Multigenerational exposure to ocean acidification during food limitation reveals consequences for copepod scope for growth and vital rates

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ABSTRACT

The copepod *Calanus finmarchicus* is a key component of northern Atlantic food webs, linking energy-transfer from phytoplankton to higher trophic levels. We examined the effect of different ocean acidification (OA) scenarios (i.e. ambient, 1080, 2080 and 3080 μatm CO_2) over two subsequent generations under limited food availability. Determination of metabolic- and feeding rates, and estimations of the scope for growth, suggests that negative effects observed on vital rates (ontogenetic development, somatic growth, fecundity) may be a consequence of energy budget constraints due to higher maintenance costs under high pCO_2-environments. A significant delay in development rate among the parental generation animals exposed to 2080 μatm CO_2, but not in the following F_1 generation under the same conditions, suggests that *C. finmarchicus* may have adaptive potential to withstand the direct long term effects of even the more pessimistic future OA-scenarios, but underlines the importance of transgenerational experiments. The results also indicate that in a more acidic ocean, increased energy expenditure through rising respiration could lower the energy transfer to higher trophic levels and thus hamper the productivity of the northern Atlantic ecosystem.

INTRODUCTION

Fossil fuel burning, altered land use and other anthropogenic activities have contributed to elevate the mean atmospheric concentration of carbon dioxide (CO_2) from a preindustrial level of approximately 280 ppm to its present level of ~390 ppm CO_2[1]. Absorbed in seawater, CO_2 lowers the pH through the production of carbonic acid - a process commonly referred to as ocean acidification (OA). As a result, the average pH of ocean surface water has been reduced by 0.1 units compared to preindustrial time. Worst-case scenario estimates based on carbon cycle models predict a CO_2 level of 970 ppm by the end of the century[2], and possibly a level of 1900 ppm by the year 2300[3].

The carbonate concentration in seawater is declining due to increased pCO_2[4], and meta-analyses indicate that calcifying organisms may be negatively affected by this phenomenon[5]. Marine metazoans rely on a positive CO_2 gradient from their body fluids to excrete metabolic CO_2 by diffusion. Elevated seawater pCO_2 can therefore lead to hypercapnia and acidosis[6] which in turn may result in a reallocation of resources away from growth and reproduction, due to mobilization of energy demanding acid-base regulatory processes to counteract internal pH reduction. Accordingly, ocean acidification has been shown to negatively affect processes such as reproduction[7], development[8] and behavior[9] in non-calcifying organisms also.

Copepods of the genus *Calanus* constitute a large part of the zooplankton biomass in the North Atlantic[10, 11]. During the development from eggs, these copepods develop through six nauplii stages (N1-N6), of which the two first (N1-2) are non-feeding, and five copepodite stages (C1-C5), before reaching maturity as either male or female[12]. These cold water species concentrate and store energy through synthesis and accumulation of lipids and therefore represent an important energy link between phytoplankton and higher trophic level predators such as fish[13, 14]

and birds[15]. In addition, calanoids contribute to the total vertical carbon flux in the oceans through the production of fecal pellets[16].

To date, the sensitivity of *Calanus* species to elevated seawater pCO_2 has predominantly been studied by assessing its effects on reproduction and development after short- or medium-term exposure (i.e. only a tiny fraction or a substantial part of the generation time, respectively)[17-23]. Findings suggest that the genus may be relatively robust against future ocean acidification scenarios. However, exposure to adverse environmental conditions may have fitness consequences which can manifest in subsequent generations[24], and responses can differ between generations[25]. In addition, physiological adjustments to new environmental conditions[26] and adaptive selection of resistant phenotypes[27] require time. Thus, the predictive power of short-term studies is limited. Transgenerational exposure studies of copepods to elevated seawater pCO_2 are rare and contradictory[7, 28]. Thus, additional studies are required to determine the long-term consequence of ocean acidification on the marine environment.

