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Contribution to Special Issue: 'Towards a Broader Perspective on Ocean Acidification Research Part 2'

Original Article

Early life stages of the Arctic copepod *Calanus glacialis* are unaffected by increased seawater pCO_2

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As the world's oceans continue to absorb anthropogenic CO_2 from the atmosphere, the carbonate chemistry of seawater will change. This process, termed ocean acidification, may affect the physiology of marine organisms. Arctic seas are expected to experience the greatest decreases in pH in the future, as changing sea ice dynamics and naturally cold, brackish water, will accelerate ocean acidification. In this study, we investigated the effect of increased pCO_2 on the early developmental stages of the key Arctic copepod *Calanus glacialis*. Eggs from wild-caught *C. glacialis* females from Svalbard, Norway (80°N), were cultured for 2 months to copepodite stage C1 in 2°C seawater under four pCO_2 treatments (320, 530, 800, and 1700 µatm). Developmental rate, dry weight, and carbon and nitrogen mass were measured every other day throughout the experiment, and oxygen consumption rate was measured at stages N3, N6, and C1. All endpoints were unaffected by pCO_2 levels projected for the year 2300. These results indicate that naupliar development in wild populations of *C. glacialis* is unlikely to be detrimentally affected in a future high CO_2 ocean.

Keywords: C:N, climate change, nauplii, ocean acidification, ontogeny, pH, respiration, zooplankton.

Introduction

Atmospheric pCO_2 has increased over the last 200 years due to anthropogenic activity and is expected to increase further. Some of this carbon dioxide is absorbed by the world's oceans (Le Quéré *et al.*, 2009), altering its carbonate chemistry, increasing hydrogen ion (H^+), bicarbonate ion (HCO_3^-), and dissolved inorganic carbon (DIC) concentrations and decreasing pH and carbonate ion (CO_3^{2-}) concentrations, a process that has been termed ocean acidification (Caldeira and Wickett, 2003; Orr *et al.*, 2005). The Arctic seas are expected to experience the largest pH decrease in the future because cold, low-salinity waters have greater uptake of CO_2, lower buffering capacity, and thus larger decreases in pH for a given pCO_2 than lower latitude seas (Steinacher *et al.*, 2008; Bellerby *et al.*, 2013). Climate warming is also expected to accelerate ocean acidification in the region, with the melting of Arctic sea ice increasing the air-to-sea flux of CO_2 and increased freshwater inflow from rivers and ice melt further decreasing the buffering capacity of seawater (Steinacher *et al.*, 2008).

Although the manner in which seawater carbonate chemistry is altered by ocean acidification poses greater problems for calcifying organisms, high pCO_2, (and associated low pH), also acts as a stressor to non-calcifying organisms (Kroeker *et al.*, 2010; Wittmann and Pörtner, 2013). Many physiological processes, including ion transport, enzyme activity, and protein function, as well as photosynthesis, rely on a specific range of intracellular pH (Whiteley, 2011). Maintaining intracellular pH can incur significant energetic costs when organisms must compensate for low extracellular pH (Pörtner, 2008; Whiteley, 2011), leaving less

energy for growth, development, and reproduction (e.g. Stumpp *et al.*, 2011).

Levels of pCO_2 projected for future oceans have limited effects on life history traits of some calanoid copepods (Kurihara and Ishimatsu, 2008; Mayor *et al.*, 2012; McConville *et al.*, 2013), whereas other calanoid species may be affected. *Pseudocalanus acuspes* experienced a 29% decrease in fecundity when exposed to pCO_2 levels predicted for the year 2100 (Thor and Dupont, 2015), and the development of *Calanus finmarchicus* was delayed at pCO_2 levels predicted for 2300 (Pedersen *et al.*, 2014a). The effect of increased pCO_2 on copepods also varies with life stage (Cripps *et al.*, 2014), with eggs and early naupliar stages being most vulnerable (Cripps *et al.*, 2014; Pedersen *et al.*, 2014b).

The calanoid copepod *Calanus glacialis* is a key component of the Arctic marine ecosystem, constituting up to 90% of the zooplankton biomass in shelf regions (Conover and Huntley, 1991; Blachowiak-Samolyk *et al.*, 2008). It is an important prey for fish, seabirds, and whales (Lowry, 1993; Karnovsky *et al.*, 2003; Hop and Gjøsæter, 2013). The few studies investigating *C. glacialis*' response to increased pCO_2 indicate that young stages may be more affected than older stages (Weydmann *et al.*, 2012; Lewis *et al.*, 2013; Hildebrandt *et al.*, 2014). While high pCO_2 does not affect subadult copepodite stage C5 and female respiration, survival, and gonad maturation rate (Hildebrandt *et al.*, 2014) or female egg production rate (Weydmann *et al.*, 2012), short-term exposure (7–9 d) to high pCO_2 delayed egg hatching (Weydmann *et al.*, 2012) and reduced naupliar survival (Lewis *et al.*, 2013). These studies focus on single developmental stages and, except Hildebrandt *et al.* (2014), exposed the copepods for <10 d. Given that effects of pCO_2 can vary with life stage, it must be emphasized to extend experiments over many life history stages to obtain an understanding of the potential effects of pCO_2 on a species. The present study investigated the effects of increased pCO_2 on early development stages of *C. glacialis*. Eggs from wild-caught *C. glacialis* were exposed to four pCO_2 levels for 2 months and development through the six naupliar stages (N1–N6) was tracked. Developmental rate, growth (dry weight, C and N mass, and C:N ratio), and respiration were measured to evaluate pCO_2 effects on these early life history stages. We hypothesized that compensating for increased pCO_2 would impose an energetic cost on developing *C. glacialis*, resulting in slowed development, reduced growth, and increased respiration.

