Contrasting physiological responses to future ocean acidification among Arctic copepod populations

Running head: Contrasting responses to ocean acidification

Peter Thor[1], Allison Bailey[1], Sam Dupont[2], Piero Calosi[3], Janne E. Søreide[4], Pierre De Wit[5], Ella Guscelli[6], Lea Loubet-Sartrou[3], Ida Marie Deichmann[7], Martin M. Candee[8], Camilla Svensen[9], Andrew L. King[10], Richard G.J. Bellerby[10,11]

[1] Norwegian Polar Institute, Fram Centre N-9296 Tromsø, Norway.

[2] University of Gothenburg, Dept. of Biological and Environmental Sciences, SE-451 78 Fiskebäckskil, Sweden.

[3]Université du Québec à Rimouski, Département de Biologie, Chimie et Géographie, Rimouski, QC G5L 3A1, Canada

[4] University Centre in Svalbard, N-9171 Longyearbyen, Norway

[5] University of Gothenburg, Dept. of Marine Sciences, SE-452 96 Strömstad, Sweden.

[6] University of Florence, 50121 Florence, Italy

[7] University of Aarhus, Department of Bioscience, DK-8000 Aarhus, Denmark

[8] Danish Technical University, DTU-AQUA, DK-2920 Charlottenlund, Denmark

[9] UiT The Arctic University of Norway, Faculty of Biosciences, Fisheries and Economics, 9037 Tromsø, Norway

[10] Norwegian Institute for Water Research, N-5006, Bergen, Norway

[11] State Key Laboratory for Estuarine and Coastal Research, East China Normal University, Shanghai 200062, China

Corresponding author: Peter Thor, tel: +47 40613027, peter.thor@npolar.no

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Abstract

Widespread ocean acidification (OA) is modifying the chemistry of the global ocean, and the Arctic is recognised as the region where the changes will progress at the fastest rate. Moreover, Arctic species show lower capacity for cellular homeostasis and acid-base regulation rendering them particularly vulnerable to OA. In the present study, we found physiological differences in OA response across geographically separated populations of *Calanus glacialis*. In copepodite stage CIV, measured reaction norms of ingestion rate and metabolic rate showed severe reductions in ingestion and increased metabolic expenses in two populations from Svalbard (Kongsfjord and Billefjord) whereas no effects were observed in a population from the Disko Bay, West Greenland. At pH_T 7.87, which has been predicted for the Svalbard west coast by year 2100, these changes resulted in reductions in scope for growth of 19% in the Kongsfjord and a staggering 50% in the Billefjord. Interestingly, these effects were not observed in stage CV copepodites from any of the three locations. It seems that CVs may be more tolerant to OA perhaps due to a general physiological reorganisation to meet low intracellular pH during hibernation. Needless to say, the observed changes in the CIV stage will have serious implications for the *C. glacialis* population health status and growth around Svalbard. However, OA tolerant populations such as the one in the Disko Bay could help to alleviate severe effects in *C. glacialis* as a species.

Introduction

Widespread ocean acidification (OA) is modifying the chemistry of the global ocean (Hoegh-Guldberg *et al.*, 2014). Driven by an increase in global atmospheric pCO_2 from 280 µatm at pre-industrial times to the present day 400 µatm (IPCC, 2013), the global ocean mean surface pH has decreased from 8.13 to the present day 8.05. Ocean models predict a continuation of this trend with a further decrease of 0.4 pH units by the year 2100 (Bopp *et al.*, 2013, Caldeira & Wickett, 2005, Cao *et al.*, 2007). Due to the chemical characteristics of Arctic sea water, the Arctic is recognised as the region where the earliest and strongest decreases in pH are expected (Fabry *et al.*, 2009, Hoegh-Guldberg *et al.*, 2014, Steinacher *et al.*, 2009). Increasing sea ice melt with low H^+ buffering capacity makes Arctic waters increasingly susceptible to OA (Yamamoto-Kawai *et al.*, 2009). Moreover, while the Arctic Ocean constitutes only 1% of the global ocean volume, it receives 11% of the riverine discharge carrying not only low H^+ buffering capacity but also significant loads of terrestrial carbon prone to conversion to CO_2 by microbial respiration (Raymond *et al.*, 2007). This input has increased by 7% since the

1930s (Peterson *et al.*, 2002). Finally, increasing inflow from the North Atlantic carries large amounts of anthropogenic CO_2 to the Arctic Ocean (Fransson *et al.*, 2001).

The magnitude of predicted chemical changes due to OA extends beyond anything experienced by most extant species (Fabry *et al.*, 2008) and significant effects are predicted for many marine animals (Dupont & Pörtner, 2013, Wittmann & Pörtner, 2013). But while effects may be severe locally, they may vary across geographic ranges and among populations (Wood *et al.*, 2016). While it has long been hypothesised that long distance dispersal of planktonic larvae and eggs in an environment with few physical barriers has rendered most marine species genetically homogeneous over long distances, recent studies of marine invertebrates, including planktonic species, show geographically structured populations and isolation on the scale of ocean basins and adjacent seas (Hellberg, 2009, Peijnenburg & Goetze, 2013, Sanford & Kelly, 2010). Such structuring increases the possibility for differential physiological responses to environmental changes to develop among hydrographic provinces (as shown at lower latitudes by Calosi *et al.*, 2017, Vargas *et al.*, 2017). Differential responses carry with them a possibility that affected species may be relieved from severe effects and extinction (Calosi *et al.*, 2016, Sunday *et al.*, 2014). Effects may be severe locally, and possibly lead to local extinction, but other enclaves may show higher tolerance.

Naturally, relief from environmental change is all the more important for the future of more environmentally sensitive species, and energetic studies suggest that the capacity to counter negative effects of OA could be particularly low in Arctic species. Contrary to cold adapted eurythermal animals, true Polar species show low energetic costs for maintenance (Clarke, 1980, Rastrick & Whiteley, 2011). While this is an evolutionary strategy to enhance growth at limited aerobic scope, lower allocation to cover maintenance costs also reduce the capacity for energy demanding cellular homeostasis and acid-base regulation (Whiteley, 2011). Moreover, because Arctic communities are characterised by simpler food webs – fewer trophic levels and fewer species occupying each trophic level – they experience reduced overall resilience to environmental changes (AMAP, 2013).

