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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 Beléhrádek 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.

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 meltwater input to the bay (Holland et al. 2008). Furthermore, from 1991 to 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*, *Calanus glacialis,* and *Calanus 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 yr 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 molting 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 (WEs), triacylglycerol (TAG), and phospholipids (PLs; Lee et al. 2006). In *C. hyperboreus* adults it is the energy-rich storage lipid WEs that dominate the lipid composition.

The eggs of *C. hyperboreus* are spawned deep in the water column, and because they are positively buoyant, they float toward the surface. Because spawning takes place during winter, nauplii have to develop without food and survive on the lipids provided with the egg until the spring bloom. When the spring bloom initiates, they usually have reached the first feeding stage and are ready to exploit the abundance of food (Melle and Skjoldal 1998). The lipid-fueled reproduction and the capability of the nauplii to survive for a period without food are advantageous in areas where the occurrence of the phytoplankton spring bloom is short and unpredictable. However, survival will probably be highly variable between different years, and changes in the timing of the spring bloom combined with increasing temperatures that increases the metabolism may have consequences for the survival of the nauplii and, thereby, the population dynamics.

A number of studies have been conducted dealing with *Calanus* spp. biology and possible effects of global warming

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on the copepod community composition (Falk-Petersen et al. 2007; Henriksen et al. 2012; Kjellerup et al. 2012). Most studies, however, have dealt with the more advanced stages and the reproduction of *Calanus,* whereas very few consider the early life stages. In Arctic areas, information on *Calanus* nauplius development times and physiology are even more limited, and only a handful of studies exist (Corkett et al. 1986; Daase et al. 2011; Grenvald et al. 2012). The success of nauplii is important for the population dynamics. Furthermore, nauplii are the most abundant metazoan in the oceans and constitute an important prey for the first feeding stages of larval fishes (Runge 1988). Development of eggs and nauplii is controlled by temperature (McLaren et al. 1969; Corkett et al. 1986), whereas food quantity and quality affect mortality rate, growth, and individual variability in development time (Hygum et al. 2000; Campbell et al. 2001; Daase et al. 2011). Even though nauplii are believed to be less affected by food limitations than later stages (Hygum et al. 2000), short periods of starvation may reduce survival and increase development time significantly (Lopez 1996). However, the lipid-rich Arctic species may have a higher starvation tolerance. *C. glacialis* nauplii were found able to survive 42 d in filtered seawater, but with mortality rates about three times higher than well-fed individuals (Daase et al. 2011). Starvation tolerance of *C. hyperboreus* nauplii is unknown, but its lifecycle suggests that it may cope even better with prolonged starvation periods.

The success of *C. hyperboreus* nauplii is sensible to changes in temperature. Nauplii must await the phytoplankton spring bloom to feed and hence even small temperature changes in the sea might affect survival of the starving nauplii as metabolism increases with temperature. Because eggs are spawned in deep waters, *C. hyperboreus* eggs and nauplii will encounter water masses at different temperatures on their way up. However, it is not known how long it takes an egg to reach the surface, in that the occurrence of positively buoyant eggs has only been described as a characteristic feature for *C. hyperboreus* (Sømme 1934; Conover 1967), and to our knowledge, no efforts have been made to measure ascent rate or determine egg density.

Earlier studies on preacclimatized *C. hyperboreus* copepodites and adult females have demonstrated a high temperature tolerance on respiration rate (Conover 1962) and egg and pellet production rates (Henriksen et al. 2012), but information on early development is lacking. To our knowledge, only Conover (1967) has measured development time, and no one has studied starvation potential and respiration rate of the nauplii. Such knowledge about the basic physiological response of nauplii is crucial to understand how global warming might affect Arctic ecosystems.

The aim of this study is to investigate the early life of *C. hyperboreus* and discuss how the early development is affected by increasing water temperatures. This is done by combining information on egg ascent rate, egg hatching, development rates of nauplii, and 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 RV *Dana* in the Greenland Sea. All other experiments were conducted in Disko Bay, western Greenland, in 2009. For those experiments, ripe *C. 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 and 800 m. They were immediately sorted 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 removed with a pipette from the top of the bottles, eggs therein counted, and new in situ water added. Experiments were conducted from 21 to 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) marked every 5 cm and 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 fresh water, 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, through which eggs were injected. Eggs 0–48 h old were transferred to a 5 mL syringe and carefully injected. A lid on top of the inner tube prevented evaporation during the experiment. Every 15 min, the water bath was stirred to prevent temperature gradients to establish. The ascent of 2–8 eggs was individually observed over 10–30 cm and timed with a stopwatch. The timing started when an egg passed the 10 cm mark and terminated when it passed the 40 cm mark or after a maximum of 4 h. At the end of an experiment, the eggs were collected, and their diameters were measured. In total, eight experiments were performed, and the ascent rates of 39 eggs spawned by four different females (1–4) were measured. On two occasions, a mix of eggs spawned by different females was used (mix 1 and mix 2). Average temperatures during the experiments varied between 3.5 ± 0.2°C and 4.0 ± 0.1°C, but because no correlation was detected between ascent rates and temperature data, they are not presented. Mean diameter of the eggs was 192 ± 7 μm (n = 36), with no significant difference between the diameters of the eggs used for the eight experiments (Kruskal–Wallis). Ascent rate was converted to egg density following the procedure described in Knutsen et al. (2001).

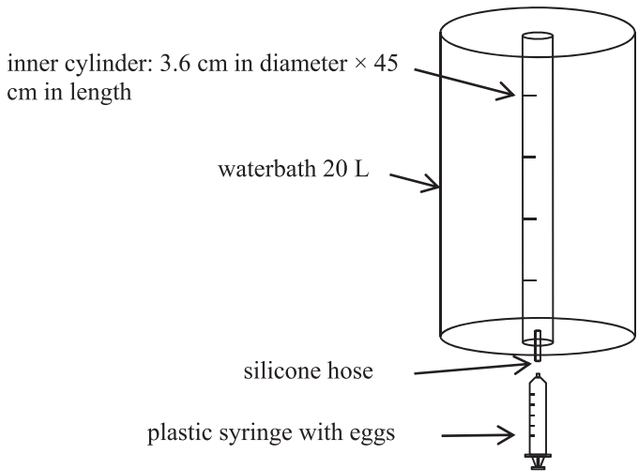

Fig. 1. Experimental setup for direct measurements of ascent rate of *Calanus hyperboreus* eggs.

