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Early development of Calanus hyperboreus nauplii: Response to a changing ocean

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Abstract:

To forecast effects of temperature changes on recruitment and population dynamics of the Arctic copepod *Calanus hyperboreus,* laboratory experiments investigating temperature and food effects on early development were performed in Disko Bay, western Greenland, in 2009, and ascent rates of *C. hyperboreus* eggs collected in east Greenland were measured in the laboratory. Ascent rates were highly variable both between and within clutches, ranging from 0.7 to 27.7 m d^{-1}, suggesting variability in the biochemical composition of the egg. Development of eggs were investigated between 0.8°C and 6.6°C, and hatching was fitted to a Bělehrádek temperature function (r^2 > 0.99) with mean development time (MDT) of eggs ranging from 2.8 to 5.8 d. MDT of fed and starved nauplii was calculated for nauplii raised at 5°C. Fed nauplii developed through the first five nauplius stages (N1–N5) during 40 d of incubation, whereas development of starved nauplii ceased at N3. Nauplii were able to survive at least 30 d of starvation. Respiration rate was measured for N1 and N3 at 0°C, 5°C, and 10°C, and it increased with development stage and temperature from 0.05 ± 0.01 to 0.29 ± 0.08 nmol O_2 $nauplii^{-1}$ h^{-1} for N1 at 0°C and N3 at 10°C, respectively. A decrease in carbon and lipid content from egg to N3 indicates that nauplii are using stored lipids to cover their metabolic costs during the nonfeeding stages. Early stages of *C. hyperboreus* seem more affected by temperature than later stages, a vulnerability that might affect future recruitment.

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Introduction

In Disko Bay, western Greenland, significant changes in hydrography and ice cover have occurred during the last decades. An inflow of deep Atlantic water occurred in 1997 (Hansen et al. 2012) causing a 1.5°C increase in bottom water temperature, and an acceleration of submarine melting of the Jacobhavns glacier has increased the melt water input to the bay (Holland et al. 2008). Furthermore, from 1991-2004 a 50% decrease in sea ice cover and an earlier breakup of sea ice have been observed (Hansen et al. 2006). Such changes in magnitude and duration of sea ice cover affect both timing and duration of the phytoplankton spring bloom (Tremblay and Gagnon 2009).

The spring bloom in Disko Bay drives the energy transfer through the marine food web with the large *Calanus* copepods as key species during spring and early summer, serving as an important link to higher trophic levels (Falk-Petersen et al. 2007). Three closely related *Calanus* species co-occur in Disko Bay; *Calanus hyperboreus*, *C. glacialis,* and *C. finmarchicus*. *C. hyperboreus* is a true Arctic species restricted to polar waters (Conover 1988) and the largest and most lipid rich of the three species (Lee et al. 2006; Swalethorp et al. 2011). *C. hyperboreus* has a 2-5 years life cycle depending on environmental conditions and food availability (Conover 1988; Falk-Petersen et al. 2007) and are believed to be multiannual-iteroparous, i.e., capable of spawning in successive years (Swalethorp et al. 2011; Hirche 2013). In Disko Bay the main spawning period of *C. hyperboreus* is from January to March (Niehoff et al. 2002; Henriksen et al. 2012) and moulting into females, maturation of gonads and production of eggs depend entirely on internal lipid reserves (Pasternak et al. 2001). The main lipid classes of zooplankton are wax esters (WE), triacylglycerol (TAG), and phospholipids (PL; Lee et al. 2006). In *C. hyperboreus* adults it is the energy rich storage lipid wax esters (WE) that dominate the lipid composition.

78 The eggs of *C. hyperboreus* are spawned deep in the water column, and as they are
79 positively buoyant, they float towards the surface. Since spawning takes place during winter,
80 nauplii have to develop without food and survive on the lipids provided with the egg until the
81 spring bloom. When the spring bloom initiates they usually have reached the first feeding stage
82 and are ready to exploit the abundance of food (Melle and Skjoldal 1998). The lipid fueled
83 reproduction and the capability of the nauplii to survive for a period without food are
84 advantageous in areas where the occurrence of the phytoplankton spring bloom is short and
85 unpredictable. However, survival will probably be highly variable between different years, and
86 changes in the timing of the spring bloom combined with increasing temperatures that increases
87 the metabolism, may have consequences for the survival of the nauplii and thereby the population
88 dynamics.
89 A number of studies have been conducted dealing with *Calanus* spp. biology, and
90 possible effects of global warming on the copepod community composition (Falk-Petersen et al.
91 2007; Henriksen et al. 2012; Kjellerup et al. 2012). Most studies however, have dealt with the
92 more advanced stages and the reproduction of *Calanus,* whereas very few consider the early life
93 stages. In Arctic areas information on *Calanus* nauplii development times and physiology are
94 even more limited and only a handful of studies exist (Corkett et al. 1986; Daase et al. 2011;
95 Grenvald et al. 2012). The success of nauplii is important for the population dynamics.
96 Furthermore, nauplii are the most abundant metazoan in the oceans and constitute an important
97 prey for the first feeding stages of larval fishes (Runge 1988). Development of eggs and nauplii
98 are controlled by temperature (McLaren et al. 1969; Corkett et al. 1986) whereas food quantity
99 and quality affect mortality rate, growth, and individual variability in development time (Hygum
100 et al. 2000; Campbell et al. 2001; Daase et al. 2011). Even though nauplii are believed to be less
101 affected by food limitations than later stages (Hygum et al. 2000) short periods of starvation may

102 reduce survival and increase development time significantly (Lopez 1996). However, the lipid
103 rich Arctic species may have a higher starvation tolerance. *C. glacialis* nauplii were found able to
104 survive 42 d in filtered seawater, but with mortality rates ~3 times higher than well-fed
105 individuals (Daase et al. 2011). Starvation tolerance of *C. hyperboreus* nauplii is unknown but its
106 lifecycle suggests that it may cope even better with prolonged starvation periods.
107 The success of *C. hyperboreus* nauplii is sensible to changes in temperature. Nauplii
108 must await the phytoplankton spring bloom to feed and hence even small temperature changes in
109 the sea might affect survival of the starving nauplii as metabolism increase with temperature.
110 Since eggs are spawned in deep waters, *C. hyperboreus* eggs and nauplii will encounter water
111 masses at different temperatures on their way up. However, it is not known how long time it takes
112 an egg to reach the surface as the occurrence of positively buoyant eggs has only been described
113 as a characteristic feature for *C. hyperboreus* (Sømme 1934; Conover 1967) and to our
114 knowledge no effort have been made to measure ascent rate or determine egg density.
115 Earlier studies on pre-acclimatized *C. hyperboreus* copepodites and adult females
116 have demonstrated a high temperature tolerance on respiration rate (Conover 1962) and egg and
117 pellet production rates (Henriksen et al. 2012), but information on early development is lacking.
118 To our knowledge only Conover (1967) has measured development time and no one has studied
119 starvation potential and respiration rate of the nauplii. Such knowledge about the basic
120 physiological response of nauplii is crucial in order to understand how global warming might
121 affect Arctic ecosystems.
122 The aim of this study is to investigate the early life of *Calanus hyperboreus* and
123 discuss how the early development is affected by increasing water temperatures. This is done by
124 combining information on egg ascent rate, egg hatching, development rates of nauplii, and
125 respiration measurements at different temperatures.

Methods

Study site- The egg buoyancy experiments were conducted at the Department of Bioscience in Denmark, November 2012 on *C. hyperboreus* eggs from females collected at a 2000 m deep station (72° 96´N, 13° 05´W) during a cruise with the research vessel R/V *Dana* in the Greenland Sea. All other experiments were conducted in Disko Bay, Western Greenland in 2009. For those experiments ripe *Calanus hyperboreus* females were sampled on 10 February 2009, approximately 2 km off the coast of Qeqertarsuaq in Disko Bay, Western Greenland (69° 15´N, 53° 33´W), at a 250-300 m deep station previously used in studies of the pelagic community (Levinsen et al. 2000; Madsen et al. 2001; Hansen et al. 2012). The laboratory studies were conducted at the Arctic Station, Copenhagen University on Disko Island.