Calanoid copepods, particularly of the *Calanus* genus, constitute keystone species in the Arctic pelagic community (Grainger, 1965, Møller *et al.*, 2006, Thor *et al.*, 2005). In most pelagic communities, these crustaceans constitute 80% of the zooplankton biomass, and they are the dominant component of prey for the larvae of most fish species (Last, 1980). Consequently, their presence is fundamental to many fish populations and studies have shown that larval survival and recruitment of such species as cod (*Gadus morhua*) and mackerel

(*Scomber scombrus*) co-vary with copepod abundance and biomass (Beaugrand *et al.*, 2003, Castonguay *et al.*, 2008, Runge *et al.*, 1999). Any negative effects of environmental changes will therefore have severe repercussions far beyond the copepod populations themselves. For instance, increase in rainfall since the 1980s and lack of intrusion of high saline water from the North Sea have affected reproduction and maturation in the copepod *Pseudocalanus elongatus* in the Baltic Sea deep basins (Möllmann *et al.*, 2003). This has forced herring (*Clupea harengus*) to revert to less favourable prey imposing serious implications for their development and population growth (Möllmann *et al.*, 2003).

In the present study we investigated the possible existence of differential responses to OA among geographically separated populations of *Calanus glacialis,* a species which dominates the shelf of the Arctic Ocean and adjacent seas (Wassmann *et al.*, 2015). We established physiological reaction norms across a pH gradient covering present and predicted future environmental pH variability for Arctic continental shelf seas. Physiological response was measured as the balance between energy intake and expenditure because it is this balance that determines energetic performance and ultimately fitness in heterotrophs (Brown *et al.*, 2004).

Methods

Collection of copepods

Copepods were caught by vertical tows of a 200 µm WP2 net equipped with a closed cod end from 100 m to the surface in the Kongsfjord, Svalbard (79.0° N, 11.7° E), the Billefjord, Svalbard (78.6° N, 16.5° E), and the Disko Bay, Western Greenland (69°15´ N, 53° 33´ W) during July 2015 (Fig. 1). On deck, the content of the cod end was diluted in 25 L seawater collected at 80 m. Copepods were then transported to cold rooms (5 °C) at either the Kings Bay Marine Laboratory (Ny-Ålesund, Svalbard) or the Arctic Station Laboratory (Qeqertarsuaq, Western Greenland). *Calanus glacialis* copepodites stages III, IV, and V (hereafter CIII, CIV, and CV) were selected under the stereomicroscope using cut off plastic Pasteur pipettes, keeping all vessels on ice to avoid high temperatures. Copepodite stages were identified by number of pleopods and abdominal segments (Mauchline, 1998). They were distinguished from *Calanus hyperboreus* and *Calanus finmarchicus* copepodites on the basis of prosome size (Arnkværn *et al.*, 2005, Thor *et al.*, 2008), by red pigmentation in the antennules, which *C. finmarchicus* most often do not have (Nielsen *et al.*, 2014), and the lack of lateral spikes on the distal prosome segment, which is a characteristic of *C. hyperboreus* (Klekowski & Weslawski, 1991).

Experimental design

We applied a regression design approach, exposing independent samples of copepods to one of seven to nine pH levels (Table 1). This approach has the advantage of enhanced predictive power compared to the character state approach, which compares effects among different distinct future climate scenarios (Havenhand *et al.*, 2010). We found CIIIs only in the Kongsfjord population, whilst CIVs and CVs were found at all three locations. However, CVs were found in very low numbers in the Billefjord population. After removal of replicates containing incorrectly stage determined individuals (as determined from photographs), individuals with very aberrant prosome length also indicative of erroneous stage determination or speciation, and individuals judged dead after incubations, a total of 153 replicates of ingestion rate measurements and 170 replicates of metabolic rate measurements remained (Table 1).

Preparation of incubation water

For the initiation of incubations and at each water change, five litre batches of incubation water for each treatment were prepared by mixing 0.3 µm filtered seawater (*fsw*) with small volumes of *fsw* acidified to ca. pH 5.5 by CO_2 bubbling (Mapcon© CO_2, Yara Praxair, Tromsø, Norway). This method for manipulating seawater carbonate chemistry has been previously described and validated (Riebesell *et al.*, 2010). The different treatments were established at target pH_T (pH on the total scale) increments of 0.2. Total alkalinity (A_T) was analysed by potentiometric titration (Dickson *et al.*, 2007) in an open cell with 0.1 M HCl using a VINDTA 042 carbonate titrator (Marianda, Germany) and total dissolved inorganic carbon (C_T) was analysed by coulometric titration (Dickson *et al.*, 2007) using a coulometer (CM5015, UIC, Joliet, IL, USA) connected to the VINDTA after acidification with 8.5 % phosphoric acid. pCO_2 and pH_T were calculated using CO2SYS (Pierrot *et al.*, 2006) with constants from Mehrbach et al. (1973) refitted by Dickson and Millero (1987) and inputs of temperature, salinity, A_T, and C_T. pH_T was monitored using a SevenGo SG2 pH meter equipped with an InLab 413 SG/2m electrode (Mettler-Toledo, Columbus, Ohio, USA) (Svalbard populations) or a HI 98183 pH/ORP meter (Hanna, Woonsocket, Rhode Island, USA) (Disko Bay population). Determination of pH_T in all incubation water batches and incubation bottles were based on a standard curve established from simultaneous measurements in water samples of electric potential (mV) with the pH electrodes and determination of pH_T from A_T and C_T with the VINDTA in the pH range 8.2-6.4. Salinity and temperature were measured using a conductimeter (Cond 340i, WTW, Weilheim, Germany).

Measured values of chemistry parameters are shown in Table 2. A_T was established only once for the Billefjord population. For food, paste of the diatom *Thalassiosira weissflogii* (Tw 1200, Reed Mariculture, Campbell, CA, USA) was added to a final concentration of ca. 10 µg Chl *a* L^{-1}. The necessary dilution of the algal paste was established from the Chl *a* content of the algal paste determined spectrophotometrically (UV-2401 PC, Shimadzu Co., Kyoto, Japan) after overnight extraction in 70% ethanol (Strickland & Parsons, 1972). Prior to incubations, the suitability of the algal paste as prey for *C. glacialis* was assured by comparing faecal pellet counts from incubations of copepodites with previous counts from copepodites incubated at similar concentrations of algae.

Copepod incubations

For each experiment, copepodites were incubated for a total of 8 d (7 d incubation plus 1 day ingestion rate measurements). For each replicate, 10 individuals were pipetted, using cut off plastic Pasteur pipettes, into a 600 mL glass Duran bottles prepared with incubation water. All bottles were closed, making sure no air bubbles were present, and placed on a slowly rotating plankton wheel (0.5 rpm) at ca. 5 °C in dim light. Every day approximately 500 mL water was replaced in each bottle by inserting a piece of pipe fitted with a 200 µm screen at the bottom, siphoning off the water from inside the tube, and replacing it with water from the pre-prepared five litre incubation water batches at the appropriate pH. Samples for A_T and C_T were taken from the incubation water batches and from water pooled from all bottles of each treatment subsequent to the incubations on days 2, 5, and 8).

