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The role of egg cannibalism for the *Calanus* succession in the Disko Bay, Western Greenland

Thomas Frank-Gopolos,[1] Eva Friis Møller,[2] Torkel Gissel Nielsen[1]*

[1]National Institute of Aquatic Resources, Section for Marine Ecology and Oceanography, Technical University of Denmark, Charlottenlund, Denmark

[2]Arctic Research Center, Department of Bioscience, Aarhus University, Roskilde, Denmark

Abstract

The present study is the first to describe egg cannibalism in the key Arctic copepod species *Calanus finmarchicus*, *Calanus glacialis*, and *Calanus hyperboreus*. Initially, a series of staining experiments evaluated the application of Neutral Red for staining *Calanus* eggs. The method was effective and applied in subsequent feeding experiments, where adult females were incubated in bottles with their own eggs. The results showed that all *Calanus* spp. ingested *C. finmarchicus* and *C. glacialis* eggs. However, consumers showed a slight preference for *C. finmarchicus* eggs when incubated with those of both species. The addition of phytoplankton even at high concentrations did not decrease clearance rates for eggs, suggesting that the presence of alternative food does not afford eggs any protection from cannibalism. To evaluate the potential impact of egg cannibalism on the succession of the three species, we calculated and compared field egg mortality rates with potential egg clearance rates for the *Calanus* complex based on rates from the experiments. Our results show that in Disko Bay cannibalism by *Calanus* spp., even at its highest level just before the spring bloom, could only account for about 10% of observed in situ egg mortality, and much less for most of the season.

Competition is widely recognized as one of the main factors shaping the abundance, distribution, and evolution of species, and it is a driver of large-scale biodiversity patterns (Townsend et al. 2002; Kaiser and Williams 2005). Resource limitation constrains the rates at which organisms can grow and proliferate, so the fecundity, growth and survival of one species is often reduced by exploitation of resources by another (Townsend et al. 2002). In a perfectly constant environment the more effective exploiters of a limiting resource would exclude less effective ones sharing the same niche (the competitive exclusion principle), but, as discussed by Hutchinson (1961) in "The paradox of the plankton," competitive exclusion rarely "runs its course." Because most environments are inherently unstable and that competitive interactions are often complicated by synergism, predation and opportunism, the coexistence of several competitors is the norm rather than the exception. For a range of species, predation regimes can be very complex and include density-dependent effects like prey-switching and cannibalism, so the basic assumptions of the competitive exclusion principle may not be met. Thus, competitive balances can shift back and forth to the rhythms of daily, seasonal, or longer-term fluctuations in environmental conditions, affecting species unequally due to differences in their optimal conditions, behavioral plasticity, and the timing of their life-history events (phenology). In Disko Bay in Western Greenland, copepods of the calanoid family Calanidae, *Calanus finmarchicus*, *Calanus glacialis*, and *Calanus hyperboreus*, dominate the zooplankton grazer community throughout the spring and early summer (Madsen et al. 2001). During the spring bloom when food is unlimited, the three species build up substantial lipid stores (Swalethorp et al. 2011), making them a valuable food source for larger organisms, including shrimp, fishes, seabirds, and baleen whales (Dale and Kaartvedt 2000; Kitaysky and Golubova 2000; Laidre et al. 2007). They are a vital link in the pelagic food web between the primary producers and higher trophic levels, passing on organic matter and energy (Falk-Petersen et al. 2002). The three species are in this way of key importance to the productivity of commercially exploited fish and shrimp stocks in Disko Bay and, in turn, to Greenland's export economy, which is almost exclusively based on fishery products.

C. finmarchicus, *C. glacialis*, and *C. hyperboreus* are all widely distributed in northern and Arctic waters. However, the core distribution of *C. finmarchicus* is in the boreal North Atlantic characterized by a yearly spring bloom periods, whereas *C. glacialis* and *C. hyperboreus* are better adapted to the unpredictability of the more extreme environments

*Correspondence: tgin@aqua.dtu.dk

typical of higher latitudes (Falk-Petersen et al. 2009). Disko Bay is located in the distributional overlap between the two "cold-water" species and that of the "warmer-water" *C. finmarchicus*. Effects of climate changes have been reported in the area since 1991, including changes in sea-ice cover, wind regime and timing of the spring phytoplankton bloom (Hansen et al. 2006). Preliminary results indicate that the proportions of *C. finmarchicus*, *C. glacialis*, and *C. hyperboreus* have changed in parallel with the observed increase in sea temperatures and reduced winter ice cover, with *C. finmarchicus* gaining in abundance relative to the other two (Nielsen and Møller unpubl.). The lipid content of *C. finmarchicus* in most life stages is half or less than that of *C. glacialis* and *C. hyperboreus* (Falk-Petersen et al. 2009), so the relative contributions of the three species determine the amount of energy available for higher trophic levels. The observed changes have raised a number of questions as to the future consequences of a warmer climate on the pelagic community and, in turn, about the overall productivity of the Disko Bay area.

In order to predict a future scenario, detailed information about the interactions of these three species is crucial. Several studies of the ecology of *Calanus* spp. have been done in the area, focusing on aspects of feeding and reproduction (Niehoff et al. 2002; Madsen et al. 2008; Swalethorp et al. 2011; Henriksen et al. 2012), on their roles in carbon flux (Møller et al. 2003; Juul-Pedersen et al. 2006) and on the plankton community structure (Madsen et al. 2001; Thor et al. 2005; Kjellerup et al. 2012; Møller et al. 2016). However, it remains unclear how competitive predation and cannibalism affect their coexistence.

The three *Calanus* spp. have phenologies adapted to high latitude environments with short, intense blooms of phytoplankton (Falk-Petersen et al. 2007; Varpe et al. 2007). The small *C. finmarchicus* and medium-sized *C. glacialis* ascend from winter hibernation at depth in early spring to spawn and graze the developing spring bloom (Madsen et al. 2001). They are followed a couple of weeks later by *C. hyperboreus* (Swalethorp et al. 2011), which spawn in deep waters during their winter diapause phase (Hirche and Niehoff 1996; Henriksen et al. 2012). *C. glacialis* initiates spawning based on stored lipid before the bloom commences, whereas the smaller *C. finmarchicus*, for which spawning in Arctic conditions is more food dependent (Niehoff and Hirche 1996), spawns when food becomes available.

During the pre- to early bloom period, the three *Calanus* species co-occur in the surface with their eggs and young nauplii. As their preferred sizes of food overlap those of their eggs and larvae (Levinsen et al. 2000), and while the level of available phytoplankton is still low, competition for food may be substantial. Egg cannibalism may then constitute a viable feeding strategy, considering the low risk of individuals ingesting their own offspring in broadcast-spawners like *Calanus*, and that the disadvantage of doing so may be counterbalanced by the nutritive value of the eggs (Kiørboe et al. 1988; Bonnet et al. 2004). Due to the winter spawning of *C. hyperboreus*, their nauplii may have grown out of the prey size spectrum of the adults (Jung-Madsen et al. 2013) before they arrive near the surface during the bloom (Henriksen et al. 2012). In contrast, *C. finmarchicus* and *C. glacialis* feed and spawn at the same time, and, since eggs are within the prey-size ranges of late copepodite stages and adults of all three species, egg cannibalism may be of major importance for those two. Phenological differences may, therefore, be significant in the coexistence of the three *Calanus*.

Cannibalism in copepods has been documented experimentally for a range of genera, including *Calanus* (Kang and Poulet 2000; Bonnet et al. 2004), *Acartia* (Landry 1978b; Lonsdale et al. 1979), *Metridia* (Sell et al. 2001), *Oithona* (Uchima and Hirano 1986), and *Temora* (Daan et al. 1988). Most studies have focused on predation on the early naupliar stages, but clearance rates on eggs have also been studied (Kang and Poulet 2000; Bonnet et al. 2004). In field studies, density-dependent egg mortality rates have been attributed to cannibalism in a number of copepod populations (Peterson and Kimmerer 1994; Ohman and Hirche 2001; Ohman et al. 2002, 2008), especially during times of low phytoplankton concentration prior to the spring bloom (Hirche et al. 2001; Ohman and Hirche 2001). In *Calanus*, most studies on the causes of egg mortality have focused their attention on *C. finmarchicus* in the North Atlantic, where egg cannibalism has been suggested as a major mechanism controlling seasonal patterns in recruitment (Hirche et al. 2001; Ohman and Hirche 2001). Head et al. (2015), however, have suggested that the role of cannibalism in situ may have been overstated and that observed mortality patterns probably result from a combination of processes including reduced egg viability, predation, sinking, and advection. The uncertainty reinforces the need for actual measurements of egg cannibalism in addition to life-table analyses.

Egg cannibalism may introduce a source of self-limitation in copepod populations, which may be of central importance in predicting long-term variability in pelagic ecosystems due to the non-linear population responses to changing environmental conditions (Ohman and Hirche 2001; Ohman et al. 2004). So, it is important to include knowledge of this source of mortality in models of zooplankton population dynamics used to predict future scenarios in the oceans. However, only a few studies of copepod mortality have focused on Arctic ecosystems (e.g., Arnkværn et al. 2005; Thor et al. 2008), and to the authors' knowledge little or no information on the mortality of eggs exists from Arctic areas.