Buoyancy of eggs- Mature females were retrieved with a Bongo net on 08 September 2012 between 500-800 m. They were immediately sorted out and incubated individually in 800 mL black Nunc bottles filled with in situ water from 800 m (salinity 34.5, -0.3°C). Females were brought to Denmark and stored dark and cold (2-4°C) until experiments began. Every 2-3 d 10 mL of the water was re moved with a pipette from the top of the bottles, eggs herein counted, and new in situ water added. Experiments were conducted from 21-23 November in a 4°C (3.8±0.2) climate room under constant light condition. The ascent rate was measured in a Plexiglas tube (3.6 × 45 cm) with marks for every 5 cm, glued to the bottom of a 20 L Plexiglas cylinder (Fig. 1). The inner tube was filled with GF/C filtered seawater from 800 m collected at the same locality as the females. The outer cylinder was filled with freshwater in order to stabilize temperature during the experiments and preventing temperature induced convection. A small piece of silicone tubing was mounted through the bottom of the inner tube

149 through which eggs were injected. Eggs 0-48 h old were transferred to a 5 mL syringe and
150 carefully injected. A lid on top of the inner tube prevented evaporation during the experiment.
151 Every 15 min the water bath was stirred to prevent temperature gradients to establish. The ascent
152 of 2-8 eggs was individually observed over 10-30 cm and time taken using a stopwatch. The
153 timing started when an egg passed the 10 cm mark and terminated when it passed the 40 cm mark
154 or after maximum 4 hours. At the end of an experiment the eggs were collected and their
155 diameter measured. In total 8 experiments were performed and ascent rate of 39 eggs spawned by
156 4 different females measured (female 1-4). On two occasions a mix of eggs spawned by different
157 females was used (mix 1 and mix 2). Average temperature during the experiments varied between
158 3.5 ± 0.2 and 4.0 ± 0.1°C but as no correlation was detected between ascent rates and temperature
159 data are not presented. Mean diameter of the eggs were 192 ± 7 μm, n=36 and there was no
160 significant difference between the diameter of the eggs used for the 8 experiments (Kruskal-
161 Wallis). Ascent rate was converted to egg density following the procedure described in Knutsen
162 et al. (2001).
163 In addition to the measured ascent rate of *C. hyperboreus* eggs from east Greenland,
164 theoretical egg buoyancy was calculated using lipid and hydrography data from the Disko Bay
165 study (*see* later sections) following the procedures in Visser and Jónasdóttir (1999). In short, an
166 egg was assumed to consist of three fractions; a lipid fraction consisting mainly of WE, a water
167 fraction, and a fraction of 'other solid material'. This last fraction was assumed to be a mixture of
168 protein and carbohydrates, and the density of this material in adult *C. finmarchicus* has been
169 estimated to 1.08-1.24 g cm^{-3} (Visser and Jónasdóttir 1999) and 1.06 g cm^{-3} (Visser and
170 Jónasdóttir 1999, from Childress and Nygaard 1974). Ascent rate of eggs was calculated
171 following the modified version of stokes equation in Visser and Jónasdóttir (1999).
172

$$w = \frac{gd^2}{18\mu}[\alpha_L(\rho_L - \rho_W) + \alpha_O(\rho_O - \rho_W)] \qquad (1)$$

Where w=ascent rate (cm s^{-1}), g=gravitational acceleration (980 cm s^{-1}), d=diameter of the egg (198 μm), μ=dynamic viscosity of seawater (0.018 g cm^{-1} s^{-1}), α_L=volume fraction of lipids, ρ_L=density of lipids (0.920 g cm^{-3}), ρ_w= density of sea water, α_O= volume fraction of other solid material, and ρ_O= density of other solid material. ρ_w and ρ_L were calculated from vertical profiles of temperature and salinity sampled on 10 February (presented in Henriksen et al. 2012). α_L was calculated to 0.15 as the volume of lipids divided into the volume of the egg. α_O was estimated by rearranging eq. 6 in Visser and Jónasdóttir (1999);

$$y = \frac{\alpha_L \times \rho_L}{\alpha_L \times \rho_L + \alpha_O \times \rho_O} \qquad (2)$$

Where y is the mass of lipid divided by the dry weight (dry wt).

Phytoplankton cultures- Rhodomomas salina used for feeding the nauplii were grown at room temperature in 15 L plastic bags filled with 0.2 μm filtered seawater added B1 medium (1 mL L^{-1}), and vitamins (0.5 mL L^{-1}). The cultures were aerated and grown in a 12:12 light:dark cycle.

Nauplii cultures- Ripe *Calanus hyperboreus* females were collected by vertical hauls with a 200 μm mesh size WP2 (working party no.2) net with a closed cod-end from 250 m and up. Animals were kept cool and at arrival to the laboratory, sorted out in ice chilled petri dishes and distributed into 10 L buckets with false net bottoms filled with 50 μm filtered

196 seawater. Thirty females were incubated in each bucket and placed in a temperature controlled
197 container at 2.5°C. One third of the water was changed every second d and eggs collected every
198 24 h. Nauplii cultures for respiration experiments, carbon and lipid analysis were established at
199 0°C, 5°C and 10°C. One hundred eggs were incubated in 600 mL polycarbonate bottles filled
200 with GF/F filtered sea water. Two third of the water was renewed every 3 d by reverse filtration
201 and from N3 they were fed *Rhodomonas salina* in a concentration of min. 15 μg Chl *a* L^{-1}.
202
203 *Egg hatching*- Two different hatching experiments were set up. The first experiment
204 (expt. 1) was initiated on 12 February. Eggs spawned within 24 h at 2.5°C were collected and
205 incubated in tissue culture trays (Nunc Multi wells) of 6 wells containing 10 mL GF/F filtered
206 surface water and 30 eggs in each. The trays were incubated at constant temperature in
207 temperature controlled thermo boxes at 0°C, 2.5°C, 5°C, 7.5°C, and 10°C. Every 6 h eggs and
208 nauplii were inspected and counted. For unknown reasons, eggs incubated at 10°C were not
209 hatching and thus not presented in the results. The second experiment (expt. 2) was initiated 03
210 March with eggs spawned within 24 h by females incubated at the 5 different temperatures for
211 13-16 d. Five×50 eggs were incubated at each temperature as described above. Temperature was
212 logged every 15 min using Hoboware thermo loggers. The actual temperatures differed slightly
213 from the intended temperatures (Table 1), but for convenience the intended temperatures will be
214 used when describing data. A Beléhrádek function relating embryonic duration to temperature
215 was fitted to data.
216 $$D=a(T-\alpha)^b \quad (3)$$
217
218 where D is mean development time (MDT) of eggs at the temperature (T) and a, α, and b are
219 constants. The coefficients a and α are fitted parameters whereas the exponent b was set to -2.05,

220 a widely used mean value for a range of copepod species (McLaren et al. 1969; Corkett et al.
221 1986; Campbell et al. 2001).
222 To model the development times during the ascent of eggs, the water column was
223 divided in to three layers reflecting different water properties. A warm bottom layer from 270-
224 152 m where average temperature were 2.8°C, a layer from 152-171 m with average temperature
225 of 1.4°C, and a cold layer from 171-0 m with an average temperature of -1.3°C (Henriksen et al.
226 2012). Development rates were then calculated according to the Beléhrádek function obtained
227 from hatching expt. 1.
228
229 *Development*- Development of nauplii from egg through the first 5 nauplii stages
230 (N1-N5) was followed at 5.0 ± 0.6°C with and without food. Six 2.6 L polycarbonate bottles
231 were filled with GF/F filtered sea water and 794 ± 19 *C. hyperboreus* eggs spawned within 24 h
232 of collection were added each bottle. Three of the bottles were spiked with the phytoplankton
233 culture *Rhodomonas salina* in a concentration of 15 μg chlorophyll a (Chl a) L^{-1}. The bottles were
234 placed in a thermo box in the temperature regulated container in constant darkness and rotated by
235 hand once a day. Every 3 day 2/3 of the water was removed by reverse filtration and 10-15
236 nauplii from each bottle were randomly sorted out and fixed in 4% formalin. Bottles were refilled
237 with filtered seawater and new food was added. Temperature was logged every 15 minutes.
238 Nauplii were staged and measured on an Olympus-CK inverted microscope. Three measures of
239 the nauplii were recorded: Length of carapace (CP), total length measured from the tip of the
240 carapace to the end of the tail (TL_1) and total length measured by adding length of CP to length of
241 tail (TL_2). For N1 and N2 only TL_1 was recorded. Mean development time (MDT) defined as the
242 time when 50% of the nauplii had moulted to a specific stage were calculated from linear
243 regression on arcsine root transformed proportion data as described by Landry (1983) and Daase

et al. (2011). Daily mortality rates were calculated according to Aksnes et al. (1997) from nauplii abundance at the start and the end of the incubation period. The Beléhrádek function obtained from hatching expt. 1 were used to calculate nauplii development times at other temperatures following the method described by Corkett et al. (1986).