Measurement of ingestion and metabolic rates

On day 7, five additional control bottles without copepods were prepared with incubation water for estimates of ingestion rates. Triplicate samples for Chl *a* determination were taken from each incubation water batch. On day 8 the content of each bottle was poured through a 20 µm sieve held in a Petri dish to remove copepods, faecal pellets, and eggs. While doing this, the water was collected in a beaker from under the Petri dish and 200 mL was filtered onto a 0.7 µm glass fiber filter (Whatman, GF/F, Maidstone, UK) which was frozen for later Chl *a* determination. The content of the 20 µm sieve was gently flushed into a Petri dish and copepods for metabolic rate measurements were collected. The rest were counted and photographed for precise determination of developmental stage under the stereoscope.

For estimates of specific metabolic rate ($\dot{M}O_2$), oxygen consumption rates were measured on individual copepodites according to Thor and Oliva (2015). One individual from each bottle

was pipetted from the Petri dish into a 1.6 mL vial fitted with fluorescent O_2 reactive foil discs (PSt3 spots, PreSens, Regensburg, Germany) and filled with *fsw*, which had been saturated with air by vigorous bubbling and adjusted to the corresponding pH. Vials were then sealed with Teflon caps and after a resting period of ca. 30 min to acclimate copepods O_2 concentrations were measured at 0, 2.5, and 5 h using an optode O_2 system (Fibox 3, PreSens, Regensburg, Germany). O_2 consumption rate (nmol O_2 ind^{-1} d^{-1}) was calculated by subtracting the average O_2 depletion rate measured in the five controls without copepods from the O_2 depletion rate in each of the copepod containing vials (nmol O_2 L^{-1} h^{-1}) and multiplying by vial volume (L) and 24 h d^{-1}. Prior testing of the optode system at 5 °C showed a 3-min 95 % reaction time, i.e. the period of time taken before the output reached within 5 % of the final O_2 concentration value (as estimated by exponential regression). Therefore, at every sampling event, O_2 concentration was read for 3 min, and an average of values read during the last minute was used for calculations. Subsequent to the measurements the copepods were transferred to Petri dishes and photographed under the stereoscope for detailed stage determination.

For estimates of ingestion rate, phytoplankton Chl *a* concentrations of all samples were determined fluorometrically. The frozen filters were extracted in 4 mL acetone overnight and fluorescence was measured on a Turner Designs 10-AU fluorometer (Strickland & Parsons, 1972). Ingestion rate (µg Chl *a* ind^{-1} d^{-1}) was calculated from the decrease in Chl *a* concentrations from all bottles containing copepods subtracted by the decrease in disappearance from the control bottles (µg Chl *a* L^{-1} d^{-1}) (Frost, 1972), multiplying by bottle volume (L), and dividing by number of copepods counted in the bottles at day 8.

To obtain weight specific rates, copepod prosome lengths were measured from the photographs using ImageJ (U. S. National Institutes of Health) and body carbon weights were calculated using a weight/length relationship of W (µgC) = 4.8L $(mm)^{3.57}$ (Madsen *et al.*, 2001). Oxygen consumption rates (nmol O_2 ind^{-1} h^{-1}) were converted to specific metabolic rate ($\dot{M}O_2$, µgC µgC^{-1} d^{-1}) by dividing by body mass (µgC ind^{-1}), multiplying by a respiratory coefficient of 0.97 mol C mol O_2^{-1} (Omori & Ikeda, 1984), multiplying by 0.012 µgC nmol C^{-1}, and multiplying by 24 h d^{-1}. Ingestion rates (ng Chl *a* ind^{-1} d^{-1}) were converted to specific ingestion rate (*IR*, µgC µgC^{-1} d^{-1}) by multiplying by 50 µgC µg Chl a^{-1} (Båmstedt *et al.*, 2000) and dividing by body mass (µgC ind^{-1}).

To avoid bias from differences in temperature among incubations, all rates were normalized to the average temperature of 5.2 °C using a Q_{10} value of 2.0 for metabolic rate in marine copepods (Ikeda *et al.*, 2001).

Data analysis and determination of reaction norms

Since treatments were evenly distributed along pH reaction norms for each population and copepodite stage, rates would be inherently non-normally distributed when reaction norms show significant slopes. For comparisons of mean rates (i.e. the average rate of all individuals from all pH treatments) among populations and stages we therefore used a 2-factor permutational analysis of variance test (PERMANOVA) on similarity matrices assembled using Euclidian distances (Anderson, 2001). Prosome lengths were similarly compared among populations and stages using a 2-factor PERMANOVA.

For each copepodite stage in each population, pH reaction norms of ingestion rate and metabolic rate were established by sequentially testing polynomial regression models of increasing order (linear, quadratic, or cubic) for the relationship between the variable and pH_T according to David et al. (1997). Best fitting models were chosen by statistically comparing sums of squares among the three models as

$$F_{1,df} = \frac{SS_{higher} - SS_{lower}}{MS_{res}}$$

where *df* is the degree of freedom of the higher degree model, SS_{higher} is the sums of squares of the higher degree model, SS_{lower} is the sums of squares of the lower degree model, and MS_{res} is the residual mean squares of the higher degree model (Rocha & Klaczko, 2012).

After assuring homoscedasticity (Levene's test), reaction norms of specific rates were compared among populations using univariate general linear model analysis (GLM) in SPSS (IBM Inc.). Differences of level among populations were detected by significant differences among populations using a pH_T + population design, and differences of slopes were detected by significant interactions of pH_T and population using pH as the covariate in a pH_T + population + population x pH_T design.

To evaluate the overall physiological effects of decreasing pH_T, scope for growth values were constructed from relationships between metabolic rate and ingestion rate in CIVs. Since metabolic rates were measured on different individuals than ingestion rate, no direct comparison was possible and we therefore calculated mean predicted scope for growth values

($\widehat{SFG}$) at each pH_T on the basis of predicted rates from the reaction norm regressions as $\widehat{SFG} = \widehat{IR} \times AE - \widehat{\dot{M}O_2}$, where *AE* is absorption efficiency, which was set at 0.6 for copepods (Thor *et al.*, 2007, Thor & Wendt, 2010).

Results

Comparison of mean rates among populations and developmental stages

Although prosome lengths were measure purely to enable calculation of weight specific rates, we found significant differences in these among populations (unrelated to pH) and therefore report the analyses here. Prosome lengths of both stage CIV and CV copepodites differed significantly among the three populations (2-factor PERMANOVA: pseudo-$F_{2,335} = 32.2$, $P < 0.001$). CIVs were significantly larger in the Kongsfjord and Disko Bay populations (2532 ± 381 µm and 2510 ± 115 µm, mean ± sd), respectively, than in the Billefjord population (2338 ± 150 µm) (2-factor PERMANOVA pair-wise test: $P < 0.001$), whereas CVs were significantly larger in the Disko Bay population (3357±144 µm) than in the Kongsfjord and Billefjord populations (2962 ± 307 µm and 2875 ± 313 µm, respectively) (2-factor PERMANOVA pair-wise test, $P < 0.001$).