Methods

Collection and acclimatization

Calanus glacialis were collected in Rijpfjorden, northeast Svalbard (80°27′54″N, 021°56′63″E, temperature −1 to 0°C), in January 2013 using a WP3 net (1 mm mesh) with a non-filtering codend. They were held on board in four aerated 100 l containers at 4°C for 13 d, after which they were transported over 12 h to the Austevoll Research Station, Institute of Marine Research, Norway (60°5′10″N, 5°15′43″E) in 50 1 l bottles packed in iced coolers at 0°C.

At the research station, the copepods, mostly stage C5, were held in two 40 l flow-through tanks at 0.6–1.1°C and pH 8.01 (±0.11 SD) for 50 d before the experiment. They were exposed to a 12L:12D light regime and fed on a mixture of algae (*Isochrysis* sp. (CCAP 927/14), *Rhodomonas baltica* (NIVA 5/91), and *Chaetocerous muelleri* (CCAP 1010/3)) at satiating concentrations (>200 μgC l^{-1}, Campbell *et al.*, 2001). During this period, C5s moulted to adults, mated, and began egg production.

Water treatments

Four pCO_2 treatments were chosen to simulate the current conditions at the collection site (low-pCO_2), ambient water at the research station (ambient), and projected conditions in Arctic seas in years 2100 (mid-pCO_2) and 2300 (high-pCO_2). The pCO_2 of seawater was altered to achieve target pHs. The present-day pH in Arctic shelf seas can be high in surface waters (from 8.1 to 8.4; Bellerby *et al.*, 2013, and sources within), and in Rijpfjorden, where the copepods were collected, it generally ranges between pH 8.1 and 8.3 throughout the water column (M. Chierici and A. Fransson, pers. comm.). Projections of Arctic ocean acidification for 2100 are $\Delta pH = -0.45$ according to the Intergovernmental Panel on Climate Change's (IPCC) RCP8.5/SRES A2 carbon emission scenario (Steinacher *et al.*, 2008; Ciais *et al.*, 2013), and $\Delta pH = -0.7$ by 2300 following the extended IPCC ERC8.5 scenario (Caldeira and Wickett, 2003; Collins *et al.*, 2013). The treatments were therefore: low-pCO_2 (pH 8.05; 320 μatm), ambient (pH 7.90; 530 μatm), mid-pCO_2 (pH 7.70; 800 μatm), and high-pCO_2 (pH 7.50; 1700 μatm).

For each treatment, pH was controlled in a 100 l mixing tank by a pH controller (Endress and Hauser, Liquiline CM 442) connected to a pH electrode (Endress and Hauser Orbisint CPS11D glass electrode) calibrated with NIST pH standards (pH_{NBS}). The controller maintained the target pH by regulating the addition of either CO_2-enriched seawater (for the high- and mid-pCO_2 treatments) or CO_2-stripped air (for the low-pCO_2 treatment) to ambient seawater in the mixing tanks. The CO_2-enriched seawater (pH ~5.5) was created by bubbling pure CO_2 continuously into ambient seawater. CO_2-stripped air was produced by forcing air through a CAS series CO_2 adsorber (Twin Tower Engineering). Seawater from each of the four mixing tanks was pumped into header tanks, each of which fed three 40 l experimental tanks. Inflow to the experimental tanks was 10–20 l min^{-1}. An 80 μm mesh covered the central outflow pipe of the experimental tanks to prevent the loss of nauplii during their earliest stages, the mesh size increasing to 300 μm from stages N5 to C1.

A constant temperature of 2°C was maintained by housing the experimental system inside a cooling container. Seawater was pumped from 160 m depth in Bjørnafjorden, sand filtered, and passed through a 20 μm Arkal™ disc filter. Once filtered, it entered the container and was cooled to 2°C and then redistributed from two 800 l storage tanks before use. Temperature was measured daily in all experimental tanks.

The pH_{NBS} of each treatment was logged by the pH electrode in each mixing tank. Water samples (100 ml) from each experimental tank were taken every few days ($n = 10$ during the experiment) for spectrophotometric measurement of total scale pH (pH_T) with the pH-sensitive indicator dye m-cresol purple (MCP; Dickson *et al.*, 2007). Water samples were collected in 100 ml gas-tight bottles and warmed to room temperature (21°C) in the dark before analysis. Analysis was carried out no more than 5 h after sample collection. For each measurement, 12 ml of water was pipetted into two quartz, 5 cm path-length cuvettes. To one of the cuvettes, 10 μl of MCP solution was added and thoroughly mixed by inverting the cuvette three times; the other cuvette served as the reference. Absorbance was measured at 578 nm (A1), 434 nm (A2), and 730 nm (background) at 21°C using a Hitachi U-2900 dual-beam spectrophotometer with a temperature controller. The pH was calculated according to Clayton and Byrne (1993) with a dye-addition correction (Dickson *et al.*, 2007: procedure 6b, section 8.3) and

adjusted from measurement temperature to *in situ* temperature according to Gieskes (1969).

