enables nauplii of *C. hyperboreus* to undergo more
78 developmental stages during the productive season and to better exploit even short lasting blooms
79 (Melle and Skjoldal 1998). *C. hyperboreus* accumulates lipids more effectively than the two others
80 (Pasternak et al. 2001; Søreide et al. 2008) and can therefore descend to deeper waters earlier,
81 sometime between June and August (Madsen et al. 2001). Furthermore, the large bodymass and
82 huge lipid reserves of *C. hyperboreus* increases its ability to arrest development and thereby survive
83 in areas with high variability in ice cover (Scott et al. 2000) like the Disko Bay area.
84
85 The temperatures in arctic have been predicted to increase 4-7˚C over the next 100 years (ACIA
86 2005). Increasing temperatures will lead to thinner sea ice and a decrease in the ice covered period.
87 Furthermore, a warmer climate will increase melt water runoff to the sea and in combination these
88 factors can be expected to lead to an earlier stabilization of the water column and as a consequence
89 an earlier onset of the arctic spring bloom (Hansen et al. 2003). An increase in temperature will not

only prolong the productive season of the phytoplankton and indirectly influence the *Calanus*-community but may also directly impact the composition of the *Calanus*-biomass. Kjellerup et al. (submitted) has shown a significant effect of temperature on egg production and feeding of *C. finmarchicus* and *C. glacialis,* including evidence that *C. finmarchicus* has a stronger positive response to increasing temperatures than *C. glacialis*. If a warmer arctic climate leads to an increase in the proportion of *C. finmarchicus* in the total *Calanus*-biomass this could also have severe consequences for predators. As *C. finmarchicus* has relatively low energy content compared to the other two *Calanus*-species (Scott et al. 2000) this may lead to starvation on higher trophic levels.

Several studies of temperature effect on production of arctic copepods have been conducted. Among these, the relationship between temperature, food concentration and reproduction has been studied for *C. finmarchicus* and *C. glacialis* (Hirche and Kwasniewski 1997; Kjellerup et al. submitted; Madsen et al. 2008b). However, information on temperature effects on *C. hyperboreus* functional biology is lacking

The aim of the present study was therefore to investigate the effect of temperature and food availability on feeding and egg production of *Calanus hyperboreus* in Disko Bay before and during the phytoplankton spring bloom. In parallel, bloom dynamics and *in-situ* egg production of *C. hyperboreus* was followed.

MATERIALS AND METHODS

Study site. Sampling was conducted from February 10 to May 25 2009 about one nautical mile off the coast of Qeqertarsuaq in Disko Bay, Western Greenland (Fig. 1), at a station previously used in studies of the pelagic community of the Bay (Madsen et al. 2001; Madsen et al 2008b; Nielsen and Hansen 1995). Sampling on February 10 and from April 17 - May 25 was carried out from boat. On all other sampling dates, samples were taken through a hole made in the sea ice.

Hydrography and phytoplankton. Temperature, salinity and fluorescence in the water column was measured using a Seabird SBE25-01 CTD and water samples from 1, 20, 50, 75, 100, 150, 200 and 250 meters were taken with a 30 l Niskin water bottle. Water samples were kept cold and dark in 10 l plastic containers and transported back to the laboratory. Here 500 ml triplicates from each depth were filtered onto GF/F filters and extracted over night in 5 ml 96 % ethanol (Jespersen and Christoffersen 1987) and fluorescence was measured on a Turner Design Model 700 fluorometer before and after acid addition. Salinity measurements were calibrated against salinity samples taken approximately once a month (n = 4) throughout the study phase, and analyzed on an 8410-Portasal salinometer (Guildline) and fluorescence were calibrated with values from chlorophyll measurements at the eight depths.

Depth distribution of *Calanus hyperboreus*. Female of *Calanus hyperboreus* were sampled on February 10 and April 17 in five 50 meter depth intervals from 250 meters to the surface. This was done using a Hydrobios Multinet (type Midi) with nets of 50 μm in mesh size. The samples from each interval were immediately preserved in buffered formalin (2 % final concentration) and later females were enumerated and the proportion of females with well ripe gonads estimated.

***In situ* egg production.** *C. hyperboreus* females were sampled from the bottom to the surface using a WP-2 net (200 μm) and a large non filtering cod-end. The samples were diluted and stored in a thermobox. In the laboratory mature females were sorted out and placed individually in 600 ml polycarbonate bottles filled with 45 μm screened surface water. The bottles were incubated at 5 ˚C for 48 hours after which the content of each bottle was concentrated on a 45 μm filter. The eggs were counted and the prosome length of the females measured. As only mature females with visible well developed gonads were incubated the EP rate measured would overestimate population EP. Therefore EP rate were corrected for maturity of the female population by multiplying the observed EP with the proportion of mature females in the population based on the biomass samples (Fig. 5a). As carbon content of the females decreased by more than 50 % over the period investigated none of the previously established length weight regressions could be used to estimate carbon content of females. An exponential decrease in dry weight over the spring has been demonstrated for *C. hyperboreus* (Conover and Sieferd 1993). Therefore average carbon content of females were estimated for each date using an exponential regression between *in situ* carbon content of females collected the 10 of February and 17 of April (Table 4). Eggs from females sampled on February 10 were collected, immediately measured and a mean egg volume was calculated assuming a spherical shape. The carbon content of eggs was estimated using a volume to carbon conversion factor for *C. glacialis* and *C. finmarchicus* of $1.10*10^{-7}$ μg C μm^{-3} (Swalethorp et al. submitted). The carbon content of females and eggs were then used to calculate specific egg production (SEP). To estimate average total fecundity of females, an exponential regression was fitted to the observed EP. Using this regression a new daily EP was estimated and summed over the period of investigation.

Laboratory experiment. The laboratory experiment was conducted twice, each time over a two week period. The first experiment was set up on February 10, before the spring bloom and the second on April 17, during the spring bloom. Females used in the experiments were collected in the same manner as for the *in situ* egg production experiment, and kept on ice during handling.

Setup – Within three hours after the females were collected in the field they were carefully sorted out and incubated at five different temperatures: 0, 2.5, 5, 7.5 and 10 ˚C. Before starting each experiment the copepods were acclimated to the temperature for 3 to 6 days. Thirty females were used at each temperature, half of which were kept starved in 0.2 μm filtered sea water and the other half kept under saturated food conditions in 0.2 μm filtered sea water with 15 μg Chl a l^{-1} of the diatom *Thalassiosira weissflogii* (equal to 680 μg C l^{-1} (Reigstad et al. 2005)). Cultures of *T. weissflogii* were grown in a 12:12 light:dark cycle (2 Osram L, 36 W/840, Lumilux cool white) placed 40 cm away in 0.2 μm filtered seawater at room temperature and B_1 medium (1 ml l^{-1}) (Hansen 1989), silicate (0.9 ml l^{-1}) and vitamins (0.5 ml l^{-1}) added every other day. The cultures were renewed every 1 to 2 weeks and were constantly aerated.

Five thermo boxes filled with freshwater were used to keep the temperatures constant. Hobo thermo loggers were used throughout the experiment to log the temperature every 15 minutes (Table 1). In each thermo box two 10 l buckets filled with 8.3 l filtered sea water (0.2 μm) were placed and in one of these *T. weissflogii* was added. In every bucket the 15 females of *C. hyperboreus* were contained in a cylinder with false bottom (400 μm mesh). Every day the cylinders were carefully transferred to new buckets with 2.5 l filtered water at the corresponding temperature. The water from the old buckets was filtered with a 45 μm filter by reverse filtration and the concentrated samples were collected and preserved in lugol (2 % final concentration). Finally 5.8 l of this filtered

water was transferred to the new buckets and phytoplankton culture added to adjust food concentration for the fed females. The eggs and pellets collected in the experiment were counted daily. Length and width of approximately 30 pellets from every temperature, both starved and fed, were measured on day 2, day 7 and day 14 for both experiments in order to calculate an average fecal pellet volume. Only pellets at least three times the length of their width were counted and measured.

Mortality in the two experiments averaged 1 % day^{-1}. During the experiment dead females were removed, their prosome length measured and subsequently replaced with new individuals previously starved and kept at 5 ˚C. The females were acclimated to the proper temperature for approximately half a day before added to the buckets.

At the end of both experiments prosome length of every individual was measured and a mean female length at each treatment was calculated.

Fecal pellet production as a proxy for grazing – All fecal pellet measurements from the starved treatments were corrected for shrinkage due to lugol fixation, as this reduces the volume of pellets from starved individuals by 2 1% (Kjellerup et al. submitted). Fecal pellet volumes for the fed and starved treatments in each experiment were then calculated from the length and width of pellets assuming that they were of a cylindrical shape. As no significant effect of temperature on pellet volume was detected a mean volume for fed or starved females was calculated (Table 2). From these values the carbon content was calculated using a conversion factor of $8.03*10^{-8}$ μg C μm^{-3} (Reigstad et al. 2005) for the fed treatment and $4.75*10^{-8}$ μg C μm^{-3} (Seuthe et al. 2007) for the starved treatment. These factors are based on experiments with comparable food concentrations to this experiment using *C. finmarchicus* and *C. glacialis*.

The carbon content of females and fecal pellets were then used to calculate a cumulated carbon specific fecal pellet production (SPP_{cum}) for each treatment in each experiment (Fig. 6).

Egg production – The mean carbon content of eggs (estimated as described for the *in situ* egg production) was, together with the female carbon contents, used to calculate the cumulated carbon specific egg production (SEP_{cum}) for each treatment in each experiment (Fig. 6)

Carbon measurements - Before each experiment 24 of the females collected in the field were washed in filtered seawater (0.2 µm), their prosome length was measured and they were placed in pre-weighed tin capsules. They were then dried for 24 hours at 60^{o}C and stored frozen (-30 ^{o}C) for 8-10 months. After re-drying the samples the carbon content of each individual was measured on a CHNS Automatic Elemental Analyzer (EA 1110 CHNS, CE Instruments). This procedure was later repeated on approximately 7 females from each treatment after the experiments had ended. The carbon content were used to make a linear interpolation between the initial weight and the weight on the last day in each treatment for both experiments. These relationships were then used to estimate the carbon weight of females for each day of the experiments and subsequently to calculate daily carbon specific egg productions (SEP) and pellet productions (SPP).