The mean specific ingestion rate of the three developmental stages (for each stage, the average rate of all individuals from all pH_T tested) were significantly different at 0.111 ± 0.042 µgC µgC^{-1} d^{-1} in CIIIs, 0.044 ± 0.021 µgC µgC^{-1} d^{-1} in CIVs, and 0.021 ± 0.011 µgC µgC^{-1} d^{-1} in CVs (2-factor PERMANOVA: pseudo-$F_{2,152} = 54.6$, $P < 0.001$). Mean rates (for each population, the average rate of all individuals from all pH_T tested) also differed significantly between the Kongsfjord and Disko Bay populations (2-factor PERMANOVA pairwise test: $P = 0.004$) mainly due to the larger size and calculated weight, and hence lower specific rates, of CVs in the Disko Bay population.

Similarly, mean specific metabolic rates were significantly different among developmental stages: 0.025 ± 0.018 µgC µgC^{-1} d^{-1} in CIIIs, 0.024 ± 0.009 µgC µgC^{-1} d^{-1} in CIVs, and 0.015 ± 0.006 µgC µgC^{-1} d^{-1} in CVs (2-factor PERMANOVA: pseudo-$F_{2,169} = 14.3$, $P < 0.001$). These differed among populations with significantly lower rates in the Disko Bay population than in the two Svalbard populations (2-factor PERMANOVA pairwise tests: $P < 0.02$).

Ingestion rate reaction norms

In CIVs ingestion rates decreased by 85% and 66% from the highest to the lowest pH_T, in the Kongsfjord and Billefjord populations respectively, but remained unchanged in CIV from the

283 Disko Bay population (Figs. 2a,b,c). Ingestion rate reaction norms showed linearly decreasing
284 rates with decreasing pH_T in CIVs from the Kongsfjord and Billefjord populations (Table 3).
285 There was no difference in slopes between the Kongsfjord and Billefjord populations (GLM,
286 comparison of slopes: $F_{1,52} = 0.61$, $P = 0.439$).

287 In CIIIs from the Kongsfjord population, ingestion rates first increased by 53% from the
288 highest pH_T to pH_T 7.337 and then decreased to 33% at the lowest pH_T compared to the rate at
289 the highest pH_T (Fig. 2d). These changes were better fitted with the second order regression,
290 $IR = maxIR + g_2(pH_T - pH_{TmaxIR})^2$, where maximum ingestion rate ($maxIR$) was 0.124
291 µgC µgC^{-1} d^{-1}, pH_T at maximum ingestion rate (pH_{TmaxIR}) was 7.41, and the slope, g_2, was -
292 0.099 ($r^2 = 0.39$, $P = 0.019$) (Fig. 2d).

293 There were no significant effect of pH_T on ingestion rates of CVs from any of the three
294 populations (Table 3; Fig 3).

295 ## Metabolic rate reaction norms

296 Metabolic rates increased by 136% and 127% from high to low pH_T in CIVs from the
297 Kongsfjord and Billefjord populations, respectively, but remained unchanged in CIVs from
298 the Disko Bay population (Figs. 2a,b,c). The metabolic reaction norms showed significant
299 linearly increasing metabolic rates in Kongsfjord and Billefjord CIVs (Table 4) but there were
300 no differences in slopes of metabolic rate reaction norms between in the Kongsfjord and
301 Billefjord population CIVs (GLM pairwise comparison of slopes: $F_{1,48} = 1.30$, $P = 0.260$),

302 Metabolic rates remained unchanged with decreasing pH_T in CIIIs (Table 4; Fig. 2d), and in
303 CVs from all three populations (Table 4; Fig. 3).

304 Temperatures were generally lower in the Disko Bay experiments. Correction for temperature
305 differences among locations changed rates by an average 8 %. These corrections did not
306 significantly affect reaction norm slopes (GLM analysis comparing slopes of all reaction
307 norms with and without temperature corrections: $P<0.05$).

308 ## Scope for growth

309 In CIVs, $\widehat{SFG}$ decreased from 0.032 µgC µgC^{-1} d^{-1} at pH_T 8.012 to -0.021 µgC µgC^{-1} d^{-1} at
310 pH_T 6.445 in the Kongsfjord population and from 0.010 at pH_T 8.041 to -0.018 µgC µgC^{-1} d^{-1}
311 at pH_T 7.036 in the Billefjord population. Thus, $\widehat{SFG}$ became negative below pH_T 7.04 in
312 CIVs from the Kongsfjord population but already at pH_T 7.67 in CIVs from the Billefjord
313 population.

In CIIIs from the Kongsfjord population predicted scope for growth ($\widehat{SFG}$) first increased from 0.025 µgC µgC^{-1} d^{-1} at pH_T 8.041 to 0.049 µgC µgC^{-1} d^{-1} at pH_T 7.333 and then decreased to -0.009 µgC µgC^{-1} d^{-1} at pH_T 6.421.

We did not calculate $\widehat{SFG}$ changes in CVs since neither ingestion rates not metabolic rates changed significantly with pH_T. Any calculated differences would stem from stochastic differences or sampling variability rather than real physiological changes.

Discussion

The balance between energy intake and energy expenditure is the prime determinant of survival in any heterotrophic organism. Energy intake has to be sufficient to cover maintenance and repair costs, as well as costs for growth or reproduction for an organism to uphold positive Darwinian fitness (Sibly & Calow, 1986). In the present study, we observed severe reductions in ingestion rate along with increased metabolic rates with decreasing pH_T in *Calanus glacialis* copepodite stage CIV from two Svalbard populations (Kongsfjord and Billefjord), but not in CIVs from the Disko Bay, West Greenland. These effects were limited to the CIV stage and there were no effects in stage CV copepodites from any of the three populations. Nevertheless, at pH_T 7.87, which has been predicted for the Svalbard west coast by the year 2100 (Bellerby *et al.*, 2012), scope for growth decreased by 19% in the Kongsfjord CIVs, while in the Billefjord CIVs it decreased by a staggering 50%. In fact, these estimates of scope for growth may be conservative since absorption efficiency may decrease with decreasing pH due to decreasing gut enzyme activity (Stumpp *et al.*, 2013). Needless to say, such changes will have serious implications for the *C. glacialis* population around Svalbard. Reductions in scope for growth on this scale will prolong stage development time and reduce the individual body size of the developing copepodites and ultimately also reduce adult body size. This effect has been observed in *Calanus helgolandicus* cohorts reared in mesocosms at low prey levels (Rey-Rassat *et al.*, 2002). The resulting reduction in adult body size will entail decreased egg production rates (Halvorsen, 2015), and there is a real risk that these effects, although possibly limited to one or a few specific copepodite stages (Kongfjord CIIIs showed a peaking ingestion rate reaction norm), may impair the general health status and growth of *C. glacialis* in this region. Accordingly, studies in the North Sea and the sub-Arctic Pacific have shown that similar changes in spring juvenile production have significant effects on overall population development. A long-term sampling series in the North Sea has shown that years with low larval growth during spring results in lower summer biomass than

346 years with higher spring larval growth (Clark *et al.*, 2003). Similar variations have been
347 observed in the sub-Arctic Pacific *Neocalanus plumchrus* population. This population
348 experiences significant inter-decadal variations in peak summer biomass, which is
349 hypothesised to stem from changes in copepodite growth rate during spring (Mackas *et al.*,
350 1998).