Carbon and lipid content- Eggs and nauplii from cultures reared at 5°C were sampled for carbon and lipid measurements. Eggs were collected after 24 h, N1 after 4 d, and N3 after 12 or 16 d of incubation. For carbon measurements, eggs or nauplii were rinsed in 0.2 μm filtered seawater and transferred to pre-combusted aluminium boats. Samples of 18-35 eggs, 15-21 N1, 6-15 N3, and 5-8 N4 were collected in 5-10 replicates, with 10 controls for each stage consisting of filtered seawater. Two types of N3 samples were collected, one after 12 d of incubation in filtered seawater and one after 16 d where N3 had been fed *R. salina* for 4 d. Samples were dried over night at 60°C and frozen until analysis. Measurements were done in an infrared gas analyser (model ADC-225 MK 3; Analytical Development Company) calibrated with oxalate. Lipid measurements were conducted on rinsed eggs and animals placed on pre-combusted GF/F filters. Samples of 70-115 eggs, 90-100 N1 and 90-100 N3 were collected in 3-6 replicates, with 5 controls for each treatment consisting of filtered seawater. Samples were placed in 1 mL Chloroform: methanol in the relationship 2:1 and frozen at -20°C until analyses. For a detailed protocol *see* Swalethorp et al. (2011).

Respiration- Respiration was measured for N1 and N3 at 0°C, 5°C, and 10°C in a closed respirometer with a Clark type O_2 micro sensor, using the micro respiratory system from Unisense A/S (Brodersen et al. 2007). Five to ten carefully rinsed nauplii (cultured at corresponding temperature and fed from N3) were placed in a 500 μL chamber filled with 0.2 μm

268 filtrated sea water. The chamber was closed by a tightfitting glass stopper with a long and slender
269 capillary hole (<0.7*13 mm) that prevented diffusion of oxygen and through which the
270 microelectrode was lowered during measurements. For each experiment 6 replicate chambers and
271 2 controls filled with 0.2 μm filtered seawater were measured. Oxygen consumption was
272 measured over 15-48 h depending on temperature. Between each measurement the chamber was
273 closed with a plug lowered through the capillary hole and nauplii were counted. Temperature was
274 logged every second minute. As for egg hatching the actual temperatures differed slightly from
275 the intended temperatures (Table 1), but the intended temperatures will be used when describing
276 data.
277
278 *Energy requirements*- Energy requirements for the nauplii were calculated based on
279 respiration and lipid measurements. As the nauplii mainly contained wax ester (WE), which is a
280 very energy and space efficient energy store, a lipid based metabolism was assumed. WE of *C.*
281 *hyperboreus* can provide 42.7 J mg lipid^{-1} (Båmstedt 1986; Auel et al. 2003) and hence the
282 energy available for the nauplii is calculated as WE content of nauplii × 42.7 J mg^{-1}. Respiration
283 rate was converted to daily energy requirements by applying an oxycaloric equivalent of 19.6 J
284 mL^{-1} typical for lipid based metabolism (Gnaiger 1983). Using these numbers theoretic stage
285 duration was calculated as energy available divided into daily energy requirement, and a theoretic
286 respiration rate calculated as energy available divided into development time. The minimum
287 carbon requirement of nauplii was calculated by applying a respiratory quotient (RQ) of 0.72
288 typical for a lipid based metabolism (Gnaiger 1983).
289

290 # Results

291 *Egg buoyancy*- The mean ascent rate recorded was 8.6 ± 7.1 m d^{-1}. However, as
292 indicated by the standard deviation the individual rates were highly variable ranging from 0.7 to
293 27.7 m d^{-1} the median being 5.6 m d^{-1} (Fig. 2). In general ascent rate varied both within a clutch
294 of eggs and between females (Fig. 3). Female 3 produced mainly fast eggs ascending at 20.2 ±
295 5.6 m d^{-1}, female 2 and 4 produced mainly slow eggs ascending at 2.3 ± 2.0 m d^{-1}, whereas
296 female 1 produced both fast and slow eggs with ascents ranging from 3-16.5 m d^{-1}. The observed
297 differences in ascent rate was significant between female 3 and female 2, 4 and mix 2 (Kruskal-
298 Wallis (p=0.001) followed by Dunns multiple comparison method). Egg density varied between
299 1.0006-1.0268 g cm^{-3} averaging 1.0194 ± 0.0063 g cm^{-3}.
300 The ascent rate for the Disko Bay eggs were calculated according to Eqs. 2 and 3.
301 Using these two equations the only unknown factor was the density of other solid material (ρ_O).
302 Calculating ascent rate using the density range of ρ_O from from Visser and Jonasdottir 1999
303 (1.06-1.24 g cm^{-3}) resulted in rates ranging from +11 m d^{-1} to -12 m d^{-1}. To obtain positive ascent
304 rates ρ_O would have to be less than 1.14 g cm^{-3}. A ρ_O of 1.08 g cm^{-3} gave an ascent rate at 8 m d^{-1}
305 equal to an egg density of 1.0191 g cm^{-3}.
306
307 *Egg hatching of Calanus hyperboreus*- In general hatching success (HS) of the eggs
308 was high in both experiments ranging between 75-83% in expt. 1 and 84-98% in expt. 2 (Table
309 2). MDT of eggs ranged between 5.8-2.8 d at 0-7°C in expt. 1 and 5.2-1.7 d at 0-10°C in expt. 2
310 with a significant effect of temperature in both experiments (2-way analysis of variance
311 (ANOVA), p<0.001). Even though temperatures were slightly lower in expt. 2 (Table 1), egg
312 hatching was significantly faster than in expt. 1 at all temperatures (Holm-Sidak Multiple
313 comparison, Fig. 4). Q_{10} calculated for the different temperature intervals in the two experiments

314 ranged between 2.6 and 5.4 with the highest values from 0-2.5°C (Table 3). Applying the
315 Beléhrádek function gave a significant fit to data (Fig. 5) in both experiments with r^2 values
316 >0.99.
317
318 *Length frequency distribution of nauplii*- Of the three measurement methods applied
319 (Tl_1, Tl_2 and CP), Tl_2 and CP where most useful in identifying distinctive length classes of
320 nauplii stages larger than N3 (Fig. 6). Coefficient of variation (CV) was in general twice as large
321 for TL_1 (6.8-5.2) as for TL_2 (2.5-3.4) and lowest for CP (1.2-2.6). Mean length of nauplii stages
322 are presented in Table 4.
323
324 *Development of nauplii*- During the 40 d experiment nauplii developed until N5 in
325 fed treatments, whereas in starved treatments development generally arrested at N3 (Fig. 7).
326 However, up to 9% of the nauplii developed to N4 in the starved treatments. The long starvation
327 period did not seem to affect the viability of nauplii since at the end of the experiment the amount
328 of dead nauplii was approximately the same in fed and starved treatments (77% and 66%,
329 respectively). There was no significant difference in MDT between fed and starved treatments
330 during the first non-feeding stages, hence average MDT of all treatments are presented in in
331 Table 5. From N3 *R. salina* was observed inside the gut of the nauplii and peristaltic movements
332 of the gut was observed. Chl *a* concentration in the bottles measured every 3 d before addition of
333 new food was 6.3 ± 0.7 and 0.2 ± 0.1 μg Chl *a* L^{-1} in fed and starved treatments, respectively.
334 Stage duration increased with development stage, the longest being N4 lasting 14.5 d. The
335 relative stage duration was used together with the Beléhrádek function for embryonic duration to
336 estimate nauplii development times at different temperatures (Fig. 8). Daily mortality rates were
337 0.09 and 0.04 d^{-1} for fed and starved nauplii, respectively.