To examine the long-term effects of future ocean acidification scenarios, cohorts of cultured *Calanus finmarchicus* (Gunnerus) were exposed for two subsequent generations to either the ambient CO_2 level (380 µatm; control) or to one of three different future CO_2 scenarios; low- (1080 µatm CO_2; a pessimistic year 2100 scenario[2]), medium- (2080 µatm CO_2; a pessimistic year 2300 scenario[3]), and high CO_2 (3080 µatm CO_2; a positive control). The main aim was to establish if elevated seawater pCO_2 causes energy-budget constraints in copepods that are sufficient to affect their vital rates in the long-term. The energy-budget (scope for growth) and vital rates (growth, development rate, fecundity and fertility) were measured among F2 generation animals to access the physiological effects of transgenerational acclimation to the imposed CO_2 conditions. Also, the effect of CO_2 exposure on the development rates in first- and

second generation animals (P- and F_1 generation, respectively) were compared to reveal possible transgenerational effects from parents to offspring. The copepods were fed algae at a restricted concentration (i.e. 200 µg C l^{-1}) to access the long-term repercussions of limited food availability, which is of relevance for future climate change scenarios where food may be in short supply[29, 30].

MATERIALS AND METHODS

Exposure. The experiment was carried out at the saltwater facilities at NTNU Centre of Fisheries and Aquaculture (SeaLab), between November 2011 and March 2012 (a total of 136 days). Two consecutive generations of *Calanus finmarchicus* were reared in a climate controlled room (at 10°C) under four different seawater pCO_2 regimes, using a custom developed flow-through exposure system with 12 exposure tanks (90L cylindro-conical polyester tanks) (see Figure 1A and Figure S1-1 in supporting information (SI)). To reach the CO_2-target concentrations, seawater was equilibrated with CO_2-enriched air in custom-built equilibration columns (for details see[22]). The CO_2-enriched air was produced by a custom-built gas mixing system (HTK Hamburg GmbH, Germany). A secondary equilibration system, integrated within each exposure tank, assisted in maintaining the target CO_2 level (Figure 1A).