351 Previous studies have shown metabolic effects of low pH on copepods, although results are
352 far from conclusive. Metabolic rate increased significantly by 28% from pH_{NBS} (National
353 Bureau of Standards scale) 8.18 to 7.83 in *Centropages tenuiremis* (no developmental stage
354 indicated) (Li & Gao, 2012) and in *Pseudocalanus acuspes* females it increased significantly
355 by 11% from pH_T 8.06 to 7.75 (Thor & Dupont, 2015). Metabolic rates doubled from pH_T
356 8.06 to pH_T 7.66 in *Acartia grani* females, although low replication rendered the difference
357 non-significant, whereas no clear effect was observed in female *A. clausi* exposed to pH_T 8.03
358 and pH_T 7.83 (Isari *et al.*, 2015, Zervoudaki *et al.*, 2014). In *Pseudocalanus acuspes* a
359 decrease from 7.95 pH_T to 7.61 showed no clear effect on metabolic rate in a population from
360 Svalbard, whereas a population from Skagerrak experienced significant changes (Thor &
361 Oliva, 2015). But these changes depended on food level and no clear response could be
362 concluded. The lack of response of *C. glacialis* CVs in the present study is corroborated by a
363 recent study in the Kongsfjord (Thor *et al.*, 2016) and has also been shown to last during
364 longer-term incubations where metabolic rates remained equal in *C. glacialis* CVs and *C.*
365 *hyperboreus* CVs and females incubated at pH_F (free scale pH) 8.13 and 7.26 for 62 days
366 (Hildebrandt *et al.*, 2014). Metabolic rates of CVs increased linearly across a range from pH_T
367 8.02 to pH_T 7.16 in a study on culture reared *C. finmarchicus* applying reaction norm statistics
368 similar to the present study (Pedersen *et al.*, 2014), whereas a later study found no effects
369 between pH_T 7.92 and pH_T 7.51 in wild caught *C. finmarchicus* CVs and females (Runge *et*
370 *al.*, 2016). Ingestion rates have been shown to be unresponsive in *A. grani and Oithona*
371 *davisae* females (Isari *et al.*, 2015). In the *Calanus* genus, *C. finmarchicus and C. glacialis*
372 CVs showed no changes in ingestion rates when exposed at pH_T 7.2 (Hildebrandt *et al.*,
373 2016).

374 Geographically specific responses to low pH exposure have been demonstrated in several
375 marine species. The metabolic response to low pH varies with latitude in the gastropod
376 *Littorina littorea* showing an upregulation in the centre of the species distribution along the
377 European continental coast but a decrease in the southern- and northern-most regions (Calosi
378 *et al.*, 2017). Such latitudinal differences also occur in the calanoid copepod *Acartia tonsa*,

larvae of the gastropod *Concholepas concholepas*, and the bivalve *Perumytilus purpuratus* along the Chilean coast (Vargas *et al.*, 2017). While ingestion rates did not change with decreased pH in *A. tonsa* originating from an estuary with low and variable pH, they decreased by 72% in individuals from a coastal ocean area with perpetual high pH (Vargas *et al.*, 2017). Geographically specific responses have been observed also in another calanoid copepod species, *Pseudocalanus acuspes*. Populations from the Kongsfjord and the Gullmarsfjord (Swedish west coast) showed differences in the relationship between ingestion rate and metabolic rate (Thor & Oliva, 2015). Low pH induced a steeper increase in metabolic rate with increasing ingestion rate in females of the Swedish population than in females of the Svalbard population. Also the isopod *Idotea balthica* has shown geographically specific OA responses. In this case, metabolic rate and osmoregulatory activity responded differently to increased pCO_2 (1000 µatm) in individuals originating from low and high salinity environments (Wood *et al.*, 2016). Likewise, larvae of the spider crab *Hyas araneus* have shown differences in growth responses between two populations from Svalbard and the North Sea (Walther *et al.*, 2010). These differences may be a reflection of a general ability of the tested species for physiological plasticity to counter pH variations. Such plasticity may originate from the environment of the individual's habitat (phenotypic plasticity) or from the environment experienced by previous generations (transgenerational plasticity). But they may also arise from genetic adaptation to different pH environments among locations. Evidence for rapid evolution in the face of fast environmental changes is increasing (Carroll *et al.*, 2007), and previous studies have shown that calanoid copepods have the capacity for fast adaptation to low pH conditions. While our experimental design, incubations for less than one generation, did not allow detection of local adaption, Thor and Dupont (2015) found adaptation causing changes in *Pseudocalanus acuspes* fecundity after only two generations at pH_T 7.54, which could be linked to observed selection in genes coding for processes involved in oxidative phosphorylation and ribosomal structure (De Wit *et al.*, 2015). Similarly, in echinoderms low pH/high pCO_2 has been observed to induce rapid selection in genes coding for biomineralization, lipid metabolism, and ion homeostasis (Pespeni *et al.*, 2013). However, in the very same study on *P. acuspes*, Thor and Dupont (2015) also found evidence of phenotypic plasticity in response to lowered pH, albeit at lower levels of pH reductions, so both mechanisms may act in concert to alleviate OA effects. Regardless of the origin of the observed geographic differences in the CIV copepodites, phenotypic plasticity, transgenerational plasticity, or local adaptation, they have specific consequences for the future of *C. glacialis* as a species. The severe reductions in scope for growth in this stage observed

in the Svalbard populations would render *C. glacialis* with little potential to survive future OA. However, the existence of enclaves or perhaps extended populations with increased tolerance, such as the Disko Bay population, could prove important as an alleviating factor to remove or at least delay future OA effects.