In addition to the measured ascent rate of *C. hyperboreus* eggs from east Greenland, theoretical egg buoyancy was calculated using lipid and hydrography data from the Disko Bay study (*see* later sections) following the procedures in Visser and Jónasdóttir (1999). In short, an egg was assumed to consist of three fractions: a lipid fraction consisting mainly of WE, a water fraction, and a fraction of ''other solid material.'' This last fraction was assumed to be a mixture of protein and carbohydrates, and the density of this material in adult *C. finmarchicus* has been estimated at 1.08–1.24 g cm^{-3} (Visser and Jónasdóttir 1999) and 1.06 g cm^{-3} (Visser and Jónasdóttir 1999, from Childress and Nygaard 1974). Ascent rate of eggs was calculated following the modified version of the Stokes equation in Visser and Jónasdóttir (1999) as

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

where w is the ascent rate (cm s^{-1}), g is gravitational acceleration (980 cm s^{-1}), d is the diameter of the egg (198 μm), μ is dynamic viscosity of seawater (0.018 g cm^{-1} s^{-1}), α_L is the volume fraction of lipids, ρ_L is the density of lipids (0.920 g cm^{-3}), ρ_W is the density of sea water, α_O is the volume fraction of other solid material, and ρ_O is the 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 be 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),

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

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 *C. hyperboreus* females were collected by vertical hauls with a 200 μm mesh WP2 (UNESCO 1968) net with a closed cod end from 250 m up. Animals were kept cool and, at arrival to the laboratory, sorted in ice-chilled petri dishes and distributed into 10 L buckets with false net bottoms filled with 50 μm of filtered seawater. Thirty females were incubated in each bucket and placed in a temperature-controlled container at 2.5°C. One third of the water was changed every second day, and eggs were collected every 24 h. Nauplii cultures for respiration experiments and carbon and lipid analysis were established at 0°C, 5°C and 10°C. One hundred eggs were incubated in 600 mL polycarbonate bottles filled with GF/F filtered sea water. Two thirds of the water was renewed every 3 d by reverse filtration, and from N3 they were fed *R. salina* in a minimum concentration of 15 μg chlorophyll a (Chl *a*) L^{-1}.

Egg hatching—Two different hatching experiments were set up. The first experiment (Expt. 1) was initiated on 12 February. Eggs spawned within 24 h at 2.5°C were collected and incubated in tissue culture trays (Nunc Multi wells) of six wells, each containing 10 mL GF/F filtered surface water and 30 eggs. The trays were incubated at constant temperature in temperature controlled thermo boxes at 0°C, 2.5°C, 5°C, 7.5°C, and 10°C. Every 6 h, eggs and nauplii were inspected and counted. For unknown reasons, eggs incubated at 10°C were not hatching and thus not presented in the results. The second experiment (Expt. 2) was initiated 03 March with eggs spawned within 24 h by females incubated at the five different temperatures for 13–16 d. Five groups of 50 eggs were incubated at each temperature as described above. Temperature was logged every 15 min using Hoboware thermo loggers. The actual temperatures differed slightly from the intended temperatures (Table 1), but for convenience, the intended temperatures will be used when describing data. A Beléhrádek function relating embryonic duration to temperature was fitted to data,

$$D = a(T - \alpha)^b \quad (3)$$

where D is MDT of eggs at temperature T, and a, α, and b are constants. The coefficients a and α are fitted parameters, whereas the exponent b was set to −2.05, a widely used mean value for a range of copepod species (McLaren et al. 1969; Corkett et al. 1986; Campbell et al. 2001).

To model the development times during the ascent of eggs, the water column was divided into three layers reflecting different water properties: a warm bottom layer from 270 to 152 m, where the average temperature was 2.8°C; a layer from 152 to 171 m, with an average temperature of 1.4°C; and a cold layer from 171 to 0 m, with an average temperature of −1.3°C (Henriksen et al. 2012). Development rates were then calculated according to the Beléhrádek function obtained from hatching Expt. 1.

Table 1. Table 1. Intended (T_{int}) and actual temperature (T ± SD) in *Calanus hyperboreus* egg hatching in Expts. 1 and 2 and in respiration experiments with N1 and N3 (RespN1, RespN3).

	T (°C)			
T_{int} (°C)	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

Development—Development of nauplii from egg through the first five nauplius stages (N1–N5) was followed at 5.0 ± 0.6°C with and without food. Six 2.6 L polycarbonate bottles were filled with GF/F filtered sea water, and 794 ± 19 *C. hyperboreus* eggs spawned within 24 h of collection were added to each bottle. Three of the bottles were spiked with an *R. salina* phytoplankton culture at a concentration of 15 μg Chl *a* L^{-1}. The bottles were placed in a thermo box in the temperature-regulated container in constant darkness and rotated by hand once a day. Every 3 d, two thirds of the water was removed by reverse filtration, and 10–15 nauplii from each bottle were randomly sorted out and fixed in 4% formalin. Bottles were refilled with filtered seawater, and new food was added. Temperature was logged every 15 min. Nauplii were staged and measured on an Olympus-CK inverted microscope. Three measures of the nauplii were recorded: length of carapace (CP); total length, measured from the tip of the carapace to the end of the tail (TL_1); and total length, measured by adding CP to the length of tail (TL_2). For N1 and N2, only TL_1 was recorded. MDT, defined as the time when 50% of the nauplii had molted to a specific stage, was calculated from the linear 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 nauplius abundance at the start and the end of the incubation period. The Beležhrádek function obtained from hatching Expt. 1 was used to calculate nauplius 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 precombusted aluminum 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, in which N3 had been fed *R. salina* for 4 d. Samples were dried overnight at 60°C and frozen until analysis. Measurements were done in an infrared gas analyzer (model ADC-225 MK 3; Analytical Development Company) calibrated with oxalate. Lipid measurements were conducted on rinsed eggs, and animals were placed on precombusted GF/F filters. Samples of 70–115 eggs, 90–100 N1, and 90–100 N3 were collected in three to six replicates, with five controls for each treatment consisting of filtered seawater. Samples were placed in 1 mL of 2 : 1 chloroform : methanol 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 microsensor, using the microrespiratory system from Unisense A/S (Brodersen et al. 2007). Five to 10 carefully rinsed nauplii (cultured at corresponding temperatures and fed from N3) were placed in a 500 μL chamber filled with 0.2 μm filtered sea water. The chamber was closed by a tight-fitting glass stopper with a long and slender capillary hole (<0.7×13 mm) that prevented diffusion of oxygen and through which the microelectrode was lowered during measurements. For each experiment, six replicate chambers and two controls filled with 0.2 μm filtered seawater were measured. Oxygen consumption was measured over 15–48 h depending on temperature. Between each measurement, the chamber was closed with a plug lowered through the capillary hole, and nauplii were counted. Temperature was logged every second minute. As for egg hatching, the actual temperatures differed slightly from the intended temperatures (Table 1), but the intended temperatures will be used when describing data.