Here we investigate the importance of egg cannibalism in *C. finmarchicus, C. glacialis*, and *C. hyperboreus* relative to the abundance of phytoplankton and evaluate its potential role in the succession of these copepod species in the Disko Bay, Western Greenland. We hypothesize that egg mortality peaks just prior to, and after the spring bloom due to

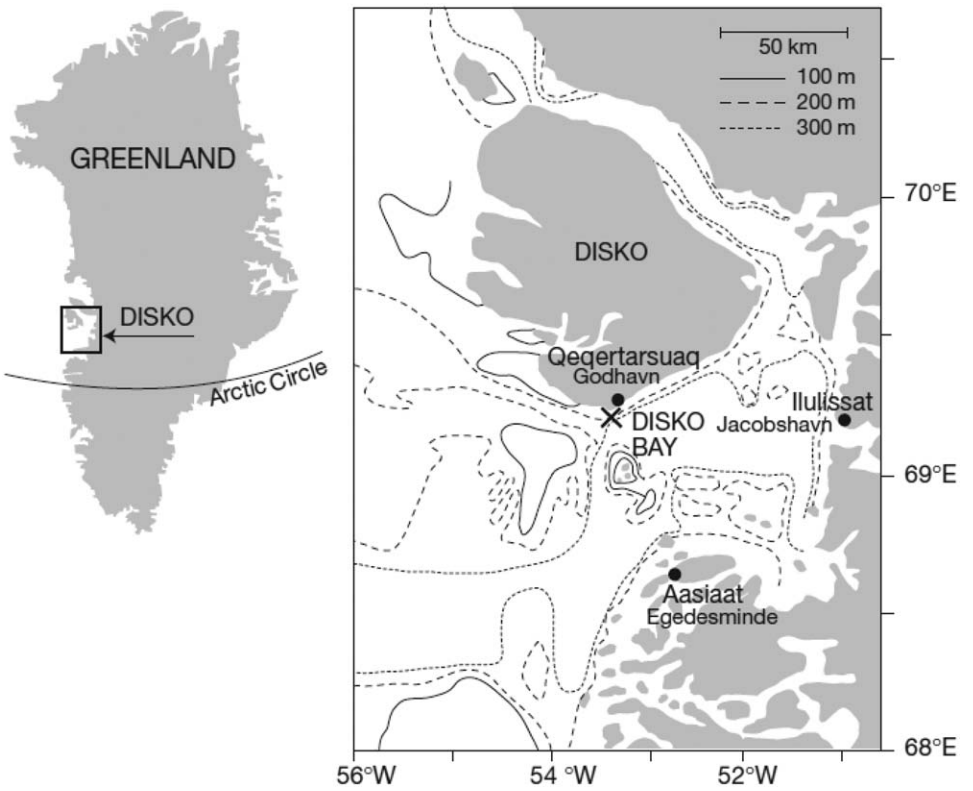

Fig. 1. Location of study site near Qeqertarsuaq (Godhavn) in the Disko Bay, Western Greenland. The sampling station used by Swalethorp et al. (2011) and the present study is marked with a cross.

cannibalism, when phytoplankton levels are low and competition for food is consequently high.

Materials and methods

Study site

The in situ part of the present study is based on data from Swalethorp et al. (2011). The data were collected at a 300 m deep monitoring station in Disko Bay, approximately 1 nautical mile outside Qeqertarsuaq, Isle of Disko (69°15′N, 53°33′W), from 21st February to 18th July 2008 (Fig. 1). All of the experiments were conducted during the period 17th April to 15th May 2012 at the Arctic Station, University of Copenhagen, situated in Qeqertarsuaq, Disko. Collection of experimental water and copepods was conducted from the Arctic Station research vessel RV "*Porsild*" at the same station as the sampling by Swalethorp et al. (2011).

Calanus cultures

The sampling was done between 14th April and 15th May 2012 using a WP-3 net (200 μm mesh size) equipped with a closing mechanism and a non-filtering cod-end. Sampling was performed by horizontal trawls in depth intervals of 25 m between 0 m and 100 m depth. Each interval was trawled for approximately 5 min at ~1.5 knots. Aboard the research vessel, zooplankton samples were stored in a thermo box at in situ temperatures and were transported to the laboratory, sorted and stored in a temperature-controlled cooler within 3–5 h. Throughout the study period the mean temperature in the cooler was $-0.3°C \pm 0.8$ (mean ± SD). *C. finmarchicus*, *C. glacialis*, and *C. hyperboreus* females with intact antennae were sorted from the samples in ice-chilled petri-dishes under a dissecting microscope. Fresh females were collected approximately twice a week.

The copepods were kept in 8 L "fecatron" buckets (see Kjellerup et al. 2012) at densities in the range of 2–5 animals L^{-1} using 50 μm filtered seawater at in situ salinity (~33). The filtering was done to ensure that the cultures were not contaminated with copepod eggs from the sampling site. The fecatron design effectively separates females from their eggs, which settle through a mesh onto the bucket floor and are thereby protected from predation by the females. The copepod cultures were stored in darkness and fed ad libitum on a mixture of cultured *Rhodomonas salina* and *Thalassiosira weissflogii*.

Eggs were harvested every 24 h to ensure that the cannibalistic feeding experiments were done with eggs of the same age and could be terminated before hatching. Egg development times at 0°C are approximately 110 h for *C. finmarchicus* and 100 h for *C. glacialis* (Grenvald et al. 2013). The egg-harvesting was done after straining the copepods on

to the mesh and moving it to a second water-filled bucket and subsequently collecting the spawned eggs on a 50 μm filter. The filtrate (eggs and fecal pellets) was transferred to a smaller container and subsequently stored in the cooler until the experiments commenced.

Phytoplankton cultures

As food for the copepod cultures, the flagellate *Rhodomonas salina* [equivalent spherical diameter (ESD) = 9 μm] and the diatom *Thalassiosira weissflogii* (ESD = 12 μm) were cultured at a ~1 : 1 ratio in 15 L plastic bags containing 0.45 μm filtered sea water. The cultures were grown at room temperature with a 12 : 12 h light/dark cycle and the light source was placed approximately 30 cm from the culture bags (2 pcs. Osram L, 36 W/840, Lumilux Cold White). Aeration was provided by a small aquarium air pump which also created the necessary turbulence to keep cells in suspension. The cultures were diluted daily with fresh 0.45 μm filtered seawater with added B1 medium (Hansen 1989), including vitamins, silicate, and trace metals to keep the cells in an exponential growth phase.

A monoculture of *T. weissflogii* used in feeding experiments was grown under the exact same conditions as the mixed cultures, but in a different room and with different equipment to avoid contamination with *Rhodomonas*.

Water sampling

Water for copepod cultures and experiments was collected below the chlorophyll maximum using a 30 L Niskin bottle. Culture water was filtered through a 50 μm filter to remove any copepods and their eggs, whereas experimental water was filtered through a 10 μm mesh and, in turn, a 0.45 μm filter to remove all potential food particles.

Staining experiments with neutral red

All experiments in this study involved staining *Calanus* eggs with the vital stain Neutral Red (synonyms: 3-Amino-7-dimethylamino-2-methylphenazine hydrochloride, Basic Red 5, toluylene red). Neutral Red is a water-soluble, non-toxic, inexpensive and simple-to-use stain which has proved effective for staining marine zooplankton, including copepods (Dressel et al. 1972; Elliott and Tang 2009; Zetsche and Meysman 2012) and copepod eggs (Dressel et al. 1972). It is weakly cationic and enters viable animal cells through the intact plasma membrane and subsequently concentrates and stores in the lysosomes (Triglia et al. 1991). Two experiments were set up; one to develop a modified protocol suitable for staining *Calanus* eggs in a timesaving manner and another to evaluate the potential effects of Neutral Red staining on egg hatching success.

In the protocol experiment exposure times were 15 min and 30 min, and Neutral Red concentrations were 15 mg L^{-1}, 30 mg L^{-1}, and 60 mg L^{-1}, six staining treatments with six replicates each. The experiment was conducted separately with *C. finmarchicus* and *C. glacialis* eggs. All treatments were based on the same Neutral Red stock solution: 0.1 g Neutral Red powder (refrigerated at 5°C) dissolved in 10 mL distilled water (10 g L^{-1}). The stock solution was made in a 50 mL, tinted, borosilicate glass vial with an air-tight screw cap, then slowly mixed on a magnetic stirrer in darkness for approximately 24 h, and finally stored in darkness at room temperature. The exposure steps for *Calanus* eggs were performed with 50 μm filtered seawater cooled to ~0°C. *Calanus* eggs harvested from the cultures were gently collected on a 50 μm filter before division into 50 mL glass vials with 10 mL seawater and approximately 50–100 eggs in each. Then, 15 μL, 30 μL, or 60 μL of Neutral Red stock solution was added, yielding 15 mg L^{-1}, 30 mg L^{-1}, or 60 mg L^{-1}, and the suspensions were mixed by gently swirling the glass vials a few times before incubating at 0°C in darkness for 15 min or 30 min. Following incubation the eggs were collected on the 50 μm filter and rinsed gently with seawater to remove excess dye. The stained eggs were then placed in NUNC™ Multi wells in ~15 mL of seawater and the staining result evaluated immediately under a dissection microscope. Based on the results (see "Staining experiments with neutral red" and "The applicability of neutral red for vital staining of *Calanus* eggs" sections for results and discussion), the staining treatment selected was 60 mg L^{-1} Neutral Red for 15 min staining time.

In the second experiment, evaluating the effect of the selected staining protocol on egg hatching success, *C. finmarchicus* and *C. glacialis* eggs were harvested and half of them stained as described above. Unstained eggs served as controls, but were treated according to the staining protocol without being exposed to Neutral Red. Stained eggs and controls were incubated in separate NUNC™ Multi wells with 10 mL of seawater for 5 d at 3°C before being fixed with Lugol's iodine. Eggs and nauplii were subsequently counted and hatching percentages calculated. The experiment was done with six replicates for *C. finmarchicus* but only three replicates for *C. glacialis* due to low egg-production rates. The numbers of eggs varied among replicates ranging from 143 to 445 in the *C. finmarchicus* experiment and from 97 to 142 in the *C. glacialis* experiment.

Egg selection experiments

The relative susceptibilities of *C. finmarchicus* and *C. glacialis* eggs to predation by adult *C. finmarchicus*, *C. glacialis* and *C. hyperboreus* females were investigated in a selection experiment using an experimental design such that the effect of staining on egg preference could be evaluated concurrently. Copepods were incubated in 1350 mL or 1750 mL bottles with 1 : 1 mixtures of *C. finmarchicus* and *C. glacialis* eggs. Each consumer species was tested in four diet treatments based on two levels of egg concentration, ~22 L^{-1} or ~38 L^{-1}, and staining of either the *C. finmarchicus* or *C. glacialis* eggs. Each treatment was done in 3–8 replicates depending on the available number of eggs from the cultures. In addition, eight controls without consumers were made, all

Table 1. Overview of all feeding experiments with indication of consumer, number of replicates (*n*), egg species (stained or unstained), total egg concentration (eggs L^{-1}), phytoplankton concentration (μg C L^{-1}), and start date of 24 h incubation.