Tolerance to certain environmental conditions is developed through pre-exposure. The few existing studies reveal a possible difference between the Disko Bay and the Svalbard fjords with respect to carbonate chemistry. While the Davis Strait outside Disko Bay exhibits similar high pH, as is common in Arctic waters (Azetsu-Scott *et al.*, 2010), the water of the Disko Bay may be somewhat special. The Disko Bay is influenced by extensive glacial discharge from the Jakobshavn glacier, and during summer the surface water are characterised by the balance between melt water production and the inflow of water from the West Greenland Current (Hansen *et al.*, 2012). Hence, the Disko Bay is very variable environment both on a seasonal and inter-annual scale. Studies from 2011 and 2012 showed that while pH_{NBS} was mostly high at the surface, it was perpetually lower than 8.0 below 50 m with values approaching 7.5 during May (Riisgaard *et al.*, 2015, Thoisen *et al.*, 2015). Frequently, low pH water was encountered throughout the water column during May in both years studied. pH_{NBS} did increase during the spring bloom but re-attained values below 8.0 immediately after the termination of the bloom (Riisgaard *et al.*, 2015). Outside the spring bloom period, pH_{NBS} was in the range 7.6-7.9 at fluorescence max depth, the depth where most copepods reside when feeding. The Kongsfjord is probably the best studied of the three, and recent investigations show high pH/low pCO_2 conditions throughout the fjord during summer and possibly also during winter (Fransson *et al.*, 2016). pH_T remained above 8.0 throughout the water column during July of the two consecutive years 2013 and 2014, and although winter data are scarcer, minimum measured winter surface water pH_T values in the Kongsfjord were 8.11 in 2013 and 8.14 in 2014 (Fransson *et al.*, 2016). To our knowledge there is no information on carbonate chemistry from the Billefjord. Thus, contrary to the Kongsfjord (and perhaps also the Billefjord), it seems that there would be a real possibility for zooplankton in the Disko Bay to be frequently exposed to low pH conditions during spring and summer, the period for copepodite growth (Yamamoto-Kawai *et al.*, 2009).

Is tolerance of low pH a special characteristic of the Disko Bay population or could we expect enclaves with similar tolerance elsewhere? While Arctic waters most often are characterised by high pH, studies show that low pH conditions do develop temporarily in some areas. Corrosive conditions have been observed in the Canada Basin connected to sea ice melt

(Yamamoto-Kawai *et al.*, 2009), and low pH/high pCO_2 conditions have also been observed in extended areas along the Siberian coast (Anderson *et al.*, 2011). Here, in the Laptev Sea, CO_2 produced from microbial decomposition of organic matter originating from river run-off has been shown to oversaturate the entire water column, even in the post spring bloom period (Anderson *et al.*, 2011). High pCO_2/low pH conditions have also been observed north of Greenland (Jutterström & Anderson, 2010). Thus, these areas could potentially function to pre-condition copepods to low or at least variable pH increasing the possibility of species wide tolerance to future OA.

Because we studied different developmental stages, our findings also contributed another important observation. While CIVs responded significantly to decreasing pH, we observed no clear change in either ingestion or metabolic rate in CVs. Also in a previous study, Thor et al. observed significant changes in the metabolic reaction to feeding at pH_T 7.73 compared to pH_T 8.11 in early copepodite stages (CII-CIII) but no changes in CVs (Thor *et al.*, 2016). Hildebrandt and colleagues found a similar lack of response of ingestion and metabolism in *C. glacialis* CVs (Hildebrandt *et al.*, 2014, Hildebrandt *et al.*, 2016). But while this led the authors to boldly conclude that shifts in seawater pH do not affect *C. glacialis* as a species, our study highlights the need to refrain from conclusions based on studies of single developmental stages. Such notion has been put forward previously by Dupont and colleagues (2010). Their meta-analysis of OA effects in echinoderms showed that larvae and juveniles mostly experience negative effects on growth and calcification while adults respond positively. In crustaceans, stage-specific metabolic responses to OA were also found for different larval stages in the European lobster (Small *et al.*, 2015). Also *Calanus* exhibits fundamental stage-specific metabolic differences, and in this respect the CV stage stands out. While somatic growth is the main goal in the preceding stages, metabolism is largely reconfigured to accommodate overwintering diapause in CVs. Ingestion rates were not much higher than metabolic expenses in this stage (Fig. 3) and it seems that CVs were entering this phase of physiological reconfiguration at the time of measurements. During diapause, *C. glacialis* CV experience extracellular pH as low as 5.5 possibly as a result of metabolic depression during hibernation (Freese *et al.*, 2015). It is therefore quite conceivable that mechanisms to counter low pH could be activated in this particular stage as part of the general physiological reconfiguration to accommodate hibernation. This would render CVs particularly unresponsive to ambient pH. If such mechanisms require energy, as most

physiological processes do, it would be evolutionarily beneficial to avoid their activation before they are needed.

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Table 1

Number of replicates *per* treatment combination: copepodite developmental stage (CIII, CIV, CV) of *Calanus glacialis* by nominal pH level according to our experimental design. When different, numbers preceding that slash refer to ingestion rate measurements and number following the slash refer to metabolic rate measurements. When only one value is indicated the number of replicates were equal. A total number of 153 samples were included in analyses of ingestion rate and a total of 170 in analyses of metabolic rates. By necessity the number of replicates varied with the number of copepodites available.

Copepodite stage	Location	Nominal pH								
		8.2	8.0	7.8	7.6	7.4	7.2	7.0	6.6	6.4
CIII	Kongsfjord	3/2	3	1/2	3	2/3	2	3	1	1
CIV	Kongsfjord	4	4	2	4/3	4	2/0	4/3	2	2
	Billefjord	4/3	4/3	2/4	3	4	3	4		
	Disko Bay	0/4	3/1	1	5	5	1	4	2/3	3/4
CV	Kongsfjord	4/3	4	2/1	3/4	4	2	4	2	1/2
	Billefjord	0/1	1	1	1	1	1	1		
	Disko Bay	0/3	6/8	2/5	4	5	1/5	3/5	2/3	2/3

Table 2

Mean ± standard deviations of carbonate chemistry parameters during incubations. pH_{nom} is nominal pH treatment, pH_T is total hydrogen scale pH, A_T is total alkalinity, and pCO_2 is CO_2 partial pressure. A_T was measured only once in the pH_{nom} 7.5 treatment once in the Billefjord population experiment.