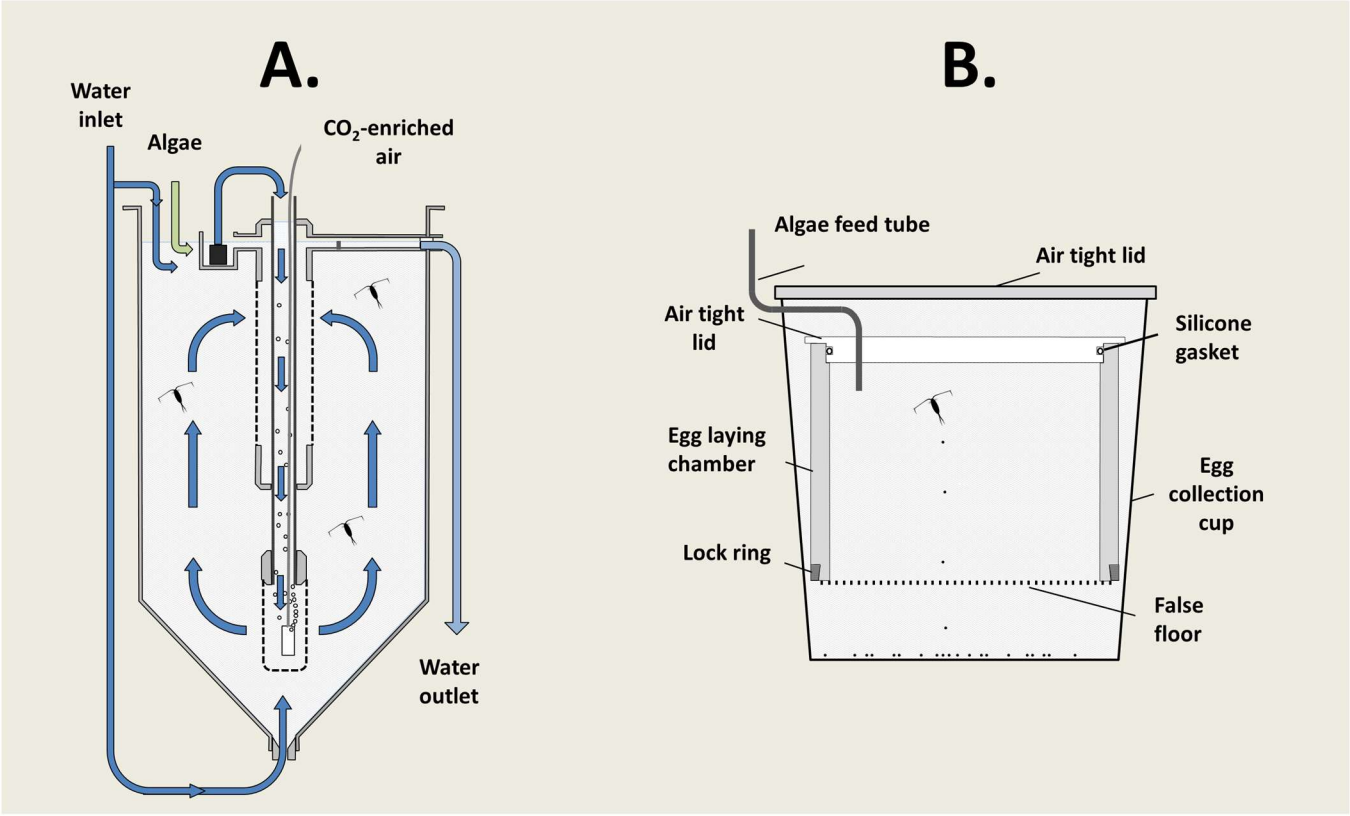

Figure 1. A: Cross section of the 90L exposure tanks. Water from the primary equilibration system (turquoise lines) entered at the base and top of the tank, providing circulation. A secondary equilibration column was mounted vertically within the tank. At the top of the column, a submersible aquarium pump caused recycling and downward movement of water (dark blue arrows), CO_2-enriched air was released at the base. The in- and outlet in this system was covered with nylon mesh (120 µm, dashed lines) to prevent animals from entering. B: Individual ovigerous females were confined to an egg-laying chamber with an airtight lid and a false nylon mesh floor (pore size: 300 µm) to determine daily egg production rate. The eggs collected on the floor of the egg collection cup. Concentrated algae feed suspension (round one; *ad libitum* feeding) or pre-equilibrated seawater with limited food availability (round two; 200 µg C l^{-1}) was continuously added through the lid of the egg laying chamber.

The temperature and total scale pH (pH_{Tot}) in the experimental tanks was measured daily using a glass thermometer and a hand held pH-meter, daily calibrated with Tris-buffer (Scripps Institution of Oceanography, La Jolla, CA, USA). More accurate spectrophotometric measurements of pH_{Tot} were also performed weekly, sensu Dixon et al[31]. Since inter-tank differences were negligible, total alkalinity determinations, using open cell titration[32], were restricted to single samples of seawater collected daily among the tanks. Accuracy was verified by analyzing certified seawater (Scripps Institution of Oceanography, La Jolla, CA, USA). Measured seawater carbonate species are presented in Table 1.

Table 1. Carbonate system speciation in the experimental treatments during the P- and F_1 generation calculated using the software CO2SYS version 2.1[33], with the dissociation constants for total scale of Mehrbach et al.[34], refitted by Dickson and Millero[35]. Values for total dissolved inorganic carbon (C_T), pCO_2 and calcium carbonate saturation state for calcite (Ω_{Ca}) were calculated from pH_{Tot} (measured by spectrophotometer) and total alkalinity (A_T). Listed values represent means ± 1 SD over the course of each generation and values listed within brackets indicate the number of replicate measurements taken at a given time point.

