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Early development of *Calanus glacialis* and *C. finmarchicus*

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Abstract

Egg and nauplii development of coexisting populations of *Calanus glacialis* and *C. finmarchicus* from Disko Bay, western Greenland, were measured in the laboratory with, and without addition of food. Egg hatching rate was measured at five temperatures from 0°C to 10°C and the fit to a Belĕhrádek equation was highly significant ($r^2 > 0.99$). There was little difference between hatching and development rates of the two species; however, the egg hatching rate at low temperatures was faster than predicted from measurements made in other areas. Also, nauplii development times at 0°C were similar, with *C. glacialis* and *C. finmarchicus* reaching nauplii stage N6 after 44 d and 48 d, respectively. The laboratory-reared nauplii could be separated accurately to species by stage and length alone. However, separation by size was not possible for the in situ community of nauplii where the influence of food, temperature, and size-dependent predation mortality, made the size distribution more variable and overlapping.

The Arctic environment is warming with reduced sea ice cover and accelerated melting of inland glaciers. These changes in forcing factors alter the physical settings for the biological processes and succession in the ocean (IPCC 2013). Therefore, a major effort has been made to investigate and predict how temperature changes impact the vulnerable Arctic marine ecosystems.

Copepods of the genus *Calanus* have a key position in the Arctic marine food web as they dominate the zooplankton community and play a central role in channeling primary production to higher trophic levels. In Arctic areas, three species of *Calanus* dominate the zooplankton. Despite many morphological similarities they differ in size, temperature preference, life cycles, and per capita lipid content. *C. hyperboreus* and *C. glacialis* are considered Arctic species, and *C. finmarchicus* a North Atlantic/subarctic species (Conover 1988). Because of the difference in their core of distribution and as they are believed to support different food webs (Falk-Petersen et al. 2007), these copepods are ideal climate indicators.

C. hyperboreus is the most lipid-rich of the three species, with reproduction occurring in winter prior to the spring bloom (Madsen et al. 2001; Henriksen et al. 2012), whereas the reproduction of the two other species is dependent on the phytoplankton spring bloom (Falk-Petersen et al. 2007; Swalethorp et al. 2011). Still, *C. glacialis* and *C. finmarchicus* represent two different life-cycle strategies. *C. glacialis* is a typical Arctic species, large, with a multiyear life cycle, which builds up lipids during summer. The lipids are used to mature gonads and initiate egg production before the phytoplankton spring bloom. Hence, *C. glacialis* matches the bloom with mature gonads and can utilize the abundant food directly for egg production. *C. finmarchicus* is a smaller, less lipid-rich species with a one-year life cycle in Arctic areas and 1–3 generations per year in temperate areas (Melle et al. 2014). The maturation of gonads and egg production of *C. finmarchicus* is food-dependent and, therefore, tightly coupled to the phytoplankton spring bloom (Falk-Petersen et al. 2007; Swalethorp et al. 2011).

The distribution of *C. glacialis* and *C. finmarchicus* overlap in Disko Bay, western Greenland, and increased ocean warming may alter the composition of the *Calanus* spp. community. Experiments considering temperature, food availability, and egg production of *C. hyperboreus* (Henriksen et al. 2012), *C. glacialis,* and *C. finmarchicus* (Kjellerup et al. 2012) suggest that increasing temperature will favor the smaller fast-developing *C. finmarchicus* at expense of the larger Arctic species, that are crucial components in the Arctic marine food web (Falk-Petersen et al. 2007).

A key to understanding population dynamics of copepods is knowledge about early development. Despite the fact that nauplii represent a bottleneck in population development, and that they are prey for most larval fishes, there have been

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few studies concerning early development of copepods, particularly for Arctic species. A few studies have investigated nauplii development of *C. glacialis* (Daase et al. 2011) and *C. finmarchicus* (e.g., Hygum et al. 2000; Campbell et al. 2001; Cook et al. 2007), however, only Corkett et al. (1986) have investigated the development in coexisting populations, and in that study the focus was only on egg and copepodite development and not nauplii development.

The use of *C. glacialis* and *C. finmarchicus* as climate indicators makes correct identification essential. Prosome length is often used to distinguish copepodites and adults of the two species. However, genetic analyses have shown a substantial overlap in prosome length of the two species over a wide geographic range (Lindeque et al. 2006; Parent et al. 2011; Gabrielsen et al. 2012). Complicating the separation further, hybrids of the two species have been found in various locations in the Arctic and North Atlantic (Parent et al. 2012). In the Disko Bay area in western Greenland, hybridization has not been observed, and live females of *C. glacialis* and *C. finmarchicus* are easily separated using size and pigmentation differences (Nielsen et al. 2014).

When the in situ community of *Calanus* spp. are investigated, only copepodite and adult stages are identified to species level. *Calanus* spp. nauplii are often pooled, as they are morphologically identical (Sømme 1934) and data on length distributions are rare and derived from different areas. Quantitative genetic methods have been used to estimate biomass of nauplii stages of a single-target species (Jungbluth et al. 2013) in a mixed sample, but genetic analyses of natural communities are time consuming and costly.

Here, we investigate how increasing temperature affects egg and nauplii development of coexisting populations of *C. glacialis* and *C. finmarchicus* from Disko Bay, western Greenland. Further, we investigate whether nauplii of the two species, cultured under similar conditions can be separated by size, and if the obtained size ranges can be applied to distinguish nauplii of the two species in the field.

Methods

Experimental setup

Females of *C. glacialis* and *C. finmarchicus* were sampled by vertical hauls with a WP2 net from 100 m to the surface on 24 April 2010, approximately one nautical mile off the coast of Qeqertarsuaq in Disko Bay, western Greenland (69° 15′N, 53° 33′W). The station was 250–300 m deep. Females were kept cold in a thermo box until arrival at the laboratory where individuals were sorted to species according to identification criteria as defined in Nielsen et al. (2014). The females were distributed into 10-L buckets with false net bottoms filled with 50 μm filtered seawater at 0°C. Every day, 1/3 of the water was renewed and females were fed surplus food of the diatom *Thalassiosira weissflogii* to promote high egg production rates.

Table 1. Intended (T_{int}) and actual temperature (°C) ± SD in egg hatching experiments with *Calanus glacialis* (*C.g*) and *C. finmarchicus* (*C.f*) eggs.

T_{int}	*C.g*	*C.f*
0	−0.3 ± 0.1	−0.3 ± 0.1
2.5	2.5 ± 0.1	2.5 ± 0.1
5	5.1 ± 0.1	5.1 ± 0.1
7.5	7.6 ± 0.0	7.7 ± 0.3
10	9.9 ± 0.2	9.9 ± 0.1

Phytoplankton cultures

The diatom *T. weissflogii* used for food was grown at room temperature in 15-L plastic bags filled with 0.2 μm filtered seawater with added B1 medium (1 mL L^{-1}), silicate (0.9 mL L^{-1}), and vitamins (0.5 mL L^{-1}) (Hansen 1989). The cultures were aerated and grown in a 12 : 12 light : dark cycle.