Carbonate chemistry and nutrient concentrations were analysed using water samples (250 ml) collected at approximately weekly intervals ($n = 8$) from one experimental tank of each treatment. The samples were poisoned with a saturated mercuric chloride solution (Riebesell *et al.*, 2010) and stored in the dark at 8°C until analysed. DIC was analysed by coulometric titration (Dickson *et al.*, 2007) using a CM5015 coulometer (UIC Inc., USA) connected to a VINDTA 043 (Marianda, Germany) after acidification with 8.5% phosphoric acid. 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 (Marianda, Germany). Reference material provided by Andrew Dickson (Scripps Institution of Oceanography, San Diego, CA, USA) was used to calibrate DIC and A_T measurements. Carbonate chemistry parameters were calculated in CO2SYS (Lewis and Wallace, 1998) using the equations and constants of Mehrbach *et al.* (1973) modified by Dickson and Millero (1987). Nutrients were measured spectrophotometrically (Bendschneider and Robinson, 1952; Grasshoff, 1965; Alpekem, 1986).

Inoculation of experimental tanks

Twelve experimental populations of copepods were established, with three replicate 40 l experimental tanks (hereafter "tanks") for each of the four treatment pHs. The tanks were inoculated with eggs produced by a common pool of 1900 female *C. glacialis,* with extra females added for the inoculation of the low-pCO_2 treatment due to some female mortality during the inoculation period. Species identification was based on female prosome lengths (mean = 3.46 mm, range = 2.99–3.96 mm, $n = 69$) and the visual verification of their red antennae (Gabrielsen *et al.*, 2012; Nielsen *et al.*, 2014). The pool of females was held in four 6 l PVC containers suspended in the tank such that the top of the container was above the waterline of the tank. The bottom of each container was covered with 500 μm mesh, allowing eggs to sink into the tank while retaining females. After 1–2 d, the containers were transferred to the next tank, allowing the females to lay eggs consecutively in all tanks over a period of 19 d. Females were fed *ad libitum* during this period with the previously described mix of *C. muelleri, R. baltica*, and *Isochrysis* sp. The number of eggs per tank was estimated by counting eggs laid by a subsample of 40 females and averaged 7039 (±1178 s.e.). During inoculations, the tanks were filled with ambient water. After the females had been removed, the pCO_2 treatment began, with tanks reaching target pCO_2 in ~4 h.

The eggs developed through N2 without food. Starting at the transition to the first-feeding stage (N3), the nauplii were fed *ad libitum* the previously described mixture of *C. muelleri, R. baltica,* and *Isochrysis* sp., with the addition of *Skeletonema costatum* (NVIA BAC-1), at a ratio of 4:3:2:1 in carbon contribution. Carbon content per cell was calculated based on the cell volume to carbon conversion equations of Montagnes *et al.* (1994). The mean cell volumes (±s.d.) of the algae were 68 ± 9, 124 ± 14, 41 ± 5, and 84 ± 15 μm^3, respectively. The algae were cultured as single species batches at 22°C with Superba™ NPK 14-4-21 and harvested three times a week during the exponential growth phase. The algal mixture was cooled to the experimental temperature and then pumped automatically into treatment tanks using peristaltic dosage pumps to maintain a minimum concentration of 600 $\mu gC\,l^{-1}$. Satiating food concentrations are 200 $\mu gC\,l^{-1}$ according to Campbell *et al.* (2001). Algal concentrations in the tanks were measured daily with either a MOXI Z automated cell counter (Orflo©) or Coulter counter (Beckman Coulter Z2®).

Data collection

Sampling of the copepod cultures began 5 days after egg laying, when eggs were expected to hatch to naupliar stage 1 (N1), and continued until at least 50 d post-hatch. Every second day, 20–40 copepod nauplii were collected from different locations in each tank using a wide-mouthed pipette to obtain a representative sample. The nauplii were then anaesthetized with MS222 (Tricaine methanesulfonate, concentration 20 $g\,l^{-1}$) and stage was determined under a Leica stereoscope (MS5). The relative stage composition was used to determine developmental rate (see the Data analysis and statistics section). Groups of nauplii of the same developmental stage were then sorted and rinsed twice in deionized water before being placed in preweighed tin cups for dry weight and CN (carbon and nitrogen) measurements, with 1–10 pooled samples per replicate tank per stage. Samples were dried at 60°C for over 24 h and then weighed to the nearest 1 μg (Mettler-Toledo UMX2 microbalance). A blank using the same volume of rinse water but without nauplii was measured in the same way for each nauplii sample, and the weight of the blank was subtracted from the sample weight. Sample weights that changed by more than 10% when corrected for the blank were removed from the analysis (33 of 252). After storage at 60°C, the carbon and nitrogen content of the samples was measured by combustion in a Costech ECS 4010 analyser with a sensitivity of ±0.3 μgC (Bigelow Analytical Services, ME, USA). For CN analyses, two blanks per day were chosen at random ($n = 86$) and their average C and N masses were subtracted as a blank from all of the nauplii C and N values.