Generation	Treatment	pH_{Tot}	A_T (µmol/ kg)	S	T (°C)	pCO_2	C_T	Ω_{Ca}
P	Control	8.00 ± 0.01 (3)	2263 ± 21 (1)	35 (1)	9.75±0.15 (3)	437 ± 17	2109 ± 19	2.86 ± 0.07
"	Low CO_2	7.64 ± 0.03 (3)	2263 ± 21 (1)	35 (1)	9.89±0.15 (3)	1102 ± 77	2233 ± 20	1.35 ± 0.08
"	Medium CO_2	7.33 ± 0.06 (3)	2263 ± 21 (1)	35 (1)	9.87±0.15 (3)	2307 ± 303	2327 ± 27	0.69 ± 0.09
"	High CO_2	7.15 ± 0.03 (3)	2263 ± 21 (1)	35 (1)	9.68±0.12 (3)	3502 ± 262	2396 ± 25	0.46 ± 0.04
F_1	Control	8.02 ± 0.01 (3)	2245 ± 16 (1)	35 (1)	9.79±0.10 (3)	421 ± 16	2091± 15	2.91 ± 0.07
"	Low CO_2	7.66 ± 0.05 (3)	2245 ± 16 (1)	35 (1)	9.94±0.10 (3)	1052 ± 111	2214 ± 17	1.40 ± 0.14
"	Medium CO_2	7.39 ± 0.04 (3)	2245 ± 16 (1)	35 (1)	9.94±0.09 (3)	2020 ± 190	2296 ± 17	0.77 ± 0.07
"	High CO_2	7.16 ± 0.08 (3)	2245 ± 16 (1)	35 (1)	9.79±0.09 (3)	3482 ± 542	2382 ± 30	0.46 ± 0.11

The animals were feed a monoculture of the cryptophyte *Rhodomonas baltica*, which have been found to be a well suited feed for *C. finmarchicus*[36]. The alga was continuously distributed from

a daily prepared stock to the tanks using a peristaltic pump with a separate channel for each experimental tank. To obtain a restricted feed regime, a nominal steady carbon concentration of 200 $\mu g\ l^{-1}$ was targeted within the tanks, which is twice the minimum requirement previously reported for *C. finmarchicus*[12]. The stock carbon content was estimated from the algae cell concentration (determined using a Multisizer TM 3 Coulter Counter®, Bechman Coulter, Inc.) and a mean carbon content of 45 $\rho g\ cell^{-1}$, empirically determined for the in-house *R. baltica* culture. Inter-tank differences in algae concentration due to varying grazing intensity were minimized by daily measurements of algae concentration in all experimental tanks and adjustments of the algae addition when necessary (see Figure S1-3 in SI).

The parent (P) generation was started from eggs derived from the continuous *C. finmarchicus* culture at the seawater facility at NTNU. Each exposure tank was inoculated by adding approximately 2000 eggs from a common pool collected following 24h incubation of ~3000 adults (i.e. median age of 12h) in 50L polyethylene tanks where the pH was close to control condition. The second (F_1) generation was obtained in the same manner, but here adults from each separate tank had to be incubated 72h (due to lower egg production), and ~2000 of the resulting eggs/nauplii (median age 36h) returned back to their original tank. Efforts were made to maintain the CO_2 level in the polyethylene tanks similar to the condition in the different exposure tanks (see Table S1-1 in SI)).

Median development time. Samples were taken from the experimental tanks to record ontogenetic development during both the P- and F_1 generation. Sampling frequency and volumes were adjusted to the duration of the different life stages (up to three samplings per day to capture the fast developing stages). The collected samples were preserved using Lugol's solution and

stored dark at 10°C in glass vials, prior to ontogenetic stage determination[37, 38]. The median development time (MDT) for the ontogenetic stages, i.e. the time from the midpoint of egg-laying to when 50% of the cohort has developed to/or past a given stage (sensu[39]), was estimated from the stage frequency against time, fitted by the least square method. The procedure followed that of Campbell et al.[12], but adopting a wider inclusion window ($y \in \langle 0.05, 0.95 \rangle$) for the regression to obtain better curve fitting. Also, a procedure described by Hu et al.[40] was adopted to reduce ´tail´-value errors.