Experiment	Consumer / control (*n*)	Egg species (stained +, unstained -)	Total egg conc. (eggs L^{-1})	Phyto conc. (μg CL^{-1})	Start date
Functional response	*C. hyp* (4), control (3)	*C. fin* (+)	5.1	0	29 Apr 2012
	C. hyp (4), control (3)	*C. fin* (+)	10.2	0	29 Apr 2012
	C. hyp (4), control (3)	*C. fin* (+)	25.5	0	29 Apr 2012
	C. hyp (4), control (3)	*C. fin* (+)	25.5	0	07 May 2012
	C. hyp (4), control (3)	*C. fin* (+)	51.1	0	29 Apr 2012
	C. hyp (4), control (3)	*C. fin* (+)	102.1	0	29 Apr 2012
	C. hyp (3), control (3)	*C. fin* (+)	204.3	0	29 Apr 2012
Phytoplankton	*C. hyp* (4), *C. gla* (4), *C.fin* (4), control (3)	*C. fin* (+)	25.5	22.5	12 May 2012
	C. hyp (4), *C. gla* (4), *C.fin* (4), control (3)	*C. fin* (+)	25.5	55.3	13 May 2012
	C. hyp (4), *C. gla* (4), *C.fin* (4), control (3)	*C. fin* (+)	25.5	142.2	11 May 2012
	C. hyp (4), *C. gla* (4), *C.fin* (4), control (3)	*C. fin* (+)	25.5	207.0	10 May 2012
	C. hyp (4), *C. gla* (4), *C.fin* (4), control (3)	*C. fin* (+)	25.5	637.5	09 May 2012
Egg preference	*C. fin* (4)	*C. fill* (+)/*C. gla* (-)	22	0	22 Apr 2012
	C. fin (1)	*C. fin* (+)/*C. gla* (-)	33	0	22 Apr 2012
	C. fin (4)	*C. fin* (+)/*C. gla* (-)	38	0	28 Apr 2012
	C. fin (4)	*C. gla* (+) /*C. fin* (-)	22	0	27 Apr 2012
	C. fin (3)	*C. gla* (+)/*C. fin* (-)	38	0	28 Apr 2012
	C. gla (2)	*C. fin* (+)/*C. gla* (-)	22	0	24 Apr 2012
	C. gla (4)	*C. fin* (+)/*C. gla* (-)	38	0	28 Apr 2012
	C. gla (1)	*C. gla* (+)/ *C. fin* (-)	19	0	26 Apr 2012
	C. gla (2)	*C. gla* (+)/*C. fin* (-)	22	0	27 Apr 2012
	C. gla (1)	*C. gla* (+)/*C. fin* (-)	24	0	27 Apr 2012
	C. gla (4)	*C. gla* (+)/*C. fin* (-)	38	0	27 Apr 2012
	C. hyp (4)	*C. fin* (+) /*C. gla* (-)	22	0	24 Apr 2012
	C. hyp (8)	*C. fin* (+)/*C. gla* (-)	38	0	28 Apr 2012
	C. hyp (8)	*C. gla* (+)/*C. fin* (-)	22	0	27 Apr 2012
	C. hyp (4)	*C. gla* (+)/*C. fin* (-)	38	0	27 Apr 2012
	Control	*C.fin* (+)/*C. gla* (-)	22	0	24 Apr 2012
	Control	*C. gla* (+)/*C. fin* (-)	22	0	28 Apr 2012

with 22 eggs L^{-1}: four with stained *C. finmarchicus* eggs and four with stained *C. glacialis* eggs (see Table 1 for overview of the full experimental setup). Each treatment egg mixture was made by picking the desired number of stained and unstained eggs individually from ice-chilled petri dishes under a dissection microscope with a mouth-operated, borosilicate glass micropipette. The treatment replicates were held on ice in 50 mL glass vials with 0.45 μm filtered seawater.

Approximately 8–12 h prior to initiating a set of experiments, females were sorted into NUNC^{TM} Multi wells with 0.45 μm filtered seawater ($\sim$15 mL per well). Females spawning a batch of eggs within this time frame were selected as consumers, thereby reducing the risk of egg-laying during experiments. The number of females screened in this manner varied throughout the study period, depending on the seasonally changing egg production rates.

One copepod was added to each bottle together with an egg mixture, and the bottles (1350 mL or 1750 mL) were incubated for approximately 24 h on a plankton wheel (1.3 rpm). The experiments were terminated by filtering the contents onto a 50 μm mesh and then rinsing the filtrate gently into a NUNC^{TM} Multi well. The consumers were immediately removed for size determination under a dissection microscope, and we carefully checked whether any eggs were accidently transferred in this process. The post-feeding number of stained and unstained eggs was counted directly

under an inverted microscope and the number of eggs ingested per day calculated for eggs of each species.

Functional responses on Calanus-egg diets

The functional responses of *C. finmarchicus*, *C. glacialis* and *C. hyperboreus* provided a diet of stained *C. finmarchicus* eggs were investigated in feeding experiments for estimating clearance and ingestion rates at 5, 10, 26, 51, 102, and 204 eggs L^{-1}. The experimental protocol included four replicate treatments and four replicate control bottles for each consumer/treatment combination, but was otherwise similar to the one applied in the egg selection experiment. Clearance rates, F [mL ind^{-1} d^{-1}], and prey ingestion rates, I [no. ind^{-1} d^{-1}] were calculated from the post-feeding egg counts according to Frost (1972):

$$F = \frac{V}{Nt} \ln\left(\frac{C_0}{C_t}\right)$$

Where V is the experimental volume, N is the number of consumers in the bottle, t is the incubation time, C_0 the initial prey concentration and C_t the post-feeding prey concentration, but with the initial prey concentration replaced by the mean end-concentration in the four controls, $\langle C_t^{control} \rangle$,

$$F = \frac{V}{Nt} \ln\left(\frac{\langle C_t^{control} \rangle}{C_t}\right).$$

Clearance rates were thus corrected for the experimental variation (error) introduced during setup and termination of experiments.

Ingestion rates were calculated as

$$I = F\langle C \rangle.$$

Here, $\langle C \rangle$ refers to the mean concentration of eggs in the experimental bottles during incubation, which was calculated as

$$\langle C \rangle = \frac{(C_t - C_0)}{\ln\left(\frac{C_t}{C_0}\right)}.$$

The calculated egg ingestion rates were finally converted to carbon units assuming a *C. finmarchicus* egg-carbon conversion factor of 0.21 μg C egg^{-1} (Swalethorp et al. 2011).

Eggs/phytoplankton mixed diet

The effects of available of phytoplankton biomass on egg cannibalism were investigated in a feeding experiment with *C. finmarchicus*, *C. glacialis*, and *C. hyperboreus* females feeding on mixtures of stained *C. finmarchicus* eggs and *T. weissflogii*. Experiments were carried out with one egg concentration, five phytoplankton concentrations and four replicates per consumer/treatment combination. The selected concentrations were within the observed natural ranges: 26 eggs L^{-1} and diatoms corresponding to (aim/actual) 25/23, 50/55, 100/142, 200/207 and 400/637 μg C L^{-1}. A Sedgewick Rafter Counting Cell was used to determine the initial cell concentration of the *T. weissflogii* monoculture, which was then converted into carbon concentration assuming 131 pg C $cell^{-1}$ (Dutz et al. 2008). The appropriate culture volume was then mixed with 0.45 μm filtered seawater in four 50 L plastic containers to obtain the experimental concentrations needed. Three water samples were taken from each experimental bottle (1350 mL or 1750 mL) after termination to determine post-experimental concentrations, and for each level of phytoplankton the mean of post-experimental concentrations in the four control bottles was used as initial concentration.

Except that this experiment included quantification of fecal pellet production during incubation (counted under a dissection microscope), the remaining protocol steps were the same as described for the other feeding experiments. Both F and I were calculated as described for the functional response experiment.

In situ calculations

Estimation of egg mortality rates during spring and summer in Disko Bay was based on data reported by Swalethorp et al. (2011). Temperature, female abundance, egg concentration sampled in situ and egg production rates (EPR) from bottle incubations were used in the analysis (See Swalethorp et al. 2011, for details on sampling and experimental procedures). The total in situ concentrations expected in the top 100 m of the water column for no mortality of eggs produced by *C. finmarchicus* and *C. glacialis* were calculated from the data and compared graphically with observed egg concentrations. These expected egg concentrations were also compared with chlorophyll within the same depth intervals to evaluate their concordance with bloom conditions. Egg mortality was later estimated according to Peterson and Kimmerer (1994).

Volume-specific egg production rates, R_0 [eggs m^{-3} d^{-1}], were calculated by multiplying the average female EPR, B [eggs $female^{-1}$ d^{-1}], on a given date with the observed female abundance, N_f [females m^{-3}], on the same date:

$$R_0 = BN_f.$$

This was done for *C. finmarchicus* and *C. glacialis*, respectively, and values of R_0 were linearly interpolated to fill out daily values between existing data points. The expected zero mortality concentrations of *C. finmarchicus* and *C. glacialis* eggs, E_0 [eggs m^{-3}], were calculated by summing R_0-values according to species- and temperature-specific egg development times, D [days] (see below):

$$E_0(t) = \sum_{n=t-D}^{t} R_0(n),$$

where $E_0(t)$ is the value of E_0 on day t, and $R_0(n)$ is the value of R_0 on day n. Estimates of E_0 for each species were finally summed to yield the total egg concentration produced by the two species. Data on female abundance was given in 50 m intervals from 0 m to 250 m depth, so female

Table 2. Results from the hatching experiments with *C. finmarchicus* and *C. glacialis* eggs summarizing the number of replicates (*n*), incubation start dates, experimental conditions, total egg and nauplii counts, hatching percentages (mean ± SE) and Chi^2 test statistics for the staining treatment vs. controls.

Species	Treatment	*n*	Temp. (°C)	Start date	Incubation time (days)	Total eggs	Total nauplii	% hatching ±SE	Chi test statistics
C. finmarchicus	Stained	(6)	3	03 May 2012	5	1033	1064	50 ± 1.0	7.15 ($p < 0.01$)
	Control	(6)	3	03 May 2012	5	491	619	56 ± 2.2	
C. glacialis	Stained	(3)	3	03 May 2012	5	75	270	78 ± 1.6	15.96 ($p < 0.01$)
	Control	(3)	3	03 May 2012	5	40	338	90 ± 0.7	

abundance in 0–100 m depth was calculated as mean abundance in the 0–50 m and 50–100 m intervals.