Lipid measurements - Approximately 20 females before the experiments and 5 females from each treatment after the experiments were placed individually in lipid test tube with a Teflon cap. One ml chloroform:methanol solution (2:1 by volume) were added and the samples stored at -30 ^{o}C for 2 to 4 months and then at -80^{o}C for 7 months. Before analyzes, additional 2 ml chloroform:methanol solution were added. The samples were kept in ice filled trays and homogenized by ultrasound. Lipids were then extracted for 24 hours at -20° C (Folch et al.1957). Polar and non-polar lipid

231 classes were separated in NH_2-SPE columns. Phospholipids being polar lipids were estimated
232 spectrometrically from the phosphate content at 660 nm and converted by applying the
233 KH_2PO_4:diheptadecanoyl phosphatidylcholine conversion factor of 5.6 reported by Madsen (2005).
234 The non-polar lipid classes Wax esters (WE), triacylglycerols (TAG) and Sterols (STE) were
235 measured on a Dionex HPLC system (Dionex P680 pump and a Dionex Gina 50 auto-sampler) with
236 a Alltech MKIII Evaporative Light-Scattering detector using the Chromeleon (v. 6.80) software
237 described in Madsen et al (2008c). For a more detailed description see Swalethorp et al.
238 (submitted).
239
240 **Data analysis.** The effects of temperature and food availability were tested with a general linear
241 model (GLM, SAS Version 9.1, SAS Institute 2004) where the response (y) equals:
242 $y = \text{intercept} + k_{temp}*\textit{temp}+k_{food}*\textit{food}$ (Eq. 1)
243 The model describes change in either SPP, SEP, carbon, nitrogen, or lipid content over the
244 incubation period, where *temp* is the temperature in the experiment and *food* is a variable that has a
245 value of zero for starved females and one for fed females. In a few occasions (e.g. Eq. 5) the time of
246 the season was included by adding a third term ($k_{expt}*\textit{season}$) where the variable *season* has a value
247 of zero in the pre-bloom experiment and a value of one in the bloom experiment. During analysis of
248 lipid content the values for triacylglycerol (TAG) at 10°C were not included in the model as those
249 were unrealistically high and therefore considered as outliers (Table 6).
250 The SPP_{rate} and SEP_{rate} were estimated as the slopes in a two phase model using an iterative non-
251 linear SAS procedure for each of the ten different treatments to estimate the coefficients that best
252 explained the observed SPP_{cum} and SEP_{cum}. A visual inspection of the time course (Fig. 6) clearly
253 showed that the cumulated production increased linearly with time but also that a shift in the rate of

254 production, both upward and downward, occurred during many of the experiments. In order to
255 model this variability a two phase model was constructed:
256 if day $\leq$ l then p = day * k_1
257 if day > l then $p = k_1 * l + k_2 * (\text{day}-l)$ (Eq. 2)
258 where p is the cumulated production of pellets or egg, k_1 and k_2 are the coefficients for the daily
259 production and l is the time where the shift from k_1 to k_2 occur (Fig. 2). To avoid k_1 or k_2 to be
260 determined based on less than three data points, bounds where placed on l so that $3 \leq l \leq 13$. Tests
261 were performed with a free estimate of l and with a constant value of l=6, and they showed only
262 minor deviations in the estimates of k_1 and k_2. The parameters were estimated with SAS proc NLIN
263 (SAS Institute 2004). Changes in k_1 and k_2 with temperature was estimated using a simple linear
264 model followed by a t-test to test if the value was significantly different from zero. Carbon specific
265 values are given in % for SPP and SEP or as % d^{-1} for SPP_{rate} and SEP_{rate} ($\mu g\ C_{egg}\ \mu g\ C_{female}^{-1}\ day^{-1}$
266 *100). Unless otherwise noted all reported means are given ± standard error (SE).
267
268 **Energy budget for females**. An energy budget was established following Auel et al (2003) for the
269 two experiments and for *in situ* development of egg production, using the observed differences in
270 total lipid content between the beginning and the end of the experiments, the number of eggs
271 spawned, the lipid content of *C. hyperboreus* eggs (0.54 ± 0.01 µg Madsen et al unpublished data),
272 an energy content of lipids on 42.7 J mg^{-1} (Båmstedt 1986, Conover 1964), an respiration rate of
273 females on 0.26 ml 0_2 g DW^{-1} h^{-1} (Auel et al. 2003) converted to 10.4 ml 0_2 g C^{-1} d^{-1} (assuming a
274 carbon content of 60 % of dry weight (Omori 1969, Plourde et al 2003). Finally, to convert
275 respiration into daily energy requirements, an oxycaloric equivalent of 19.64 J ml^{-1} typical for lipid
276 based metabolism (Ikeda et al. 2000) was assumed. The energy budget for *in situ* egg production
277 were calculated by multiplying average female fecundity over the season with lipid content of eggs

and comparing it with the loss of female lipids occurring in the same period. Potential *in situ* egg production (egg female^{-1} d^{-1}) were calculated as:

$$EP_{potential} = \frac{(TL_{loss} * 42.7\ J\ mg^{-1} - 10.4\ ml\ O_2\ g\ C^{-1}\ d^{-1} * C_{females} * 19.64\ J\ ml^{-1} * 66\ d) / 42.7\ J\ mg^{-1}}{5.4*10^{-4}\ mg\ egg^{-1}} \quad \text{Eq. 3}$$

Where TL_{loss}= loss of total lipids (mg) and $C_{females}$= average carbon content of females (g) during the period. $C_{females}$ were estimated by averaging the carbon content of females calculated for each day over the period of 66 days (d) assuming an exponential relationship between measurements on the 10 of February and 17 of April.

RESULTS

Hydrography and phytoplankton. In February there was a clear pycnocline just below 100 meters. The temperature increased from about -1.6 ˚C in the surface layers to 3 ˚C in the bottom layers and the salinity varied from 32.9 in the surface to 34.2 at 250 m (Fig. 3A). The Chlorophyll a (Chl a) concentration was very low throughout the water column with values increasing toward the surface reaching a maximum concentration at 0.05 µg l^{-1} in 24 m. Due to malfunction of the CTD, no CTD cast from April can be presented. Instead Fig. 3B show point measurements of temperature, salinity and chl a done at 8 depths. In April a weak pycnocline at about 40 meters was present but the main pycnocline was still situated just below 100 meters. The temperature at the bottom was as in February, just around 3 ˚C. Chlorophyll a was found from the surface and down to 150 m showing that the phytoplankton spring bloom was well on the way. Highest concentrations were found above 50 m, peaking at 1 m at 2.2 µg l^{-1}.

Depth distribution of *Calanus hyperboreus.* From February to mid April the majority of the female population was found in the deepest strata (Fig. 4) at rather constant temperatures (3 °C) and very low food concentration. At the beginning of the second experiment on April 17, 10 % of the females were found in the surface waters indicating that the ascent towards the surface had just begun. By late April the majority of the females had ascended to surface waters to exploit the developing phytoplankton bloom.

***In situ* egg production.** *In situ* egg production (EP) and the proportion of ripe females were measured between February 10 and April 17. Egg diameter was 198 ± 7 µm, giving an egg volume of 40.8 ± 5 * 10^5 um^{-3} (n = 110, mean ± standard deviation (SD)). The measurements of *in situ* EP showed that EP was independent of the chlorophyll a concentration of the water (Fig. 5). Mean population EP was 54 ± 8 eggs $female^{-1}$ day^{-1} before the spring bloom and declined as the proportion of mature females declined, until the 17th of April at the beginning of the spring bloom were spawning was terminated. Clutch size was quite variable ranging between 9-227 egg pr clutch. During the main spawning event (February - March) average clutch size ranged between 52 ± 9 and 85 ± 20 eggs, whereas in April when EP had seized, clutch size averaged 16 ± 5 eggs.

Mean specific egg production (SEP) started at 3.5 ± 0.5 % d^{-1} and declined to 0.06 ± 0.03 % d^{-1} on April 8 until it reached zero on April 17. During the same period the integrated chlorophyll a concentration down to 100 meters increased from 3.2 mg Chl a m^{-2} to 76.9 mg Chl a m^{-2}.

Laboratory experiment. Surprisingly, no positive effect of temperature on neither egg nor fecal pellet production in the pre-bloom or bloom period was observed. Food had a clear positive effect on fecal pellet production whereas the effect on egg production was less clear (Fig 6).

326 *Pellet production as a proxy fro grazing* –The mean cumulated specific pellet production (SPP_{cum})
327 after two weeks varied from 0.1 to 7.9 % in the four groups of experiments. The separate GLM
328 models for the pre-bloom and bloom experiments showed a strong positive effect of food for both
329 periods (Table 3). Also in the experiment without food a pellet production was observed, and even
330 though the intercept in the GLM model (estimated value at 0 ^{o}C without food) was not significantly
331 positive, the mean SPP_{cum} after 2 weeks at higher temperatures were significantly different from
332 zero (Table 3). The pellets produced by starved females were clear and empty "ghost type" pellets
333 (Seuthe et al. 2007; Kjellerup et al. submitted). There was no significant effect of temperature on
334 SPP_{cum} but both coefficients where positive (with and without food, Table 3) and the temperature
335 coefficient in a model for just the pre-bloom experiment without food was significantly positive:
336 $SPP_{cum} = 10.3 \pm 2.9$ (p=0.038) + 1.57 ± 0.47 (p=0.046)*temp, r^2=0.78 (Eq. 4)
337 The effects of temperature and season were also significant in a common GLM-model for all
338 experiments:
339 $SPP_{cum} = 8.9 \pm 5.1$ (p=0.1) + 1.8 ± 0.6 (p=0.01)*temp + 60 ± 4.6 (p<0.0001) *food -17.0 ± 4.6
340 (p=0.0019) *season , r^2=0.92, (Eq. 5)
341 Thus, overall there was a tendency to a positive effect of temperature on SPP_{cum}
342 In Figure 7, a more detailed pattern for the relationship between SPP_{rate}, time, temperature and food
343 availability is shown. For the pre-bloom experiment there was an increase in the SPP_{rate} over time
344 as k_2 was higher than k_1, where as the opposite was observed in the bloom experiment with food
345 (Fig.7 A+B). The SPP_{rate} in the pre-bloom experiment ranged from 0.16 % d^{-1} (k_1 at 7.5 °C) to 1.1
346 % d^{-1} (k_2 at 7.5 °C) for fed females and from 0.046 % d^{-1} (k_1 at 2.5 °C) to 0.48 % d^{-1} (k_2 at 10 °C)
347 for starved females. During the bloom experiment the SPP_{rate} for fed females ranged from 0.2 (k_2 at
348 0°C) to 0.8 % d^{-1} (k_1 at 5 °C). In the starved treatments almost no fecal pellets were produced, thus
349 specific values were always lower than 0.019 % d^{-1} (k_1 at 10 °C). Changes in k_1 and k_2 with