Feeding. Feeding- and oxygen consumption rate was measured in the F_1 generation copepodites (six individuals from each tank). Sub-adults (C5) were selected to avoid discrimination between sexes. Measurements were staggered over a seven day period. Individual animals were randomly collected from the tanks and transferred to separate 50 mL glass bottles, filled with seawater from the respective tanks. A bottle with only 32 µm filtered water from each of the same tanks served as controls. The bottles were incubated under dim light conditions at 10°C on a rotating plankton wheel (~1 rpm). Filtration- and ingestion rates were estimated as clearance rate by measuring the algal concentration (delta cells L^{-1}) at the end of the incubation (mean time 20 h) in the control and experimental bottles, using a Coulter counter. Feeding rate (cells ind^{-1} h^{-1}) was derived from filtering rate (F, mL ind^{-1} h^{-1}), sensu Frost[41], normalized to dry weight (dw), and converted to carbon equivalents (µg C $dw^{-1}h^{-1}$), using the carbon content of our in-hose *R. baltica* culture (45 ρg C $cell^{-1}$).

Respirometry. Following the feeding measurements, each animal was transferred into separate 2 mL glass respiration vials prefilled with 32µm filtered seawater from the same tank as the

185 animals were originally collected. The copepodites were also pre-washed in this water prior to
186 transfer. The vials (16 in total) were closed and incubated ~8 hours in a water bath maintained at
187 10 ± 0.1°C under dim light. The oxygen concentration in the vials was recorded hourly, using
188 oxygen sensitive patches and a fiber-optic oxygen meter (Fibox 3 LCD trace, Precicion Sensing
189 GmbH). The first reading was taken one hour after transfer to allow recovery from handling
190 stress. Oxygen consumption rates were constant during the incubations and the oxygen levels
191 never dropped to less than 60% of the initial value. Following the measurements, all individuals
192 were checked for viability.

193

194 **Morphometry and dry weight** Following the respirometry, each measured animal was
195 photographed under a microscope to perform morphometric measurements (body (prosome)
196 length, lipid sac- and prosome area) (see Pedersen et al.[22]). The volumes of the lipid storage sac
197 and the prosome were calculated from the area and length, sensu Miller et al.[42] Dry weight was
198 determined on the photographed animals sensu Williams and Robins[43].

199

200 **Scope for growth.** Feeding- and metabolic rates were converted to energy equivalents to
201 estimate the daily energy input and the respiratory energy loss. Due to lack of data on egestion
202 and excretion, assimilated food was estimated assuming a general assimilation efficiency of 80%
203 for herbivorous feeding copepods[44]. The assimilated food was converted to dry weight using a
204 general ratio, C:dw = 0.45, for phytoplankton[45] and converted to energy equivalents, assuming
205 an energy content of 19.45 kJ g^{-1} dw in *R. baltica*[36]. The oxygen consumption rate was converted
206 to energy equivalents using the oxyenthalpic equivalents for lipid and protein, assuming an equal

contribution of both classes (484 kJ mol O_2^{-1})[46]. Scope for growth (SfG) was calculated sensu Widdows and Johnson[47]: SfG (Joule*mg dw^{-1}*h^{-1}) = A - R, where A is the assimilated energy, and R represents the energy lost due to respiration.

Egg production and hatching success. Daily egg production rate was measured in ovigerous F_1 generation females randomly collected from their exposure tanks over a seven day period. Daily, the single female in each egg-laying chamber was gently transferred to a new egg-collection cup (Figure 1B). The spawned eggs were transferred to an 8 mL glass vial, incubated under dark conditions (96h, 10°C), before further development was arrested using Lugol's solution. The number of non-hatched eggs and nauplii in the samples were determined using an inverted microscope. The egg production during the first day was ignored to reduce handling stress influence. At the end of the incubation period, pH_{Tot} and temperature in the egg-collection chambers and incubation vials were measured for each CO_2-treatment, using a pH-meter (for water parameters see SI Table S1-1).

The egg production of ovigerous F_1 generation females was measured at two feeding regimes and repeated twice to obtain data from six females per tank. In the first round, the females were offered *R. baltica* algae at *ad libitum* concentrations (1087 ± 370 µg C L^{-1}) throughout the incubation period, by continuous addition of algal suspension through an inlet in the lid of the egg laying chamber. In the second round, daily prepared seawater, with limited food (i.e. 200 µg C l^{-1}), and adjusted pCO_2- levels, was prepared in four header tanks and distributed to the egg laying chambers using peristaltic pumps, at a flow rate corresponding to a full water exchange

per day. Due to gas exchange, the pCO_2 conditions in the egg-laying cups dropped below the target values in the second egg laying round (see Table S1-1, SI)