Oxygen consumption rate (OCR) was used to assess metabolic rates of stages N3, N6, and C1. Copepods in one replicate tank in each of the treatments did not develop to C1 and thus were only measured at N3 and N6. Copepods were collected from each treatment tank and sorted by stage in a Petri dish on ice under a stereoscope. They were then transferred into 4.5 ml respiration chambers filled with water from their treatment tank, leaving no air space within the chamber. Experimental chambers were sealed with a ground glass top equipped with a small hole (0.4 mm) to accommodate the oxygen-sensitive microelectrode. The number of individuals per chamber was 30–51 for N3, 20–50 for N6, and 3–35 for C1. Water in the chamber was mixed using a glass magnetic stirrer in the bottom of the chamber separated from the copepods by a fine metal mesh. Control chambers without copepods were also run to correct for respiration by bacteria or algae. The chambers were submerged in a water bath (ThermoScientific Model A10B with thermostat SC100) at 2°C (±0.01°C) for 15 min before measurement began. Dissolved oxygen concentrations were measured every 2 s with a Unisense respirometry system connected to a Clark-type oxygen microelectrode (Unisense; Aarhus, Denmark) for 1.5 h. Oxygen concentration in the chambers never decreased to <20% of the initial saturation. The OCR, measured in 1–5 pools of animals per tank per stage, was corrected for oxygen consumption in the control chamber. The oxygen electrode was calibrated before measurement with seawater at 100% oxygen saturation (0.2 μm filtered seawater bubbled vigorously with air for a minimum of 1 h) and 0% oxygen saturation (a sealed silicone tube of seawater immersed in a solution of 0.1 M sodium ascorbate and 0.1 M sodium hydroxide for over 4 h). OCRs per individual ($nmolO_2\,ind.^{-1}\,h^{-1}$) were normalized to the average dry weight (DW) of the given developmental stage ($nmolO_2\,\mu gDW^{-1}\,h^{-1}$).

The average dry weight was calculated from all pCO_2 treatments pooled, as there were no significant differences between pCO_2 treatments (see the Results section).

Data analysis and statistics

The developmental rate was measured as the median development time (MDT) to a given developmental stage. This was calculated as the number of days from the midpoint of the egg-laying period for each tank to when 50% of the copepods reached the given stage. To calculate this, the percentage of the population that had reached or surpassed a given stage was plotted against time (Supplementary Figure S1). A linear regression was fitted to the linear portion of the curve (excluding the tails, <10 and $>90\%$) and the day the population reached 50% was extracted (Landry, 1975; Campbell *et al.*, 2001). Stage duration was calculated as the time between the MDT of two successive stages.

To account for small differences in temperature between tanks, the development time was corrected for temperature effects using Bělehrádek's (1935) equation, $D = a(T - \alpha)^b$, where D is the development time (in days) and T is temperature (°C). Using literature values of parameters b (-2.05 for copepod species; McLaren *et al.*, 1969) and α (-13.04 for *C. glacialis*; Corkett *et al.*, 1986) and the average temperature in a given tank from egg until a given stage, we fit the equation to our data (MDT and tank temperature from all pCO_2 treatments) to determine the stage-specific parameter a for each naupliar stage (Supplementary Figure S2). The effect of temperature was then removed from all MDTs.

The effect of pCO_2 treatment on the eight response variables (MDT, stage duration dry weight, C and N mass, C:N ratio, respiration rate, and specific respiration rate) was tested for each developmental stage. Two types of statistical models were used, depending on whether multiple measurements were taken per tank. For MDT and stage duration, with one measurement per tank per stage, one-way ANOVAs were used to test for differences among pCO_2 treatments. Dry weight, C and N mass, C:N ratio, and respiration rates, with multiple measurements per tank per stage, were analysed by linear mixed-effects models (LMM) using the lme4 package (version 1.1-10) and lmerTest package (version 2.0-30), with pCO_2 treatment as a fixed effect factor and tank as a random effect to account for the dependence of measurements taken within the same tank. If pCO_2 had a significant effect on the response variable, *post hoc* multiple comparison tests were performed using Tukey's pairwise comparisons (for LMMS: multcomp package, version 1.4-1). Initial tests on each response variable showed that incorporating all developmental stages did not reveal significant differences between pCO_2 treatments in seven of the eight response variables. pCO_2 significantly influenced C:N ratio, but the direction of the effect varied by stage. Therefore, stage-specific test results are presented. To account for increasing variability in older stages in these initial tests, a *VarIdent* variance–covariate structure was added to the models (generalized least-squares for MDT and stage duration, and LMM for the other variables) using the nlme package (version 3.1-122), which allowed variance to differ by stage. Differences in temperature, algae concentration, and carbonate system parameters (NO_2^-, NO_3^-, PO_4^{2+}, SiO_4^{4-}, and A_T) between pCO_2 treatments were tested with one-way ANOVAs. All statistics and graphs were done using R 3.2.2 (R Core Team, 2015) and the graphics package ggplot2, version 2_1.0.1. Means are presented with ± standard error (s.e.).