350 temperature were analyzed with linear regression. The only experiment with a significant
351 relationship between SPP_{rate} and temperature was the pre-bloom experiment without food. Here the
352 SPP_{rate} increased by 0.044% ± 0.011 $°C^{-1}$ (p=0.026). For all other experiments the relationships with
353 temperature were positive but not significant (data not presented), however, as shown in eq. 4 and 5
354 the cumulated SPP after 2 weeks was significantly positively related with temperature.
355
356 *Egg production* – Values for egg production only exists for the pre-bloom experiment as the females
357 had stopped spawning at the beginning of the bloom experiment (Fig. 5). The cumulated specific
358 egg production (SEP_{cum}) over 2 weeks was independent of both temperature and food availability
359 (Table 3). Although food availability had no effect on SEP_{cum} it had a pronounced effect on the time
360 course of egg production (Fig. 7 C+D). In general fed females had a lover SEP_{rate} at all temperatures
361 in the first part of the experiment (k_1) compared to starved females, whereas the rate values were
362 reversed in later part of the incubation (k_2) so that after 14-15 days there was no effect of food.
363 SEP rates varied from 0-1.1% d^{-1}. Maximal SEP rates were found at the lower temperatures for
364 starved females (1.07 and 1.02 % d^{-1}, k_1 at 0 and 2.5°C respectively) and at high temperatures for
365 fed females (1.11 and 0.87 % d^{-1}, k_2 at 7.5 and 10°C, respectively). Nevertheless there was not a
366 significant effect of temperature on SEP_{rate} for neither fed nor starved females (Table 3).
367
368 *Carbon content* – Overall *C. hyperboreus* lost carbon during most of the experiments (Fig. 8). The
369 loss was most pronounced in the pre-bloom experiment where the average loss for both fed and
370 starved females after two weeks was 34 % of the initial carbon content. In the bloom experiment the
371 initial carbon content of the females had decreased by 58 % compared to the pre-bloom experiment.
372 After two weeks incubation a significant difference between fed and starved treatments was
373 observed (Table 4). Fed females were able too maintain their starting weight or even gain weight

374 during the experiment, whereas starved females showed a net loss of 17 % carbon. Food availability
375 had a positive effect on the carbon content in the bloom experiment (p=0.0013) whereas the effect
376 was insignificant in the pre-bloom experiment (p=0.68). The effect of temperature on final carbon
377 weight was not significant in either of the two experiments when tested separately or when tested
378 with a GLM model across the two periods. There was, however, a tendency to a negative effect of
379 temperature of about 1 % $^{o}C^{-1}$ in both experiments (Table 4).
380
381 *Nitrogen content* – The overall pattern for changes in nitrogen content resembled that of carbon.
382 There was a loss in nitrogen content at all temperatures between 8 and 20 % except for the bloom
383 experiments with food where the nitrogen content increased by 22 %. There was no effect of
384 temperature or food on the nitrogen loss in the pre-bloom experiment, whereas in the bloom
385 experiment there is a clear positive effect of food availability (Table 4).
386
387 *Lipid content* – Similar to the pattern described for carbon and nitrogen content, there was an
388 overall loss of total lipids during the experimental periods that in general were not related to either
389 food or temperature (Table 5, Fig. 9). The lipid content of the females was analyzed in five groups:
390 Total lipids (TL), wax esters (WE), triacylglycerol (TAG), phospholipids (PL) and sterols (STE).
391 As sterols constituted less than 2 % of total lipids and no significant change during the experiments
392 were observed, results are not included in this section. However data for sterol content is available
393 in Table 6. In the pre-bloom experiment total lipid content (TL) of the females decreased with 45-
394 70% in starved treatments and 30-52% in fed treatments (Fig. 9, Table 6). The lipid composition
395 was dominated by WE which on average constituted 78-92% of total lipids in all treatments. The
396 trend in WE therefore clearly mimicked the trend in total lipids (Fig. 9 A+B). TAG constituted less
397 than 3 % of total lipids. PL constituted on average 9-18% of total lipids in all treatments. There was

398 a clear positive effect of food on the PL content where PL increased in fed females and decreased in
399 starved females. There was no significant effect of temperature (Table 5).
400 From pre-bloom to bloom experiment, the *in situ* content of lipids decreased by 74%. Despite this
401 large decrease in TL the amount of TAG remained the same (Table 5) The lipid composition of the
402 females at the end of each experiment was similar to what was found in the pre-bloom experiment.
403 WE dominated with 72-89% of total lipids, followed by PL (8-24%) and with TAG constituting less
404 than 2 % (Table 6). Again the trend in WE mimicked the trend in total lipids where no significant
405 trend related to either temperature or food was apparent (Fig. 9 E+F, Table 5). The amount of TAG
406 decreased significantly in all treatment ranging from an 82-75% loss. The decrease was independent
407 of temperature and food. The amount of PL increased for both fed and starved females at low
408 temperatures, but at temperatures >5 °C, PL of starved females decreased whereas PL in fed
409 females continued to increase to a maximum of 181% at 10 °C (Fig 8 H). The effect of food was as
410 in the pre-bloom highly significant whereas the effect of temperature was not (Tabel 6).
411
412 DISCUSSION
413
414 ***In situ* condition.** The spring bloom in 2009 was well on the way in mid-April when spawning of
415 *C. hyperboreus* was terminated (fig. 5). This confirms that egg production in *C. hyperboreus* is
416 uncoupled from the phytoplankton spring bloom, which has previously been shown in Disko Bay
417 (Madsen et al. 2001), the Greenland Sea (Hirche and Niehoff 1996) and the Barents Sea (Melle and
418 Skjoldal 1998). The relative distribution of *C. hyperboreus* females showed that they were at over-
419 wintering depths in February and had only just started their ascent in mid-April when chlorophyll
420 content of the water was rising, in agreement with the assumption that *C. hyperboreus* over-winters

421 in the near bottom layers and ascend to the surface when the spring bloom develops to feed on the
422 high phytoplankton concentrations.
423
424 *Egg production* - The *in situ* egg production showed a maximum of 54 eggs female^{-1} day^{-1} in
425 February, after which EP decreased steadily until mid-April where spawning ended. Madsen et al.
426 (2001) measured *in situ* EP of *C. hyperboreus* in Disko Bay on one occasion in the middle of March
427 1997 and found EP to be 33.3 ± 3.4 eggs female^{-1} day^{-1}. In this study EP in March ranged between
428 10 and 21 eggs female^{-1} day^{-1}. In the Greenland Sea a maximum production of 23 eggs female^{-1} day^{-1}
429 was found in February 1988 and 1989 whereas data from November and December showed an EP
430 as high as 148 eggs female^{-1} day^{-1} (Hirche and Niehoff 1996). Generally, higher EP rates were
431 found in November and December with values decreasing towards March. This corresponds well
432 with what were shown in our study; a clear reduction in egg production as spring approached. When
433 average female fecundity was estimated from February to April a total number of 1164 eggs female^{-1}
434 were found. During the same period a decrease in lipid content of 74 % were seen. This number
435 compares well with previous studies where female fecundity was measured in the laboratory.
436 Conover (1967) found female egg production ranging from 429-3397 eggs female^{-1} year^{-1}, while
437 other studies have observed average fecundity between 762-1500 eggs female^{-1} (Plourde et al. 2003;
438 Conover and Sieferd 1993; Hirche and Niehoff 1996) and a carbon loss over the same period of 81
439 % (Plourde et al. 2003). Comparing the number of eggs laid over the spawning period with the
440 amount of TL lost in that same period and knowing the TL content of eggs, it was calculated that 86
441 % of the lost lipids should be converted into eggs. This number however is leaving too little energy
442 to cover metabolic costs. If instead a potential EP was calculated based on the lipid loss subtracted
443 the energy needed for sustaining metabolism during the period (assuming a respiration rate of 0.26
444 ml 0_2 g DW h^{-1} and carbon content to be 60% of DW), potential egg production would be only 693

445 eggs female^{-1}, which equals 51 % of the lost lipids and compares well with the assumption that 42
446 % of an observed loss in *C. hyperboreus* female dry weight would be converted into reproductive
447 products (Conover and Sieferd 1993).
448
449 **Laboratory experiments.** *Egg production* - The specific egg production rate in the laboratory
450 experiment showed no significant temperature or food dependence indicating that EP is determined
451 by the lipid content of the female, and not affected by environmental conditions during the
452 spawning phase. As a positive effect of temperature was documented for the arctic *C. glacialis*
453 (Kjellerup et al. submitted) it was somewhat surprising not to observe a similar temperature
454 response in *C. hyperboreus*. Kjellerup (submitted) showed that SEP_{rate} of *C. glacialis* in a pre-
455 bloom situation peaked at 7.5˚C. The SEP_{rate} would be expected to increase with temperatures until
456 a certain limit where high temperatures would no longer be beneficial. However, the results suggest
457 that *C. hyperboreus* is a strictly arctic species that does not benefit from higher temperatures.
458
459 As *C. hyperboreus* spawns prior to the spring bloom when no food is available, the lack of a
460 positive effect of food on EP is as expected. The two other *Calanus*-species in Disko Bay do not
461 spawn until the beginning of the bloom (Madsen et al. 2001; Madsen et al 2008b) and therefore
462 shows a completely different food-response. A significantly lower EP have been found in starving
463 females for both *C. glacialis* and *C. finmarchicus* (Madsen et al. 2008b; Kjellerup et al. submitted).
464 Even though no significant effect of food was found in this study after the 2 week period,
465 differences in the course of production was observed, where SEP_{rate} increased for fed females and
466 decreased for starved females in the last part of the experiment ($k_{2,}$ Fig. 7C & D). Therefore there
467 might have been a positive effect of food if the experiment had continued for a longer period of
468 time. A possible explanation for the initial lower EP of fed females is that the animals need to

prepare their metabolism to feeding when exposed to food, and that this take resources away from egg production. Hence the effect of food on EP rate may depend on the pre-feeding history of the animals. This may explain the opposing results on the effects of food on egg production of *C. hyperboreus* that have been found previously. Some studies have found EP to be independent of food (Conover 1967; Plourde et al. 2003) whereas other studies conducted later in the season have found *C. hyperboreus* females to produce more eggs when food was available as a supplement to internal lipids (Melle and Skjoldal 1998; Sømme et al 1934; Niehoff 2007 Fig. 9). In general EP rates measured in the laboratory experiment was lower than the *in situ* rates measured at the same time. As different incubation methods were used the values found should not be compared directly. The handling method was rougher in the laboratory experiment where a large amount of water was concentrated on a small sieve which increased the risk of breaking and disintegrating eggs. Because of the large lipid content, eggs of *C. hyperboreus* have been shown to be rather fragile. Furthermore, neither of the methods prevented cannibalism of eggs as eggs of *C. hyperboreus* are positively buoyant and hence does not sink through the sieve. Therefore egg production in this study may be underestimated. Though average SEP in the laboratory experiment were found to be rather low (ranging between 0.3-0.6% d^{-1} in the 15 day period) it is still comparable with what was reported in another laboratory study where SEP were 0.7% d^{-1} measured over a nine day period (Hirche and Niehoff 1996).