Transfer experiment; the influence of CO_2-exposure history on the hatching success. F_2-generation eggs spawned at the respective pCO_2 levels were obtained by transferring F_1 males and females and 64 µm filtered tank water to separate 50L polyethylene tanks. Newly spawned eggs (median age; 6h) collected from each tank were transferred for hatching under identical- or altered seawater pCO_2 hatching conditions (fully crossed design) in glass scintillation vials (10 eggs per vial, six sub-replicates per seawater pCO_2 hatching condition) (for water parameters see SI Table S1-1). Following a 96h incubation under dark conditions at 10°C, further development was arrested by conserving the samples with 10 µL Lugol´s solution and the number of non-hatched eggs and nauplii determined as described above.

Data analysis. Morphometric characters (dry weight, body length and volume percent oil-sac) and energetic variables (feeding rate, metabolic rate, and scope for growth) were analyzed by least square linear regression. Correlation with measured pCO_2 values were investigated by Pearson's product moment correlation. Mean values of sub-replicates from the complete dataset were investigated (except for feeding rate and scope for growth, where three sub-replicate values from the 1080 µatm treatment were considered as outliers and were removed). In five cases slightly negative feeding values were set to zero (one in control, one in 1080 µatm, and three in the 3080 µatm treatment). The effect of CO_2 exposure on MDT and fecundity was analyzed using a two way repeated-measures ANOVA, where generation and pCO_2 was the "between-

subjects" factors. Prior to the analysis missing MDT values (9 of a total of 250 values) were replaced using the expectation maximization procedure in SPSS, since the requirements were fulfilled (Little's Missing Completely at Random test[48]). The examined parameters were tested for potential deviations from the assumption of homogenous variation using Levene's test. All MDT values were log10 (x+10) transformed prior to statistical treatment. The effect of seawater pCO_2 on hatching success during egg production and hatching was examined with a two way ANOVA. Significant differences between treatments were identified using Dunnett's *post hoc*-test. P-values > 0.05 were treated as significant. Analysis and graphical treatment was performed using R version 3.0.0 and SPSS version 20.