Energy requirements—Energy requirements for the nauplii were calculated on the basis of respiration and lipid measurements. Because the nauplii mainly contained WE, which is a very energy- and space-efficient storage medium, a lipid-based metabolism was assumed. WE of *C. hyperboreus* can provide 42.7 J (mg lipid)$^{-1}$ (Båmstedt 1986; Auel et al. 2003); hence, the energy available for the nauplii is calculated as WE content of nauplii × 42.7 J mg^{-1}. Respiration rate was converted to daily energy requirements by applying an oxycaloric equivalent typical for lipid-based metabolism of 19.6 J mL^{-1} (Gnaiger 1983). Using these numbers, theoretic stage duration was calculated as energy available divided into daily energy requirement, and a theoretic respiration rate was calculated as energy available divided into development time. The minimum carbon requirement of nauplii was calculated by applying a respiratory quotient (RQ) typical for lipid-based metabolism of 0.72 (Gnaiger 1983).

Results

Egg buoyancy—The mean ascent rate recorded was 8.6 ± 7.1 m d^{-1}. However, as indicated by the standard deviation, the individual rates were highly variable, ranging from 0.7 to 27.7 m d^{-1}, the median being 5.6 m d^{-1} (Fig. 2). In general, ascent rate varied both within a clutch of eggs and between females (Fig. 3). Female 3 produced mainly fast eggs ascending at 20.2 ± 5.6 m d^{-1}; females 2 and 4 produced mainly slow eggs ascending at 2.3 ± 2.0 m d^{-1}, whereas female 1 produced both fast and slow

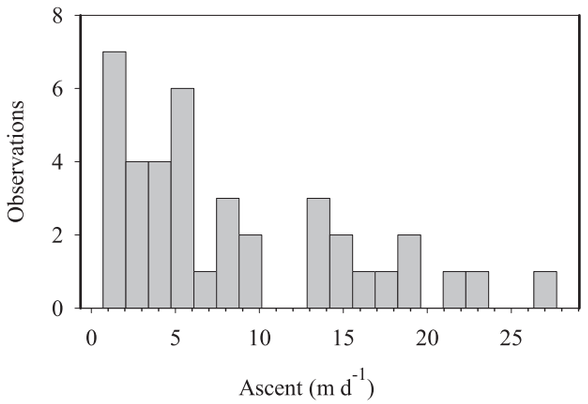

Fig. 2. Histogram presenting ascent rates of 39 *Calanus hyperboreus* eggs, spawned by six different females.

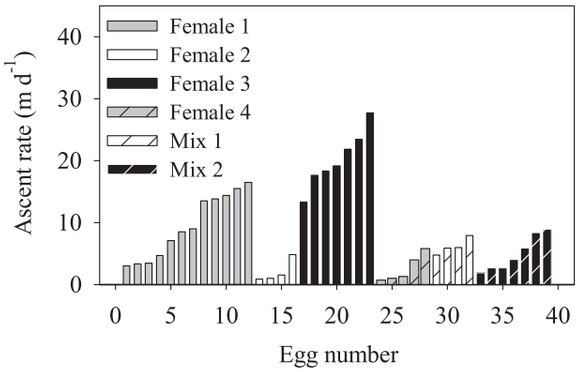

Fig. 3. Ascent rate of *Calanus hyperboreus* eggs grouped by female. On two occasions, mix 1 and 2, eggs spawned by two females were mixed.

eggs, with ascents ranging from 3 to 16.5 m d^{-1}. The observed differences in ascent rate were significant between female 3 and females 2 and 4 and mix 2 (Kruskal–Wallis, $p = 0.001$, followed by Dunn's multiple comparison method). Egg density varied between 1.0006 and 1.0268 g cm^{-3}, averaging 1.0194 ± 0.0063 g cm^{-3}.

The ascent rate for the Disko Bay eggs were calculated according to Eqs. 2 and 3. Using these two equations, the only unknown factor was the density of other solid material (ρ_O). Calculating ascent rate using the density range of ρ_O from Visser and Jónasdóttir (1999; 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 rates, ρ_O would have to be < 1.14 g cm^{-3}. A ρ_O of 1.08 g cm^{-3} gave an ascent rate at 8 m d^{-1} equal to an egg density of 1.0191 g cm^{-3}.

Egg hatching of C. hyperboreus—In general, hatching success of the eggs was high in both experiments, ranging between 75% and 83% in Expt. 1 and 88% and 98% in Expt. 2 (Table 2). MDT of eggs ranged between 5.8 and 2.8 d at 0–7°C in Expt. 1 and 5.3 and 1.7 d at 0–10°C in Expt. 2, with a significant effect of temperature in both experiments (two-way ANOVA, $p < 0.001$). Even though temperatures were slightly lower in Expt. 2 (Table 1), egg hatching was significantly faster than in Expt. 1 at all temperatures (Holm–Sidak Multiple comparison; Fig. 4). Temperature coefficient (Q_{10}) calculated for the different temperature intervals in the two experiments ranged between 2.6 and 5.4, with the highest values from 0°C to 2.5°C (Table 3). Applying the Beléhrádek function gave a significant fit to data (Fig. 5) in both experiments with $r^2 > 0.99$.

Length frequency distribution of nauplii—Of the three measurement methods applied (TL_1, TL_2, and CP), TL_2 and CP where most useful in identifying distinctive length classes of nauplius stages larger than N3 (Fig. 6). Coefficient of variation was in general twice as large 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 nauplius stages are presented in Table 4.

Development of nauplii—During the 40 d experiment, nauplii developed until N5 in fed treatments, whereas in starved treatments, development generally arrested at N3 (Fig. 7). However, up to 9% of the nauplii developed to N4 in the starved treatments. The long starvation period did not seem to affect the viability of nauplii since, at the end of the experiment, the amount of dead nauplii was approximately the same in fed and starved treatments (77% and 66%, respectively). MDT between fed and starved treatments was not significantly different during the first nonfeeding stages; hence, average MDT of all treatments are presented in Table 5. From N3, *R. salina* was observed inside the gut of the nauplii, and peristaltic movement of the gut was observed. Chl *a* concentration in the bottles measured every 3 d before addition of new food was 6.3 ± 0.7 and 0.2 ± 0.1 μg Chl *a* L^{-1} in fed and starved treatments, respectively. Stage duration increased with development stage; the longest, N4, lasted 14.5 d. The relative stage duration was used together with the Beléhrádek function for embryonic duration to estimate nauplius development times at different temperatures (Fig. 8). Daily mortality rates were 0.09 and 0.04 d^{-1} for fed and starved nauplii, respectively.