Fecal Pellet production - As could be expected the fecal pellet production showed significant higher rates in fed females both before and during the spring bloom. In the first experiment there seemed to be a lag phase in SPP that could be due to the fact that these females were collected long before the spring bloom and needed some time before they reached a maximal intake of food. As a result of this, the highest production was not reached until six to seven days into the experiment.

493 The opposite tendency was observed in the second experiment where the pellet production started
494 out high and then leveled of. The reason for this opposite tendency is unknown. Kjellerup
495 (submitted) found a lag phase for both *C. glacialis* and *C. finmarchicus* not only before the bloom
496 but also during it. SPP_{rate} was higher before the spring bloom than after at all temperatures for both
497 fed and starved females. One explanation for this decrease over the spring could be differences in
498 assimilation efficiency related to the lifecycle of the females. In the first experiment the females
499 may not be ready to feed as they are dwelling in deep waters where ambient food concentration is
500 very low. As they would normally not encounter food at this time of year, they may not be able to
501 assimilate the ingested food as effectively as later in the season when the bloom is developing. This
502 might also explain the difference in food response observed between the prebloom and bloom
503 experiment. Even though fed females seemed to be grazing in both periods an effect of food on
504 bodyweight was only obvious in the prebloom experiment (Fig. 8).
505 In the second experiment the bloom is underway and ingested food provide energy to regeneration
506 of gonads and lipid stores, which have been exhausted by the lipid-fueled spawning over the winter.
507 Indeed initial carbon and lipid content had decreased 2 and 4 times respectively between the two
508 experiments (Fig 8 + 9). These stores would need to be refilled if the females were to reproduce
509 another season. Iteroparity is likely to occur in *C. hyperboreus* (Conover and Sieferd 1993; Hirche
510 1997) as it has been suggested for the closely related *C. glacialis* in the White Sea (Kosobokova
511 1999), in the Barents Sea (Tande et al. 1985) as well as in the Disko Bay area (Kjellerup et al.
512 submitted).
513 Furthermore, *in situ* investigations from Disko Bay in 2008 showed a 3.5 fold increase in carbon
514 content and 4.7 fold increase in lipid content of *C. hyperboreus* females over the summer, indicating
515 such a refueling process (Swalethorp et al. submitted). As the second experiment was conducted
516 very early in the bloom and only a slight increase in carbon and lipid content was observed for fed

females, it is likely that the animals had just started feeding *in situ* and the rebuilding of lipids stores had not yet begun.

Another explanation for the lower SPP_{rate} in the bloom experiment could be that the spent females are about to die (Head and Harris 1985). This could also explain why k_2 is consistently lower than k_1 in the second experiment as dying females would slowly stop all feeding. The feeding of the females in the first experiment could in such a scenario be explained by a need to attain some additional energy for the egg production (Melle and Skjoldal 1998; Niehoff 2007; Takahashi 2002). Even though no effect of food on EP was seen in this experiment the finding of a higher EP rate in the last part of the experiment for fed females makes this a likely explanation. Further studies of the fate of the spawning females should be made to confirm such theories. In general we would expect to see the same temperature dependency in pellet production as in egg production; a low production at low temperatures, a temperature optimum and a decline at temperatures too high. As was the case for the SEP_{rate} no convincing effect of temperature was observed for SPP_{rate} neither before nor during the spring bloom in the temperature range investigated here.

The measured SPP rates ranging from 0.003-1.1 % d^{-1} was low compared to values obtained for *C. finmarchicus* and *C. glacialis* in a similar designed experiment from 2008 were values were ranging from 0.006-20.4 % d^{-1} (Kjellerup et al. submitted) but comparable to *in situ* values measured for *C. hyperboreus* in the area during the same year which ranged from 0.01-0.46 % d^{-1} (Swalethorp et al. submitted). The fecal pellets produced in the starved treatments are not due to grazing but due to forced elimination of the intestine epithelium (Besiktepe and Dam 2002) fueled by the stored lipids as also shown by Kjellerup et al. (submitted).

The carbon and lipid content over the course of the experiment. The female loss of carbon and lipids during the pre-bloom experiment, as well as the loss observed *in situ* between the pre-bloom and the bloom experiment, are partly due to the production of eggs during this period. Comparing mean lipid loss (462 µg), mean number of eggs laid (211) and knowing the lipid content of an egg (0.54 µg Jung-Madsen et al. unpublished data) it was found that in average 26 % of the lipid loss during the incubation was channeled directly into egg production. This is however most likely underestimated because of the underestimated egg production rate (see earlier discussion). On the other hand, if assuming an EP rate equal to the *in situ* rate (54 eggs female^{-1} day^{-1}) over the same period (15 days), then 96% of the lipids should have gone into reproduction, leaving too little energy to cover metabolic costs. The 26 % however fits better with what was calculated for the *in situ* situation and what was estimated by Conover and Sieferd (1993).

Temperature effects on *Calanus hyperboreus*. The temperature interval of 0 ˚C to 10 ˚C that the females were exposed to in this study did not reveal a temperature response in the monitored rates. Comparable studies of temperature effect on both SPP and SEP for *C. hyperboreus* is not available but temperature related studies investigating egg production and lifecycle patterns exist. Conover (1962) investigated the respiration of *C. hyperboreus* over a range of 2˚C to 8˚C and found the species to regulate well over this interval if previously acclimatized to the temperature. Ringuette et al. (2002) found chlorophyll a concentration and not temperature to have the greatest impact on recruitment of *C. hyperboreus* copepodites, whereas they found the recruitment of *C. glacialis* to be more temperature dependent. On the other hand Plourde et al. (2003) investigated egg production at a temperature interval of 0˚C and 8˚C for *C. hyperboreus* and concluded that high temperatures could reduce the reproductive output of *C. hyperboreus* with 30% and shorten the spawning period

564 significantly. Hirche (1987) studied respiration and mortality at increasing temperatures (-0.8 to 17
565 °C) and found *C. hyperboreus* to be the least temperature tolerant of the three *Calanus* species.
566 Both *C. glacialis* and *C. finmarchicus* have been shown to have a positive response to higher
567 temperatures on pellets and egg production rates (Kjellerup et al. submitted). Thus, the finding that
568 *C. hyperboreus* shows no temperature response suggest potential future changes in composition of
569 the *Calanus*-community in Disko Bay. In a warmer climate the fact that *C. finmarchicus* has a clear
570 advantage of temperatures up to at least 10˚C while *C. glacialis* increases production rates up to
571 7.5˚C could give these two species a competitive advantage over *C. hyperboreus*.
572
573 Other opposing and more indirect effects of a warmer climate will also influence the future
574 biomass-composition. This is illustrated in two studies by Ringuette et al. (2002) and Plourde et al.
575 (2003). Ringuette et al. (2002) suggested that a longer productive season in the arctic as a
576 consequence of a warmer climate could result in an earlier recruitment of *C. glacialis* and *C.*
577 *hyperboreus* and a possibility for them to complete their lifecycles in fewer seasons and thereby
578 increase their population sizes. Plourde et al. (2003), however, showed that a warmer climate would
579 lead to a shorter winter-spawning season for *C. hyperboreus* and a subsequent mismatch between
580 the development from egg to the first feeding nauplii stage and the phytoplankton spring bloom
581 which could lead to a decrease in population size. Hence, it is very difficult to predict exactly how
582 the composition of the *Calanus*-biomass will change with increasing temperature in the future.
583
584 In conclusion, this study demonstrates the winter-spawning strategy of *C. hyperboreus* where
585 reproduction is coupled to the spring bloom with a time lag of one year. Furthermore, it was
586 documented that temperature had no positive effect on neither pellet nor egg production of *C.*
587 *hyperboreus*. This finding suggests that this high-energy *Calanus*-species will loose in competition

588 with the two smaller *Calanus* species in a future warmer climate because of their ability to exploit
589 the higher temperature to increase grazing and egg production rates.
590
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595
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Figure 1. Map of the study site in Disko Bay

Figure 2. Illustration of the model used to establish specific fecal pellet production rate (SPP_{rate}) and specific egg production rate (SEP_{rate}) from the cumulated production. k_1 (% C of body C day^{-1}) lasts from day 1 to *l* (the intercept between the fitted lines) and k_2 (% C of body C day^{-1}) last from *l* to day 14.

Figure 3. Hydrography of Disko Bay on February 10 (A) and April 17 (B), 2009. Thick line = salinity, dotted line = temperature (°C), and thin line = chl a (µg l^{-1}). Fig. A = CTD data, Fig B = point measurements of parameters in 8 depths (cross-symbols) due to malfunction of CTD on April 17^{th}.

Figure 4. Relative depth distribution of *Calanus hyperboreus* females and integrated chlorophyll a (shaded area) in the different depths from February 10 to May 25. First Y-axis show the relative distribution of females, second Y-axis integrated chlorophyll a. Note different scale on second Y-axis.

Figure 5. A: Percentage of mature females, B: I*n situ* egg production (EP) and C: Specific *in situ* egg production (SEP) ± SE, between February and April 2009. The shaded area is integrated chlorophyll a down to 100 meters.

Fig 6. Cumulated specific egg production (SEP_{cum}) and cumulated specific fecal pellet production (SPP_{cum}) for *C. hyperboreus* before and during spring bloom at 0˚C, 2.5˚C, 5˚C, 7.5˚C and 10˚C.

The filled circles are fed females and the empty circles are starved females. Modeled values of production (Eq. 2) used for estimating k_1 and k_2 are indicated as thin lines.