Development times (D) in days as a function of temperature (T) in °C were calculated from Belehrádek's temperature function (Belehrádek 1935):

$$D = a\ (T - \propto)^b$$

with empirically fitted constants a and α from Corkett et al. (1986) and the exponent b from McLaren et al. (1969) yielding

$$D = 691\ (T + 10.6)^{-2.05}\ (C.\ finmarchicus)$$
$$D = 975\ (T + 13.04)^{-2.05}\ (C.\ glacialis).$$

The egg development times were calculated using those equations and the mean temperatures measured in the upper 50 m of the water column.

Abundance of eggs (120–200 μm), E [eggs m^{-3}], was extracted from the dataset, assuming they were exclusively *C. finmarchicus* and *C. glacialis* eggs (egg diameter = 153 ± 10 μm and 178 ± 12 μm, respectively (Swalethorp et al. 2011)), as no other copepod species in the area produces eggs in that size-range. Concentrations were extracted for the depth intervals 0–50 m and 50–100 m, and the 0–100 m concentration was calculated as the mean.

Mortality rates, m [d^{-1}], were calculated iteratively from data on EPR, female abundance, observed egg concentrations and estimates of egg development times according to Peterson and Kimmerer (1994) by the equation

$$\frac{E}{BN_f} = \frac{1 - \exp(-mD)}{m}. \quad (1)$$

In addition, mortalities were calculated with a modification described by Head et al. (2015), taking into account the fact that egg-hatching success is often less than 100%:

$$\frac{E}{BN_f} = \frac{1 - v * \exp(-mD)}{m}. \quad (2)$$

where v is hatching success. Data on chlorophyll *a* (Chl *a*) was also extracted from the Swalethorp et al. (2011) dataset. Only chlorophyll > 10 μm was included, which is assumed to be the size fraction grazed by females of all three *Calanus* spp. (Juul-Pedersen et al. 2006; Swalethorp et al. 2011).

Results

Staining experiments with neutral red

In all staining treatments, *C. finmarchicus* and *C. glacialis* eggs stained inconsistently, with colors varying from unstained or pale pink to deep magenta. The strength of staining showed a positive relationship with increasing staining time and increasing Neutral Red concentration. The effect of concentration was most significant in the 15 min treatment, in which only 60 mg L^{-1} yielded relatively homogenous egg staining. In comparison, the 30 min treatment showed much less variation among concentrations. At 60 mg L^{-1}, staining saturated within 15 min, with the 15 min and 30 min treatments giving similar results. To minimize staining time, the 15 min staining time at 60 mg L^{-1} Neutral Red concentration treatment was selected for further use. The protocol described in "Staining experiments with neutral red" section was applied to batches of up to ~2000 eggs during the subsequent feeding experiments with no significant impairment of the staining results.

Eggs of the two species stained quite differently. *C. glacialis* eggs generally were more homogeneously stained, with a much smaller proportion of unstained or pale pink eggs. However, as the egg production rate of *C. glacialis* cultures was expected to decrease towards the end of the study period, whereas *C. finmarchicus* is known to produce eggs for a prolonged period as long as food is available, *C. finmarchicus* eggs were used in subsequent feeding experiments, for which only one egg species required staining.

A comparison of the hatching percentages of stained and unstained (control) *Calanus* eggs showed a difference between hatching success of *C. finmarchicus* and *C. glacialis* eggs. For *C. finmarchicus*, hatching percentages of 50% ± 1.0% and 56% ± 2.2% were observed in the stained and control treatments respectively, whereas percentages of 78% ± 1.6% and 90% ± 0.7% were found for *C. glacialis*. A X^2 two-sample test (1 degree of freedom) with Yates' Correction for Continuity was used to test for association of observed frequencies in stained vs. controls and revealed significant differences on the 1% level for both species (Table 2).

Egg selection experiments

All three *Calanus* spp. fed on stained as well as unstained eggs of both *C. finmarchicus* and *C. glacialis*. In Fig. 2, ingestion rates are plotted relative to the 1 : 1 line. Selection for stained or non-stained eggs will result in the displacement of

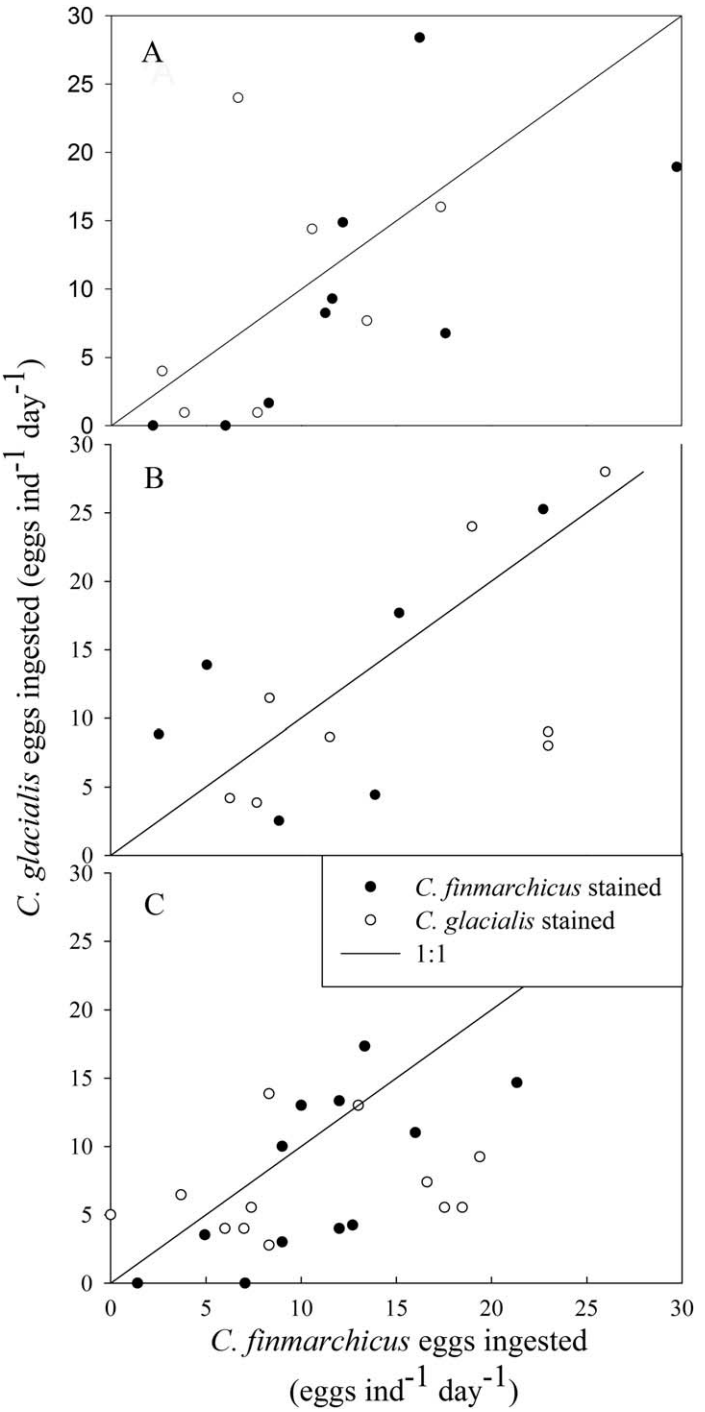

Fig. 2. Results of the egg preference experiments plotted relative to the 1 : 1 line. Each point represents the result from one experimental bottle, with the number of *Calanus finmarchicus* eggs ingested as the *x*-coordinate and the number of *C. glacialis* eggs ingested as the *y*-coordinate. The egg consumers were **A**: *C. finmarchicus*, **B**: *C. glacialis* and **C**: *C. hyperboreus*.

the black (*C. finmarchicus* stained) and white (*C. glacialis* stained) sets of data points relative to each other. Selection for *C. finmarchicus* egg over *C. glacialis* eggs or vice versa would result in deviation from the 1 : 1 line.

For each copepod, a generalized linear model (GLM) was fitted using R (v. 2.14.1). The model used number of consumed and surviving eggs as response variables assuming a binomial distribution. Staining (yes/no) and egg species entered into the model as explanatory variables using a logit link function. In each model, i.e., for each consumer, the effect of staining was not significant (*p*-values between 0.06 and 0.47). However, *C. glacialis* and *C. hyperboreus* consumers showed a preference for *C. finmarchicus* over *C. glacialis* eggs ($p = 0.023$ and $p = 1.47 \times 10^{-06}$ respectively), the former with a 6.8% greater chance of eating a *C. finmarchicus* egg and the latter with 9.7% greater chance of eating a *C. finmarchicus* egg. *C. finmarchicus* consumers showed the same tendency, but the result was not significant ($p = 0.088$).

Functional responses on *Calanus*-egg diets

Most of the *Calanus* consumers fed actively on eggs during incubation. However, feeding rates were highly variable, with some bottles yielding negative clearance and ingestion rates—those were included in mean calculations, as they represent variation linked to the methods used. Results for the three species were plotted together (Figs. 3, 4). However, only for *C. hyperboreus* were enough data available to fit functional response curves. The response was assumed to follow a Holling type II function (Holling 1959):

$$F = \frac{\beta}{1+\beta\tau C}$$

and

$$I = FC = \frac{\beta C}{1+\beta\tau C}.$$

where F and I are the concentration-dependent clearance and ingestion rates respectively, β the encounter rate kernel (or the maximum clearance rate), τ the prey handling time and C the concentration of prey. The equations were fitted to the data for mean clearance and ingestion rates for *C. hyperboreus*. The regression line found for clearance was $y = a/(1 + x/b)$, $R^2 = 0.125$, with $a = 582.6 \pm 221.1$ ($p = 0.034$) and $b = 31.7 \pm 58.2$ ($p = 0.603$). That fit was not significant (ANOVA, corrected for the mean of the observations, $p = 0.3498$). For ingestion, the regression line was $y = ax/(1 + bx)$, $R^2 = 0.696$, with $a = 0.37 \pm 0.15$ ($p = 0.044$) and $b = 0.026 \pm 0.025$ ($p = 0.332$). That fit was significant (ANOVA, corrected for the mean of the observations, $p = 0.0052$).