pH_{nom}	T °C	S	pH_T	A_T µmol kg^{-1}	pCO_2 µatm
			Kongsfjord		
8.1	6.1 ± 0.9	34.0±0.1	8.012±0.064	2 347±11	450±95
7.9	6.0 ± 0.7	34.0 ± 0.1	7.851 ± 0.062	2 351 ± 14	712 ± 134
7.7	5.9 ± 0.6	33.9 ± 0.1	7.618 ± 0.092	2 354 ± 9	1 213 ± 346
7.5	6.3 ± 0.8	34.0 ± 0.1	7.442 ± 0.088	2 353 ± 21	1 973 ± 460
7.3	6.4 ± 0.7	34.0 ± 0.1	7.318 ± 0.067	2 348 ± 11	2 414 ± 308
7.1	6.3 ± 0.8	34.0 ± 0.1	7.160 ± 0.063	2 353 ± 5	3 546 ± 543
6.9	6.4 ± 0.7	34.0 ± 0.1	6.998 ± 0.044	2 350 ± 16	5 132 ± 526
6.6	6.5 ± 0.5	34.0 ± 0.1	6.636 ± 0.050	2 337 ± 8	11 534 ± 1 368
6.4	6.3 ± 0.4	34.0 ± 0.1	6.445 ± 0.039	2 332 ± 6	18 567 ± 2 163
			Billefjord		
8.1	6.2 ± 0.7	34.0 ± 0.1	8.041 ± 0.056		446 ± 93
7.9	6.5 ± 0.7	34.0 ± 0.1	7.851 ± 0.033		683 ± 49
7.7	6.5 ± 0.4	34.0 ± 0.1	7.644 ± 0.047		1 119 ± 178
7.5	6.9 ± 0.7	34.0 ± 0.1	7.497 ± 0.034	2 322 ± 3	1 536 ± 154
7.3	6.5 ± 0.4	34.0 ± 0.1	7.337 ± 0.036		2 319 ± 257
7.1	6.5 ± 0.5	34.0 ± 0.1	7.180 ± 0.043		3 336 ± 392
6.9	6.4 ± 0.4	34.1 ± 0.1	7.036 ± 0.041		4 526 ± 499
			Disko Bay		
8.1	3.9 ± 0.3	34.4 ± 0.1	8.001 ± 0.059	2 280 ± 0	436 ± 64
7.9	3.9 ± 0.4	34.4 ± 0.1	7.805 ± 0.050	2 286 ± 7	721 ± 91
7.7	3.6 ± 0.6	34.4 ± 0.1	7.627 ± 0.046	2 293 ± 0	1 112 ± 128
7.5	3.9 ± 0.7	34.4 ± 0.1	7.431 ± 0.056	2 287 ± 7	1 774 ± 250
7.3	3.6 ± 0.5	34.6	7.262 ± 0.036	2 287 ± 7	2 642 ± 233
7.1	3.5 ± 0.5	34.6	7.099 ± 0.020	2 293 ± 0	3 865 ± 194
6.9	3.6 ± 0.6	34.6	6.920 ± 0.040	2 287 ± 7	5 878 ± 517
6.6	4.5 ± 0.8	34.6	6.562 ± 0.037	2 280 ± 0	13 325 ± 1 130
6.4	4.1 ± 0.8	34.6	6.403 ± 0.077	2 280 ± 0	19 456 ± 3 521

Table 3

Ingestion rate reaction norms of *Calanus glacialis* copepodite stage CIV. Results of the first order regression model, $IR = \overline{IR} + g(pH_T - \overline{pH_T})$ (David *et al.*, 1997), where $\overline{IR}$ is mean ingestion rate, g is the slope, and $\overline{pH_T}$ is mean pH_T.

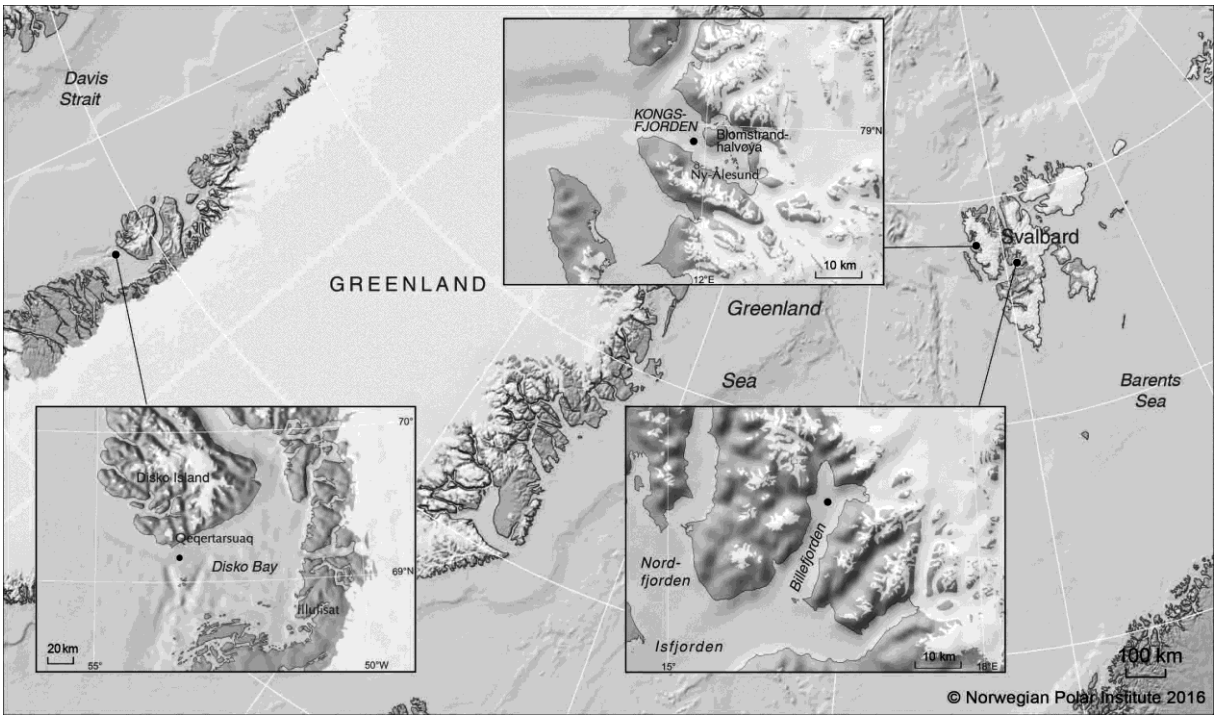
Stage	Location	$\overline{IR}$ µgC µgC^{-1} d^{-1}	$\overline{pH_T}$	g x10^{-3}	r^2	P
CIII	Kongsfjord	0.1006	7.30	17.6	0.05	0.369$^+$
CIV	Kongsfjord	0.0474	7.30	39.4	0.41	<0.001
	Billefjord	0.0398	7.62	28.0	0.19	0.031
	Disko Bay	0.0456	7.30	7.13	0.04	0.323
CV	Kongsfjord	0.0271	7.24	13.8	0.11	0.111
	Billefjord	0.0234	7.51	-2.23	0.02	0.808
	Disko Bay	0.0121	7.31	-0.91	0.02	0.540

$^+$ Ingestion rates of CIIIs were best fitted with the second order regression model (see text).