Egg hatching

Eggs of *C. glacialis* and *C. finmarchicus* spawned within 24 h at 0°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 50 eggs. The trays were incubated at a constant temperature in temperature-controlled thermo boxes at 0°C, 2.5°C, 5°C, 7.5°C, and 10°C. Every six hours, eggs and nauplii were inspected and counted. 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 hereafter. Mean development time (MDT), defined as the time when 50% of the eggs had moulted to the next stage, was calculated from linear regression on arcsine-root-transformed proportion data as described by Landry (1983) and Daase et al. (2011). A Belĕhrádek function relating egg hatching to temperature was fitted to data.

$$D = a(T-\alpha)^b \quad (1)$$

where D is mean development time (MDT) of eggs at the temperature (T, °C) 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).

Development

Development of nauplii from nauplii stage 1 (N1) to copepodite stage 1 (C1) was followed at 0.0 ± 0.3°C with and without food. Eggs 0–24 h old where collected from *C. glacialis* and *C. finmarchicus* on 4 May and incubated in 2.6-L polycarbonate bottles with GF/F filtered seawater. N1 nauplii

hatched on 10 May (*C. finmarchicus*) and 11 May (*C. glacialis*) were used for further development studies. For each species seven polycarbonate bottles were filled with GF/F filtered surface water and N1 nauplii were added in a concentration of 397 L^{-1} for *C. finmarchicus* and 117 L^{-1} for *C. glacialis*. From 13 May, the diatom *T. weissflogii* was added to four of the bottles in a concentration of 20 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 every eight hours. Temperature was logged every 15 min with a Hoboware thermo logger. Every second day, 1/3 of the water was removed by reverse filtration and 10–15 nauplii from each bottle were randomly sorted out into an ice-chilled Petri dish. The nauplii were placed individually in a drop of water on a glass slide and photographed with a digital camera (640 × 480 USB CCD digital camera) mounted on a Olympus SZX12 stereo microscope in the cooling container set to 2.5°C. During development from N1 to N2, nauplii were fixed in 4% buffered formalin for later stage determinations, whereas from N3 onward, nauplii were returned to the bottle after the picture was taken. The experiment continued in the laboratory at the Arctic Station, Greenland for 50 d. For *C. finmarchicus*, however, only one replicate bottle contained C1 at this time and development to C1 was therefore based on that bottle only.

Mean development time (MDT) of nauplii was calculated and the Belĕhrádek function obtained from the egg hatching experiments was used to calculate nauplii development times at other temperatures following the method described by Corkett et al. (1986).

Mortality

Mortality was estimated to assess the suitability of experimental conditions by counting a subsample from each bottle every fourth day. These counts were corrected for the artificial mortality introduced by removing nauplii for preservation during the experiment using a stage-based model from the Template Model Builder "TMB" package in R (Kristensen 2013).

Size

Preserved samples were measured and staged on a "Nikon Diafot 200" inverted microscope at 20 × magnification, whereas the photos were processed using Image J (version 1.43u, http://rsb.info.nih.gov/ij/) software. Length of nauplii was recorded and for 92% of the photos, stage could be directly determined. In the few cases where this was not possible, length was used to assign the correct stage. Two measurements of the nauplii were recorded: length of carapace (CP) and length of tail. Together these give the total length (TL) of the nauplii. For N1 and N2, TL was measured from the tip of the carapace to the end of the tail. The size distribution of *Calanus* eggs was obtained from seasonal studies in Disko Bay in 2008 (Swalethorp et al. 2011) and 2009 (Henriksen et al. 2012) and are included in Fig. 5 for comparison.

Table 2. Hatching success (HS, %) and mean development time (MDT, days) ± SD of *C. glacialis* and *C. finmarchicus* eggs at five temperatures.

	Calanus glacialis		*Calanus finmarchicus*	
Temp (°C)	HS (%)	MDT (d)	HS (%)	MDT (d)
0	93 ± 5	4.4 ± 0.1	93 ± 4	4.5 ± 0.1
2.5	86 ± 8	3.3 ± 0.0*	94 ± 2	3.2 ± 0.0*
5	88 ± 6	2.2 ± 0.1	78 ± 7	2.3 ± 0.1
7.5	75 ± 13	1.9 ± 0.1*	84 ± 4	1.6 ± 0.0*
10	86 ± 8	1.6 ± 0.2*	81 ± 3	1.4 ± 0.0*

*Indicate significant difference in MDT between species.

To identify the length that best separated the species, a logistic regression model was fitted to length-frequency data using the Regression Modelling Strategies "RMS" package in R (Harrell 2014).

In situ sampling of nauplii community

From 29 March to 6 July, weekly samples of nauplii were collected at the permanent station from 0 m to 250 m, using a Hydrobios Multinet (type midi, opening 0.25 m^2), equipped with nets of 50 μm mesh size. Samples were preserved in buffered formalin (4% final concentration) and in the laboratory *Calanus* spp. eggs and nauplii were staged and measured. All nauplii where pooled by stage, and histograms where constructed to show the length distributions within each stage of the natural *Calanus* spp. nauplii community.

Results

Egg hatching

There was no significant pattern relating hatching success (HS) of eggs to temperature. However, for both species HS was highest at 0°C (Table 2). Mean development time (MDT) of eggs decreased with temperature (Fig. 1; Table 2). At low temperatures, MDT of eggs of both species was similar, whereas at higher temperatures *C. finmarchicus* eggs were hatching significantly faster than eggs of *C. glacialis* (*t*-test with bonferroni correction, Table 2). Q_{10} ranged between 1.8 and 4.5 for *C. glacialis* and 2.4–3.8 for *C. finmarchicus* (Table 3). Applying the Belĕhrádek function to the observed MDT gave a significant fit to data (Fig. 2A) in both experiments with r^2 values > 0.99.

Development

During the 50 d experiment nauplii developed until C1 when fed (Fig. 3), whereas when starved, development was arrested at N3 (Fig. 4). However, in *C. finmarchicus* a few starved individuals developed past N3 and toward the end of the experiment both N4 and N6 occasionally appeared but in numbers too low for proper analyses. Only in one replicate bottle did fed *C. finmarchicus* nauplii develop to C1 on

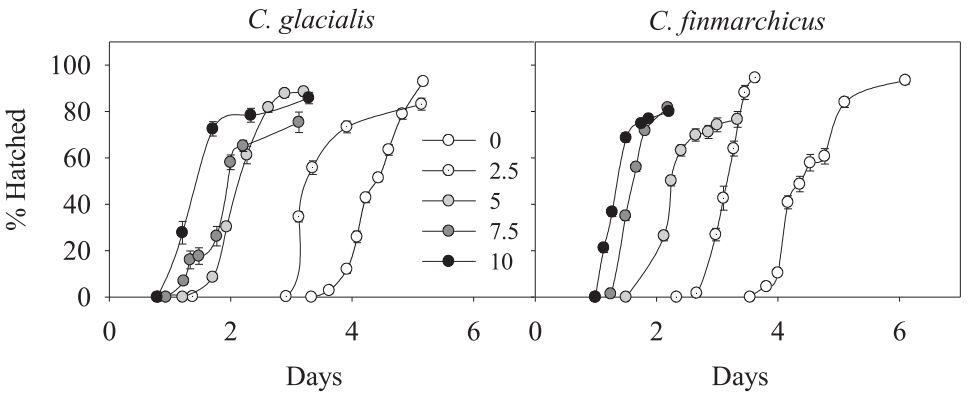

Fig. 1. Egg hatching of *C. glacialis* and *C. finmarchicus* at five temperatures from 0°C to 10°C. Values are mean ± SE of 6–10 replicates.

the last day of the experiment. MDT calculated from that bottle was 57.7 d. MDT of nauplii was similar in both species during the first stages, whereas from N4, *C. finmarchicus* seemed to develop a bit slower (Table 4). However, a significant difference in MDT between species was only detected between N2, N6, and C1 (Two-Way Analysis of Variance (ANOVA) on ranks, followed by Dunns multiple comparison).