338

339 *Carbon and lipid content*- There was a clear trend of decreasing carbon content
340 during the non-feeding stages (egg-N3) followed by increasing carbon content during the feeding
341 stages ($N3_{fed}$- N4) (Fig. 9) even though variation between replicates were high. However, only in
342 N3 vs. N4 and egg vs. N3 the observed difference in carbon content was significant (one-way
343 ANOVA). The N3 fed for 4 d ($N3_{fed}$) had 59% higher carbon content than starved N3. Total lipid
344 content of *C. hyperboreus* eggs (diameter 198 ± 7 μm, Henriksen et al. 2012) was 0.55 μg $eggs^{-1}$
345 amounting to approximately 72% of carbon content and 46% of egg dry wt when using a volume
346 to dry wt conversion 0.29 pgC μm^3 (calculated from the dry wt and diameter of eggs measured by
347 Conover 1967). WE was the main lipid class representing 85-90% of total lipids in all stages. The
348 amount of TAG was considerably lower accounting for 3-4%, PL represented 6-12% of total
349 lipids, whereas concentration of sterols were under the detection limit. A general decrease in lipid
350 amounts from egg-N3 was seen for WE and TAG but not PL (Fig. 9). The decrease was most
351 profound in WE, where 47% was used from egg-N3. Hatching accounted for a 12% decrease
352 (3.8% d^{-1}) whereas 35% of WE stored in the egg were used during development from N1-N3 (4%
353 d^{-1}). For TAG the trend was a bit more blurred with an initial rise in concentration from egg-N1
354 followed by a decrease from N1-N3. Overall there was a significant effect of stage, lipid class
355 and stage × lipid class ($p<0.001$, 2-way ANOVA). The difference between stages was only
356 significant within WE and the difference between lipid classes significant between WE and PL
357 and WE and TAG (Holm-Sidak multiple comparison).

358

359 *Respiration*- Respiration rate increased with temperature and stage (Fig. 10). There
360 was a significant effect of temperature ($p<0.001$) and stage ($p=0.05$) on respiration rate and the
361 difference between stages was significant at 5°C (2-way ANOVA, followed by Holm-Sidak

362 multiple comparison). Calculated Q_{10} values for the different temperature intervals was high
363 ranging between 2.6-7.8 (Table 6). Minimum carbon requirements for covering basic metabolism
364 at 0-10°C were calculated to be between 1.8-8.8 and 3.9-12.8% of bodyweight individual^{-1} (ind.)
365 d^{-1} for N1 and N3, respectively (Table 6).
366
367 *Energy requirements*- To evaluate how our different measurements corresponded,
368 theoretical stage duration was calculated from the decrease in lipids from N1 to N3 and the
369 respiration rate for N1 at 5°C. This theoretic stage duration was 6.3 d, whereas the measured
370 stage duration was 6.8 d. However, the actual difference in age between N1 (age 4 d) and N3 (age
371 12 d) used for lipid analysis was 8 d (*see* Method section). Therefore, using the actual time of
372 lipid consumption (8 d) and the decrease in lipids, a theoretical respiration rate for N1 was
373 calculated to 0.086 nmol O_2 h^{-1} which is 21% lower than the one measured. Accordingly in order
374 for the measure to add up, respiration rate should be 21% lower or lipid content 27% higher,
375 which is considered within the error margin of the measurements. With a N3 respiration at 0.172
376 nmol O_2 h^{-1} the maximum timespan that N3 could starve was calculated to 6 d, whereas the
377 observed starvation potential of N3 was at least 30 d.
378

379 # Discussion

380 *The egg*- The spawning patterns of the three co-occurring *Calanus* spp. are
381 different; *C. hyperboreus* spawn at depth prior to the spring bloom and the lipid rich eggs float to
382 the surface, while *C. glacialis* and *C. finmarchicus* spawn in the surface layer in association with
383 the spring bloom with eggs settling after spawning (Sømme 1934; Knutson et al. 2001). These
384 differences in phenology are reflected in the morphology and biochemical composition of the

385 eggs. Where eggs of *C. glacialis* and *C. finmarchicus* have similar appearance being transparent
386 with a robust egg shell and therefore easy to handle, eggs of *C. hyperboreus* are bright orange and
387 have a very fragile egg shell that breaks easily during handling (pers. observation). The specific
388 carbon content of eggs differs considerably between species being 0.11 pg C μm^{-3} for *C. glacialis*
389 and *C. finmarchicus* (Swalethorp et al. 2011) and 0.19 pg C μm^{-3} for *C. hyperboreus.* The higher
390 specific carbon content of *C. hyperboreus* eggs is most likely due to their very high lipid content
391 since lipids are ~80% carbon (Jónasdóttir 1999). The lipid content of *C. hyperboreus* eggs was
392 46% of egg dry wt, with the main lipid class being WE. In comparison, lipid content of *C.*
393 *finmarchicus* eggs has been estimated to 15% of dry wt with PL being the dominant lipid class
394 (Lee et al. 2006). These differences corroborate the different life strategies of the two species
395 with *C. finmarchicus* spawning when food is abundant in contrast to *C. hyperboreus* spawning in
396 the absence of food. In the latter case the nauplii must survive on the lipids from the egg until
397 food becomes available. As a result the main lipid in *C. hyperboreus* eggs are storage lipids
398 consisting of the highly energy rich and condensed WE in contrast to the easy mobilised TAG
399 and PL of *C. finmarchicus* eggs.
400 A side effect of having lipid rich eggs is that it makes them positively buoyant.
401 With a calculated ascent rate of Disko Bay eggs at 8 m d^{-1} it takes 34 d to reach the surface which
402 means that all eggs have developed to nauplii on arrival. The average temperature of the bottom
403 water (270-152 m) was 2.8°C. At this temperature eggs hatch after 4.3 d having moved 35 m
404 from the spawning depth. As lipid content of the nauplii decrease with stage the ascent rate also
405 decrease with stage and nauplii reaches N3 after 13.8 d in a depth of 159 m. Based on the lipid
406 content of N3 and an estimated nauplii volume calculated as the volume of an ellipsoid, ascent
407 rate of N3 was calculated to 4.4 m d^{-1}. At this rate it take another 36 d to reach the surface
408 meaning that the nauplii arrive to surface waters 50 d after the eggs were spawned. Using the

409 measured range of ascent rates however the fastest eggs hatch in a depth of 150 m and reach the
410 surface as N1 after 10 d, whereas the slowest eggs reach the surface after 386 d.
411 There was good agreement between the calculated ascent rate of eggs (8 m d^{-1}) and
412 the mean value from the direct measurements (8.6 m d^{-1}). This rate seems reasonable as passive
413 ascent rate of adult *C. finmarchicus* has been estimated to 13-18 m d^{-1} (Visser and Jónasdóttir
414 1999). However, as there was a large variation in ascent rate of eggs coming from the same
415 clutch, it seems likely that the eggs also differ in other aspects. Kjesbu et al. (1991) found that the
416 buoyancy of cod eggs was related to their diameter. This may also be true for the *C. hyperboreus*
417 egg. However, the variation in ascent rate caused by differences in diameter is rather small
418 (Eq.1), compared to the huge variability in ascent rate observed, and hence it will easily be
419 masked by other factors influencing the ascent rate. Such factors could be differences in lipid
420 content which directly influence the buoyancy, in the hatching rate, or in the metabolic activity of
421 the egg. Viability of the eggs used for the experiment was not investigated, but from expt. 1 we
422 saw that around 20% of the eggs did not hatch. As the eggs were discarded after 5-10 d it cannot
423 be excluded that some of the eggs might have hatched later. Delayed hatching eggs (DHE) are
424 described in various Atlantic copepod species (Engel 2005) and most likely produced as a
425 response to unfavorable environmental conditions (Drillet et al. 2011). DHE are not believed to
426 be as important in Arctic areas where copepods have alternative strategies to cope with
427 environmental variability such as lipid storage capacity and dormancy of copepodite stages
428 (Engel 2005). However, it may be an advantage for *C. hyperboreus* to spread out the hatching
429 period of eggs to maximise the chance that some of the developing nauplii are matching the
430 spring bloom. Plasticity in hatching times can also be a way to conserve energy as the metabolic
431 activity of eggs (embryos) most likely is less than for nauplii. Furthermore, as the eggs are
432 positively buoyant they are not lost to the sediment but slowly make their way towards the