Figure 7. Specific fecal pellet production rate (SPP_{rate}) ± SE before and after the bloom (A+B) and specific egg production rate (SEP_{rate}) ± SE before the bloom (C+D), as a function of temperature. k_1 represent the first, and k_2 the last, part of the experiment. The filled symbols are fed females and the empty symbols are starved females

Figure 8: Carbon content at the end of the incubation period for the pre-bloom and bloom experiment at temperatures from 0-10 °C. Values are given in % of start content ± SE. The filled circles are fed females, the empty circles are starved females, the solid line represent an unchanged carbon content and the cross is the carbon value at the beginning of each experiment. The initial carbon value is also given at the bottom of each figure in µg C $female^{-1}$.

Figure 9. Total lipid (TL), wax ester (WE), triacylglycerol (TAG) and phospholipids (PL) at the end of the incubation period for the pre-bloom and bloom experiment at temperatures from 0-10°C. Values are given per female as % of start content ± SE. The filled circles are fed females, the empty circles are starved females, the solid line represents unchanged lipid content and the cross is the lipid value at the beginning of each experiment. The initial lipid value is also given at the bottom of each figure in µg lipid $female^{-1}$.

Figure 1.

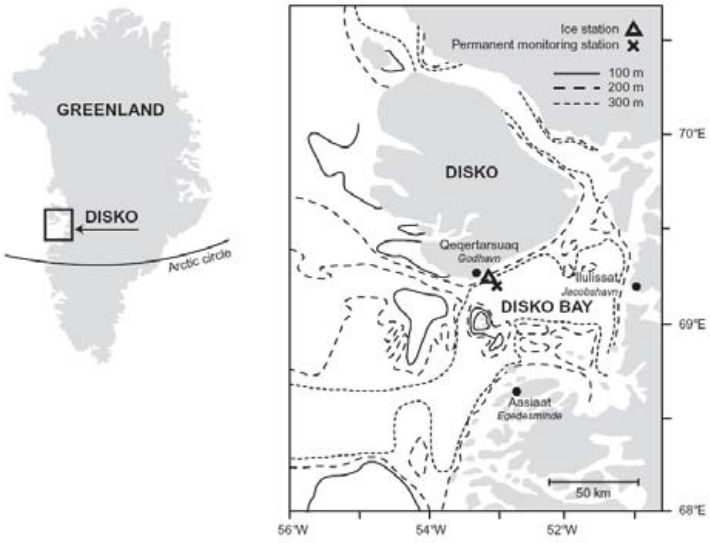
GREENLAND
DISKO
Arctic circle
Ice station
Permanent monitoring station
100 m
200 m
300 m
DISKO
Qeqertarsuaq
Godhavn
Ilulissat
Jacobshavn
DISKO BAY
Aasiaat
Egedesminde
50 km
70°E
69°E
68°E
56°W
54°W
52°W

Figure 2.

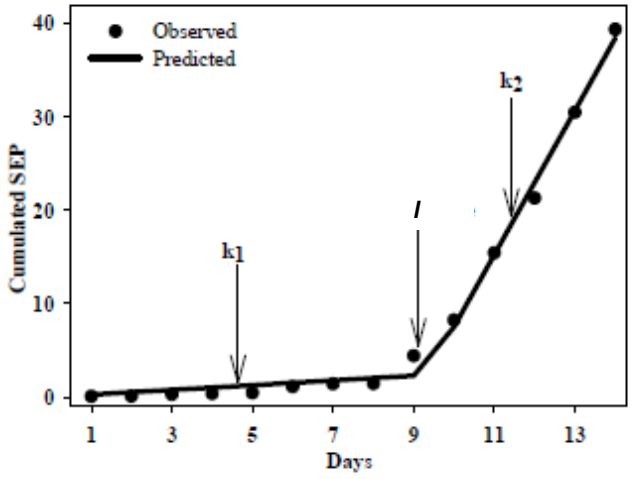
Observed
Predicted
k1
k2
I
Cumulated SEP
Days
0
10
20
30
40
1
3
5
7
9
11
13

Figure 3.

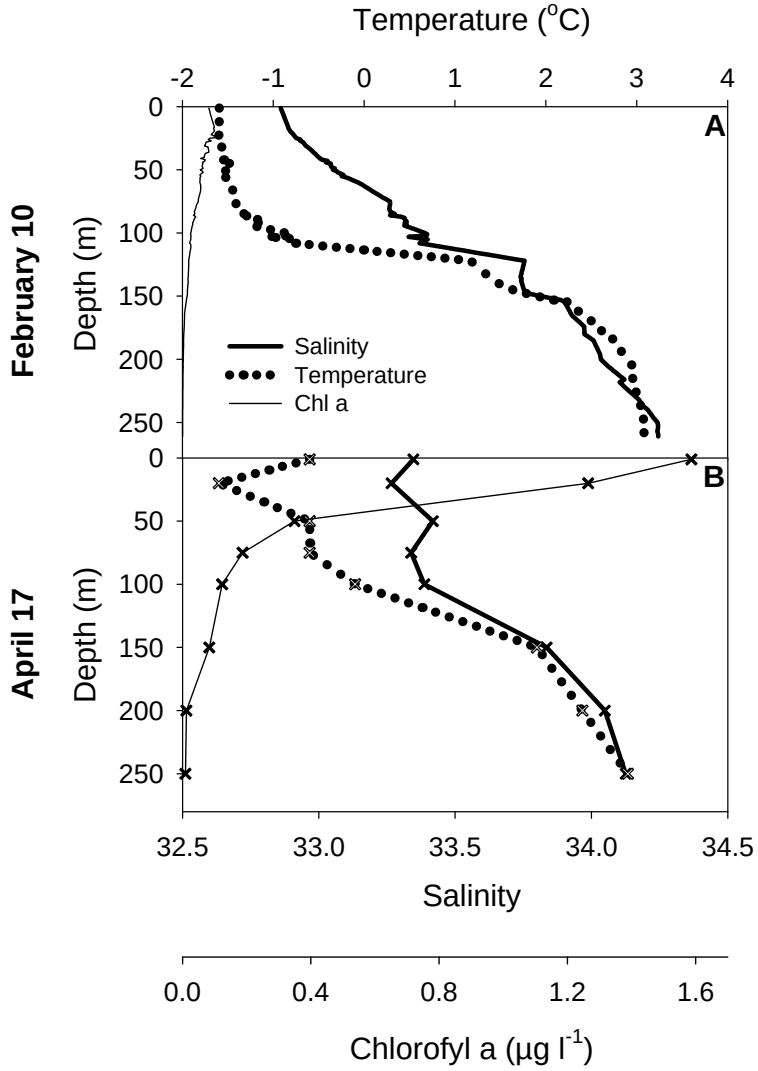
Temperature (°C)
-2
-1
0
1
2
3
4
A
B
February 10
April 17
Depth (m)
0
50
100
150
200
250
Salinity
Temperature
Chl a
32.5
33.0
33.5
34.0
34.5
Salinity
0.0
0.4
0.8
1.2
1.6
Chlorofyl a (µg l-1)

Figure 4.

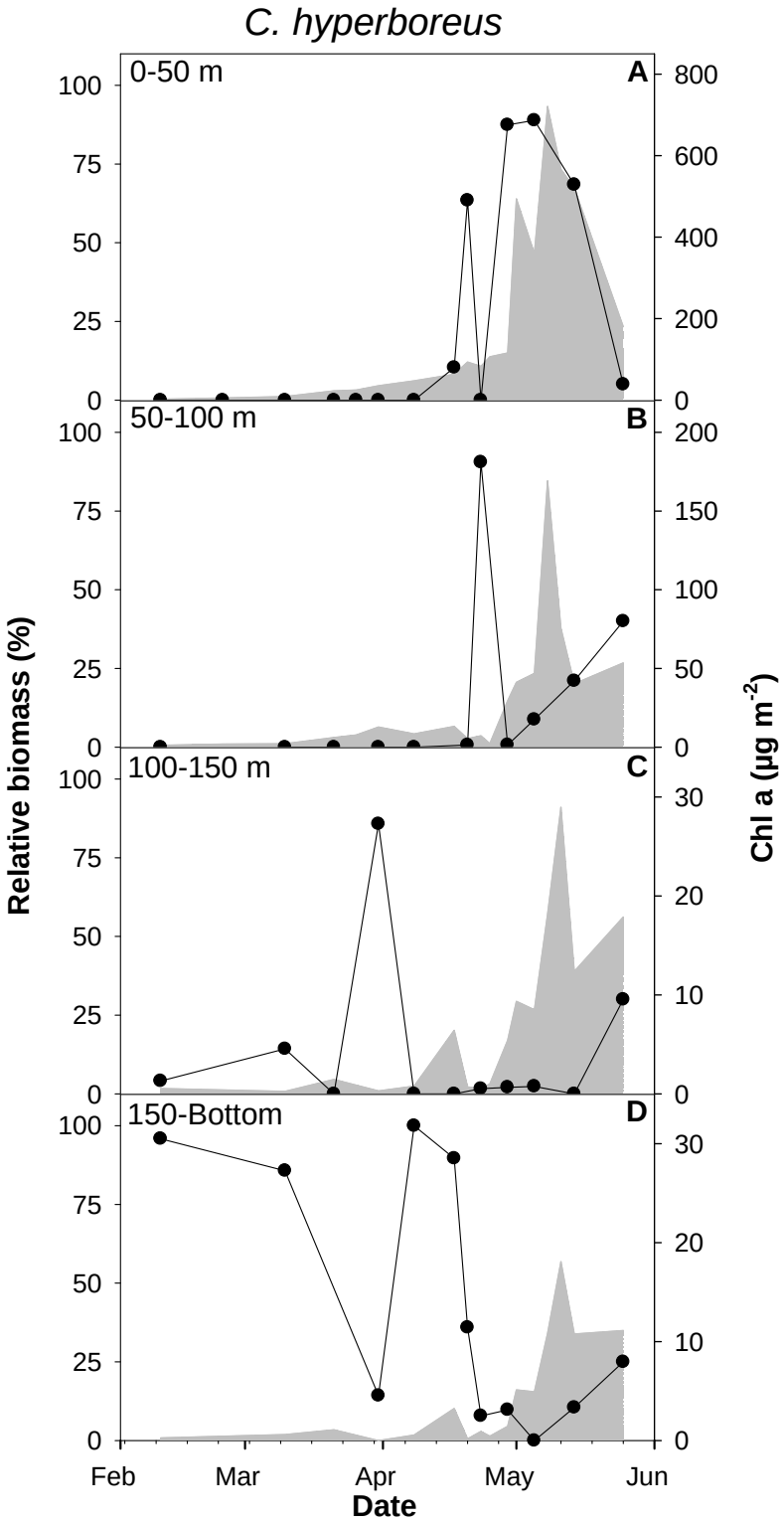
C. hyperboreus
0-50 m
A
50-100 m
B
100-150 m
C
150-Bottom
D
Relative biomass (%)
Chl a (µg m-2)
Feb
Mar
Apr
May
Jun
Date

Figure 5.