Assuming equiproportional development, the Beléhrádek function for egg hatching was used together with the relative stage duration at 0°C to estimate MDT of nauplii at other temperatures (Corkett et al. 1986). Estimated *a*-values are given in Table 5.

Table 3. Q_{10} for egg hatching of *C. glacialis* (*C.g*) and *C. finmarchicus* (*C.f*) at different temperature intervals.

Temp (°C)	*C.g*	*C.f*
0–2.5	2.8	3.5
0–5	3.5	3.4
0–7.5	2.9	3.5
0–10	2.7	3.2
2.5–5	4.5	3.3
2.5–7.5	2.9	3.5
2.5–10	2.6	3.1
5–7.5	1.8	3.8
5–10	2.0	3.0
7.5–10	2.2	2.4

Mortality

Mortality estimates from all bottles ranged between 0.04 d^{-1} and 0.07 d^{-1}, averaging 0.04 ± 0.004 d^{-1} and 0.06 ± 0.006 d^{-1} for fed and starved *C. glacialis,* and 0.05 ± 0.002 d^{-1} and 0.07 ± 0.003 d^{-1} for fed and starved *C. finmarchicus,* respectively.

Size

Even though the egg diameter of *C. glacialis* overlapped with both *C. finmarchicus* and *C. hyperboreus* (Fig. 5), the mean ± SD of egg diameter did not (0.178 ± 0.012 mm, 0.153 ± 0.01 mm, and 0.198 ± 0.07 mm for *C. glacialis*, *C. finmarchicus* (Swalethorp et al. 2011), and *C. hyperboreus* (Henriksen et al. 2012), respectively). Also, all nauplii stages of the three species could be separated by length (Figs. 5, 6). Even though the total range of measurements overlapped in some stages (Table 6) the range of measurements excluding outliers did not (Fig. 7). Especially for N5 and N6, the clearest separation of stages was obtained by measuring the carapace (CP), and this had a lower coefficient of variation than for total length (TL).

To evaluate the applicability of the size classes obtained from the laboratory as criteria for the three species in a natural population of *Calanus* nauplii, we compared the laboratory size classes with those retrieved from in situ sampling (Fig. 8). This was done under the assumption that formalin preservation did not affect length of nauplii as demonstrated by Põllupüü (2007). The clear patterns from the laboratory cultures were not at all apparent in situ. In the early stages of N1–N3, no clear separation between stages was detectable, whereas in the late stages a tendency toward a bimodal/trimodal size distribution of the nauplii was observed. However, the laboratory length distribution did not match observed in situ length distributions and peak in situ observations were often falling between peaks of the laboratory measurements separating the species (Fig. 8).

Discussion

Egg and nauplii development

Coexisting populations of *C. glacialis* and *C. finmarchicus* are found in Disko Bay (Madsen et al. 2001). Despite *C. finmarchicus* being at its northern border (Møller et al. unpubl.), eggs of *C. finmarchicus* hatched successfully at 0°C and nauplii developed at a rate similar to *C. glacialis*.

Egg development was controlled by temperature for both species, as previously documented (e.g., Corkett et al. 1986;

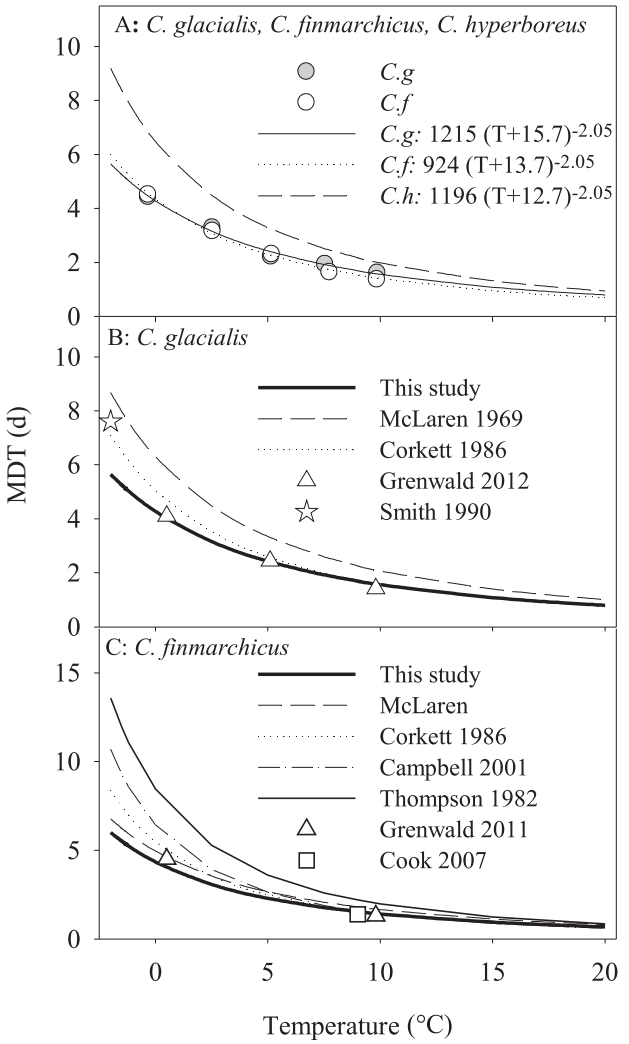

Fig. 2. (A) Belĕhrádek function (lines) fitted to mean development time (MDT, days) of *C. glacialis* and *C. finmarchicus* eggs at five temperatures (circles). The Belĕhrádek function of *C. hyperboreus* eggs from the same area are from Jung-Madsen et al. (2013). Belĕhrádek function (lines) of embryonic duration of *C. glacialis* (B) and *C. finmarchicus* (C) from different areas. McLaren et al. (1969) is from Frobisher Bay, Canada (*C. glacialis*, 0–8°C) and Tromsø, Norway (*C. finmarchicus*, 0–20°C), Corkett et al. (1986) is from Nova Scotia, Canada (2–10°C), Campbell et al. (2001) from waters off Cape Cod, U.S.A. (4–12°C) and Thompson (1982) is for a mixture of *C. finmarchicus* and *C. helgolandicus* from the North Sea (4.5–15°C). Some single-point measurements of MDT (symbols) are from Disko Bay (Grenvald et al. 2012), Fram Strait (Smith 1990), and from the North Sea (Cook et al. 2007).