The significant estimate of the parameter a obtained from the ingestion regression corresponds to a maximum clearance rate for *C. hyperboreus*

$$\beta = 370 \pm 150\ \text{mL d}^{-1}$$

A linear curve fit was additionally done for the ingestion data (not shown), to test the assumption of food saturation inherent in the Holling Disc equation. The regression line $y = 1.0 + 0.160x$ had an R^2 value practically equal to that

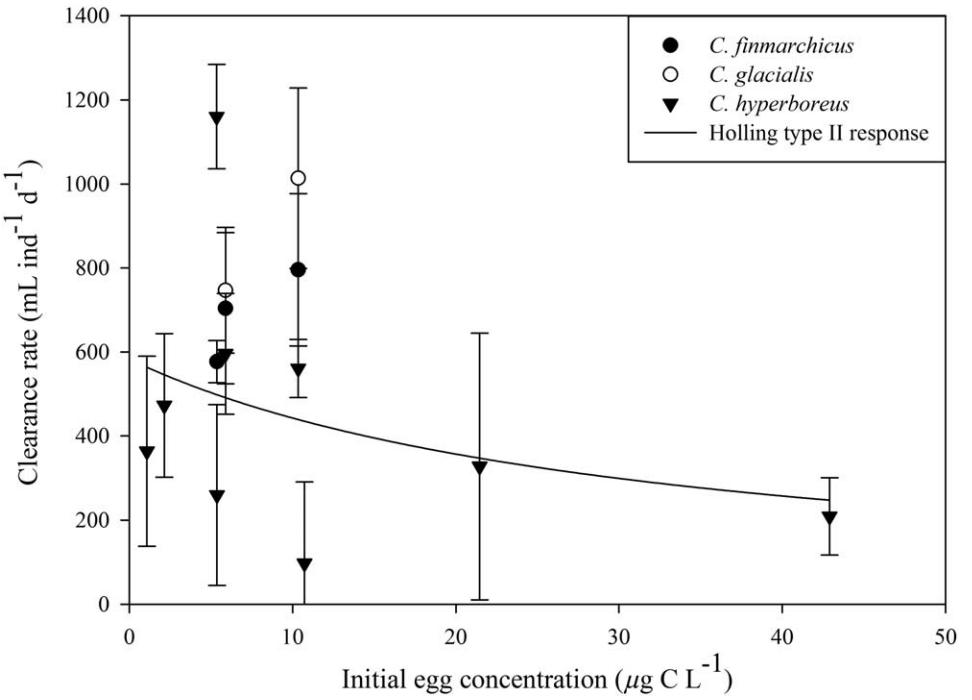

Fig. 3. Clearance rates of *C. hyperboreus* females feeding on *C. finmarchicus* eggs. Regression line is $y = 582.6/(1 + x/31.7)$, $R^2 = 0.125$. A few data points with *C. finmarchicus* and *C. glacialis* consumers are shown but not included in the fit. Values are mean ± SE.

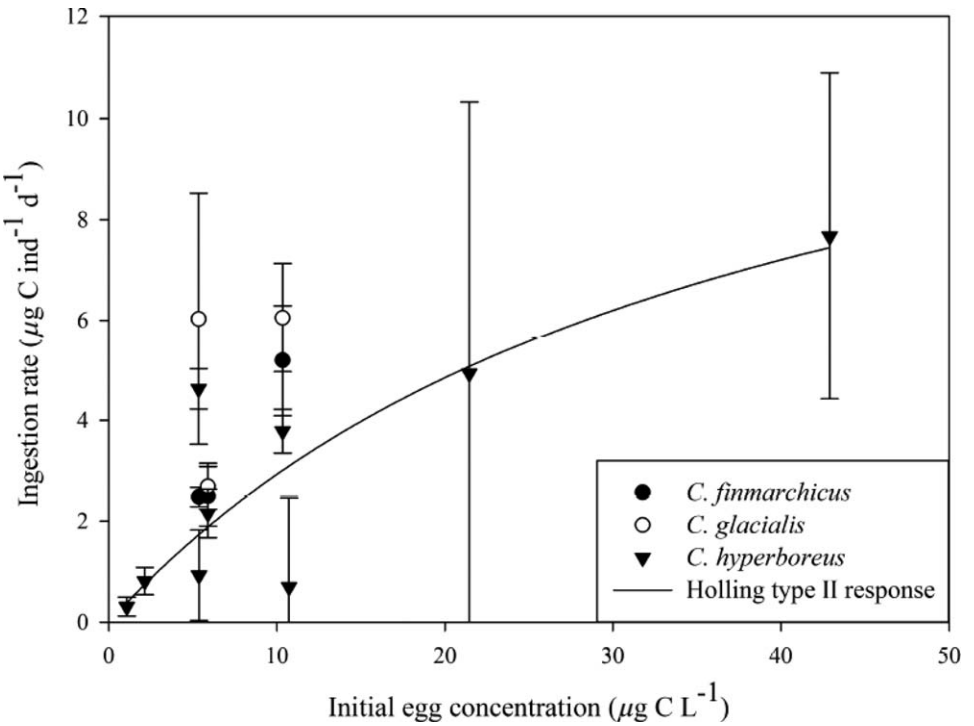

Fig. 4. Ingestion rates of *C. hyperboreus* females feeding on *C. finmarchicus* eggs. Regression line is $y = 0.37/(1 + x/0.026)$, $R^2 = 0.696$. A few data points with *C. finmarchicus* and *C. glacialis* consumers are shown but not included in the fit. Values are mean ± SE.

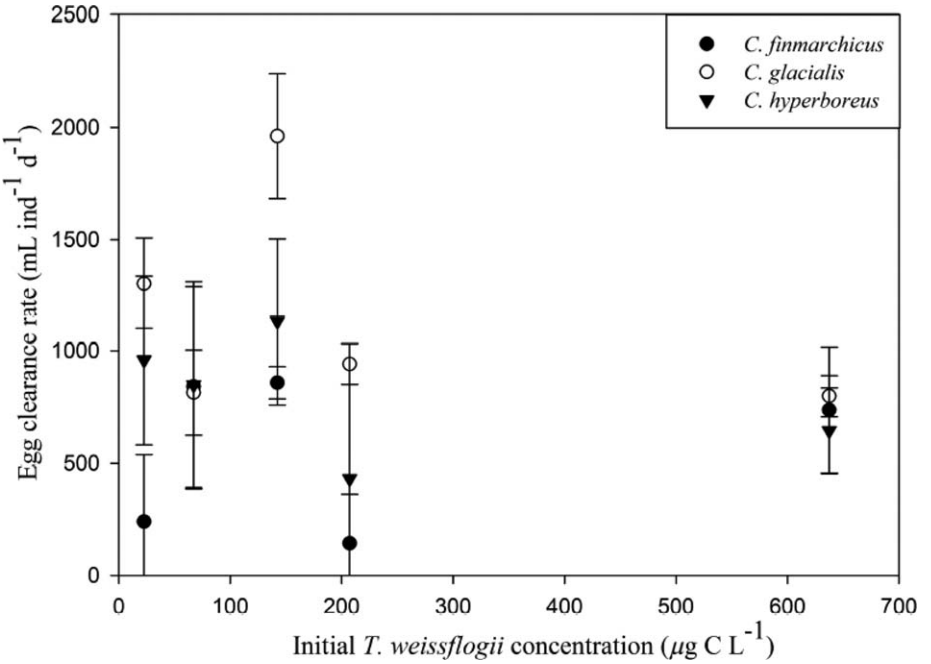

Fig. 5. Egg clearance rates of *C. finmarchicus*, *C. glacialis*, and *C. hyperboreus* females at increasing *Thalassiosira weissflogii* concentration and constant egg concentration (5.4 μg C L^{-1}). Values are mean ± SE.

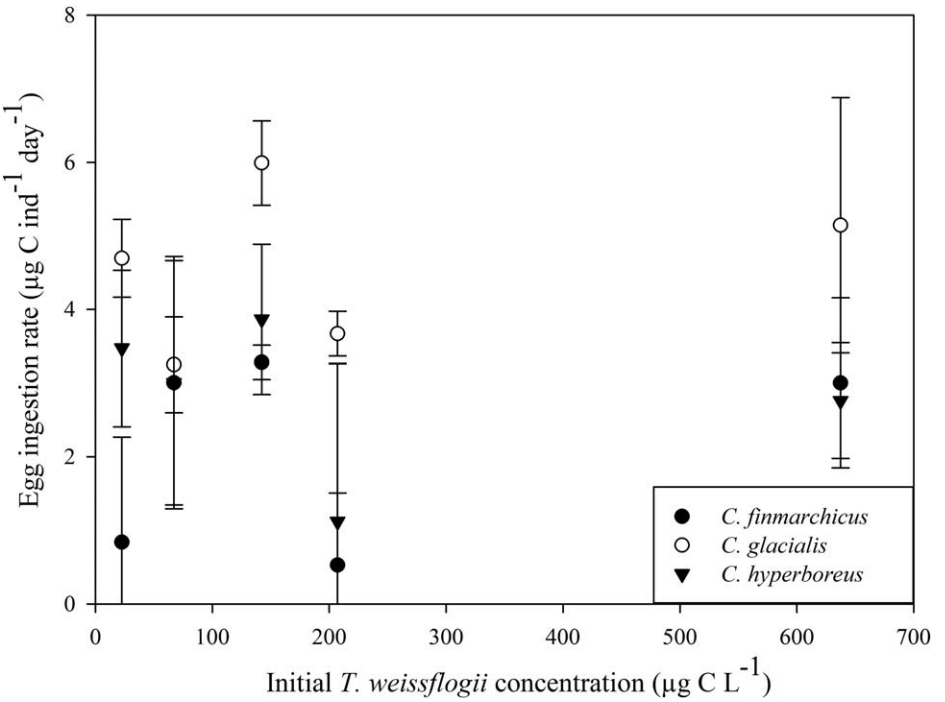

Fig. 6. Egg ingestion rates of *C. finmarchicus*, *C. glacialis*, and *C. hyperboreus* females at increasing *Thalassiosira weissflogii* concentration and constant egg concentration (5.4 μg C L^{-1}). Values are mean ± SE.

obtained from the Disc equation fit (0.701 vs. 0.696), indicating that the offered maximum egg concentration of 43 μg C L^{-1} (204 eggs L^{-1}) was likely below the critical concentration and thus that ingestion was not saturated.