433 surface. The ascent experiments were performed in the very beginning of the spawning cycle of
434 *C. hyperboreus* where only a small fraction of the females were producing eggs. It therefore
435 remains to be investigated if there is an effect of season on the ascent rate of the eggs, if
436 buoyancy and viability of the eggs are connected as suggested by Conover (1967), and if there is
437 plasticity in the hatching pattern of eggs. The large observed difference in buoyancy properties of
438 the egg was also noted by Conover (1967) who observed both sinking and floating eggs during
439 his egg production experiment. He found that the amount of floating eggs varied between 40-
440 100% and were decreasing with time. However, from his data it is not possible to separate
441 variation between or within clutches of eggs.
442 After the eggs are spawned in deep waters and have started ascending they develop
443 towards hatching. The egg hatching rate of *C. hyperboreus* eggs was measured two times with 19
444 d apart. In expt. 1 the eggs were spawned at 2.5°C (which is close to bottom water temperatures
445 in Disko Bay, Hansen et al. 2012) over a period of 24 h and incubated at a range of experimental
446 temperatures. Hence, eggs have been affected by this low temperature both during oogenesis and
447 maturation in the female gonads and 0-24 h after spawning. This might underestimate hatching
448 rate in incubations above 2.5°C whereas at 0°C hatching rate could be slightly overestimated. The
449 observed temperature dependence of hatching rate was similar to the one found in a comparable
450 experiment by McLaren et al. in 1969 (Fig. 5). Expt. 1 mimics the current situation where eggs
451 are spawned in the warm bottom water and float up through water masses of different
452 temperatures. In the present situation they end up in the cold surface water. But if surface
453 temperatures are increasing as predicted in future climate change scenarios, they meet a warmer
454 water layer.
455 In expt. 2 eggs were spawned by females preconditioned to the 5 experimental
456 temperatures and final maturation and spawning of eggs therefore occurred at the same

457 temperature. This experiment mimics a situation where also the bottom water temperatures have
458 increased due to climate change. If the observed differences in hatching rate only were a matter
459 of method, hatching in the two experiments should be identical at 2.5°C (where the method are
460 the same), at 0°C slightly faster in expt. 1, and for temperatures above 2.5°C faster in expt. 2.
461 Since hatching at all temperatures took significantly longer in expt. 1 than in expt. 2, there might
462 be an effect of season, i.e., that eggs produced early develop slower than eggs produced later in
463 the season. This could be explained by a change in egg quality concerning biochemical
464 composition with season. If lipid content of eggs declined as the lipid content of females declined
465 (Henriksen et al. 2012) less lipid rich eggs may hatch and develop faster in order to reach the first
466 feeding stage sooner. Conover (1967) noted that the first batches of eggs laid by *C. hyperboreus*
467 were floating whereas later produced egg batches sank. As it is lipids that make the eggs
468 positively buoyant it suggests that the lipid content of eggs actually was changing, but it remains
469 to be experimentally verified. Differences between clutches of eggs spawned by the same female
470 was also observed by Hirche (2013) who found the viability of eggs to vary considerably from
471 clutch to clutch, however no temporal trend in the variation was detected.
472
473 *Nauplii*-The nauplii stages of *C. hyperboreus* could be divided into specific size
474 classes (Fig. 6, Table 4). The present nauplii were slightly larger than reported by Conover
475 (1967), and overlapped in size with measures of *C. glacialis* from Daase et al. (2011). It may be a
476 problem to compare size distribution of nauplii from different populations as size variation
477 between populations from different environments has been found among nauplii (Table 6 in
478 Daase et al. 2011) as well as among copepodites and adults (Kwasniewski et al. 2003;
479 Weydmann and Kwasniewski 2008). To be able to distinguish between different species of
480 *Calanus* nauplii from in situ samples, body size measurements have to be from the same area.

481 Furthermore, it is also important to consider which measure to use (Sømme 1934). When
482 studying specific nauplii species and development stages are CP, TL_2, or both measures often
483 recorded. However, when nauplii are not in focus and a mixed biomass samples are analysed
484 often TL_1 is the measure that is taken, as measuring TL_2 is both tedious and time consuming.
485 Consequently the nauplii of closely related *Calanus* species cannot be separated based on
486 literature reports of stage and body length alone. Therefore we call for a standardised routine
487 measuring procedure of CP instead of TL_1 which would not be more time consuming and would
488 allow future comparative analysis of the nauplii communities.
489 In the present study development of *C. hyperboreus* was followed to N5. N3 is
490 normally considered to be the first feeding stage for most copepod species, but for *C.*
491 *hyperboreus* also N5 has been suggested (Conover 1962). Our study corroborates the findings of
492 Conover (1967) and the suggestions by Melle and Skjoldal (1998) that N3 is the first feeding
493 stage of *C. hyperboreus*. The development time from egg to N1 was comparable with that found
494 by Conover (1967) (Table 5). After N1 development proceeded slower and by the time the
495 nauplii reached N5, development time was twice as long as suggested by Conover. Part of this
496 difference might reflect differences in incubation method and food quality offered, as Conover
497 was feeding the nauplii with the diatom *Thalassirosira fluviatilis* and we were feeding them with
498 the chryptophyte *Rhodomonas salina*. Mortality during the experiment was high, 77% of the fed
499 and 66% of the starved nauplii died during the incubation period of 40 d. However, this is
500 comparable with mortality of *C. glacialis* nauplii measured over 42 d during which 78 and 95%
501 of the nauplii died in fed and starved treatments, respectively (Daase et al. 2011). As argued in
502 Daase et al. the reason for the high mortality is the long duration of the incubation, as when it
503 comes to daily mortality rates (0.09 and 0.04 d^{-1} for fed and starved nauplii, respectively) they are
504 similar to what has been found for other *Calanus* species (Daase et al. 2011; Grenvald et al.

505 2012). The lower mortality of starved *C. hyperboreus* in the present study probably reflects the
506 high lipid content of the nauplii which enable them to endure starvation and obtain a higher
507 survival rate than *C. glacialis.* The decrease in lipid from egg to N3 documented that nauplii were
508 indeed metabolising lipid to cover their energy requirements during moulting (Fig. 9).
509 Nauplii of *C. hyperboreus* seem to be more sensible to temperature changes than
510 later development stages (Henriksen et al. 2012; Conover 1962), which render recruitment the
511 most sensible parameter in the future. Increasing temperature raised respiration rate dramatically
512 and even though nauplii were raised at the experimental temperatures they were not able to
513 regulate their respiration as has been shown for pre-acclimatised adults measured between 2-8°C
514 (Conover 1962). It seemed that N1 were more affected by elevated temperature than N3 as Q_{10}
515 for N3 was 1/3 of that for N1. A future earlier warming of the surface water in early spring will
516 therefore significantly increase the carbon requirements to cover the basic metabolism and
517 thereby increase the need for earlier feeding opportunities. At 0°C N3 were using 3.8% of their
518 bodyweight d^{-1} to cover basic metabolism giving them 26 d before they had used all their
519 reserves, whereas at 5°C that would take only 13 d. However, the gap found between observed
520 age of starved N3 (30 d) and calculated maximal starvation potential of N3 (6 d) indicates that the
521 nauplii must be able to reduce their metabolism further in response to long starvation periods e.g.,
522 by metabolic down regulation. To verify this respiration measurements should be done on
523 starving N3 and not only on fed N3 as was the case in this study.
524 Very few data exist for nauplii respiration at low temperature. However, Ikeda et al.
525 (2001) compiled data on metabolic rate from 35 zooplankton species within the temperature
526 range of -1.7- 29°C and developed a model relating dry wt (mg) and temperature (T, °C) to
527 metabolic rate of zooplankton (y, μL O_2 $ind.^{-1}$ h^{-1}):
528

529 $$Ln(y) = -3.99 + 0.801 \times \ln(\text{dry wt}) + 0.069 \times T \quad (4)$$

530

531 When our results are compared with the value calculated from the observed dry wt and
532 temperature, all measured values were well below this general one. Respiration of *Acartia tonsa*
533 eggs at 10°C have been measured to 0.09 nmol O_2 egg^{-1} h^{-1} corresponding to 769 nmol O_2 mg dry
534 wt^{-1} h^{-1} or 364 nmol O_2 mg dry wt^{-1} h^{-1} at 0°C (Q_{10}=2.51, Nielsen et al. 2007) whereas respiration
535 of *Eucalanus pileatus* nauplii at 21°C have been measured to 0.82 nmol O_2 $nauplii^{-1}$ h^{-1} or 653
536 nmol O_2 mg dry wt^{-1} h^{-1}(Köster et al. 2008). In comparison the mass specific respiration of N1 in
537 the present study was just 51 and 251 nmol O_2 mg dry wt^{-1} h^{-1} at 0°C and 10°C, respectively. This
538 indicates metabolic acclimatization or more likely an overall adaptation to living in the arctic
539 with general low water temperatures.