Campbell et al. 2001). MDT of *C. glacialis* and *C. finmarchicus* eggs was relatively similar, but with a tendency of *C. finmarchicus* eggs to hatch faster at temperatures above 5°C (Fig. 2B,C), whereas eggs of the larger co-occurring *C. hyperboreus* (Fig. 2A) was hatching significantly slower at all temperatures (Jung-Madsen et al. 2013). The same pattern was observed in Corkett et al. (1986), who compared egg hatching of the three species from Nova Scotia, Canada. However, eggs from Disko Bay developed faster at temperatures below 5°C than eggs from any previously studied area, e.g., at 0°C development time is up to two days and four days faster for *C. glacialis* and *C. finmarchicus*, respectively. This confirms the observation by Corkett et al. (1986) that there is a regional difference in hatching rate between populations of copepods. When comparing hatching rate from different areas it is evident that *C. finmarchicus* populations inhabiting warmer waters such as the North Sea (Thompson 1982), and waters between Cape Cod and Georges Bank (Campbell et al. 2001) are less-adapted to cold temperatures than populations inhabiting colder areas, such as Nova Scotia (McLaren et al. 1969), Tromsø (McLaren et al. 1969), and Disko Bay (this study), suggesting cold adaption in this species (Fig. 2B,C). It might be an advantage to speed up egg development in cold areas as the sinking rate of eggs are high, and development at low temperatures slow, so by hatching faster the vertical displacement from the relatively shallow surface food layer is minimized.

Nauplii development was equiproportional and followed the pattern described for copepods in general (Landry 1983; Daase et. al. 2011), with two short nonfeeding stages and a longer first-feeding stage N3, lasting around 30% of the total development time to C1. Similar to the findings of Daase et al. (2011) and Campbell et al. (2001), we found stage duration of N5 to be the shortest of the feeding stages, in contrast to Hygum et al. (2000) and Cook et al. (2007) who found N6 to be the shortest stage (Table 7). At low temperatures, the development time of eggs from Disko Bay was faster than eggs from other areas, in contrast to the nauplii development which was similar or longer than observed in other studies (Fig. 2; Table 7). This seems counterintuitive and we were puzzled to only observe shorter development time in the egg stage. The reason for this observation is not known, but as argued above, fast hatching may be very crucial for success of the nauplii in cold waters, especially in relatively shallow habitats like the Disko Bay.

The observed MDT of *C. glacialis* of 48 d (from N1 to C1) was longer than previous estimates at 45 d and 46 d and also our measurements of *C. finmarchicus* development were longer than predicted from studies conducted in other areas (Table 7). As experimental setups can vary between studies, differing in incubation method and type of food offered, these differences would likely influence development rates. However, even in the nonfeeding stages of N1–N3, which should be unaffected by rearing conditions, a longer development time was observed. Furthermore, the mortality estimated during this study (*see* Results) was equal to the mortality rates in other studies, e.g., Daase et al. 2011 (0.04 d^{-1}), Hygum et al. 2000 (0.04 d^{-1}) indicating similar rearing conditions. Together these observations support the conclusion of an overall longer development rate of nauplii in the Disko Bay area.

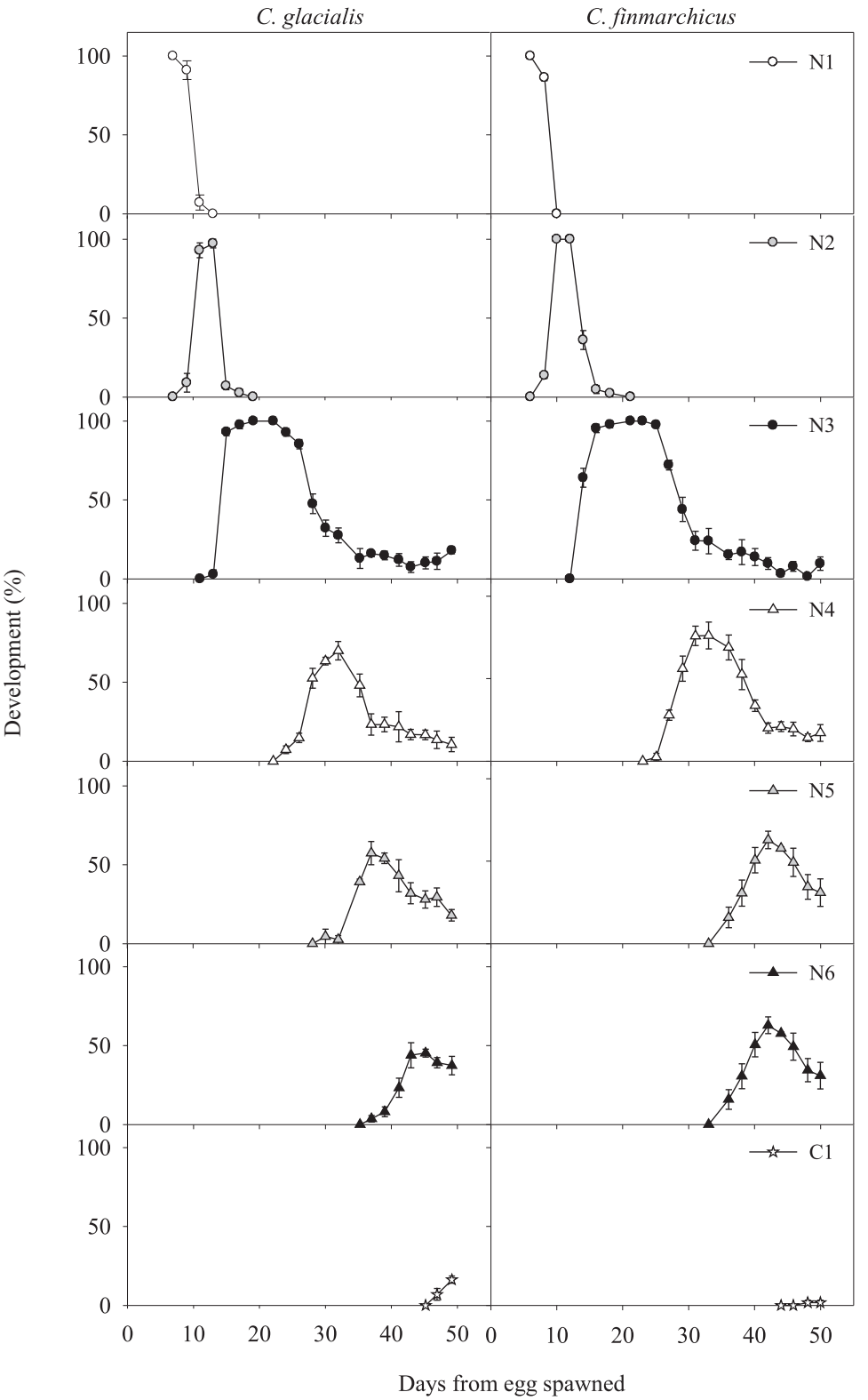

Fig. 3. Development of fed *C. glacialis* and *C. finmarchicus* nauplii at 0°C. Values are means ± Standard error (SE) of 3–4 replicates.