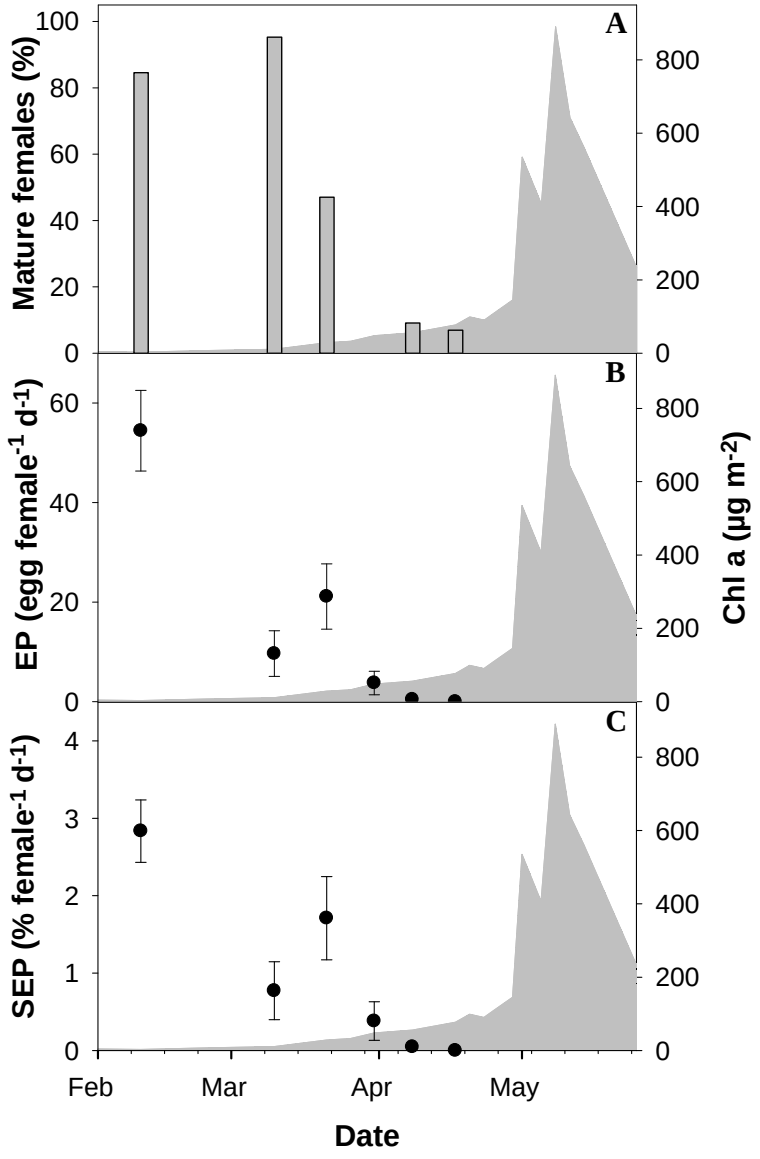
A
B
C
Mature females (%)
EP (egg female-1 d-1)
SEP (% female-1 d-1)
Chl a (µg m-2)
Feb
Mar
Apr
May
Date

Figure 6.

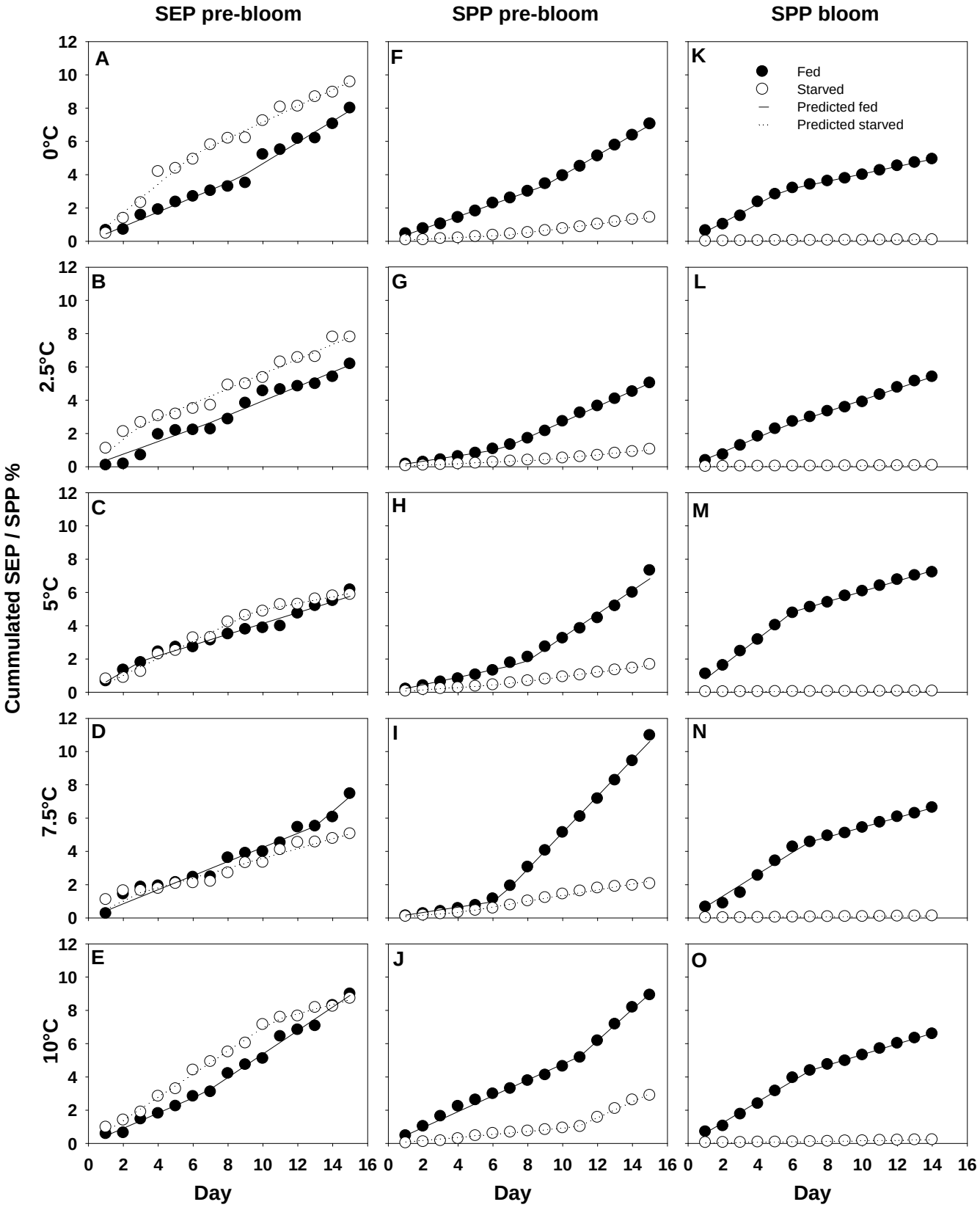
SEP pre-bloom
SPP pre-bloom
SPP bloom
Fed
Starved
Predicted fed
Predicted starved
Cummulated SEP / SPP %
0°C
2.5°C
5°C
7.5°C
10°C
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
Day
Day
Day

Figure 7.

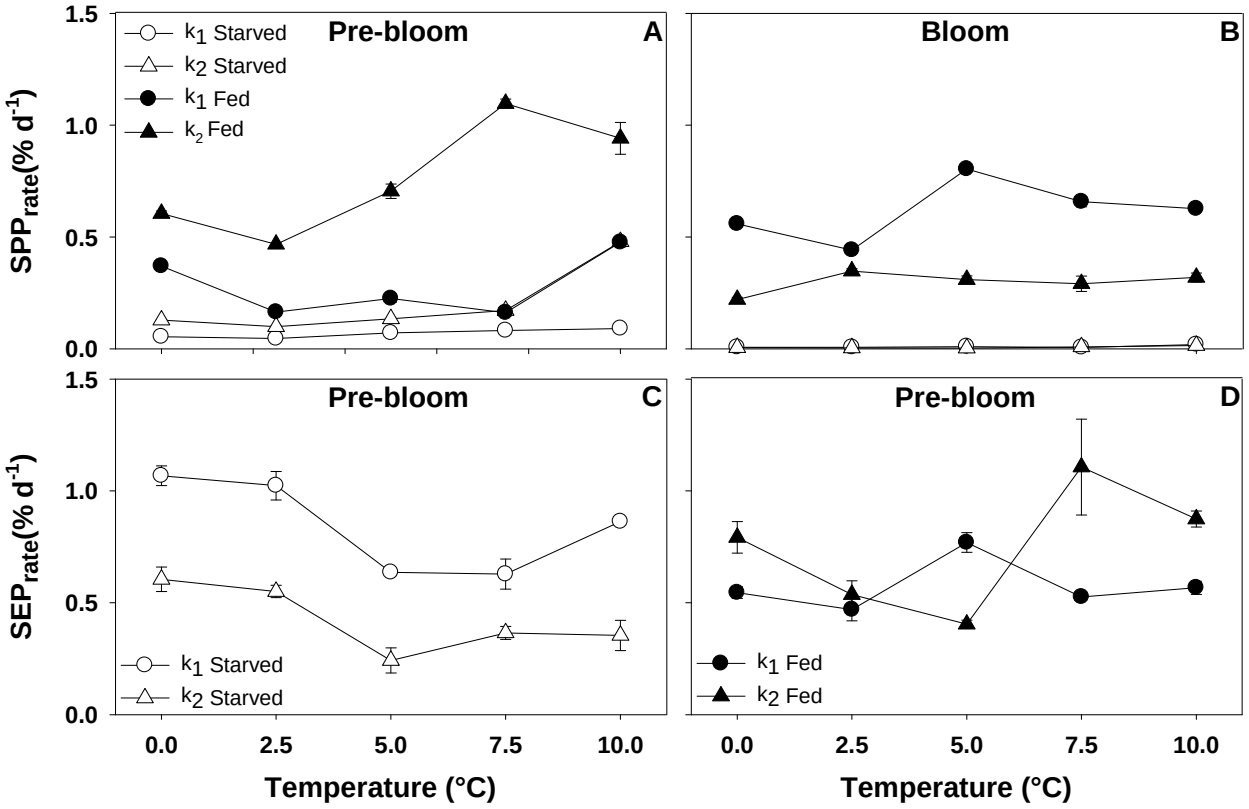
Pre-bloom
A
Bloom
B
Pre-bloom
C
Pre-bloom
D
k1 Starved
k2 Starved
k1 Fed
k2 Fed
SPPrate(% d-1)
SEPrate(% d-1)
1.5
1.0
0.5
0.0
0.0
2.5
5.0
7.5
10.0
Temperature (°C)

Figure 8.