540 The station where the eggs were spawned is 270 m deep. Hence eggs spawned at
541 this depth must be positively buoyant to reach the productive surface layers. With both of the
542 present estimated ascent rates (8 or 8.6 m d^{-1}) nauplii would have developed to the first feeding
543 stage well before arrival to the surface waters. As the eggs were spawned from January to March
544 and the spring bloom peaked in the beginning of May (Henriksen et al. 2012, Fig. 5) nauplii
545 would face starvation for at least a month during ascend and arrive to the surface layer prior to
546 the developing bloom. However, from early April Chl *a* concentration exceeded 1 μg L^{-1} in the
547 upper 50 m (equal to 30 μg C L^{-1}, applying the Chl *a* to a carbon conversion of 30 reported by
548 Dünweber et al. 2008 in Disko Bay) meaning that some food was available for the nauplii at this
549 time. Alternatively ice algal blooms may form patches of higher food concentration at the ice
550 water interface. The present starvation experiment showed that even at 5°C N3 were able to
551 survive at least 30 d without food, equivalent to a period of 69 d at surface water temperatures of
552 -1.5°C (Applying a Q_{10} of 3.6 from hatching expt. 1, Table 3). If the large variations in ascent

553 rate also are representative for Disko Bay, some eggs would reach the surface layer after only 10
554 d and some not at all. However, to get to the surface faster slow ascending nauplii could engage
555 in swimming. Swimming speed for *Calanus helgolandicus* N5 have been measured to 325 m d^{-1}
556 (Titelman and Kiørboe 2003). As *C. helgolandicus* only spend 80% of the time swimming and
557 does not swim in a straight line (Titelman and Kiørboe 2003) this would roughly be equal to
558 around 87 m d^{-1}. Assuming swimming speed scale with size N3 of *C. hyperboreus* would swim
559 around 69 m d^{-1}. As a conservative estimate this means that nauplii could reach the surface in less
560 than a week. However, the passive ascent conserves energy and as no food is available for the
561 first month, there would not be any reason for the early spawned nauplii to use energy on
562 swimming. Nauplii produced towards the end of the spawning season may benefit from reaching
563 the surface water earlier as some food might already be available. In both cases however,
564 swimming activity would also increase the risk of being eaten (Tiselius and Jonsson 1997,
565 Titelman 2003). Another important adaptive benefit of buoyant eggs is that it separates the eggs
566 from the females, thereby avoiding the cannibalistic mothers. Conover (1967) observed that
567 females ingested eggs during an egg production experiments. Likewise, observations of egg
568 laying females producing orange pellets indicates feeding on own eggs (S. Jung-Madsen unpubl.
569 2009).
570 The Disko bay is a rather shallow habitat compared to the Greenland Sea or Arctic
571 Ocean. In the Greenland Sea and Fram Strait *C. hyperboreus* may overwinter at depths below
572 2000 m (Hirche et al. 2006; Auel et al. 2003). At such depth active swimming will be necessary
573 in order to reach the surface in time for the spring bloom. However, during November to March
574 when *C. hyperboreus* is reproducing (Hirche and Niehoff 1996), the main part of the female
575 population is situated in 1000-1500 m and they move upwards during the period (Hirche and
576 Niehoff 1996; Hirche 1997). Nauplii originating from eggs produced at this depth should be able

577 to reach the spring bloom. Increasing ocean temperatures will affect not only development and
578 energy requirement of nauplii but also the timing and duration of the springbloom that they feed
579 on. An earlier occurring bloom would counteract some of the metabolic effects of warmer water
580 if spawning occurs at the same time. The factors controlling when *C. hyperboreus* initiates
581 spawning still remains to be identified (Hirche 2013). However, if females use their lipid stores
582 faster due to higher metabolism, this could be hypothesized to cause earlier spawning.
583 In summary, ascent rate of eggs is highly variable indicating production of eggs
584 with different biochemical properties, which could be a strategy to enhance chances that some of
585 the offspring successfully matches the phytoplankton springbloom. As for other copepod species
586 the embryonic development of *C. hyperboreus* was mainly controlled by temperature, but there
587 also seemed to be an effect of the season when eggs were produced. The early stages of *C.*
588 *hyperboreus* seem to be more affected by temperature than older stages. The high lipid content of
589 eggs and nauplii enhance survival in a food limited environment. However, increasing
590 temperature decreases the development time and increase the carbon requirements of nauplii, and
591 will therefore affect their possibility of matching the phytoplankton spring bloom. This may in a
592 future warmer climate have large implication for the recruitment of *Calanus hyperboreus*.
593

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737

Tables

Table 1: Intended (T_{int}) and actual temperature (°C) ± Standard deviation (SD) in *Calanus hyperboreus* egg hatching expts. 1 and 2 and in respiration experiments with N1 and N3 (RespN1, RespN3).

T_{int}	Expt. 1	Expt. 2	RespN1	RespN3
0.0	0.8 ± 0.6	0.0 ± 0.3	0.5 ± 0.2	0.5 ± 0.1
2.5	2.6 ± 0.2	2.6 ± 0.2		
5.0	5.5 ± 0.5	4.9 ± 0.6	4.8 ± 0.3	4.9 ± 0.3
7.5	6.6 ± 0.4	7.1 ± 0.2		
10.0	10.0 ± 0.3	9.9 ± 0.4	10.2 ± 0.1	10.3 ± 0.1

Table 2: Mean development time (MDT, days) and hatching success (HS, %) of *Calanus hyperboreus* eggs in hatching expts. 1 and 2 at 5 different temperatures.

	Expt. 1		Expt. 2	
T (°C)	MDT (days)	HS (%)	MDT (days)	HS (%)
0	5.8 ± 0.07	83 ± 8	5.3 ± 0.16	88 ± 6
2.5	4.2 ± 0.12	78 ± 10	3.7 ± 0.14	94 ± 4
5	3.2 ± 0.02	75 ± 8	2.9 ± 0.05	91 ± 4
7	2.8 ± 0.05	79 ± 5	2.3 ± 0.05	98 ± 2
10			1.7 ± 0.20	95 ± 2

Table 3: Q_{10} of *Calanus hyperboreus* egg hatching at different temperature intervals in expts. 1 and 2.

T	Expt. 1	Expt. 2
0-2.5	5.4	4.1
0-5	3.6	3.3
0-7	3.4	3.1
0-10		3.2
2.5-5	2.8	2.6
2.5-7	2.7	2.6
2.5-10		2.9
5-7	2.7	2.7
5-10		3.1
7-10		3.4

Table 4: Mean length (μm) ± SD, sample size (*n*) and range of *Calanus hyperboreus* nauplii carapace (CP) and total length of nauplii (TL_1 for N1-N2 and TL_2 for N3-N5).

	CP (*n*)	Range (CP)	TL (*n*)	Range (TL)
N1			260 ± 13 (69)	217-279
N2			318 ± 10 (70)	279-341
N3	314 ± 8 (540)	279-341	441 ± 11 (158)	397-471
N4	375 ± 10 (159)	341-403	550 ± 19 (87)	502-595
N5	432 ± 5 (34)	415-440	673 ± 9 (33)	651-694

Table 5: MDT and stage duration (days) at 5°C, for *Calanus hyperboreus* N1-N5 in this study and according to Conover (1967).

	This study		Conover 1967	
	MDT	duration	MDT	duration
N1	3.1 ± 0.5	2.5	2.8	2.3
N2	5.6 ± 0.4	4.3	5.0	3.0
N3	9.9 ± 0.7	12.3	8.0	6.0
N4	22.2 ± 2.6	14.5	14.0	4.0
N5	36.7		18.0	

Table 6: Respiration rate ± SD and No. of replicates (*n*) of *Calanus hyperboreus* N1 and N3 at 3 temperatures, corresponding carbon requirements in % of bodyweight (body wt) and Q_{10} for temperature intervals.