When comparing development of *C. finmarchicus* at 0°C from various areas a difference between the northwestern Atlantic and the North Sea was observed. Nauplii from the North Sea area (Hygum et al. 2000; Cook et al. 2007) were estimated to reach C1 15–18 d faster than nauplii from Disko Bay and the northwestern Atlantic (this study; Campbell et al. 2001; Corkett et al. 1986) (Table 7). Comparisons between rates obtained at different temperatures can be biased as the temperature coefficient Q_{10} used to correct the rates to 0°C depends on the interval from which it is calculated (Conover 1978). When comparisons are made at the relevant temperatures (Fig. 9), the differences are not as large although *C. finmarchicus* from this study still developed slower at warm temperatures than populations from the more southern areas. Complicating the discussion further is the possibility that some previous studies might be biased by inclusion of either hybrids or small *C. glacialis* (Parent et al. 2011, 2012; Gabrielsen et al. 2012). For the development of *C. Finmarchicus*, this should cause an underestimation of development rate, as a mix of characters properly would make them more cold-adapted. Hence, we would expect the rates to be faster at lower temperatures than those of a pure *C. finmarchicus* stock. This problem, together with the fact that measurements were done at different temperatures, make direct comparisons of results more difficult. That set aside, genetic analyses suggest, that there are up to four distinct *C. finmarchicus* populations in the North Atlantic, with the greatest genetic difference observed between the northeastern and northwestern/central Atlantic Ocean (Unal and Bucklin 2010). These two areas coincide with the areas where development of *C. finmarchicus* is most different. Other differences between populations of *C. finmarchicus* are lipid content, where North Atlantic populations contain ~ 18% less lipid than the Arctic populations (Kattner and Hagen 2007), and size, where populations of the northeastern

Table 4. Mean development time (MDT, days) ± SD and stage duration (d) from egg spawning to C1 of *C. glacialis* and *C. finmarchicus* nauplii at 0°C.

	Calanus glacialis		*Calanus finmarchicus*	
Stage	**MDT (d)**	**Duration (d)**	**MDT (d)**	**Duration (d)**
N1*	4.3	5.7	4.3	4.1
N2	10 ± 0.6	4.4	8.7 ± 0.3	5.7
N3	14.4 ± 0.5	14.2	14.3 ± 0.3	14.6
N4	28.6 ± 0.7	8.9	28.9 ± 0.8	11.1
N5	37.5 ± 1.5	6.6	40.0 ± 1.4	7.5
N6	44 ± 1.3	8.2	47.5 ± 1.6	10.2
C1	52.3 ± 1.2		57.7†	

*MDT N1 is calculated from the Beléhrádek function.
†C1 was only found in one bottle at the time the experiment ended.

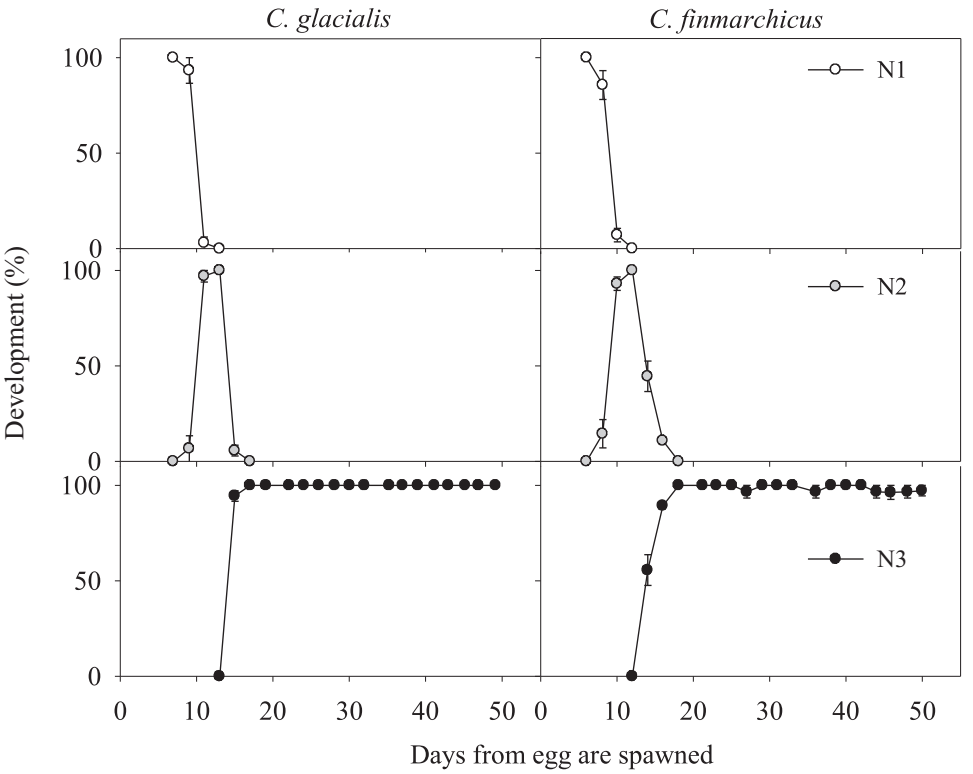

Fig. 4. Development of starved *C. glacialis* and *C. finmarchicus* nauplii at 0°C. Values are means ± SE of three replicates.

Atlantic are smaller than those from the northwestern Atlantic (Melle et al. 2014). Furthermore, maximum egg production rate can be up to two times higher in the northwestern Atlantic compared to the northeastern Atlantic where *C. finmarchicus* also seems to be adapted to a lower food concentration as the maximum egg production is reached at a lower Chl *a* concentration than in the northwestern Atlantic (Melle et al. 2014).

Table 5. Effect of temperature on mean development time (MDT) from egg to a given stage of *C. glacialis* and *C. finmarchicus*. MDT in days can be calculated by inserting the given *a*-value into the Belĕhrádek function for egg hatching, i.e., $D = a \times (T + 15.7)^{-2.05}$ and $D = a \times (T + 13.7)^{-2.05}$ for of *C. glacialis* and *C. finmarchicus*, respectively.

	C. glacialis	*C. finmarchicus*
	a	***a***
N1	1215	924
N2	2836	1840
N3	4091	3042
N4	8113	6138
N5	10643	8507
N6	12510	10105
C1	14846	12285

Size

The laboratory cultures of *C. glacialis*, *C. finmarchicus*, and *C. hyperboreus* nauplii could be separated by carapace size. However, when compared with sizes of nauplii from other areas, the separation is not evident, in that *C. glacialis* nauplii from Svalbard were consistently larger than those from Disko Bay (Daase et al. 2011, Fig. 1B.), and overlapped with sizes of *C. hyperboreus* nauplii from Lofoten in northern Norway (Sømme 1934; Table 7 in Daase et al. 2011). This emphasizes the importance of making size comparisons of co-occurring *Calanus* spp. populations, i.e., originating from the same area.