Table 1. Mean (±s.e.) pH, temperature (°C), nutrients, and carbonate chemistry in experimental tanks, averaged over the experiment by tank and by treatment.

pCO_2 treatment	Tank	$pH_{T,spec}$	Temp (°C)	$pH_{T,spec}$	$pH_{T,calc}$	pCO_2 (µatm)	Salinity	A_T (µmol kg^{-1})	HCO_3 (µmol kg^{-1})	CO_3 (µmol kg^{-1})	CO_2 (µmol kg^{-1})	Nitrite (µmol l^{-1})	Nitrate (µmol l^{-1})	Phosphate (µmol l^{-1})	Silicate (µmol l^{-1})
Low	A	8.07 ± 0.01	2.09 ± 0.06	8.05 ± 0.01	8.12 ± 0.02	322 ± 15	34.2 ± 0.1	2324 ± 2	2022 ± 11	118.9 ± 4.5	18.7 ± 0.9	0.08 ± 0.01	7.09 ± 1.55	1.98 ± 0.53	6.63 ± 0.39
	B	8.04 ± 0.02	2.07 ± 0.05												
	C	8.04 ± 0.02	2.04 ± 0.05												
Ambient	A	7.86 ± 0.01	1.9 ± 0.06	7.86 ± 0.01	7.93 ± 0.02	530 ± 27	34.2 ± 0.1	2324 ± 1	2122 ± 8	79.3 ± 3.0	30.9 ± 1.5	0.06 ± 0.01	9.23 ± 0.19	1.63 ± 0.21	6.18 ± 0.22
	B	7.86 ± 0.02	1.94 ± 0.07												
	C	7.85 ± 0.02	1.88 ± 0.07												
Mid	A	7.7 ± 0.01	1.87 ± 0.06	7.69 ± 0.00	7.77 ± 0.05	808 ± 76	34.2 ± 0.1	2325 ± 1	2172 ± 21	60.1 ± 8.5	47.2 ± 4.4	0.07 ± 0.01	7.35 ± 1.21	1.4 ± 0.21	6.05 ± 0.15
	B	7.69 ± 0.01	1.99 ± 0.07												
	C	7.68 ± 0.01	2.06 ± 0.06												
High	A	7.47 ± 0.01	1.69 ± 0.07	7.47 ± 0.00	7.44 ± 0.01	1698 ± 37	34.2 ± 0.1	2324 ± 2	2253 ± 2	27.4 ± 0.5	99.3 ± 2.0	0.06 ± 0.01	7.33 ± 1.22	1.53 ± 0.31	6.14 ± 0.19
	B	7.47 ± 0.00	1.85 ± 0.08												
	C	7.47 ± 0.01	1.79 ± 0.07												

Total scale pH measured spectrophotometrically ($pH_{T,spec}$) and calculated from DIC and A_T using CO2SYS ($pH_{T,calc}$) are presented.

Results

Tank conditions

Carbonate system parameters and salinity were stable throughout the 2-month experiment (Table 1), although small, significant differences in temperature between tanks became evident (one-way ANOVA: $F_{(3,476)} = 9.95$, $p < 0.001$) with high-pCO_2 tanks being colder (by 0.2–0.3°C) than low-, ambient-, and mid-pCO_2 treatments. Algal concentrations varied, but with no significant differences between treatments (one-way ANOVA: $F_{(3,304)} = 2.01$, $p = 0.113$); concentrations were rarely below 200 μgC l^{-1}. Nutrient concentration (NO_2^-, NO_3^-, PO_4^{2+}, or SiO_4^{4-}) and total alkalinity (A_T) did not differ between the tanks (one-way ANOVAs: NO_2^-, $F_{3,34} = 0.61$, $p = 0.61$; NO_3^-, $F_{3,34} = 0.81$, $p = 0.50$; PO_4^{2+}, $F_{3,34} = 0.57$, $p = 0.64$; SiO_4^{4-}, $F_{3,34} = 1.06$, $p = 0.38$; and A_T, $F_{3,34} = 0.09$, $p = 0.97$).

Development

Frequent sampling allowed tracking of development from N1 to N6, but also meant that few tanks reached stage C1 with >200 individuals. Consequently, C1 was excluded from the development analyses. The mean MDT was 7.2 ± 0.2 d for stage N2, 10.6 ± 0.2 d for N3, 21.1 ± 0.3 d for N4, 27.8 ± 0.4 d for N5, and 34.7 ± 0.4 d for N6. There were no significant differences in MDT or stage duration between pCO_2 treatments for any stage (N2–N6; Figure 1a and b; Supplementary Table S1). Sample sizes, means, and standard errors for all endpoints are provided in Supplementary Tables S2 and S3.

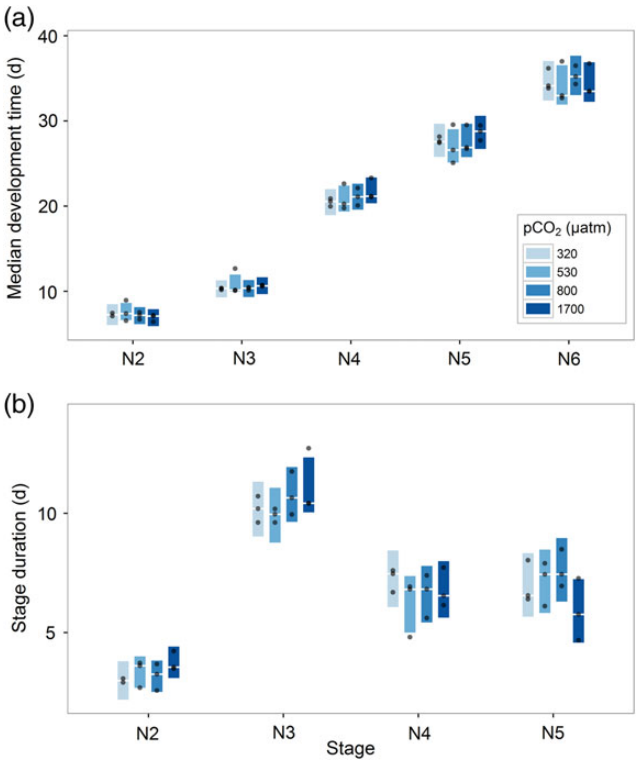

Figure 1. Effect of pCO_2 treatment on median development time (MDT) (a) and stage duration (b) for different developmental stages of *C. glacialis*. Shaded bars span 5–95% CI, with the mean indicated by a white line. All data are depicted as grey points. Treatments within a stage did not differ significantly. This figure is available in black and white in print and in colour at *ICES Journal of Marine Science* online.