Observations of maximum clearance rates were 796 ± 181 mL ind^{-1} d^{-1} (n = 7) at 38 eggs L^{-1} for *C. finmarchicus*, 1013 ± 215 mL ind^{-1} d^{-1} (n = 8) for *C. glacialis*, also at 38 eggs L^{-1}, and 1160 ± 124 mL ind^{-1} d^{-1} (n = 4) for *C. hyperboreus* at 25 eggs L^{-1}.

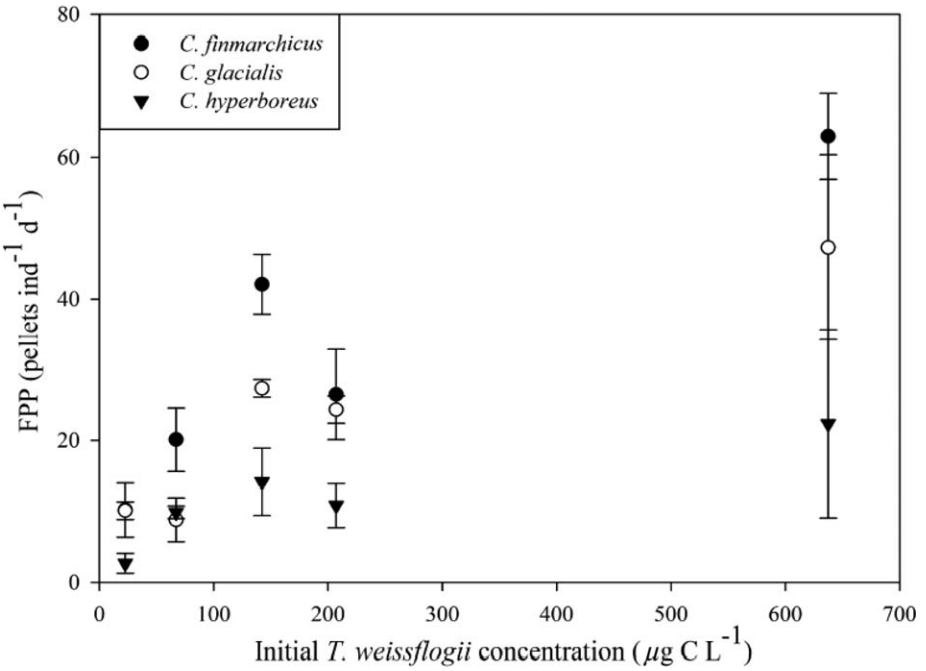

Fig. 7. Fecal pellet production rates (FPP) of *C. finmarchicus*, *C. glacialis*, and *C. hyperboreus* females at increasing *Thalassiosira weissflogii* concentration and constant egg concentration (5.4 μg C L^{-1}). Values are mean ± SE.

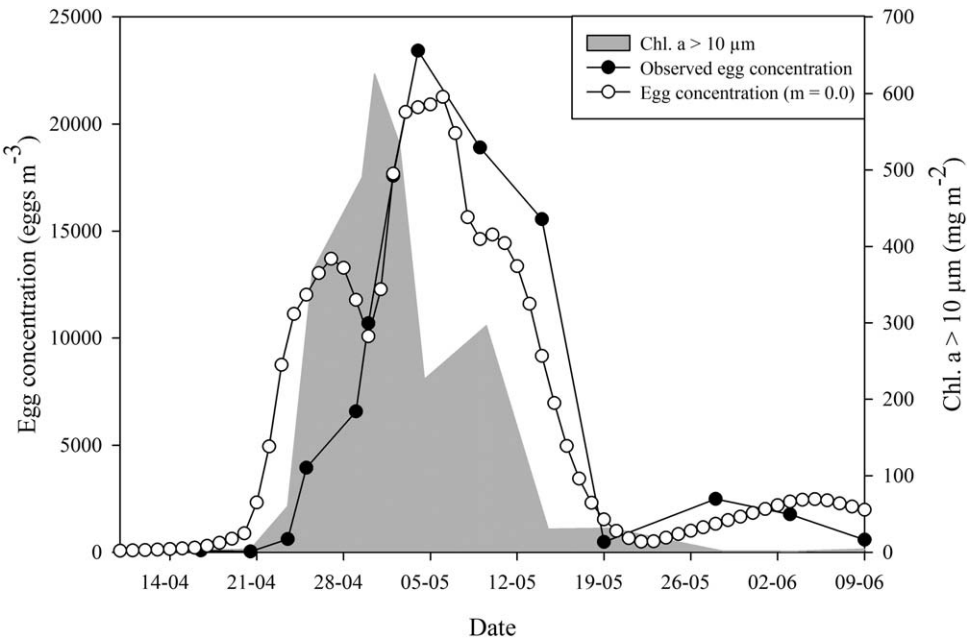

Fig. 8. Chl *a* concentrations from the large phytoplankton fraction (cell sizes ≥ 10 μm) integrated over the top 100 m of the water column (shaded area) are shown together with observed *C. finmarchicus* and *C. glacialis* total in situ egg concentrations (black data series) and calculated egg concentrations, assuming zero mortality (white data series). Data is from Swalethorp et al. (2011) and collected during the productive season 2008.

Eggs/phytoplankton mixed diet

The females fed on both eggs and the diatom *Thalassiosira weissflogii* in the mixed diet incubations, but egg clearance and ingestion rates (Figs. 5, 6) showed no clear dependence on phytoplankton concentration. *C. glacialis* females had the highest clearance rates ranging from 799 ± 91 to 1961 ± 278 mL ind^{-1} d^{-1}, while clearance rates ranged from 143 ± 218 to 858 ± 72 mL ind^{-1} d^{-1} and from 432 ± 600 to 1132 ± 372 mL ind^{-1} d^{-1} in *C. finmarchicus* and *C. hyperboreus*, respectively. Maximum egg clearance

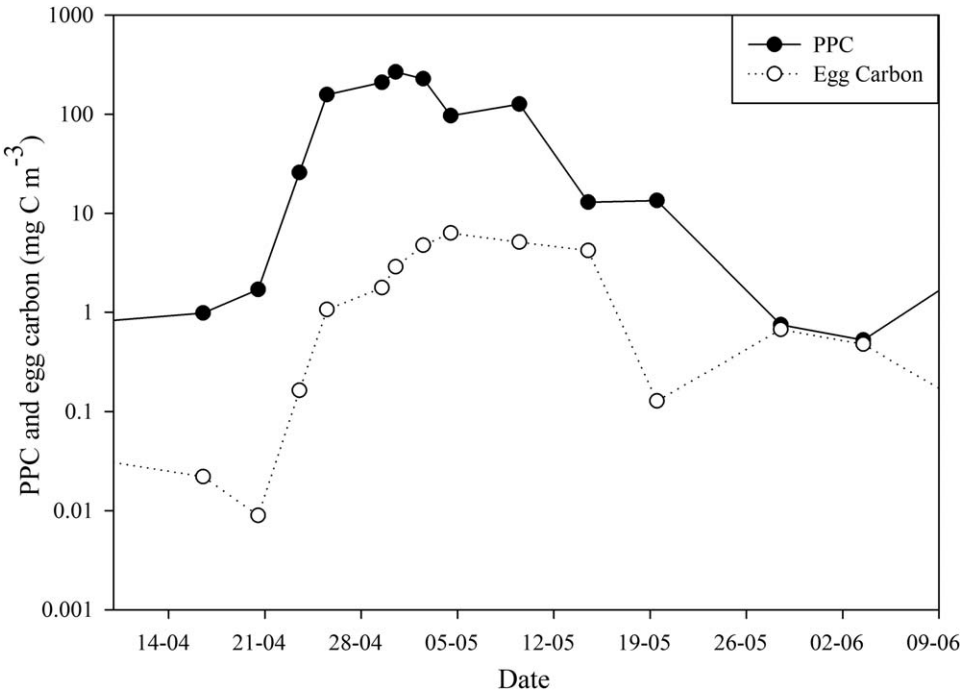

Fig. 9. Observed concentrations of phytoplankton carbon, PPC, and egg carbon. Values are calculated from the data depicted in Fig. 8, assuming a 1 : 1 ratio of *C. finmarchicus* and *C. glacialis* eggs in addition to carbon conversion factors of 0.21 and 0.33 μg C egg^{-1}, respectively (Swalethorp et al. 2011). A factor of 42.8 is used for the conversion of Chl *a* to PPC (Juul-Pedersen et al. 2006).

rates were observed at an initial phytoplankton concentration of 142 μg C L^{-1} for all three species, corresponding to maximum egg ingestion rates of 6.0 ± 0.6 μg C ind^{-1} d^{-1} for *C. glacialis*, 3.3 ± 0.2 μg C ind^{-1} d^{-1} for *C. finmarchicus*, and 3.9 ± 1.0 μg C ind^{-1} d^{-1} for *C. hyperboreus*. These maxima at 142 μg C L^{-1} phytoplankton were probably induced by some effect linked with the experimental protocol. To account for this source of correlation in the data a linear mixed model was fitted (function "lmer" from the lme4 package in R (R version 3.2.5)) with egg clearance rate as response variable and species and phytoplankton as explanatory variables. Species entered the model as a fixed effect, while phytoplankton entered both as fixed and random effects. In this model phytoplankton was borderline non-significant ($p = 0.058$) and thus was dropped from the fixed effects. A log likelihood ratio of 0.221 (df = 5) between this and a simplified model with *C. finmarchicus* and *C. hyperboreus* merged to one level showed that the two species were not significantly different and the simplified model was accepted. The clearance estimates from this model were 684 ± 144 (SE) mL ind^{-1} d^{-1} for *C. finmarchicus* and *C. hyperboreus* and 1180 ± 178 (SE) mL ind^{-1} d^{-1} for *C. glacialis*.

Fecal pellet production rates, FPP, (Fig. 7) showed increasing ingestion rates (eggs and phytoplankton) with increasing phytoplankton concentration.

In situ calculations

The spring bloom commenced in 2008, with a progressive, exponential increase in Chl *a* from 4 mg m^{-2} to 60 mg m^{-2} between 20th April and 23rd April, and peaking on 30th April at a maximum value of 625 mg m^{-2} (Fig. 8). Then Chl *a* abundance decreased rapidly, with a second but smaller peak on 09th May. The spring bloom ended around 14th May, reaching a low but steady Chl *a* level.