Table 4

Metabolic rate reaction norms of *Calanus glacialis* copepodite stage CIV. Results of the first order regression model, $\dot{M}O_2 = \bar{\dot{M}}O_2 + g(pH_T - \overline{pH_T})$ (David *et al.*, 1997), where $\bar{\dot{M}}O_2$ is mean metabolic rate, g is the slope, and $\overline{pH_T}$ is mean pH_T.

Stage	Location	$\bar{\dot{M}}O_2$ µgC µgC^{-1} d^{-1}	$\overline{pH_T}$	g x10^{-3}	r^2	P
CIII	Kongsfjord	0.0210	7.29	15.1	0.16	0.080
CIV	Kongsfjord	0.0206	7.30	-6.81	0.15	0.043
	Billefjord	0.0254	7.62	-10.2	0.23	0.014
	Disko Bay	0.0258	7.31	-2.92	0.03	0.359
CV	Kongsfjord	0.0170	7.30	2.51	0.04	0.354
	Billefjord	0.0149	7.62	-6.18	0.12	0.456
	Disko Bay	0.0231	7.31	-3.81	0.04	0.236



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741 Figure 1. Study sites in Kongsfjord, Billefjord (Svalbard), and Disko Bay (West Greenland).

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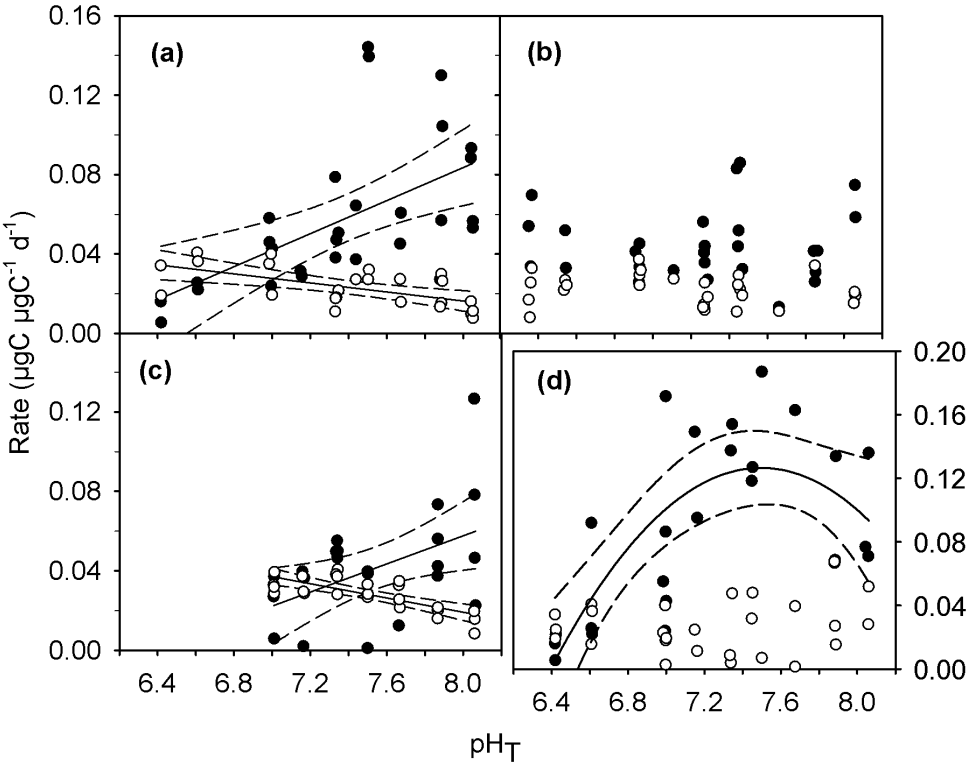
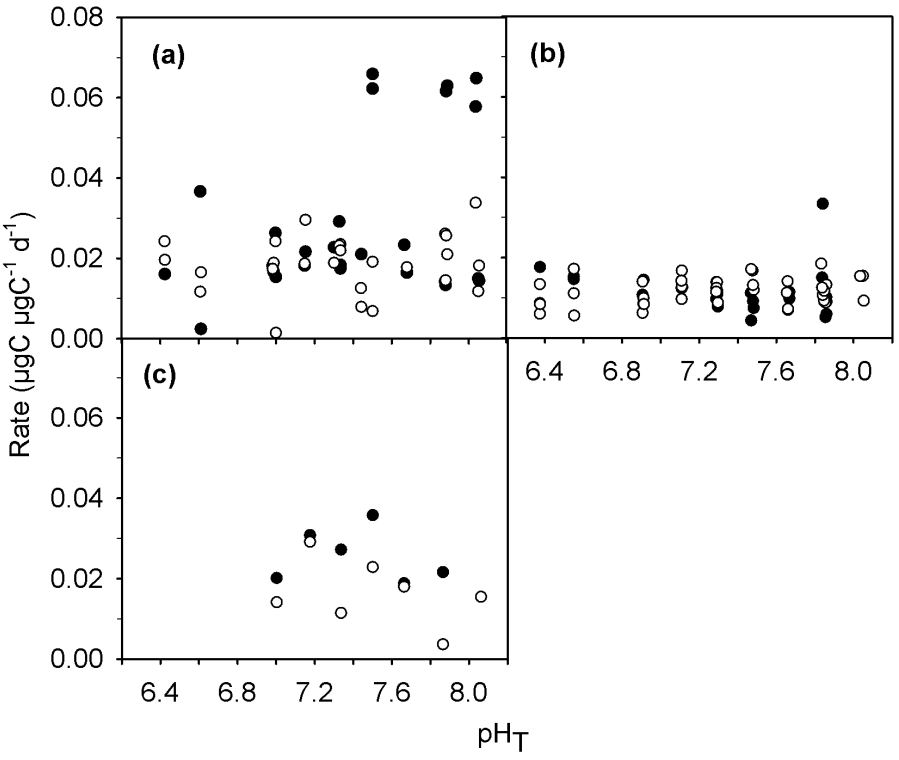


743

744 Figure 2. *Calanus glacialis* copepodite stages CIII and CIV. Ingestion rates (filled circles) and
745 metabolic rates (open circles) vs. seawater pH_T in the three populations. a) Kongsfjord CIVs,
746 b) Disko Bay CIVs, c) Billefjord CVs, and d) Kongsfjord CIIIs. Lines depict first or second
747 order reaction norms. Solid lines show predicted values and hatched lines show 95%
748 confidence limits. Reaction norm parameters and statistics are shown in Tables 3 and 4.

749



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751 Figure 3. *Calanus glacialis* copepodite stage CV. Ingestion rates (filled circles) and metabolic
752 rates (open circles) *vs.* seawater pH_T in the three populations investigated. a) Kongsfjord, b)
753 Disko Bay, and c) Billefjord.