Dry weight, carbon and nitrogen mass

The mean dry weight increased from 1.5 ± 0.1 μg ind.$^{-1}$ at N3 to 7.1 ± 0.2 μg ind.$^{-1}$ at N6 (Figure 2a). Concurrently, the average carbon body mass increased from 0.3 ± 0.0 μgC ind.$^{-1}$ at N3 to 2.3 ± 0.0 μgC ind.$^{-1}$ at N6 and nitrogen mass increased from 0.1 ± 0.0 to 0.6 ± 0.0 μgC ind.$^{-1}$ (Figure 2c and d). The dry weights of N5 nauplii were significantly lower in the high-pCO_2 treatment than in the ambient ($\Delta = -0.97 \pm 0.32$ μg ind.$^{-1}$, $p < 0.02$), though no treatment differences were observed at stages N3, N4, or N6. Neither carbon nor nitrogen body mass varied significantly between treatments at any stage. The C:N ratio increased slightly with stage, from 3.9 ± 0.1 at N3 to 4.2 ± 0.2 at N4 (Figure 2b, Supplementary Table S1). The C:N ratio of N6s was significantly lower in the low-pCO_2 treatment than both the mid-pCO_2 treatment ($\Delta = -0.35 \pm 0.11$, $p < 0.01$) and the ambient treatment ($\Delta = -0.31 \pm 0.10$, $p < 0.02$), though C:N did not differ between treatments at stages N3, N4, or N5 (Supplementary Table S2).

Metabolic rate

Oxygen consumption increased from 2.6 ± 0.5 to 20.3 ± 2.2 nmolO_2 ind.$^{-1}$ h^{-1} during development from N3 to C1

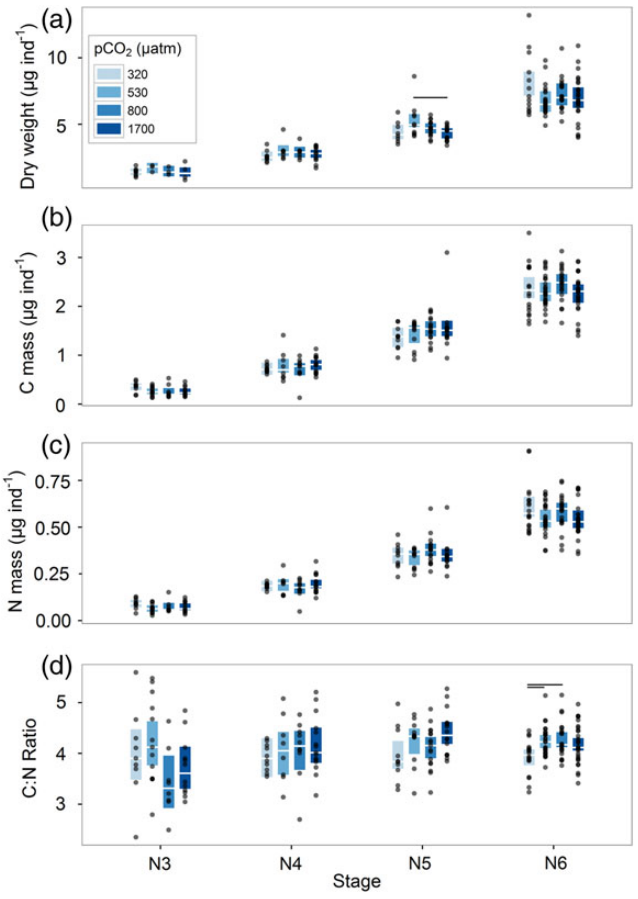

Figure 2. Effect of pCO_2 treatment on dry weight (a), C:N ratio (b), carbon mass (c), and nitrogen mass (d) for different developmental stages of *C. glacialis*. Shaded bars span 5–95% CI, with the mean indicated by a white line. All data are depicted as grey points. Significant differences between treatments within a stage are indicated by horizontal lines. This figure is available in black and white in print and in colour at *ICES Journal of Marine Science* online.