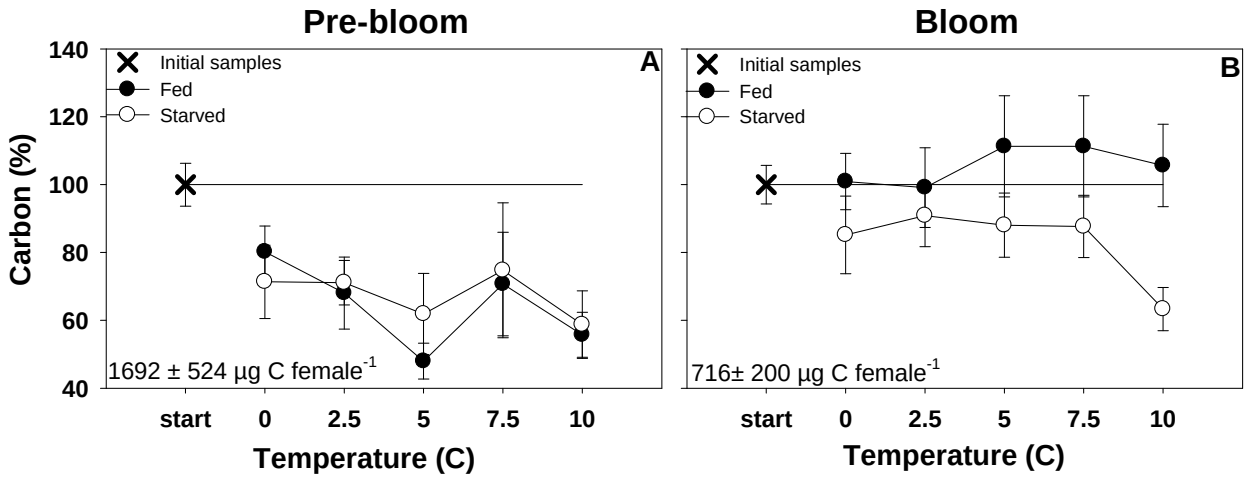
Pre-bloom
Bloom
Initial samples
Fed
Starved
A
B
Carbon (%)
140
120
100
80
60
40
1692 ± 524 µg C female-1
716± 200 µg C female-1
start
0
2.5
5
7.5
10
Temperature (C)

Figure 9.

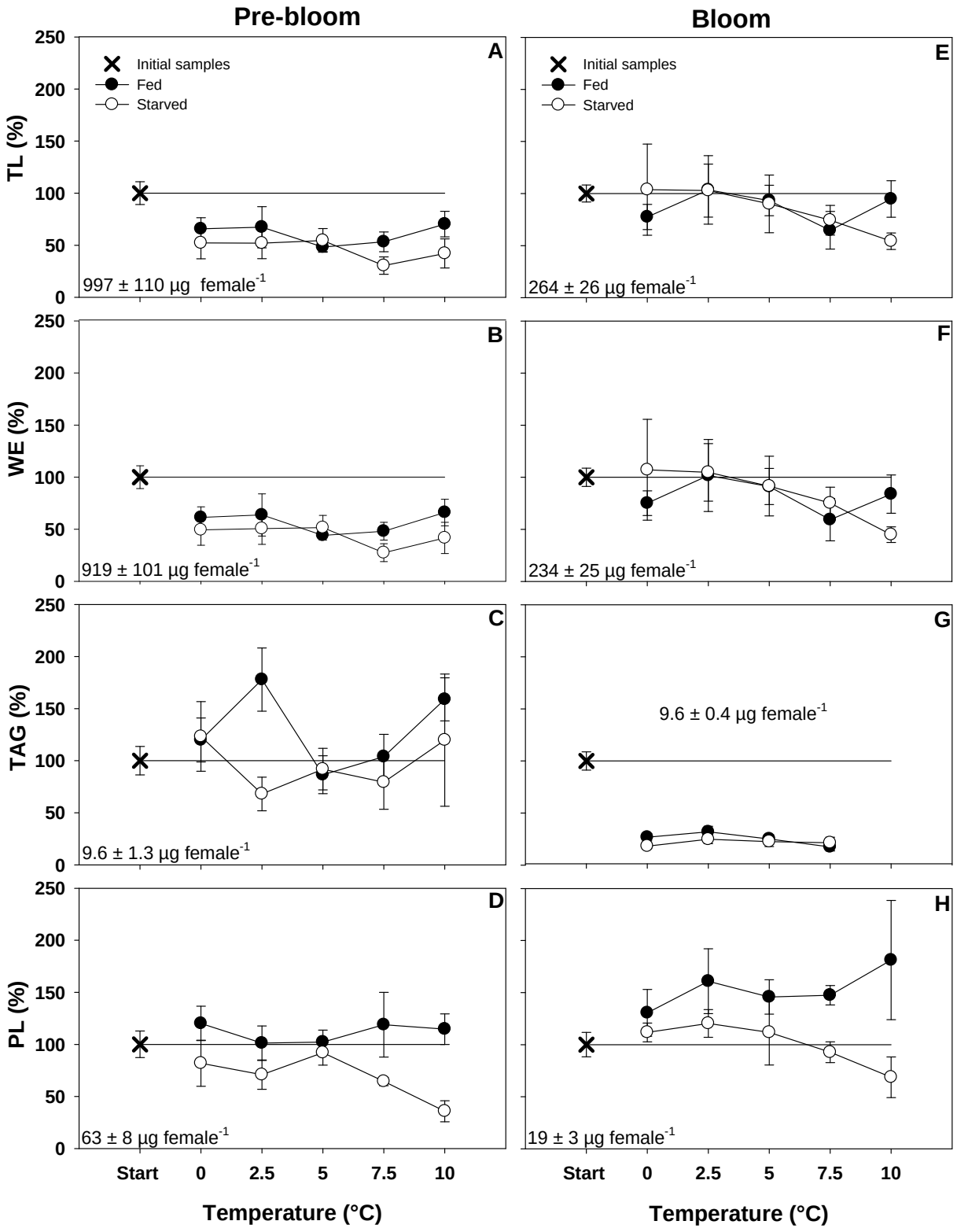
Pre-bloom
Bloom
Initial samples
Fed
Starved
A
B
C
D
E
F
G
H
TL (%)
WE (%)
TAG (%)
PL (%)
997 ± 110 µg female-1
919 ± 101 µg female-1
9.6 ± 1.3 µg female-1
63 ± 8 µg female-1
264 ± 26 µg female-1
234 ± 25 µg female-1
9.6 ± 0.4 µg female-1
19 ± 3 µg female-1
Start
0
2.5
5
7.5
10
Temperature (°C)

Table 1. Mean temperature ± SD in laboratory experiments logged every 15 minutes.

Intended temperature (°C)	Mean temperature ± SD (˚C)	
	Pre-bloom	**Bloom**
0	0.5 ± 0.7	0.7 ± 1.3
2.5	2.6 ± 0.3	2.7 ± 0.2
5	5.1 ± 1.0	5.8 ± 0.8
7.5	7.3 ± 0.7	7.5 ± 0.4
10	10.0 ± 0.4	10.1 ± 0.5

Table 2. Mean fecal pellet volume ± SD for fed and starved females in each experiment

	Pre-bloom		**Bloom**	
	n	**Pellet volume (10^5 µm^{-3})**	**n**	**Pellet volume (10^5 µm^{-3})**
Fed	460	48.1± 23.7	456	32.9 ± 13.0
Starved	425	36.6 ± 20.7	174	12.3 ± 6.5

Table 3: Statistics for the cumulated specific pellet and egg production (SPP_{cum}/SEP_{cum}) of *Calanus hyperboreus* at the end of each experiment. Intercept and coefficients for GLM-models (Eq. 1) as a function of temperature and food availability are given for the two periods of the season. Mean values are calculated across five experiments at temperatures from 0 to 10 ^{o}C (n=5) and are as all other values given ± SE. Significant p-values are highlighted.

		Mean values (%)		**Glm model parameters (%)**		
		Fed	**Starved**	**Intercept**	**Temp**	**Food**
SPP_{cum}	**Pre-bloom**	7.9 ± 1.0	1.8 ± 0.3	0.46 ± 0.84 p=0.60	0.27 ± 0.12 C^{-1} p=0.057	6.0 ± 0.84 **p=0.0002**
	Bloom	6.2 ± 0.4	0.1 ± 0.03	-0.37 ± 0.37 p=0.35	0.01 ± 0.5 C-1 p=0.11	6.0 ± 0.37 **p<0.0001**
SEP_{cum}	**Pre-bloom**	7.4 ± 0.5	7.4 ± 0.9	7.5 ± 1 **p=0.002**	-0.03 ± 0.19 C^{-1} p=0.88	-0.05 ± 1.3 p=0.97

Tabel 4: Statistics for total carbon- (C) and nitrogen- (N) content in *Calanus hyperboreus* at the end of each experiment. Initial values (µg female^{-1}) represent values at day 0 (n=24). Mean end values are means (µg female-^{1}) and change in percent of the initial value (Δ %) across five experiments at temperatures from 0 to 10 ^{o}C (n=34-36). Intercept (%) and coefficients for GLM-models (Eq. 1) for the changes in percent of start values as a function of temperature (% °C^{-1}) and food availability (%) are also given for the two periods of the season. All values given ± SE. Significant p-values are highlighted.