Carbon and lipid content—A clear trend of decreasing carbon content was observed during the nonfeeding stages (egg–N3) followed by increasing carbon content during the feeding stages (N3 fed for 4 d [$N3_{fed}$]–N4) (Fig. 9, Table 6), even though variation between replicates was high. However, only in N3 vs. N4 and egg vs. N3 were the observed differences in carbon content significant (one-way ANOVA). The $N3_{fed}$ had 59% higher carbon content than starved N3. Total lipid content of *C. hyperboreus* eggs (diameter 198 ± 7 μm; Henriksen et al. 2012) was 0.55 μg egg^{-1}, amounting to approximately 72% of carbon content and 46% of egg dry wt when using a volume to dry wt conversion of 0.29 pg C μm^3 (calculated from the dry wt and diameter of eggs measured by Conover [1967]). WE was the main lipid class, representing 85–90% of total lipids in all stages. The amount of TAG was considerably lower, accounting for 3–4%; PL represented 6–12% of total lipids, whereas the concentration of sterols was under the detection limit. A general decrease in lipid amounts from egg to N3 was seen for WE and TAG, but not PL (Fig. 9, Table 6). The decrease was most profound in WE, in which

Table 2. Mean development time (MDT) and hatching success (HS) of *Calanus hyperboreus* eggs in hatching Expts. 1 and 2 at five different temperatures.

	Expt. 1		Expt. 2	
T (°C)	MDT (d)	HS (%)	MDT (d)	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

47% was used from egg to N3. Hatching accounted for a 12% decrease (3.8% d^{-1}), whereas 35% of WE stored in the egg was used during development from N1 to N3 (4% d^{-1}). For TAG, the trend was a bit more blurred, with an initial rise in concentration from egg to N1, followed by a decrease from N1 to N3. Overall, effect of stage, lipid class, and stage × lipid class was significant ($p < 0.001$, two-way ANOVA). The difference between stages was only significant within WE, and the differences between lipid classes were significant between WE and PL and WE and TAG (Holm–Sidak multiple comparison).

Respiration—Respiration rate increased with temperature and stage (Fig. 10). The effect of temperature ($p<0.001$) and stage ($p=0.05$) on respiration rate was significant, and the difference between stages was significant at 5°C (two-way ANOVA, followed by Holm–Sidak multiple comparison). Calculated Q_{10} values for the different temperature intervals was high, ranging between 2.6 and 7.8 (Table 7). Minimum carbon requirements for covering basic metabolism at 0–10°C were calculated to be between 1.8–8.8% and 3.9–12.8% of bodyweight individual^{-1} (ind.) d^{-1} for N1 and N3, respectively (Table 7).

Energy requirements—To evaluate how our different measurements corresponded, theoretical stage duration was calculated from the decrease in lipids from N1 to N3 and the respiration rate for N1 at 5°C. This theoretic stage duration was 6.3 d, whereas the measured stage duration was 6.8 d. However, the actual difference in age between N1 (age 4 d) and N3 (age 12 d) used for lipid analysis was 8 d (*see* Method section). Therefore, using the actual time of lipid consumption (8 d) and the decrease in lipids, a theoretical respiration rate for N1 was calculated to 0.086 nmol O_2 h^{-1}, which is 21% lower than the one measured. Accordingly, for the measure to add up, respiration rate should be 21% lower or lipid content 27% higher, which is considered within the error margin of the measurements. With N3 respiration at 0.172 nmol O_2 h^{-1}, the maximum time span N3 could starve was calculated to be 6 d, whereas the observed starvation potential of N3 was at least 30 d.

Discussion

The egg—The spawning patterns of the three co-occurring *Calanus* spp. are different; *C. hyperboreus* spawn

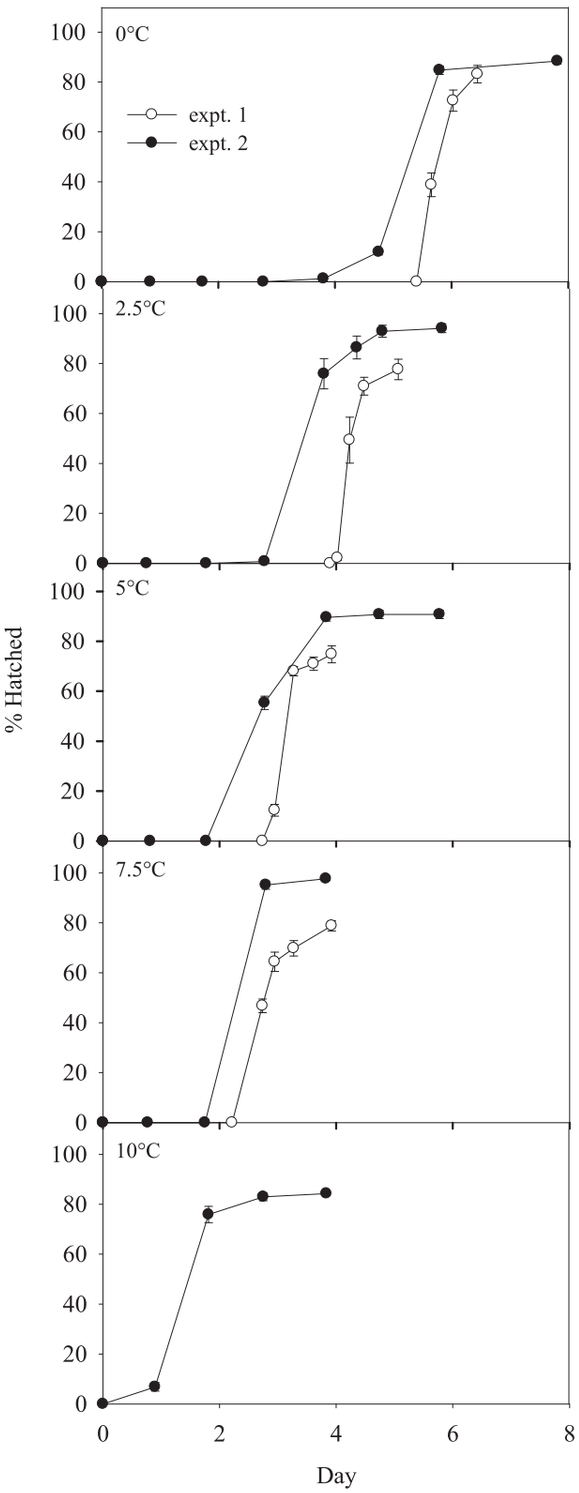

Fig. 4. Mean proportion of hatched *Calanus hyperboreus* eggs (%) ± standard error (SE) as a function of time (d) at five temperatures in Expt. 1, in which females were incubated at 2.5°C, and Expt. 2, in which females were incubated at the same temperatures as the eggs.