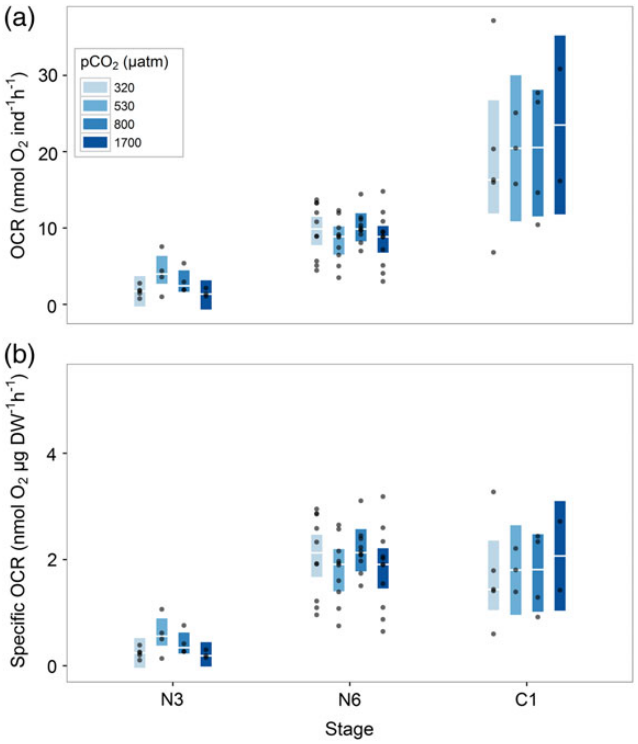

Figure 3. Effect of pCO_2 treatment on oxygen consumption rate (OCR) (a) and specific OCR (b) for different developmental stages of *C. glacialis*. Shaded bars span 5–95% CI, with the mean indicated by a white line. All data are depicted as grey points. Treatments within a stage did not differ significantly. This figure is available in black and white in print and in colour at *ICES Journal of Marine Science* online.

(Figure 3a). Specific respiration rate was stable over time, being 1.7 ± 0.3 nmolO_2 µgDW^{-1} h^{-1} for N3 and 1.8 ± 0.2 nmolO_2 µgDW^{-1}h^{-1} for C1 (Figure 3b; Supplementary Table S1). Individual and specific respiration rates did not vary significantly between treatments (Supplementary Table S2).

Discussion

The early life stages (N1–N6) of *C. glacialis* were unaffected by increased pCO_2 levels predicted for the year 2300: naupliar developmental rate, growth (dry weight, carbon body mass, C:N ratio), and metabolic rate were indistinguishable over the range of 320–1700 µatm pCO_2. The variability in the data was not affected by pCO_2 but did increase with developmental stage, although this was normalized for by testing for pCO_2 effects within each stage. The few significant differences between pCO_2 treatments were isolated to single developmental stages and not part of a consistent trend with pCO_2: we interpret them as stochastic rather than physiological findings. These results do not support our original hypothesis of increased energetic costs of pCO_2 during early development. Consistent with the findings for early developmental stages of *C. finmarchicus* (Pedersen *et al.*, 2014b; Runge *et al.*, 2016) and older stages of *C. glacialis* (Hildebrandt *et al.*, 2014), the results indicate that *C. glacialis* will maintain normal development, growth, and metabolic rate in a high pCO_2 ocean.

Maintaining acid–base balance between intra- and extracellular fluids under high pCO_2 increases the energetic cost of homeostasis for some marine organisms (Pörtner, 2008; Saba *et al.*, 2012; Stapp *et al.*, 2015). Increased maintenance costs under high pCO_2 can reduce the energy that marine organisms are able to allocate to growth and reproduction (Stumpp *et al.*, 2011; Saba *et al.*, 2012). This can result in slower development, smaller body size, or reduced reproductive output in some species (Stumpp *et al.*, 2011; Pedersen *et al.*, 2014a). Copepods reduce developmental rate, body size, and C:N ratio in response to limited energy for growth, due to increased homeostatic activity at high salinity (Devreker *et al.*, 2007) or food limitation (Campbell *et al.*, 2001). In *C. glacialis*, food limitation slowed developmental rate but left body length unaffected (Daase *et al.*, 2011), indicating that changes in developmental rate may be more sensitive than body size to alterations of the energy budget. Nonetheless, we found no effect of pCO_2 on development, growth (dry weight, C, and N mass), or C:N ratio, indicating that the treatment pCO_2 levels did not affect the energy available for growth and development in *C. glacialis* nauplii.

Calanus glacialis nauplii may have increased their feeding to compensate for a moderate increase in maintenance costs due to high pCO_2 (i.e., Li and Gao, 2012). By increasing their total energy input, the nauplii exposed to high pCO_2 might have been able to allocate the same amount of energy to growth and development as control nauplii, resulting in indistinguishable development and growth. As feeding was not measured, we cannot answer this directly. However, if this was the case, we would expect that non-feeding stages (egg–N2) would not have been able to compensate, and would have exhibited reduced developmental rates or growth; this was not the case. In feeding stages (N3–C1), compensatory feeding at high pCO_2 would be expected to result in higher metabolic rates due to specific dynamic action, as has been suggested for other species in high pCO_2 treatments (Li and Gao, 2012; Saba *et al.*, 2012). However, this was also not the case: specific respiration was indistinguishable in the different pCO_2 treatments. Thus, we found no evidence of compensatory feeding at high pCO_2. This is consistent with findings for older life history stages: neither *C. glacialis* C5 nor *C. finmarchicus* C5 or females increase their feeding at high pCO_2 (Pedersen *et al.*, 2014a; Hildebrandt *et al.*, 2015; Runge *et al.*, 2016).