		Initial value (µg female^{-1})	**Mean end values** (µg female^{-1} / Δ%)		**GLM model parameters** (%)		
		In -situ	**Fed**	**Starved**	**Intercept**	**Temp**	**Food**
C	**Pre-bloom**	1692 ± 107	1091 ± 77	1140 ± 91	-25.8 ± 7.0	-1.4 ± 1.0	2.9 ± 7.0
			-36% ± 5	-33% ± 5	**p=0.0005**	p=0.18	p=0.68
	Bloom	716 ± 41	746 ± 34	592 ± 31	-14.0 ± 6.4	-0.6 ± 0.9	21 ± 6.4
			4% ± 5	-17% ± 4	**p=0.03**	p=0.48	**p=0.0013**
N	**Pre-bloom**	206 ± 11	178 ± 8	165 ± 10	-14.2 ± 6.2	-1.1 ± 0.9	6.1 ± 6.2
			-14% ± 4	-20% ± 5	**p=0.02**	p=0.20	p=0.33
	bloom	127 ± 4	155 ± 5	116 ± 3	-10.0 ± 4.4	0.3 ± 0.6	31 ± 4.4
			22% ± 4	-8% ± 2	**p=0.03**	p=0.61	**p<0.0001**

Table 5: Statistics for total lipids (TL), wax esters (WE), triacylglycerol (TAG) and phospholipids (PL) in *Calanus hyperboreus* at the end of each experiment. Initial values (µg female^{-1}) represent values at day 0 (n=15). Mean end values are means (µg female^{-1}), and change in percent of the initial value (Δ %), across five experiments at temperatures from 0 to 10 ^{o}C (n=22-33). Intercept (%) and coefficients for GLM-models (Eq. 1) for the changes in percent of start values as a function of temperature (°C) and food availability are also given for the two periods of the season. All values given ± SE. Significant p-values are highlighted.

		Initial value	**Mean end values**		**GLM model parameters**		
		(µg female-1)	**(µg female-1 / Δ%)**		**(%)**		
		In -situ	**Fed**	**Starved**	**Intercept**	**Temp**	**Food**
TL	**Pre-bloom**	997 ± 110	606 ± 54	478 ± 59	-47.9 ± 7.6	-1.0 ± 1.2 C^{-1}	13.6 ± 8.4
			-39% ± 5	-52% ± 6	**p<0.0001**	p=0.43	p=0.11
	Bloom	264 ± 26	230 ± 24	221 ± 29	-2.4 ± 14.6	-2.66 ± 2.0 C^{-1}	1.8 ± 14.2
			-16% ± 9	-25% ± 9	p=0.87	p=0.19	p=0.90
WE	**Pre-bloom**	919 ± 101	518 ± 50	419 ± 55	-50.4 ± 7.7	-0.9 ± 1.2 C^{-1}	11.5 ± 8.5
			-44% ± 5	-54% ± 6	**p<0.0001**	p=0.45	p=0.18
	Bloom	234 ± 25	194 ± 23	194 ± 28	2.02 ± 15.6	-3.6 ± 2.2 C^{-1}	2.5 ± 15.2
			-21% ± 9	-23% ± 10	p=0.90	p=0.10	p=0.85
TAG	**Pre-bloom**	9.6 ± 1.3	12.4 ± 1.1	9.3 ± 1.3	-1.9 ± 17.6	-0.3 ± 2.8 C^{-1}	33.3 ± 19.6
			30% ± 12	-3.2% ± 14	p=0.91	p=0.92	p=0.09
	Bloom	9.6 ± 0.4	2.5 ± 0.2	2.1 ± 0.2	-76.1± 3.2	-0.5 ± 0.6 C^{-1}	3.7 ± 3.2
			-74% ± 2.3	-78% ± 2.1	**p<0.0001**	p=0.35	p=0.26
PL	**Pre-bloom**	63 ± 8	70 ± 5	47 ± 5	-19.9 ± 9.9	-1.3 ± 1.6 C^{-1}	37.7 ± 11.1
			11% ± 8	-25% ± 7	**p=0.05**	p=0.43	**p=0.0013**
	Bloom	19 ± 3	29 ± 2.5	19 ± 1.7	-3.1 ± 16.4	-0.6 ± 2.3 C^{-1}	52.3 ± 16.0
			52 %± 13	-0.2% ± 9	p=0.85	p=0.78	**p=0.0026**

Table 6: Mean ± SE of carbon, nitrogen and lipids, at the beginning and the end of each experiment in the pre-bloom and bloom period and mean ± SE of pellet and egg production in the different incubations. Here n = number of replicates, Length= prosome length of females in mm, Carbon (C), Nitrogen (N) and Total lipids (TL) in µg female^{-1}, Wax esters (WE), Triacylglycerol (TAG), Phospholipids (PL) and Sterols (STE) in % of TL, and Pellet production (PP) and Egg production (EP) in pellet / egg female^{-1} day^{-1}.

	Carbon and Nitrogen					Lipids							Pellet and egg production		
	n	Length mm	C µg	N µg	C/N	n	Length mm	TL µg	WE %	TAG %	PL %	STE %	n	PP	EP
Pre-bloom															
Initial	24	6.2 ± 0.04	1692 ± 107	206 ± 11	8.1	18	6. 2 ± 0.05	997 ± 110	92 ± 0.4	1.0 ± 0.1	6.4 ± 0.4	0.7±0.1	-	-	-
0-	7	6.3 ± 0.13	1207 ± 183	174 ± 21	6.8	8	6.2 ± 0.08	521 ± 152	86 ± 0.6	2.6 ± 0.5	10.4 ± 0.8	0.7±0.2	15	7.0 ± 0.8	18.1 ± 4.2
0+	7	6.4 ± 0.04	1356 ±129	210 ± 13	6.4	4	6.4 ± 0.2	656 ± 105	86 ± 0.7	1.8 ± 0.3	11.7 ± 0.5	0.9±0.1	16	16.1 ± 1.6	15.2 ±3.5
2.5-	7	6.4 ± 0.1	1203 ±111	179 ± 9	6.7	7	6.0 ± 0.09	518 ± 149	88 ± 1.6	1.3 ± 0.2	10.0 ± 1.4	0.8±0.4	15	4.9 ± 0.5	15.2 ± 3.6
2.5+	7	6.4 ± 0.04	1151 ±179	167 ± 10	6.7	5	6.4 ± 0.2	671 ± 197	85 ± 2.6	3.1 ± 0.6	11.2 ± 1.8	1.0 ±0.4	15	10.9 ± 1.3	11.8 ± 3.2
5-	8	6.4 ± 0.03	1047 ± 202	147 ± 14	6.8	8	6.3 ± 0.09	544 ±113	85 ± 2.1	1.6 ± 0.1	12.4 ± 2.1	0.9 ± 0.2	16	7.3 ± 0.6	12.3 ± 3.1
5+	7	6.4 ± 0.15	811 ± 90	153 ± 9	5.3	5	6.3 ± 0.02	480 ± 43	84 ± 2	1.7 ± 0.3	13.7 ± 1.8	0.7 ± 0.2	15	12.4 ± 1.2	11.0 ± 2.1
7.5-	7	6.4 ± 0.14	1264 ±336	186 ± 42	6.4	5	6.4 ± 0.1	303 ± 83	78 ± 5.4	2.6 ± 0.3	17.7 ± 4.9	1.5 + 0.3	15	10.9 ± 1.5	10.2 ± 2.9
7.5+	7	6.5 ± 0.11	1196 ±258	188 ± 30	6.1	5	6.4 ± 0.1	531 ± 95	84 ± 3.1	2.0 ± 0.5	13.4 ± 3.5	0.9 ± 0.2	15	20.0 ± 3.3	13.6 ± 3.3
10-	7	6.2 ± 0.08	993 ± 168	141 ± 13	6.9	5	6.3 ± 0. 08	420 ± 140	88 ± 2.8	2.3 ± 0.6	8.7 ± 2.9	1.0 ± 0.4	14	12.2 ± 3.0	16.9 ± 3.1
10+	7	6.3 ± 0.05	943 ± 112	170 ± 16	5.5	5	6.4 ± 0.1	700 ± 123	85 ± 2.3	2.5 ±. 0.6	11.1 ± 1.5	1.0 ± 0.5	14	18.4 ± 1.9	16.2 ± 2.8
Bloom															
initial	24	6.4 ± 0.04	716 ± 41	127 ±4	5.6	16	6.3 ± 0.07	264 ± 26	88 ± 1.1	4.1 ± 0.4	7.5 ± 0.9	0.7 ± 0.2	-	-	-
0-	7	6.3 ± 0.06	610 ± 82	113 ± 9	5.3	4	6.4 ± 0.07	274 ± 116	88 ± 3.3	0.9 ± 0.3	11.3 ± 3.1	0.1 ± 0.1	13	0.7 ± 0.1	-
0+	7	6.4 ± 0.07	722 ± 60	140 ± 6	5.1	5	6.5 ± 0.1	205 ± 32	86 ± 0.9	1.6 ± 0.5	12.1 ± 0.9	0.7 ± 0.3	16	9.0 ± 1.3	-
2.5-	6	6.5± 0.08	651 ± 65	124 ± 4	5.2	4	6.5 ± 0.2	272 ± 67	88 ± 3.3	1.0 ± 0.2	10.5 ± 3.4	0.5 ± 0.3	14	0.6 ± 0.1	-
2.5+	7	6.4 ± 0.08	710 ± 84	155 ± 9	4.5	5	6.4 ± 0.01	274 ± 87	84 ± 2.6	1.5 ± 0.3	13.9 ± 2.5	0.8 ± 0.4	15	10.0 ± 0.8	-
5-	7	6.4 ± 0.05	630 ± 68	120 ± 7	5.2	5	6.3 ± 0.1	238 ± 73	89 ± 1.8	1.1 ± 0.2	10.0 ± 1.8	0.1 ± 0.1	17	0.5 ± 0.1	-
5+	7	6.3 ± 0.07	797 ± 107	160 ± 13	4.9	4	6.4 ± 0.2	247 ± 39	85 ± 3.8	1 ± 0.1	13.0 ± 3.8	1.3 ± 0.1	15	13.5 ± 1.7	-
7.5-	7	6.4 ± 0.12	628 ±66	121 ± 6	5.1	5	6.3 ± 0.05	197 ± 38	89 ± 1	1 ± 0.2	9.7 ± 1.1	0.6 ± 0.4	15	0.9 ±0.1	-
7.5+	7	6.4 ± 0.08	743 ± 40	160 ± 9	4.7	4	6.4 ± 0.1	171 ± 48	73 ± 10	1.2 ± 0.4	23.8 ± 8.9	2.2 ± 1.3	15	13.4 ± 2.1	-
10-	7	6.3 ± 0.12	453 ± 46	103 ± 6	4.3	5	6.3 ± 0.08	143 ± 21	72 ± 2.9	17.9 ± 8.3	8.5 ± 2.1	2.1 ± 0.8	15	1.6 ± 0.4	-
10+	7	6.6 ± 0.17	756 ± 87	158 ± 13	4.8	4	6.4 ± 0.2	251 ± 46	77 ± 4.7	8.3 ± 3.9	13.0 ± 3.4	1.7 ± 0.2	14	13.1 ± 1.5	-