Egg sizes of the three species overlapped substantially, so morphological features should also be applied to distinguish eggs of the three species. *C. hyperboreus* eggs are easily

Table 6. Mean size of carapace (CP, μm) and total length (TL, μm) ± SD, sample size (*n*) and range of measurements of *C. glacialis* and *C. finmarchicus* N1–C1. N3 is divided into fed ($N3^+$) and starved individuals ($N3^-$).

	Calanus glacialis		*Calanus finmarchicus*	
	CP (*n*) range	**TL (*n*) range**	**CP (*n*) range**	**TL (*n*) range**
N1	—	197 ± 7 (147) 184–209	—	172 ± 7 (255) 153–189
N2	185 ± 6 (107) 173–204	234 ± 9 (141) 214–260	153 ± 5 (87) 138–168	192 ± 8 (173) 179–214
$N3^-$	250 ± 9 (382) 213–272	377 ± 14 (267) 333–411	208 ± 9 (572) 179–229	323 ± 13 (359) 286–354
$N3^+$	255 ± 11 (337) 220–280	384 ± 15 (217) 351–419	209 ± 9 (349) 184–229	321 ± 14 (230) 275–353
N4	315 ± 11 (189) 293–339	485 ± 16 (160) 445–531	259 ± 9 (250) 236–279	404 ± 15 (200) 374–446
N5	384 ± 17 (205) 345–421	607 ± 33 (187) 531–674	310 ± 11 (216) 283–329	509 ± 20 (188) 463–555
N6	446 ± 12 (169) 412–479	746 ± 32 (154) 668–832	359 ± 18 (130) 328–421	621 ± 40 (120) 557–726
C1	823 ± 70 (141) 637–1063	—	676 ± 40 (102) 580–748	—

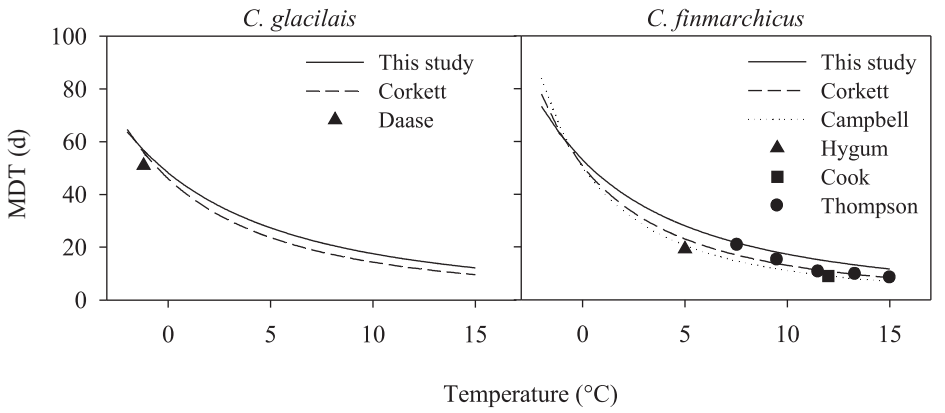

Fig. 5. Comparison between mean development time (MDT, days) from N1–C1 of *C. glacialis* and *C. finmarchicus* in this study, according to Corkett et al. (1986), and Campbell et al. (2001). Point measurements of MDT are by Daase et al. (2011), Hygum et al. (2000), Cook et al. (2007), and Thompson (1982).

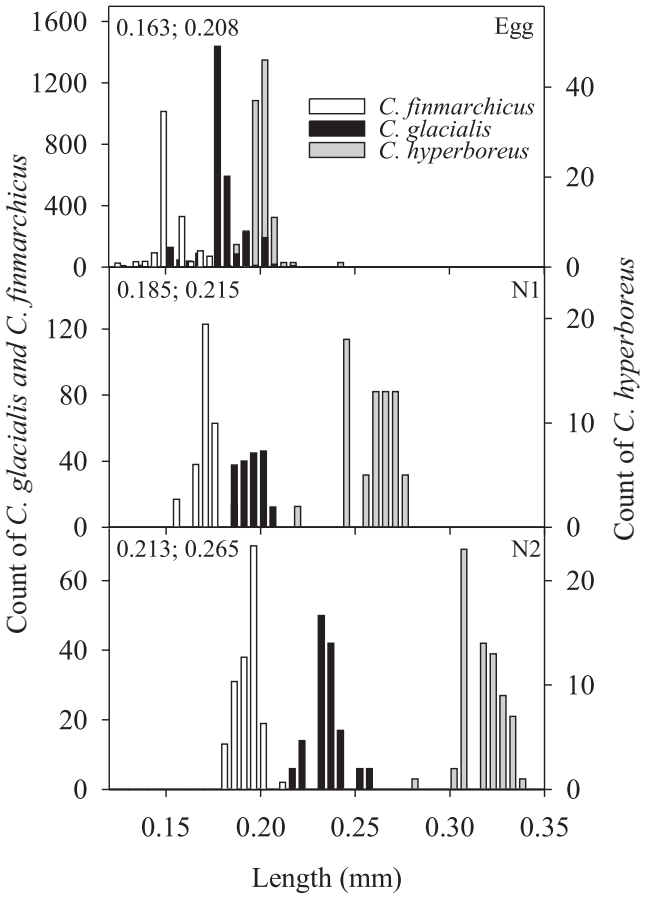

Fig. 6. Egg diameter and total length (TL, mm) of *C. glacialis* and *C. finmarchicus*. For comparison, lengths of *C. hyperboreus* collected in the same area (Jung-Madsen et al. 2013) are presented. Egg measurements are from Swalethorp et al. (2011) and Henriksen et al. (2012). In the left corner are the lengths separating the species, i.e., minimum and maximum size of *C. glacialis* as determined by a logistic regression model.