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

at depth before the spring bloom, and the lipid-rich eggs float to the surface, whereas *C. glacialis* and *C. finmarchicus* spawn in the surface layer in association with the spring bloom, with eggs settling after spawning (Sømme 1934; Knutson et al. 2001). These differences in phenology are reflected in the morphology and biochemical composition of the eggs. Whereas eggs of *C. glacialis* and *C. finmarchicus* have similar appearance, being transparent with a robust egg shell and therefore easy to handle, eggs of *C. hyperboreus* are bright orange and have a very fragile egg shell that breaks easily during handling (pers. obs.). The specific carbon content of eggs differs considerably between species, being 0.11 pg C μm^{-3} for *C. glacialis* and *C. finmarchicus* (Swalethorp et al. 2011) and 0.19 pg C μm^{-3} for *C. hyperboreus.* The higher specific carbon content of *C. hyperboreus* eggs is most likely because of their very high lipid content, seeing that lipids are ~ 80% carbon (Jónasdóttir 1999). The lipid content of *C. hyperboreus* eggs was 46% of egg dry wt, with the main lipid class being WE. In comparison, lipid content of *C. finmarchicus* eggs has been estimated to 15% of dry wt, with PL being the dominant lipid class (Lee et al. 2006). These differences corroborate the different life strategies of the two species, with *C. finmarchicus* spawning when food is abundant, in contrast to *C. hyperboreus* spawning in the absence of food.

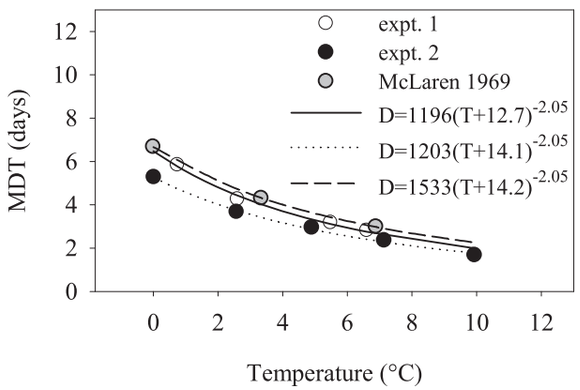

Fig. 5. Belĕhrádek's function (lines), relating mean development time (MDT) of *Calanus hyperboreus* eggs to temperature, fitted to data from the two hatching experiments and to egg hatching data from McLaren et al. (1969; circles).

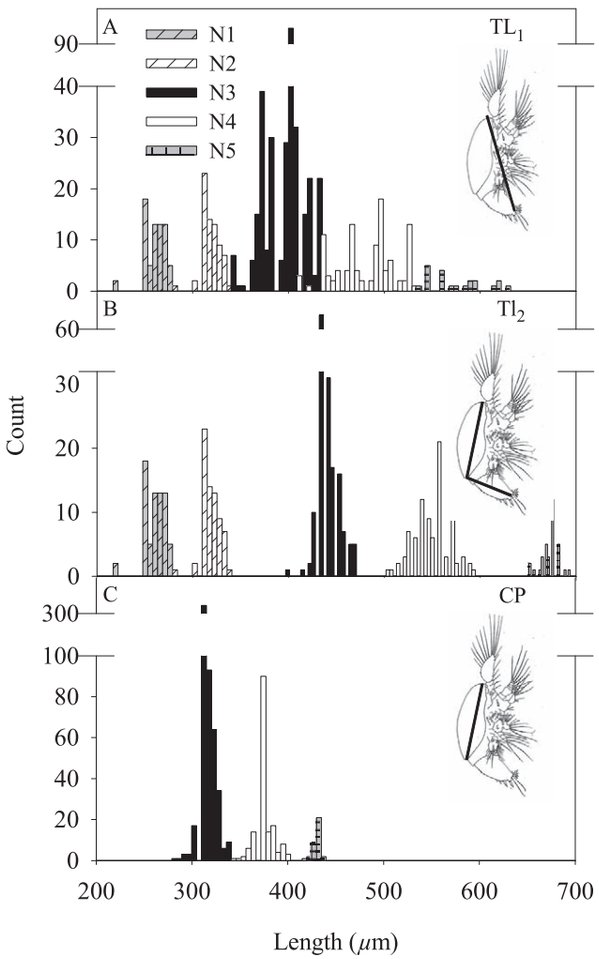

Fig. 6. *Calanus hyperboreus* nauplius measurements arranged in 5 μm bins; (A) total length 1 measure (TL_1), (B) total length 2 measure (carapace + tail, TL_2), and (C) length of carapace (CP). Drawing of nauplii from Sømme et al. (1934).

In the latter case, the nauplii must survive on the lipids from the egg until food becomes available. As a result, the main lipid in *C. hyperboreus* eggs are storage lipids consisting of the highly energy rich and condensed WE, in contrast to the easily mobilized TAG and PL of *C. finmarchicus* eggs.

Table 4. Table 4. Mean ± SD nauplius carapace (CP) and total nauplius lengths (TL_1 for N1 and N2, and TL_2 for N3–N5) and ranges, and sample size (*n*) of *Calanus hyperboreus*.

	Length (μm)			
	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

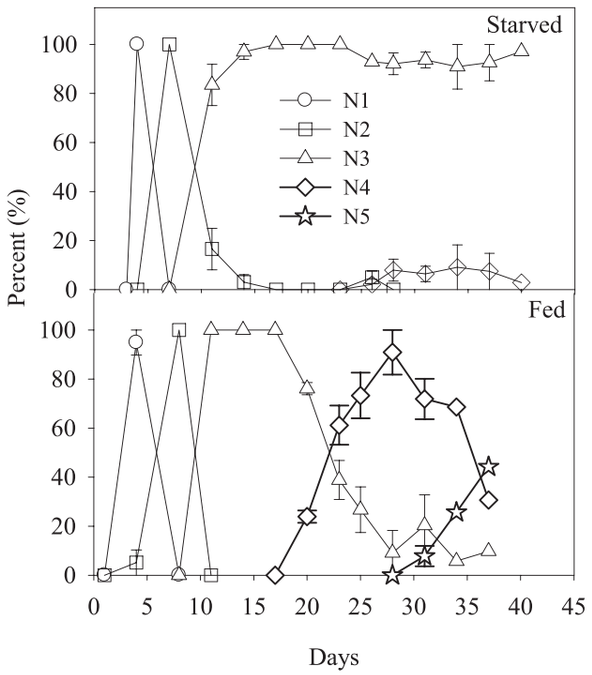

Fig. 7. Development of starved and fed *Calanus hyperboreus* nauplii at 5°C. Values are mean ± SE (n = 3).