RESULTS

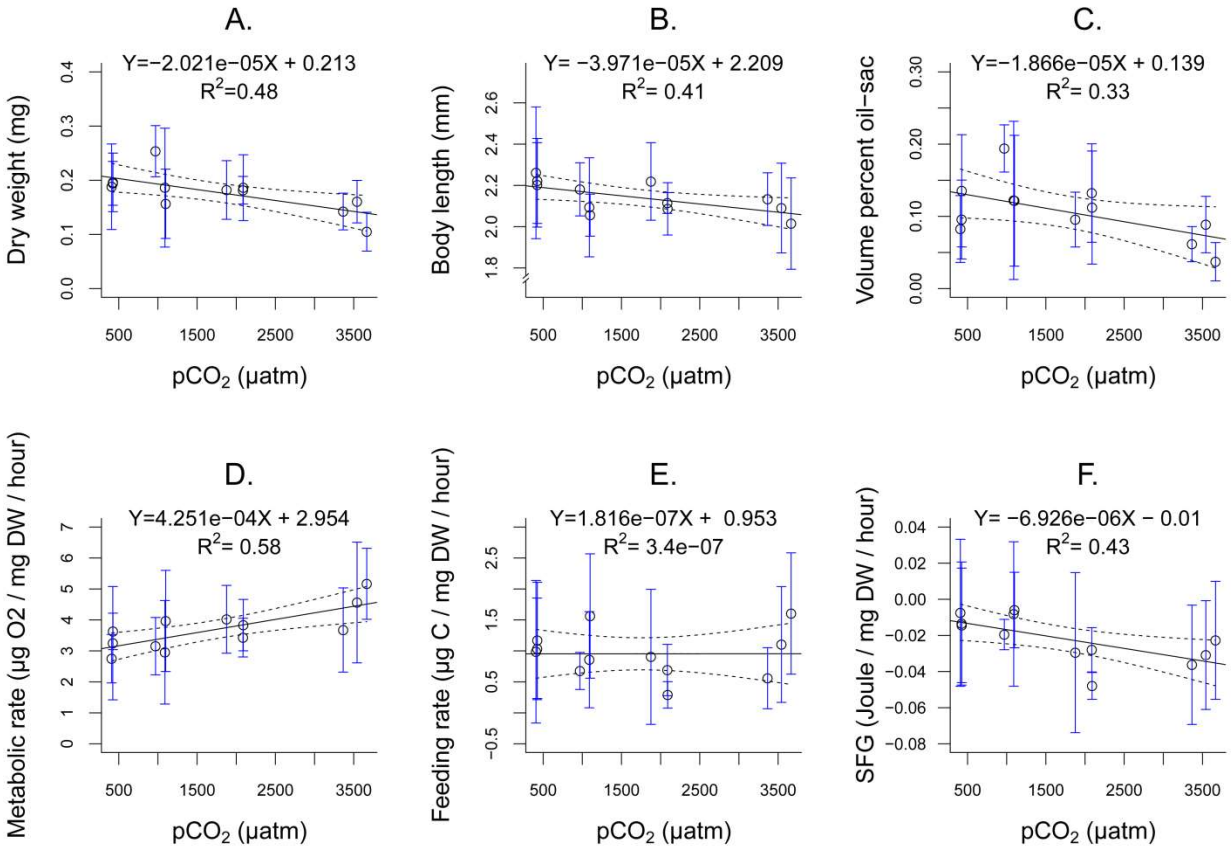

Figure 2. Relationship between pCO_2 and different parameters measured on F_1 generation sub-adults (C5): Dry weight (mg) ($F_{1,10}$=9.38, p= 0.012) (**A**). Body length (mm) ($F_{1,10}$= 6.88 p=0.026) (**B**). Volume percent oil-sac (%) ($F_{1,10}$ = 4.85, p= 0.052) (**C**). Weight-specific metabolic rate (µg O_2 mg^{-1} dry weight h^{-1}) ($F_{1,10}$ = 13.93, p= 0.004) (**D**). Feeding rate (µg carbon dry $weight^{-1}$ h^{-1}) ($F_{1,10}$ = 3.35e-06, p= 1.00) (**E**). Scope for growth (Joule mg^{-1} dry weight h^{-1}) ($F_{1,10}$= 7.53, p= 0.021) (**F**). Symbols represent mean and whiskers SD (n = 6) of one experimental tank. Solid lines show least square linear regression based on the mean values while the stippled lines indicate the 95% confidence intervals for the correlations.

Morphometric characters. The size of the F_1 generation sub-adult C5 animals was inversely related to pCO_2 both in terms of dry weight and body length (prosome length), indicating a ~33 and ~5% reduction in the high pCO_2 treatment when compared to the control, respectively (Figure 2A, B). A close

283 to significant negative relationship between lipid sac volume (vol. % of body) and pCO_2 was also
284 observed (p= 0.052), indicating ~50% reduction in the high pCO_2 treatments compared to the control
285 (Figure 2C).

286 **Scope for growth.** Mean dry weight specific oxygen consumption rate of control F_1 sub-adult
287 C5 copepodites was 3.21±1.37 µg O_2 dw^{-1} h^{-1} (Figure 2D). Seawater pCO_2 increased oxygen
288 consumption rate in a linear fashion (p=0.004, R^2=0.58). Mean dry weight specific feeding rate
289 of controls was 1.063±0.923 µg carbon dw^{-1} h^{-1} (Figure 2E). Feeding rate did not correlate with
290 seawater pCO_2 (p=1.00, R^2<0.001). A significant inverse linear relationship was observed
291 between estimated scope for growth and seawater pCO_2 (p=0.021, R^2=0.43) (Figure 2F).