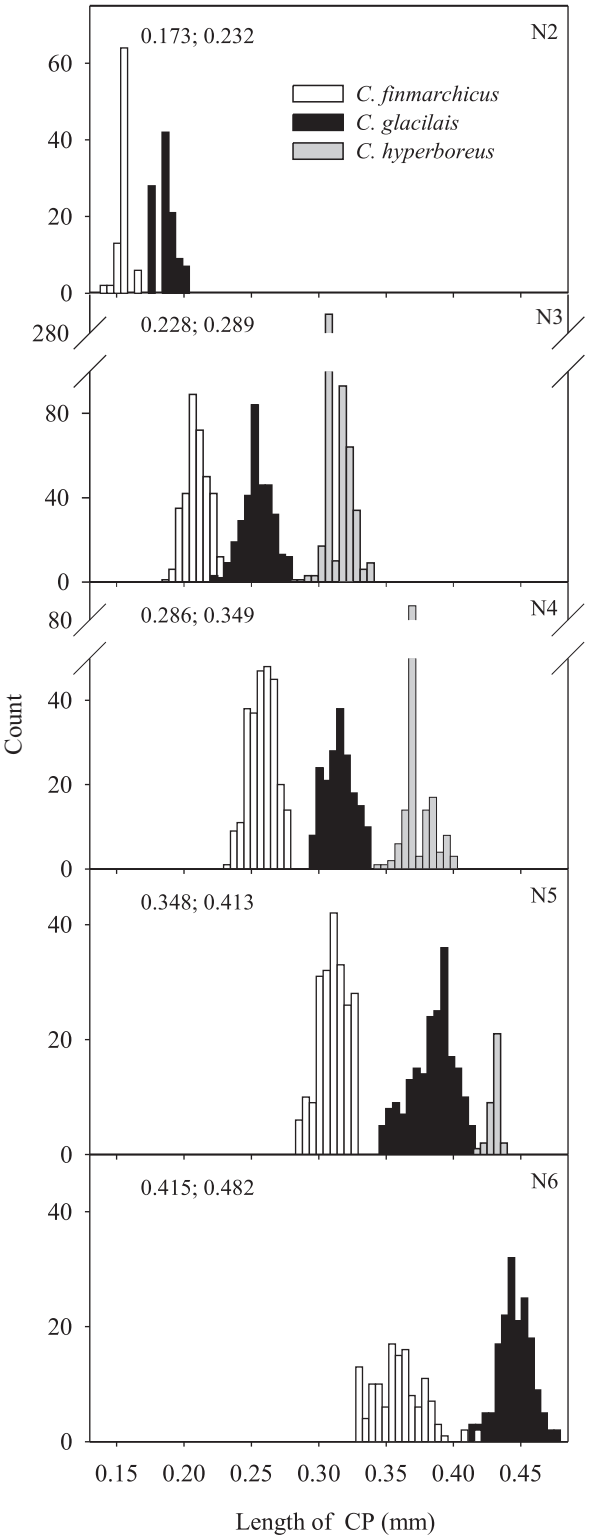

Fig. 7. Carapace length (CP, mm) of *C. glacialis* and *C. finmarchicus*. For comparison, lengths of *C. hyperboreus* collected in the same area are presented (Jung-Madsen et al. 2013). In the left corner are the length separating the species, i.e., min and max size of *C. glacialis* as determined by a logistic regression model.

distinguished from the two other species as the eggs are bright orange, with a smooth membrane and of a completely different texture (Sømme 1934), and because they are spawned during winter, *C. hyperboreus* eggs do not overlap in time with eggs of the other two species. *C. glacialis* and *C. finmarchicus* eggs are more similar but a clear discriminator of live eggs is the outer membrane of *C. glacialis* eggs, which is absent in *C. finmarchicus* (Werner and Hirche 2001).

The discrepancies between the length of laboratory-raised and natural populations of *Calanus* nauplii were disappointing but not unexpected. The laboratory-raised populations experienced stable conditions in terms of high food concentrations and constant temperature, in contrast to the in situ nauplii where food, temperature, and size-dependent predation mortality experienced by both the parents and the offspring were changing during the investigation. Campbell et al. (2001) investigated temperature effects on length of *C. finmarchicus* and found significant differences in temperature

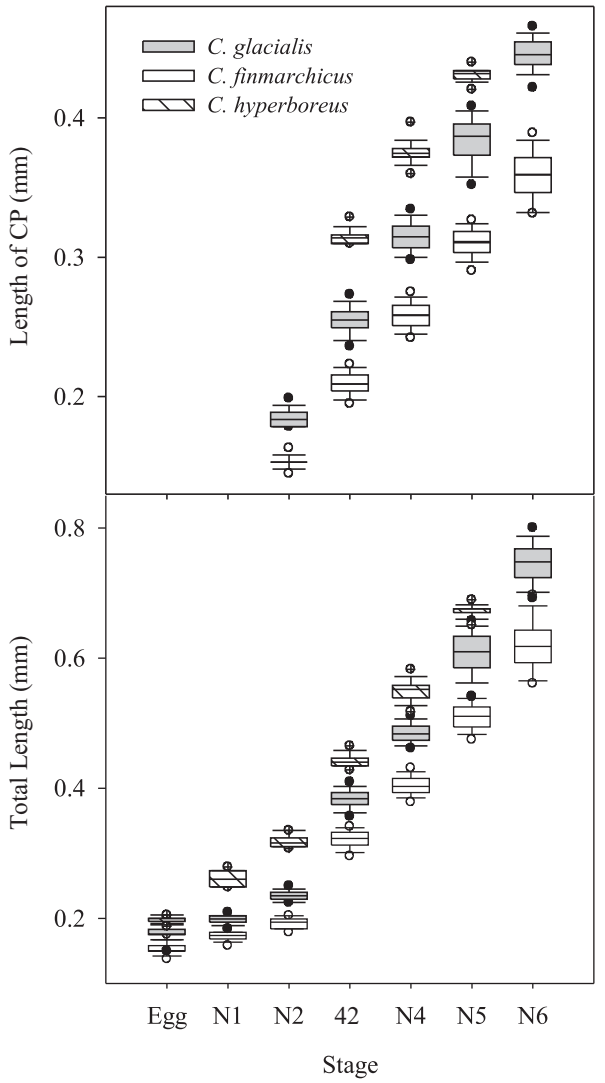

Fig. 8. Boxplot of carapace length (CP, mm) and Total length (TL, mm) of *C. glacialis* and *C. finmarchicus* egg and nauplii. For comparison, lengths of *C. hyperboreus* nauplii collected in the same area (CP; N3–N5 and TL; N1–N5) (Jung-Madsen et al. 2013) are presented. Egg measurements are from Swalethorp et al. (2011) and Henriksen et al. (2012). Horizontal lines show the median. The bottom and the top of the box show the 25th and 75th percentiles. Whiskers extend 1.5 × interquartile range of the sample. Dots show the 5th and 95th percentile.