A side effect of having lipid-rich eggs is that it makes them positively buoyant. With a calculated ascent rate of Disko Bay eggs at 8 m d^{-1}, it takes 34 d to reach the surface, which means that all eggs have developed to nauplii on arrival. The average temperature of the bottom water (270–152 m) was 2.8°C. At this temperature, eggs hatch after 4.3 d, having moved 35 m from the spawning depth. As lipid content of the nauplii decreases with stage, the ascent rate also decreases with stage, and nauplii reach N3 after 13.8 d at a depth of 159 m. On the basis of the lipid content of N3 and an estimated nauplius volume, calculated as the volume of an ellipsoid, ascent rate of N3 was calculated as 4.4 m d^{-1}. At this rate, it would take another 36 d to reach the surface, meaning the nauplii arrive in surface waters 50 d after the eggs are spawned. Using the measured range of ascent rates, however, the fastest eggs hatch in a depth of 150 m and reach the surface as N1 after 10 d, whereas the slowest eggs reach the surface after 386 d.

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

	This study		Conover 1967	
	MDT (d)	Duration (d)	MDT (d)	Duration (d)
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	—

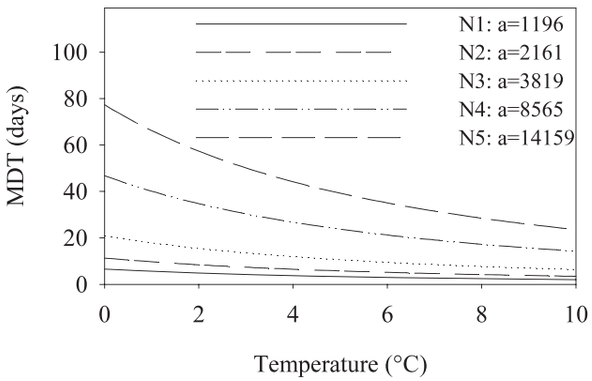

Fig. 8. Mean development time (MDT) of *Calanus hyperboreus* nauplius stages N1–N5 predicted by Belĕhrádek's function $D = a(T + 12.7)^{-2.05}$, relating MDT to temperature assuming equiproportional development.

Agreement was good between the calculated ascent rate of eggs (8 m d^{-1}) and the mean value from the direct measurements (8.6 m d^{-1}). This rate seems reasonable because passive ascent rate of adult *C. finmarchicus* has been estimated at 13–18 m d^{-1} (Visser and Jónasdóttir 1999). However, because of a large variation in ascent rate of eggs coming from the same clutch, it seems likely that the eggs also differ in other aspects. Kjesbu et al. (1992) found that the buoyancy of cod eggs was related to their diameter. This may also be true for the *C. hyperboreus* egg. However, the variation in ascent rate caused by differences in diameter is rather small (Eq. 1) compared with the huge variability in ascent rate observed; hence, it will easily be masked by other factors influencing the ascent rate. Such factors could be differences in lipid content, which directly influence buoyancy; in the hatching rate; or in the metabolic activity of the egg. Viability of the eggs used for the experiment was not investigated, but from Expt. 1, we saw that around 20% of the eggs did not hatch. Because the eggs were discarded after 5–10 d, we cannot exclude the possibility that some of the eggs might have hatched later. Delayed hatching eggs (DHEs) are described in various

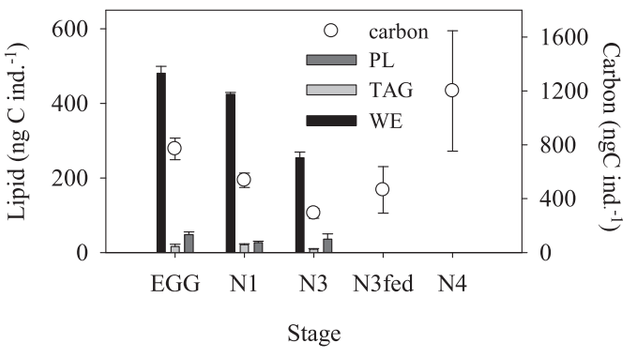

Fig. 9. Carbon (open circles) and lipid (bars) content of *Calanus hyperboreus* eggs and nauplii. Values are mean ± SE of carbon (C), phospholipids (PL), triacylglycerol (TAG), and wax esters (WE). $N3_{fed}$ was fed for 4 d before collection. n = 3–6 samples per measurement.

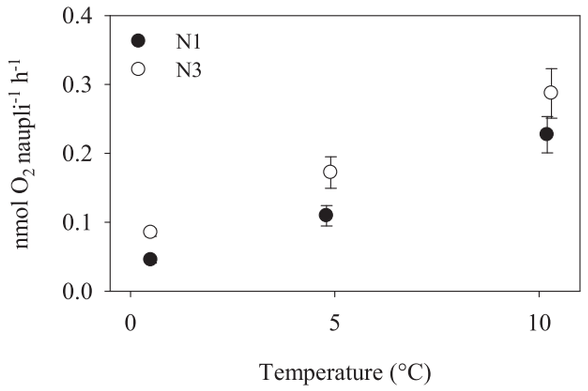

Fig. 10. Respiration of *Calanus hyperboreus* N1 and N3. Values are mean ± SE.

Atlantic copepod species (Engel 2005) and are most likely produced as a response to unfavorable environmental conditions (Drillet et al. 2011). DHEs are not believed to be as important in Arctic areas where copepods have alternative strategies to cope with environmental variability, such as lipid storage capacity and dormancy of copepodite stages (Engel 2005). However, it may be an advantage for *C. hyperboreus* to spread out the hatching period of eggs to maximize the chance that some of the developing nauplii are matching the spring bloom. Plasticity in hatching times can also be a way to conserve energy, in that the metabolic activity of eggs (embryos) most likely is less than for nauplii. Furthermore, because the eggs are positively buoyant, they are not lost to the sediment but slowly make their way toward the surface. The ascent experiments were performed in the very beginning of the spawning cycle of *C. hyperboreus*, during which only a small fraction of the females were producing eggs. It therefore remains to be investigated whether season has an effect on the ascent rate of the eggs; whether buoyancy and viability of the eggs are connected, as suggested by Conover (1967); and whether plasticity in the hatching pattern of eggs is present. The large observed difference in buoyancy properties of the egg was also noted by Conover (1967), who observed both sinking and floating eggs during his egg production experiment. He found that the number of floating eggs varied between 40% and 100% and decreased with time. However, from his data, it is not possible to separate variation between or within clutches of eggs.