The response of *C. finmarchicus*, a species that is closely related and morphologically similar to *C. glacialis*, to high pCO_2 has been thoroughly studied. While *C. finmarchicus* appears tolerant of realistic future pCO_2 levels, there are some conflicting results. Development time (MDT) to C5 was delayed by ~11% at 2080 µatm pCO_2 in the parental generation (but not the F_1 generation) and the metabolic rate of C5s increased (142%) at 3080 µatm pCO_2 (Pedersen *et al.*, 2014a). In contrast, Runge *et al.* (2016) reported no developmental delay and no change in metabolic rate in N3, N6, C5 and females at 1910 µatm pCO_2. Pedersen *et al.* (2014a) ran their experiment under food-limiting conditions (200 µgC l^{-1}), whereas Runge *et al.* (2016) used satiating food concentrations (600 µgC l^{-1}). Using non-limiting food concentrations can mask an effect of high pCO_2, as some organisms are able to compensate for an energetic cost of high pCO_2 by increasing feeding (Thomsen *et al.*, 2013). However, neither Pedersen *et al.* (2014a) nor Runge *et al.* (2016) found any difference in feeding rate, indicating that non-limiting food conditions were not masking effects of high pCO_2. Additionally, the copepods used by Pedersen *et al.* (2014a) were from a population that had been cultured in the laboratory for >33 generations, whereas Runge *et al.* (2016) used animals freshly collected from the wild. Continuously cultured lab populations will have adapted to the relatively stable environmental

conditions, and may, therefore, have a narrower tolerance for changing environmental conditions (Drillet *et al.*, 2008), including pCO_2 (Fitzer *et al.*, 2012; Cripps *et al.*, 2014). This highlights the necessity of using wild-caught organisms in experiments investigating the response of populations to environmental change.

If there had been high mortality in the high pCO_2 treatments, our sampling would have focused on the performance of the tolerant portion of the population, and this would have become more prevalent over time. Mortality is very difficult to track in flow-through systems such as the one used in this experiment and was therefore not quantified. However, the majority of the wild-caught population used in this experiment survived a more acute pCO_2 change than that which is expected to occur over a decadal time-scale in the wild, supporting the interpretation that *C. glacialis* is tolerant of pH down to at least 7.5 and pCO_2 of 1700 μatm.

Small differences in water temperature between tanks may have influenced developmental rate, but this does not affect our conclusions. The 0.4°C difference in water temperature observed between tanks (1.8–2.2°C) would produce a 1.8 d difference in development time to N6 according to Bělehrádek's (1935) equation with parameters fit to *C. glacialis* (Supplementary Figure S2). As this is similar to the delay in development seen in *C. finmarchicus* exposed to high pCO_2 (Pedersen *et al.*, 2014a), correcting for the effect of temperature on development was necessary (see the Methods section). Nonetheless, the temperature correction did not change our findings: the naupliar developmental rate was similar among pCO_2 treatments before and after temperature correction.

The natural variability in pH experienced by *C. glacialis* may also partly explain its tolerance to high pCO_2. Acidification of extracellular fluids to regulate buoyancy during dormancy at depth results in haemolymph pHs of overwintering C4s and females being as low as pH 5.4 ± 0.4 and 6.7 ± 0.8, respectively (Freese *et al.*, 2015). Organisms are expected to tolerate the range of environmental variability that they encounter (Lynch and Gabriel, 1987), with species inhabiting highly variable environments expected to have wider tolerance ranges than those inhabiting stable environments (e.g., Aguilera *et al.*, 2015). Organisms inhabiting coastal areas, where pH varies on diurnal and seasonal scales (Duarte *et al.*, 2013; Almén *et al.*, 2014), or those migrating horizontally and/or vertically through water masses of variable pH, may be adapted to perform well over a range of pHs (Maas *et al.*, 2012; Almén *et al.*, 2014). Lewis *et al.* (2013) applied this reasoning to Arctic copepods, suggesting that the diel vertical migration of older stages of *C. glacialis* and *Calanus hyperboreus* through water masses of differing pH ($\Delta pH = 0.3$) explained why they were less affected by lower pH than the naupliar stages of *C. glacialis* and *C. hyperboreus*, as well as the adult stages of *Oithona similis* which do not migrate vertically and may experience greater mortality under experimental high pCO_2 exposure. Additionally, *C. glacialis* overwinters at depth for 3–6 months, where the pH is often considerably lower than that of surface waters ($\Delta pH = -0.4$) (Bellerby *et al.*, 2013). Adding to the vertical variability in pH experienced by *C. glacialis* are seasonal changes in pH. Both sea ice dynamics and the strong pelagic spring bloom in Arctic shelf seas add seasonal variability in surface waters on the order of pH ~0.2, with sea ice production and melt reducing pH directly under the ice (<5 m) and algal photosynthesis increasing pH in the upper 50 m (Fransson *et al.*, 2013; Shadwick *et al.*, 2013). These changes are similar to the magnitude of changes in pH expected to occur over the next 100 years. Thus, *C. glacialis* may already be adapted to tolerate variable pH and pCO_2 due to the seasonal and spatial variability of its natural Arctic habitat.

Our study, which tracked *C. glacialis* through nearly half of its life cycle at various pCO_2 levels, indicates that the early development of the species will not be detrimentally affected by increases in pCO_2 expected in the next 200 years. Together with the findings of other studies that focused on single stages of *C. glacialis* which were either younger (Weydmann *et al.*, 2012) or older (Hildebrandt *et al.*, 2014, 2015) than those in our study, this makes a strong case for the tolerance of this key Arctic species to ocean acidification.

Supplementary data

Supplementary material is available at the *ICESJMS* online version of the manuscript.

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