T (°C)	Respiration rate nmol O_2 ind.$^{-1}$ hour^{-1}		Carbon requirements % body wt ind.$^{-1}$ day^{-1}		T (°C)	Q10	
	N1	N3	N1	N3		N1	N3
0	0.045 ± 0.01 (7)	0.085 ± 0.01 (5)	1.8	3.8	0-5	7.8	5.0
5	0.109 ± 0.04 (6)	0.172 ± 0.06 (6)	4.2	7.7	0-10	5.3	3.5
10	0.227 ± 0.06 (5)	0.287 ± 0.08 (6)	8.8	12.8	5-10	3.9	2.6

Table 7: *Calanus hyperboreus* lipid and carbon content in ng ind.$^{-1}$ ± SD and No. of replicates (*n*) of wax esters (WE), triacylglycerol (TAG), phospholipids (PL), and carbon (C). $N3_{fed}$ has been fed *R. salina* for 4 days.

	Egg	N1	N3	$N3_{fed}$	N4
WE (ng)	481 ± 45 (6)	424 ± 8 (3)	254 ± 37 (6)		
TAG (ng)	16 ± 12 (4)	21 ± 4 (3)	9 ± 4 (6)		
PL (ng)	48 ± 12 (3)	27 ± 6 (3)	36 ± 28 (4)		
Carbon (ng)	769 ± 255 (10)	536 ± 130 (6)	292 ± 75 (4)	465 ± 386 (5)	1200 ± 893 (4)

Figure text.

Fig. 1. Experimental setup for direct measurements of ascent rate of *Calanus hyperboreus* eggs.

Fig.2. Histogram presenting ascent rates of 39 *Calanus hyperboreus* eggs, spawned by six different females.

Fig.3. Ascent rate of *Calanus hyperboreus* eggs grouped by female. At two occasions, mix 1 and 2, eggs spawned by two females were mixed.

Fig.4. Proportion of hatched *Calanus hyperboreus* eggs (%) ± Standard error (SE) as a function of time (days) at 5 temperatures in expt. 1 where females were incubated at 2.5°C and expt. 2 where females were incubated at the same temperatures as the eggs.

Fig.5. Beléhrádeks function (lines), relating mean development time (MDT) of *Calanus hyperboreus* eggs to temperature, fitted to data from the two hatching experiments and to egg hatching data from McLaren et al. (1969) (circles).

Fig.6. *Calanus hyperboreus* nauplii measurements arranged in 5 μm bins; (A) Total length 1. measure (TL_1), (B) Total length 2. measures (carapace + tail, TL_2), and (C) Length of carapace (CP). Nauplii drawing from Sømme et al. (1934).

Fig.7. Development of starved and fed *Calanus hyperboreus* nauplii at 5°C. Values are means ± SE (n=3).

Fig.8. Mean development time (MDT) of *Calanus hyperboreus* nauplii stage N1-N5 predicted by Beléhrádeks function $D= a\times(T+12.7)^{-2.05}$, relating MDT to temperature assuming equiproportional development.

Fig 9. Carbon (open circles) and lipid (bars) content of *Calanus hyperboreus* eggs and nauplii. Values are mean ± SE of carbon (C), phosphorlipids (PL), triacylglycerol (TAG), and wax esters (WE). $N3_{fed}$ was fed for 4 days before collection. *n*=3-6 samples per measurement.

Fig.10. Respiration of *Calanus hyperboreus* N1 and N3. Values are means ± SE.

Fig.1.

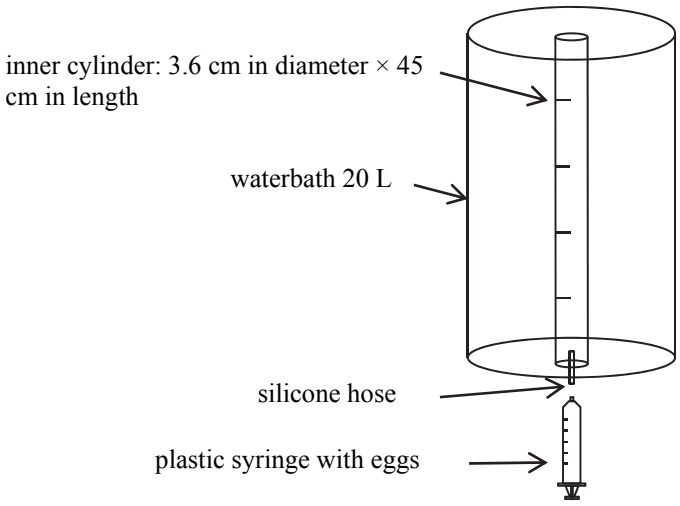
inner cylinder: 3.6 cm in diameter × 45 cm in length
waterbath 20 L
silicone hose
plastic syringe with eggs

Fig.2.

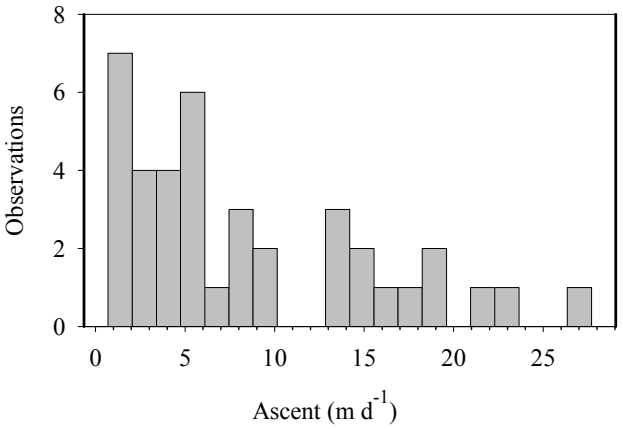

Fig.3.

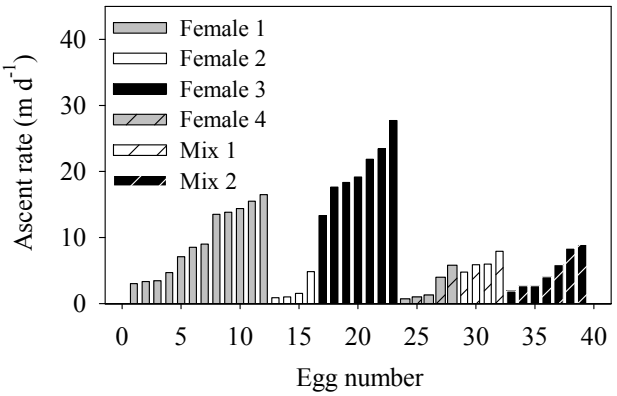
Female 1
Female 2
Female 3
Female 4
Mix 1
Mix 2
Ascent rate (m d-1)
0
10
20
30
40
0
5
10
15
20
25
30
35
40
Egg number

Fig.4.

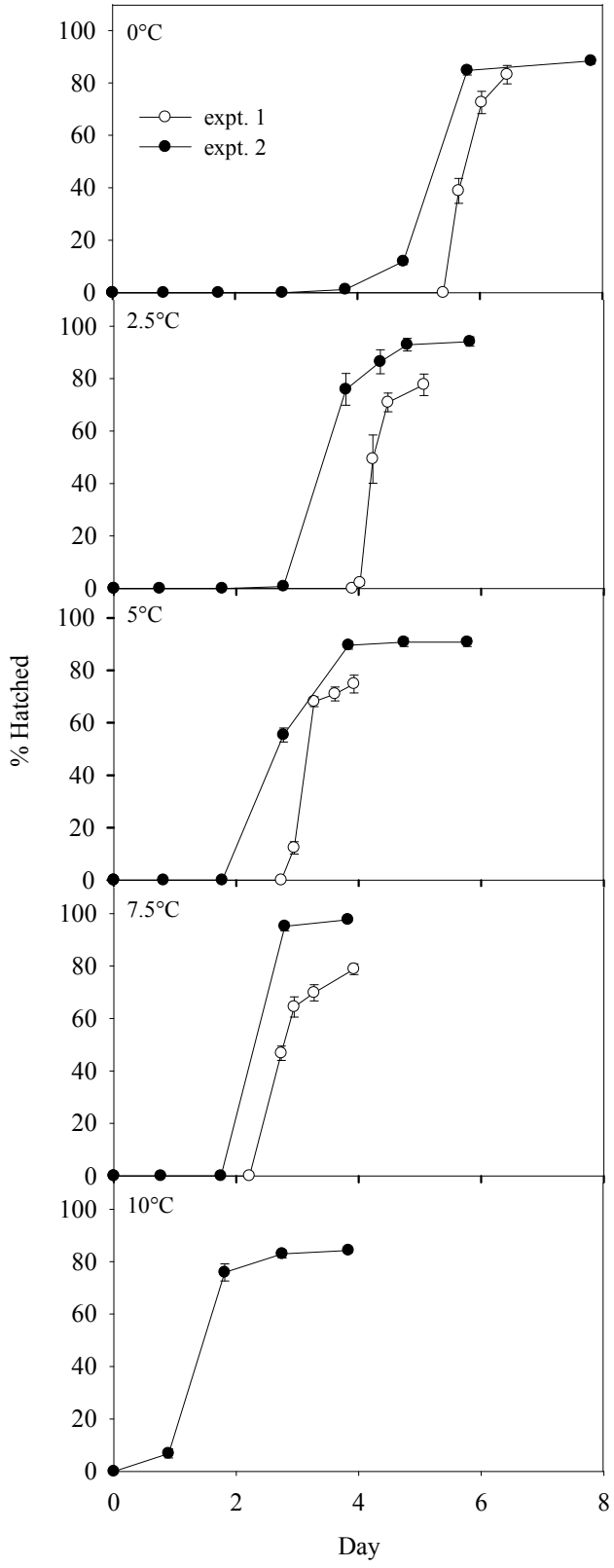
0°C
expt. 1
expt. 2
2.5°C
5°C
7.5°C
10°C
% Hatched
Day
0
20
40
60
80
100
0
2
4
6
8