After the eggs are spawned in deep waters and have started ascending, they develop toward hatching. The egg hatching rate of *C. hyperboreus* eggs was measured two times 19 d apart. In Expt. 1, the eggs spawned at 2.5°C (which is close to bottom water temperatures in Disko Bay; Hansen et al. 2012) over a period of 24 h were incubated at a range of experimental temperatures. Hence, eggs have been affected by this low temperature both during oogenesis and maturation in the female gonads and 0–24 h after spawning. This might underestimate the hatching rate in incubations > 2.5°C, whereas at 0°C, hatching rate could be slightly overestimated. The observed temperature dependence of hatching rate was similar to that found in a comparable experiment by McLaren et al. in 1969 (Fig. 5). Expt. 1 mimics the current situation in which eggs are spawned in the warm bottom water and float up through water masses of different temperatures. In the present situation, they end up in the cold surface water, but if surface temperatures are increasing, as predicted in future climate change scenarios, they meet a warmer water layer.

In Expt. 2, eggs were spawned by females preconditioned to the five experimental temperatures, and final maturation and spawning of eggs therefore occurred at the same temperature. This experiment mimics a situation in which the bottom water temperatures also have increased because of climate change. If the observed differences in hatching rate were a matter of method only, hatching in the two experiments should be identical at 2.5°C (where the methods are the same), slightly faster in Expt. 1 at 0°C, and faster for temperatures above 2.5°C in Expt. 2. Because hatching at all temperatures took significantly longer in Expt. 1 than in Expt. 2, season might exert an effect (i.e., eggs produced early develop slower than eggs produced later in the season), which could be explained by a change in egg quality concerning biochemical composition with season. If lipid content of eggs declined as the lipid content of females declined (Henriksen et al. 2012), less lipid-rich eggs may hatch and develop faster, to reach the first feeding stage sooner. Conover (1967) noted that the first batches of eggs laid by *C. hyperboreus* were floating, whereas egg batches produced later sank. Lipids make the eggs positively buoyant, which suggests that the lipid content of eggs was changing, although it remains to be verified experimentally. Differences between clutches of eggs spawned by the same female were also observed by Hirche (2013), who found the viability of eggs to vary considerably from clutch to clutch; however, no temporal trend in the variation was detected.

Nauplii—The nauplius stages of *C. hyperboreus* could be divided into specific size classes (Fig. 6, Table 4). The present nauplii were slightly larger than reported by Conover (1967) and overlapped in size with measures of *C. glacialis* from Daase et al. (2011). It may be a problem to

Table 6. *Calanus hyperboreus* mean lipid and carbon content ± SD and No. of replicates (*n*) of wax esters (WE), triacylglycerol (TAG), phospholipids (PL), and carbon (C). $N3_{fed}$ was fed *R. salina* for 4 d.

Content (ng ind.$^{-1}$)	Egg	N1	N3	$N3_{fed}$	N4
WE	481±45(6)	424±8(3)	254±37(6)	—	—
TAG	16±12(4)	21±4(3)	9±4(6)	—	—
PL	48±12(3)	27±6(3)	36±28(4)	—	—
Carbon	769±255(10)	536±130(6)	292±75(4)	465±386(5)	1200±893(4)

Table 7. Mean respiration rate ± SD and No. of replicates (*n*) of *Calanus hyperboreus* N1 and N3 at three temperatures, corresponding carbon requirements in percent bodyweight, and Q_{10} for temperature intervals.

	Respiration rate (nmol O_2 ind.$^{-1}$ h^{-1})		Carbon requirements (%body wt ind.$^{-1}$ d^{-1})			Q_{10}	
T (°C)	N1	N3	N1	N3	*T* (°C)	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

compare size distribution of nauplii from different populations, because size variation between populations from different environments has been found among nauplii (*see* table 6 in Daase et al. 2011), as well as among copepodites and adults (Kwasniewski et al. 2003; Weydmann and Kwasniewski 2008). To be able to distinguish between different species of *Calanus* nauplii from in situ samples, body size measurements have to be from the same area. Furthermore, it is also important to consider which measure to use (Sømme 1934). When studying specific nauplius species and development stages, CP, TL_2, or both measures are often recorded. However, when nauplii are not the focus and a sample of a mixed biomass is analyzed, often TL_1 is the only measure taken because measuring TL_2 is both tedious and time consuming. Consequently, the nauplii of closely related *Calanus* species cannot be separated on the basis of literature reports of stage and body length alone. Therefore, we call for a standardized procedure for measuring CP instead of TL_1, which would not be more time consuming and would allow future comparative analysis of the nauplius communities.

In the present study, development of *C. hyperboreus* was followed to N5. N3 is normally considered to be the first feeding stage for most copepod species, but for *C. hyperboreus* N5 also has been suggested (Conover 1962). Our study corroborates the findings of Conover (1967) and the suggestions by Melle and Skjoldal (1998) that N3 is the first feeding stage of *C. hyperboreus.* The development time from egg to N1 was comparable to that found by Conover (1967) (Table 5). After N1, development proceeded slower, and by the time the nauplii reached N5, development time was twice as long as suggested by Conover. Part of this difference might reflect differences in incubation method and food quality offered: Conover was feeding the nauplii with the diatom *Thalassiosira fluviatilis*, and we were feeding them with the chryptophyte *R. salina*. Mortality during the experiment was high; 77% of the fed and 66% of the starved nauplii died during the incubation period of 40 d. However, this is comparable to mortality of *C. glacialis* nauplii measured over 42 d, during which 78% and 95% of the nauplii died in fed and starved treatments, respectively (Daase et al. 2011). As argued in Daase et al. (2011) the reason for high mortality is the long duration of incubation, whereas daily mortality rates (0.09 and 0.04 d^{-1} for fed and starved nauplii, respectively) are similar to the rates found for other *Calanus* species (Daase et al. 2011; Grenvald et al. 2012). The lower mortality of starved *C. hyperboreus* in the present study probably reflects the high lipid content of the nauplii, which enables them to endure starvation and obtain a higher survival rate than *C. glacialis.* The decrease in lipid from egg to N3 documented that nauplii were indeed metabolizing lipid to cover their energy requirements during molting (Fig. 9).