Both observed egg concentration and expected egg concentration calculated from the in situ egg production roughly followed the Chl *a* curve with a 5–6 d delay, peaking at 23,400 and 21,300 eggs m^{-3} of all species in the 04th May and 06th May samplings (Fig. 8). Calculated expected values were generally slightly lower than observed during the period. However, a marked discrepancy was seen during the pre- and early bloom period from 16th April to 30th April, with calculated values exceeding observed by a factor up to ~26-fold (20th April), an ~8100 eggs m^{-3} difference in concentrations (23rd April).

Observed concentrations of phytoplankton carbon exceeded egg carbon concentrations by a factor of 10–100 during most of the pre-bloom and bloom period, with maximum values of 267.6 mg phytoplankton C m^{-3} and 6.3 mg egg carbon m^{-3} on 30th April and 04th May, respectively (Fig. 9).

Egg mortality rates during the same period for the top 100 m are shown in Fig. 10, together with fraction of the water column cleared by larger stages of the *Calanus* community with assumed egg clearance rates equal to that of adult females. These are here defined as stages ≥ *C. finmarchicus* C5

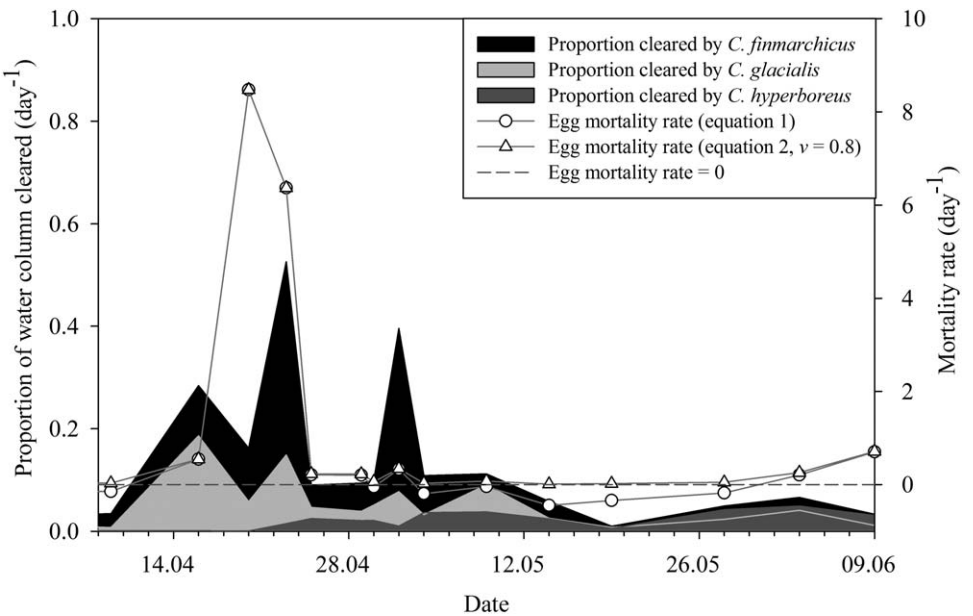

Fig. 10. Egg mortality rates (scatter plot) calculated *a*. according to Peterson and Kimmerer (1994) and *b*. according to Head et al. (2015), assuming 80% egg hatching success ($v = 0.8$), are shown together with *Calanus* community clearance (proportion of water column cleared d^{-1}), calculated from clearance rates obtained in lab experiments (area plot).

(mean prosome length ± SE = 2304 ± 6 μm, $n = 864$), i.e., C5, adult males (AM) and adult females (AF) in *C. finmarchicus*, C4-C5, AM and AF in *C. glacialis*, and finally C3-C5 and AF in *C. hyperboreus* (no males present in the surface layers). Mortalities calculated from the Peterson and Kimmerer Eq. 1 generally ranged from -1.0 d^{-1} to 0.7 d^{-1}, but with very distinct peak values of 8.5 d^{-1} and 6.4 d^{-1} on 20th April and 23rd April, respectively, corresponding to the largest calculated to observed egg concentration ratios (Fig. 8). Mortalities calculated with the Head et al. Eq. 2, assuming an egg hatching success of 0.8, largely overlapped with those from the other method and ranged from 0.0 d^{-1} to 0.7 d^{-1} with peak values equal to those calculated from Eq. 1.

The proportion of the water column cleared of phytoplankton by grazing of the *Calanus* community in the top 100 m reached a maximum of 69% on 23rd April (Fig. 10), which was calculated using the mixed-model clearance estimates from the experiments with varying food concentration.

Discussion

The applicability of neutral red for vital staining of *Calanus* eggs

Neutral Red vital stain is often applied in viability assessments, i.e., discrimination between live and dead plankton organisms, and it has been reported to stain live copepods vividly (Dressel et al. 1972; Elliott and Tang 2009, 2011; Zetsche and Meysman 2012). However, its application for staining copepod eggs has only been vaguely described in the literature (e.g., Dressel et al. 1972). In the present study, the Neutral Red staining protocol, which was adapted from the one described by Elliott and Tang (2009), proved effective for staining *C. finmarchicus* and *C. glacialis* eggs at temperatures around 0°C. Its application greatly facilitated the sorting and counting of eggs and allowed for easy differentiation of eggs from species with overlapping morphology in mixed-diet feeding experiments. In addition, the method was applied to single species experiments, where it allowed for the detection of eggs spawned during incubation.

We found the relationship reported by Dressel et al. (1972) for live adult copepods to be valid for copepod eggs, that the degree of staining depends on stain concentration and duration of exposure. However, egg coloration varied substantially within egg batches, which is also consistent with the findings of Dressel et al. (1972). The application of Neutral Red in viability assessment is based on the fact that stain uptake relies on cellular activity (Zetsche and Meysman 2012). Therefore, uptake rates can be expected to reflect the metabolic rates of the organisms exposed to the stain. Eggs failing to absorb the dye are most likely resting or nonviable. That is supported by the larger proportion of unstained or pale pink eggs observed in batches of stained *C. finmarchicus* eggs compared with those of *C. glacialis* eggs, which fits well with the observed difference in egg hatching percentages (*C. finmarchicus*/control = 56% ± 2.2% and *C. glacialis*/control = 90% ± 0.7%).

Color retention of adult copepods following vital staining was reported by Dressel et al. (1972) to depend on stain exposure time and temperature, with color loss retarded by cooling or freezing. In the present study color loss was observed, but not quantified. Most eggs were counted within

approximately 24–28 h after staining, and although most batches showed considerable reduction in coloration, the stain was clearly detectable under a dissection microscope. Color retention was, again, inconsistent in *Calanus* eggs, probably also reflecting varying rates of metabolic activity in the eggs. In similar experiments conducted with other species and at higher incubation temperatures, the stain appeared to be excreted at higher rates, making color loss a factor that should be taken into account in experimental designs.

The hatching success of *C. finmarchicus* reported here (control = 56% ± 2.2% after 5 d at 3°C) is lower than the values reported by Ohman et al. (2002) and Grenvald et al. (2013) of 88.9% after 3 d at 5–7°C and > 80% after 5 d at 0°C, respectively. The low hatching success observed in this study likely reflects low egg viability, which Jónasdóttir et al. (2009) associated with low food quality. Staining with Neutral Red showed a small but significant effect on egg hatching success in both *C. finmarchicus* and *C. glacialis*. For our experiments, however, this would not affect a successful application of the staining. For example, the selection experiment showed no significant effect of staining on egg preference in either of these consumers, which allows the projection of feeding rates on stained eggs obtained in experiments to in situ conditions.

Cannibalistic feeding rates

Consistent with reports of cannibalistic egg feeding in a range of marine copepods (e.g., Bonnet et al. 2004), the results of the present study show that *C. finmarchicus, C. glacialis*, and *C. hyperboreus* are capable of eating the eggs of *C. finmarchicus* and *C. glacialis*, but do so at variable rates. Studies on egg predation are few, and this is the first report of egg feeding rates estimated for these species. When accounting for size, the obtained maximum clearance rates were comparable with values found in the literature for other species feeding on *Calanus* eggs. Clearance rates of 320 mL ind^{-1} d^{-1} were reported for *Calanus helgolandicus* (Bonnet et al. 2004) when feeding on their own eggs. For *Metridia lucens* and *Centropages typicus* clearance rates of 190 mL ind^{-1} d^{-1} and 270 mL ind^{-1} d^{-1}, respectively, were reported when feeding on *C. finmarchicus* eggs (Sell et al. 2001). Even higher cannibalistic feeding rates have been reported that included predation on nauplii, which has been investigated in a larger number of studies. Clearance rates of up to ~660 mL ind^{-1} d^{-1} were reported for *Calanus pacificus* feeding on their own nauplii (Landry 1980), and values as great as ~2700 mL ind^{-1} d^{-1} have been estimated for the more carnivorous species *Labidocera trispinosa* feeding on *C. pacificus* nauplii (Landry 1978a).

In our experiments, *C. hyperboreus* seemed to ingest eggs in direct proportion to their availability and showed no evidence of saturation, even at an egg concentration of 43 μg C L^{-1} (204 eggs L^{-1}). That is well above the maximum in situ egg concentration observed during the 2008 spawning season, ~23 L^{-1} (6.3 μg C L^{-1}), indicating that saturating egg concentrations are likely never attained in the field. Maximum clearance rates can, therefore, be applied to the range of in situ egg concentrations observed within the study area. Assuming the maximum clearance rates obtained in the experiments without phytoplankton (Figs. 3, 4), egg ingestion corresponds to 5.0, 6.4, and 7.3 μg C ind^{-1} d^{-1} for *C. finmarchicus*, *C. glacialis*, and *C. hyperboreus*, respectively, a few days after the bloom peak. From the pre-bloom to early–bloom period length-to-carbon regression for *Calanus* from Swalethorp et al. (2011), and applying mean prosome lengths, the average carbon weights of adult females can be calculated as ~113, 338, and 1153 μg C ind^{-1}, which gives weight-specific egg ingestion rates of 0.6–4% d^{-1}. Estimates by Dünweber et al. (2010) of weight-specific ingestion by the *Calanus* species in Disko Bay from April to June on a natural diet averaged 10.3% ± 2.2% d^{-1} from gut fluorescence and 6.7% ± 0.7% d^{-1} from in situ fecal pellet production. Thus, egg cannibalism alone would be unlikely to sustain metabolic costs and egg production in *Calanus*, but may constitute a high quality nutritive supplement to the phytoplankton diet.