Fig.5.

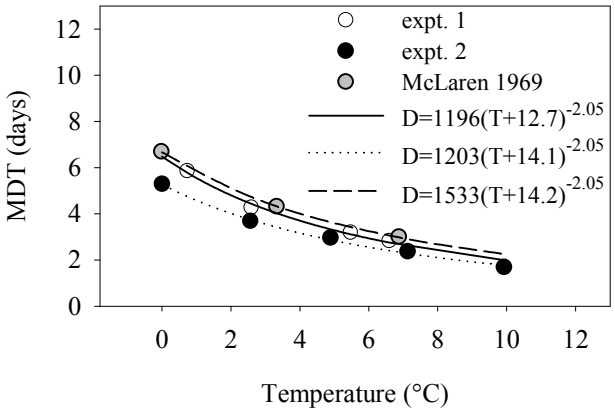
MDT (days)
Temperature (°C)
expt. 1
expt. 2
McLaren 1969
D=1196(T+12.7)-2.05
D=1203(T+14.1)-2.05
D=1533(T+14.2)-2.05
0
2
4
6
8
10
12

Fig.6.

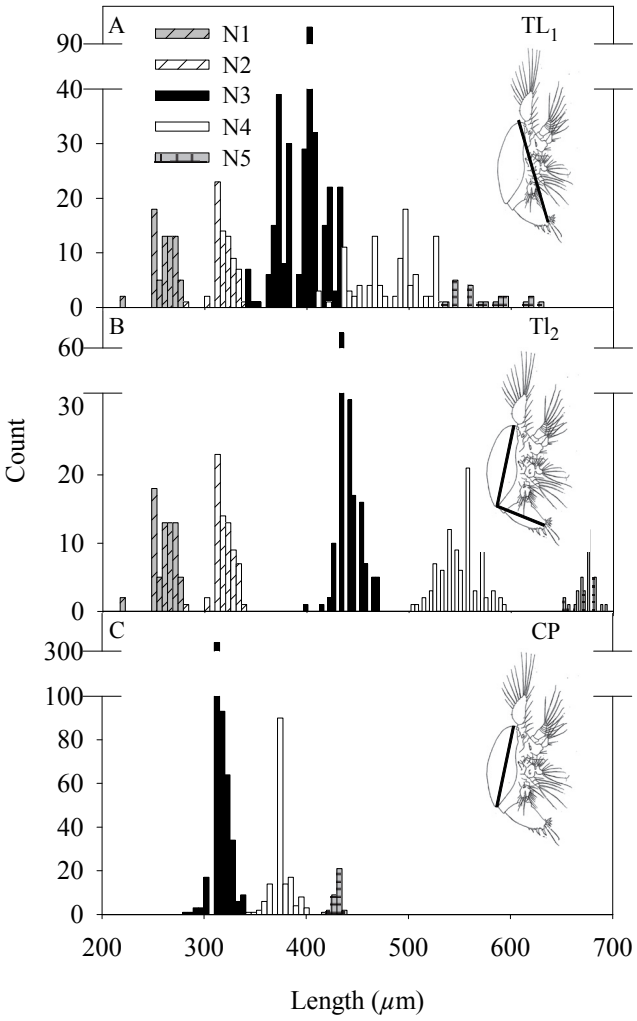

Fig.7.

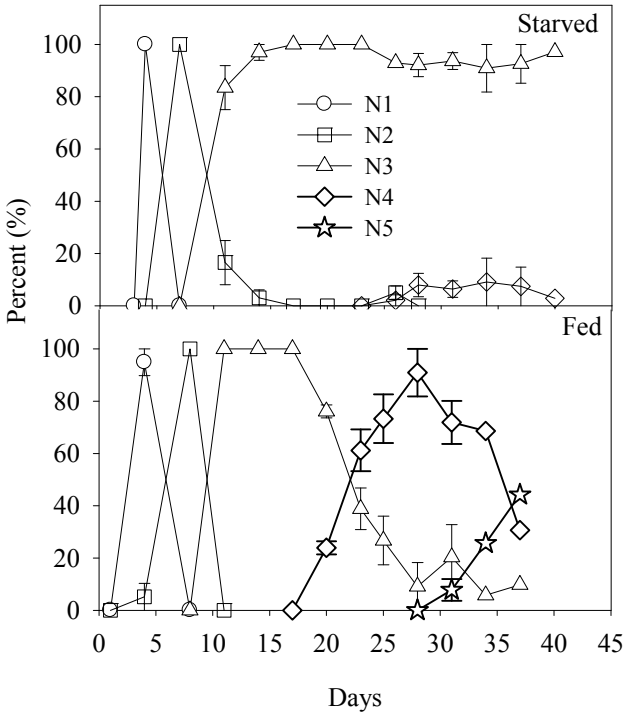
Starved
Fed
N1
N2
N3
N4
N5
Percent (%)
Days
0
20
40
60
80
100
0
5
10
15
20
25
30
35
40
45

Fig. 8.

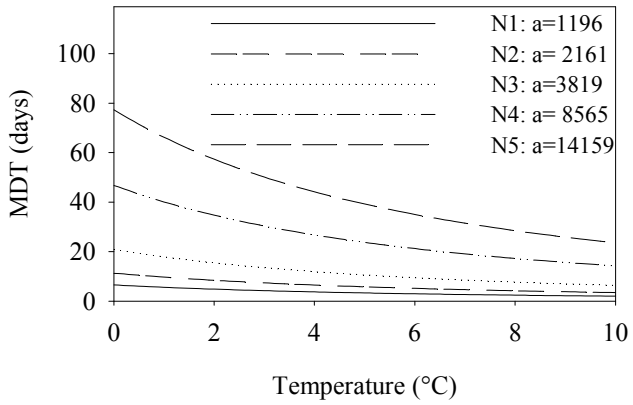
N1: a=1196
N2: a= 2161
N3: a=3819
N4: a= 8565
N5: a=14159
MDT (days)
0
20
40
60
80
100
0
2
4
6
8
10
Temperature (°C)

Fig.9.

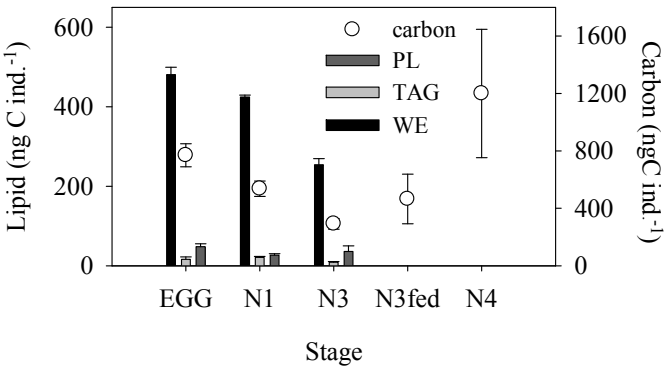
Lipid (ng C ind.-1)
600
400
200
0
carbon
PL
TAG
WE
1600
1200
800
400
0
Carbon (ngC ind.-1)
EGG
N1
N3
N3fed
N4
Stage

Fig.10.