Nauplii of *C. hyperboreus* seem to be more sensible to temperature changes than later development stages (Conover 1962; Henriksen et al. 2012), which renders recruitment the most sensible parameter in the future. Increasing temperature raised respiration rate dramatically, and even though nauplii were raised at the experimental temperatures, they were not able to regulate their respiration, as has been shown for preacclimatized adults measured between 2°C and 8°C (Conover 1962). It seemed that N1 were more affected by elevated temperature than N3, in that Q_{10} for N3 was one third that for N1. A future earlier warming of the surface water in early spring will therefore significantly increase the carbon requirements to cover basic metabolism and thereby increase the need for earlier feeding opportunities. At 0°C, N3 were using 3.8% of their bodyweight per day to cover basic metabolism, giving them 26 d before they had used all their reserves, whereas at 5°C, that would take only 13 d. However, the gap found between observed age of starved N3 (30 d) and calculated maximal starvation potential of N3 (6 d) indicates that the nauplii must be able to reduce their metabolism further in response to long starvation periods (e.g., by metabolic down regulation). To verify this, respiration measurements should be done on starving N3, not only on fed N3 as was the case in this study.

Very few data exist for nauplius respiration at low temperature. However, Ikeda et al. (2001) compiled data on metabolic rate from 35 zooplankton species within the temperature range of −1.7°C to 29°C and developed a model relating dry wt (mg) and temperature (*T*, °C) to metabolic rate of zooplankton (*y*, μL O_2 ind.$^{-1}$ h^{-1}):

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

When our results are compared with the value calculated from the observed dry wt and temperature, all measured values were well below that of Eq. 4. Respiration of *Acartia tonsa* eggs at 10°C have been measured as 0.09 nmol O_2 egg^{-1} h^{-1}, corresponding to 769 nmol O_2 (mg dry 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 of *Eucalanus pileatus* nauplii at 21°C have been measured as 0.82 nmol O_2 nauplii^{-1} h^{-1}, or 653 nmol O_2 (mg dry wt)$^{-1}$ h^{-1} (Köster et al. 2008). In comparison, the mass specific respiration of N1 in 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 indicates metabolic acclimatization or, more likely, an overall adaptation to living in the Arctic with generally low water temperatures.

The station where the eggs were spawned is 270 m deep. Hence, eggs spawned at this depth must be positively buoyant to reach the productive surface layers. With both of the present estimated ascent rates (8 or 8.6 m d^{-1}), nauplii would have developed to the first feeding stage well before arrival to the surface waters. Because the eggs were spawned from January to March and the spring bloom peaked at the beginning of May (Henriksen et al. 2012; Fig. 5), nauplii would have faced starvation for at least a month during ascent and arrived at the surface layer before the developing bloom. However, from early April, Chl *a* concentration exceeded 1 μg L^{-1} in the upper 50 m (equal to 30 μg C L^{-1}, applying the Chl *a* to a carbon conversion of 30 reported by Dünweber et al. [2010] in Disko Bay), meaning that some food was available for the nauplii at this time. Alternatively, ice algal blooms can form patches of higher food concentration at the ice–water interface. The present starvation experiment showed that even at 5°C, N3 were able to survive at least 30 d without food, equivalent to a period of 69 d at surface water temperatures of −1.5°C (applying a Q_{10} of 3.6 from hatching Expt. 1; Table 3). If the large variations in ascent rate also are representative for Disko Bay, some eggs would reach the surface layer after only 10 d, and some not at all. However, to get to the surface faster, slow ascending nauplii could engage in swimming. Swimming speed for *Calanus helgolandicus* N5 have been measured at 325 m d^{-1} (Titelman and Kiørboe 2003). Because *C. helgolandicus* only spend 80% of their time swimming and do not swim in a straight line (Titelman and Kiørboe 2003), this would roughly be equal to around 87 m d^{-1}. Assuming swimming speed scales with size, N3 of *C. hyperboreus* would swim around 69 m d^{-1}. As a conservative estimate, this means that nauplii could reach the surface in less than a week. However, passive ascent conserves energy, and because no food is available for the first month, there would not be any reason for the early-spawned nauplii to use energy swimming. Nauplii produced toward the end of the spawning season may benefit from reaching the surface water earlier because some food might already be available. In both cases, however, swimming activity would also increase the risk of being eaten (Tiselius and Jonsson 1997, Titelman 2003). Another important adaptive benefit of buoyant eggs is separation of the eggs from the females, thereby avoiding the cannibalistic mothers. Conover (1967) observed during an egg production experiment that females ingested eggs. Likewise, observations of egg-laying females producing orange pellets indicate feeding on their own eggs (S. Jung-Madsen unpubl., 2009).

Disko Bay is a rather shallow habitat compared with the Greenland Sea or Arctic Ocean. In the Greenland Sea and Fram Strait, *C. hyperboreus* may overwinter at depths < 2000 m (Auel et al. 2003; Hirche et al. 2006). At such a depth, active swimming will be necessary to reach the surface in time for the spring bloom. However, from November to March, when *C. hyperboreus* is reproducing (Hirche and Niehoff 1996), the main part of the female population is situated in 1000–1500 m of water, and they move upward during the period (Hirche and Niehoff 1996; Hirche 1997). Nauplii originating from eggs produced at this depth should be able to reach the spring bloom. Increasing ocean temperatures will affect not only development and energy requirement of nauplii, but also the timing and duration of the spring bloom on which they feed. An earlier occurring bloom would counteract some of the metabolic effects of warmer water if spawning occurred at the same time. The factors controlling when *C. hyperboreus* initiates spawning still remain to be identified (Hirche 2013). However, females using their lipid stores faster because of higher metabolism could be hypothesized to cause earlier spawning.

In summary, ascent rate of eggs is highly variable, indicating production of eggs with different biochemical properties, which could be a strategy to enhance chances that some of the offspring successfully matches the phytoplankton spring bloom. As in other copepod species, the embryonic development of *C. hyperboreus* was mainly controlled by temperature, but also an apparent effect of the season when eggs were produced. The early stages of *C. hyperboreus* seem to be more affected by temperature than older stages. The high lipid content of eggs and nauplii enhance survival in a food-limited environment. However, increasing temperature decreases the development time and increases the carbon requirements of nauplii and will therefore affect their possibility of matching the phytoplankton spring bloom. This may in a future warmer climate have large implications for the recruitment of *C. hyperboreus*.

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