Egg cannibalism in relation to phytoplankton abundance

Maximum egg clearance rates without phytoplankton ranged from 796 mL ind^{-1} d^{-1} in *C. finmarchicus* to 1160 mL ind^{-1} d^{-1} in *C. hyperboreus* and were higher when phytoplankton was added, ranging from 858 mL ind^{-1} d^{-1} in *C. finmarchicus* to 1961 mL ind^{-1} d^{-1} in *C. glacialis*.

Egg clearance rates remained fairly constant over the range of phytoplankton concentrations offered, so that the presence of alternative food (phytoplankton) even at high concentration, did not reduce the feeding rate on eggs. Even at 637 μg C L^{-1} phytoplankton and 5 μg egg-C L^{-1}, egg clearance for the three species was still in the range of 645–799 mL ind^{-1} d^{-1}, corresponding to ingestion of 414–513 μg C ind^{-1} d^{-1} assuming non-selective feeding. If we assume mean pellet volumes of 1.3×10^6 μm^3 for *C. finmarchicus* and *C. glacialis* and 2.1×10^6 μm^3 for *C. hyperboreus*, mean pellet carbon content of 43×10^{-9} μg C μm^{-3} (Swalethorp et al. 2011) and an assimilation coefficient of ~2/3 (Conover 1966), total ingestion in the three species would fall in the range of 6–11 μg C ind^{-1} d^{-1} at maximum phytoplankton concentrations (taken as 637 μg C L^{-1}). This is clearly less than the ingestion of 414–513 μg C ind^{-1} d^{-1} inferred if we assume the phytoplankton were cleared at the same rate as eggs. Assuming nonselective feeding, a total ingestion of 6–11 μg C ind^{-1} d^{-1} translates to clearance rates in the range of 9–17 mL ind^{-1} d^{-1}. Hence, it seems that consumers were feeding selectively on eggs during incubation, and that high phytoplankton concentrations associated with bloom conditions offer no protection for *Calanus* eggs with respect to cannibalism.

Can egg cannibalism explain the observed in situ egg mortality?

The majority of planktonic copepods are free-spawners, i.e., they spawn their eggs freely into the water. Feeding experiments have shown that copepods are capable of selecting particles of certain sizes or food quality from incubations with mixed prey (Frost 1977; Poulet and Marsot 1978). Thus, given the high nutritive value of an egg, the free-spawning strategy exposes the eggs to predation by a range of pelagic predators and suspension feeders, including the mothers themselves, making them subject to periods of substantial predation mortality (Kiørboe et al. 1988). In Disko Bay, the calculated egg mortality rates were generally low during most of the study period, but with a marked peak during a few days in the early bloom phase when values were 6–8 d^{-1}. While the Peterson and Kimmerer (1994) equation gave several negative values, these became positive yet close to zero when including the modification by Head et al. (2015) and assuming an egg hatching success of 80%. However, the two approaches showed very similar scenarios. The maximum rates of egg mortality calculated here, imply an average life expectancy of an egg of only 1/8 to 1/6 of a day or 3–4 h. With an egg development time of ~6.5 d, only fractions on the order of $\sim 10^{-17}$ to $\sim 3 \times 10^{-23}$ $\left(e^{-mD}\right)$ of the eggs would survive to first nauplius stage, which corresponds to a daily survival of ~0.2% to ~0.03%. Published estimates of egg mortality rates are few, but they are generally similar. Beckman and Peterson (1986) reported *Acartia tonsa* egg mortality rates of up to 4.7 d^{-1} in Long Island Sound. Kiørboe et al. (1988) reported mortality rates of up to 9.1 d^{-1} in *A. tonsa* and *Paracalanus parvus* eggs from Danish waters, but noted that these estimates should only be interpreted as general magnitudes.

One problem with the application of the Peterson and Kimmerer equation, with or without the Head et al. modification, in these calculations is that its derivation is based on the assumption that the period between sampling dates is shorter than the egg development time. In other words "steady state" is assumed to exist around each sampling date. This assumption is not met for all dates in the present data set, which may introduce a source of error to the calculations. The most reliable method in the present study is, however, the one proposed by Head et al. (2015), as it incorporates egg hatching success. The hatching percentage found for *C. finmarchicus* was actually very low (56%), so if this observation reflects in situ conditions, the bias of omitting an estimate of hatching success would be significant.

One of the main objectives in the present study was to investigate whether egg cannibalism could explain the observed egg mortality rates. The peak in mortality (6.4–8.5 d^{-1}) coincided with the highest proportion of the water column cleared by the *Calanus* community, 0.69 d^{-1}. Thus, egg cannibalism may, at most, account for roughly 10% of observed mortality at this point. During the remainder of the season mortality and community clearance showed a low degree of correlation, and overall, the data indicate that cannibalism has little effect on egg mortality. Correlations between female abundance and egg mortality rates have sometimes been interpreted as evidence of cannibalism (e.g., Ohman and Hirche 2001) but, as noted by Head et al. (2015), these relationships are ambiguous, because the variables being correlated are not independent, since mortality rates are calculated using female abundances. Nevertheless, the peak mortality rates in the early bloom phase represent an extreme deviation from the otherwise low values and may, at least in part, be caused by cannibalism. With phytoplankton levels still relatively low and the *Calanus* females aggregating in the surface, spawning and eating concurrently, the prerequisites are certainly present. Consumption by krill may provide another possible explanation. A recent study by Agersted et al. (2011) from the Godthåbsfjord, SW Greenland, showed that the dominant krill species, *Thysanoessa raschii*, was capable of exploiting plankton in a remarkable size range of 5–400 μm. Thus, the schooling and migratory behavior of krill could cause short, intense bursts of high feeding pressure in the euphotic zone, potentially affecting survival of copepod eggs.

One weakness in the calculation of community clearance is the disproportionately low clearance rate for adult *C. hyperboreus* females. In a large copepod species like *C. hyperboreus*, a lower size-specific feeding rate is to be expected due to the general relationship of decreasing metabolic rate with increasing size in an organism. However, the broad average of *absolute* clearance rate of the large *C. hyperboreus* is only roughly 60% that of *C. finmarchicus* in the present study. Similar observations was made by Henriksen et al. (2012) who reported that female *C. hyperboreus* collected during the pre-bloom period took some days to develop maximal faecal pellet production (FPP) rates when fed in the laboratory, while FPP rates for females collected during the bloom tended to decrease over time. The reason for these observations is not known, however, we propose that physiologic responses to capture and handling or a higher threshold for entering into a feeding mode may have caused the low average clearance rate in *C. hyperboreus*, a rate not representative of its actual feeding activity during bloom conditions.

The trend after the bloom of high observed relative to expected egg concentrations in the study of Swalethorp et al. (2011) might be associated with a number of factors. The large nonviable proportion of *C. finmarchicus* eggs observed in the laboratory may be part of the explanation. The calculations of expected zero mortality egg concentrations involved integrating the daily volume-specific egg production over the development time. In reality, the nonviable fraction of the eggs would accumulate according to in situ predation, sinking and decomposition rates, rather than to development time. Assuming the former to be slower than

the latter, the bias inferred could cause the observed pattern. Alternatively, the observations here could be caused by advective "egg enrichment" of the parcel of water studied. The drift of organisms with ocean currents and the vertical shear caused by different rates of advection over the depth distribution of a focal species can potentially complicate time series data obtained from a fixed geographic location, because in- and out-fluxes of organisms are virtually impossible to account for (Aksnes and Ohman 1996). This explanation may not apply for Disko Bay, however, where females and eggs seem to share similar vertical distributions. Thus, in 2008 85% ± 8% of the eggs and 55% ± 12% of the females in the 0–100 m layer were located within the upper 50 m, while in 1997 and 2001 eggs, nauplii and females were concentrated just below the pycnocline in the 25–30 m depth layer (Juul-Pedersen et al. 2006; Munk et al. 2015). Eggs could also be lost via sedimentation, but this is probably of minor importance for viable eggs. Viable eggs should hatch within 2–3 d (Greenvald et al. 2013), while settling at rates of 25–35 m d^{-1} (Knutsen et al. 2001), so that eggs laid in the 0–50 m layer should hatch before they reach 100 m, allowing the nauplii to swim back to the productive surface layer.

Potential influence of egg cannibalism on *Calanus* succession

The present study is the first to describe cannibalistic feeding in Arctic *Calanus* through the combination of feeding experiments and a comprehensive in situ data set. It shows that all three Arctic *Calanus* species are ingesting the eggs of *C. finmarchicus* and *C. glacialis*. However, even at the highest egg concentrations observed, in situ egg cannibalism could not sustain metabolic costs and egg production in *Calanus*, but may constitute a high quality nutritive supplement to the phytoplankton diet.

The present findings also suggest that *C. finmarchicus* eggs may be more vulnerable to predation than the similar *C. glacialis* eggs, an observation that may be related to the difference in their size. The smaller *C. finmarchicus* eggs may simply be easier to handle and ingest. As long as sufficient food levels are maintained, *C. finmarchicus* is capable of eating and producing eggs throughout the summer. *Calanus glacialis* and *C. hyperboreus* have a very short and intensive feeding phase centered around the spring bloom, and thus have no influence on *C. finmarchicus* egg mortality throughout the remainder of the season. The potential impact of cannibalism on *C. finmarchicus* may, therefore, concentrate in the short bloom period when all three species are present in the surface layer.

The eggs of *C. hyperboreus* are spawned in deep water during winter and subsequently float to the surface. Therefore, their exposure to cannibalism by their own or other *Calanus* may be limited. Upon arrival in the surface *C. hyperboreus* females tend to remain deeper in the water column than the two other species, and are thus spatially separated from most of the eggs. They are therefore probably only minor contributors to cannibalistic egg mortality. Unlike the two others, spawning in *C. glacialis* is focused entirely around the bloom during the aggregation of females. It could be expected to be the species most affected by cannibalism.

In conclusion, cannibalism may explain some of the observed egg mortality, but our results suggest that other factors play a role. Especially during the early phase of the spring bloom, the observed high mortality rates cannot be attributed to *Calanus* cannibalism alone. Additional causes of mortality probably include predation by other zooplankton (e.g., krill and chaetognaths), advective effects, and the production of non-viable eggs (and their subsequent loss via sedimentation, predation etc.), which should all be considered in future studies of *Calanus* egg mortality.

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Conflict of Interest

None declared.

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