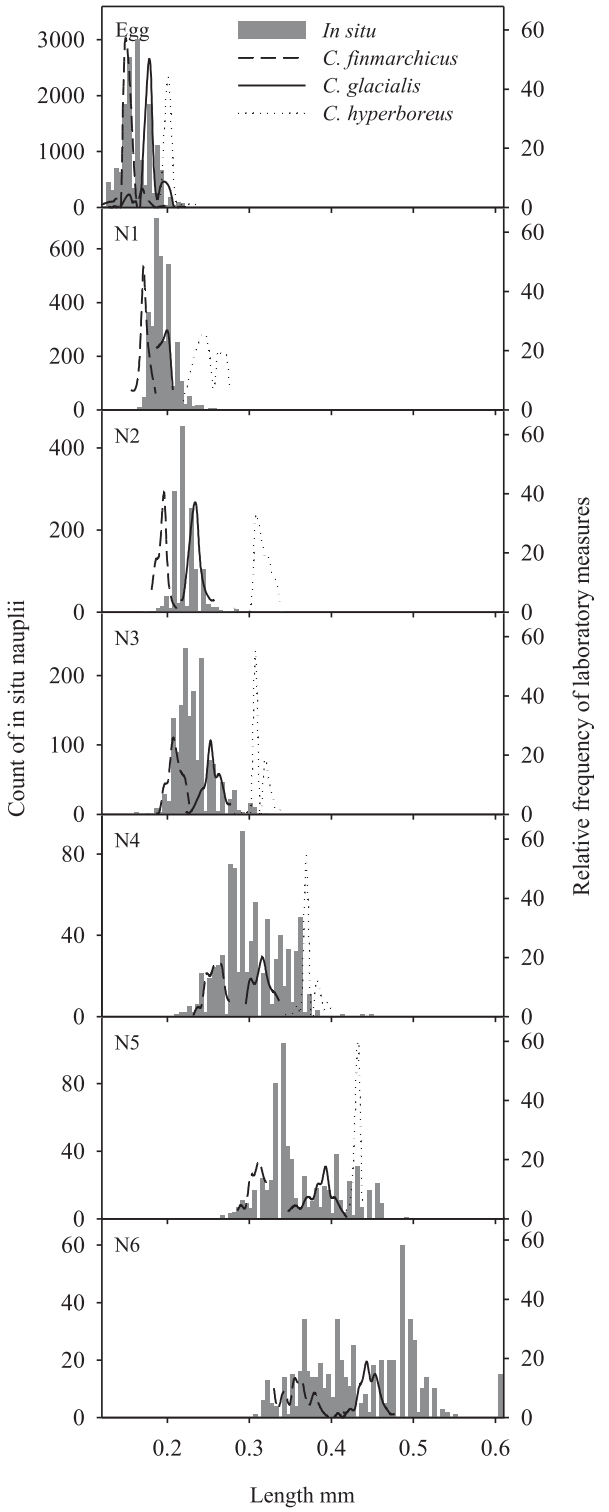

Fig. 9. Size distribution (grey bars) of egg and nauplii collected in Disko Bay from 29 March to 7 June from 0 m to 250 m (first *y*-axis). Lines represent relative size distribution of *C. finmarchicus*, *C. glacialis*, and *C. hyperboreus* nauplii obtained from Figs. 5, 6 (second *y*-axis). From Egg-N2, total length is measured whereas from N3–N6 it is length of carapace. Note different scale on *y*-axis.

coefficients (Q_{10}) between growth and development of copepodites, whereas Q_{10} were more similar for nauplii stages. At the temperature range of −1.3°C to +7.2°C found in Disko Bay, this would mean a change in body size of N3 and N6 of ∼1% and ∼5%, respectively. However, narrowing the dataset by including only −1.5°C to +1.5°C did not improve the separation of species (data not presented).

Food quantity and quality may also add to the observed variation in length of the in situ community. Nauplii developing after the bloom experience patchy food distributions concentrated around the pycnocline (Madsen et al. 2001), so

Table 7. Stage duration of *C. glacialis* and *C. finmarchicus* nauplii at 0°C in this and other studies (Daase et al. 2011 [−1.2°C], Corkett et. al. 1986 [2–10°C], Campbell et al. 2001 [4–12°C], Cook et al. 2007 [12°C], Hygum et al. 2000 [∼ 5°C]). Temperature corrected by applying the given Belëhrádek function or the relevant Q_{10} from Table 3.

	Calanus glacialis			*Calanus finmarchicus*				
Stage	**This study**	**Daase**	**Corkett**	**This study**	**Campbell**	**Cook**	**Hygum**	**Corkett**
N3	14.2	15.2		14.6	15.0	10.9	10.7	
N4	8.9	8.2		11.1	8.2	6.1	5.3	
N5	6.6	5.1		7.5	7.7	6.5	5.5	
N6	8.2	8.0		10.2	9.1	5.2	4.8	
N1–N3	10.1	8.5		10.0	10.5	7.7	9.0	
N1–C1	48.0	45.1	45.9	53.4	50.4	36.4	35.0	50.8

there might be substantial differences in the conditions experienced by the individual nauplii depending on their vertical positions. However, most studies conclude that food quantity (Hygum et al. 2000; Campbell et al. 2001) and food quality (Daase et al. 2011) have little effect on size in nauplii, whereas Cook et al. (2007) found both to interact with temperature and impact size in nauplii from stage N3. Also, the effects of food quantity and quality might be more severe at low temperatures where grazing rates are impaired (Møller et al. 2012). In conclusion, it seems that there is no easy way to use size to separate the mixed *Calanus* spp. nauplii in situ. Instead, we have to rely on genetic tools being developed. A promising method is the use of quantitative PCR to identify and estimate the abundance of nauplii from mixed samples (Jungbluth et al. 2013).

Impact of higher temperatures on the Arctic plankton communities

Arctic *C. finmarchicus* accumulate more lipids (Kattner and Hagen 2007) and their eggs hatch faster (this study) than what is observed in more southern populations. Nevertheless, successful recruitment is still very dependent on matching the spring bloom as they depend on food to mature gonads and initiate egg production. The combination of a higher lipid content in Arctic populations of *C. finmarchicus* and increasing water temperature seems to induce prebloom spawning similar to *C. glacialis* (Kjellerup et al. 2012). This would cause an even larger overlap in reproduction period and increase potential competition between the two species. Because of the low temperatures in Disko Bay, *C. finmarchicus* populations in the spring are reproducing at 15–20% of their maximum potential (Møller pers. comm.). An earlier warming of surface waters will increase reproduction significantly (Kjellerup et al. 2012) and speed up development. So far, only one generation per year has been observed in Disko Bay (Madsen et al. 2001) and other Arctic areas (Melle et al. 2014), but with the measured development rates a second generation of *C. finmarchicus* would theoretically be possible. However, the success of the second generation depends on the food availability in autumn, which most likely would be too low to reach a state suitable for overwintering (Ji et al. 2012). Nevertheless, increasing ocean temperatures might shift the balance between the *Calanus* species resulting in dominance of *C. finmarchicus*. Interestingly, this and other studies point toward a threshold temperature of 5°C at which it seems *C. finmarchicus* will have a reproductive advantage and develop faster than *C. glacialis* and *C. hyperboreus* (This study; Henriksen et al. 2012; Kjellerup et al. 2012). Alcaraz et al. (2014) investigated the carbon balance of *C. glacialis* and suggested 6°C to be the upper boundary of the successful population development. Beyond this temperature, carbon loss by respiration exceeded carbon gained by ingestion. Likewise, the observation that ingestion by *C. hyperboreus* did not increase significantly with temperatures between 0°C and 5°C (Jung-Madsen et al. 2013), whereas respiration in the same temperature interval almost doubled (Hildebrandt et al. 2014) implies that the carbon balance of *C. hyperboreus* may also be severely affected in a future warmer ocean.

To be able to model changes in the *Calanus* spp., composition parameterization of models is crucial. Even slight differences in rates and temperature responses between species can have major consequences for their predicted contributions (e.g., Maar et al. 2013). The present study provides new knowledge on development at low temperatures of two *Calanus* species, which is essential for modeling of secondary production in Arctic and subarctic ecosystems.

This is the first study to measure temperature-dependent egg and nauplii development of coexisting *C. glacialis* and *C. finmarchicus* populations and determine size criteria to distinguish nauplii stages of the two species. As the developing in situ nauplii are affected by variable environmental parameters, the laboratory-reared size classes could not be applied to separate the two species in the field. We found early development rates of the two species to be similar, however, *C. glacialis* reached N6 four days faster at 0°C, whereas at temperatures above 5°C *C. finmarchicus* developed faster, supporting the suggested dominance of *C. finmarchicus* in a future warmer Disko Bay (Kjellerup et al. 2012).

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