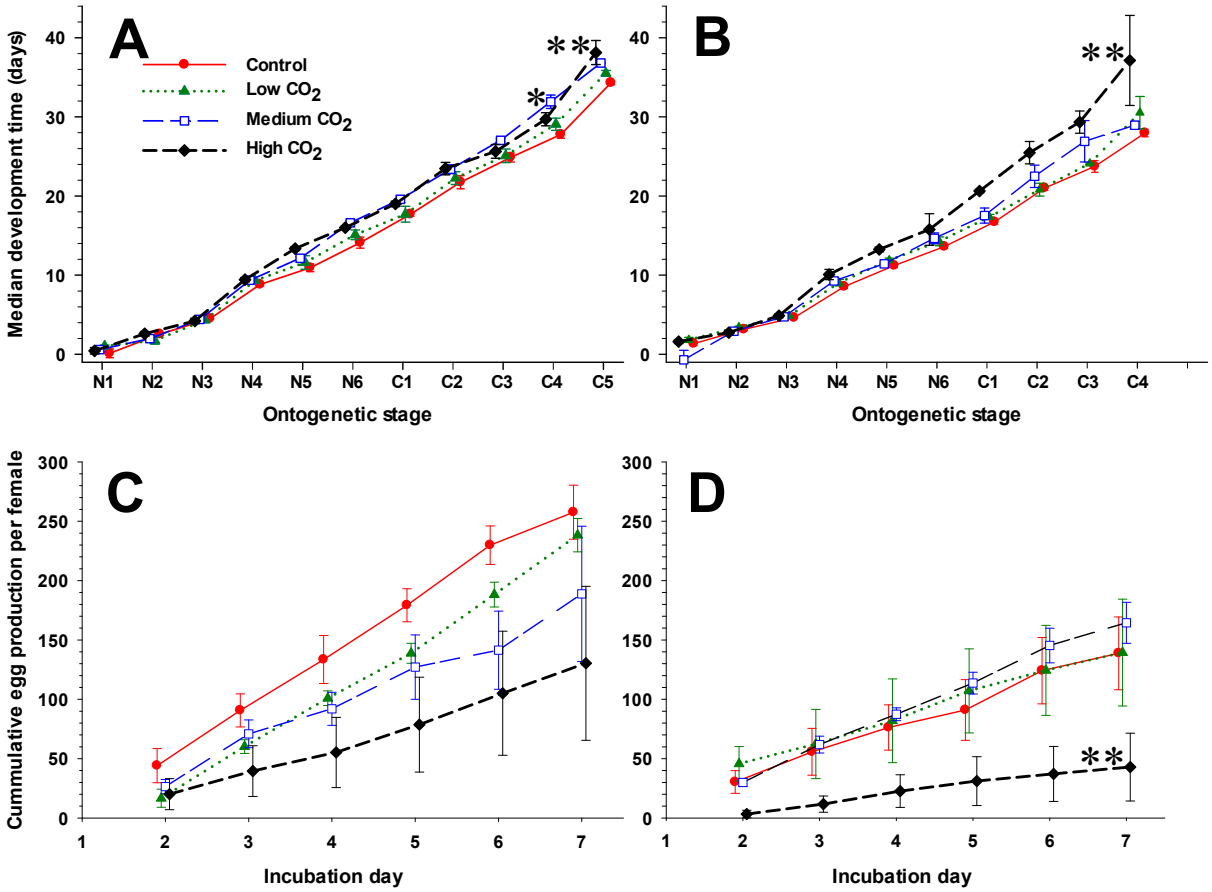

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Figure 3. Median development time (days) for nauplius- (N1-N6) and copepodite stages (C1-C6) of the P generation (A) and the F_1 generation (B) exposed nominally to either 380 (control), 1080 (low), 2080 (medium) or 3080 (high) µatm CO_2. Symbols represent mean and whiskers SD for each ontogenetic stage (n=3 replicates). Significant differences (p<0.05) between control- and elevated seawater pCO_2 treatments in median development time for the entire generation are highlighted by * for control vs. medium CO_2 and ** for control vs. high CO_2. Effects of food abundance on the cumulative number of eggs produced over a six day period per F_1 generation female exposed nominally to either 380 (control), 1080 (low), 2080 (medium) or 3080 (high) µatm CO_2: (C) algae *ad libitum*; (D) algae level corresponding to 200 µg carbon L^{-1}. Symbols represent mean, whiskers SE (n=3 replicates, where each replicate was derived from individual egg counts of six ovigerous females randomly selected from each exposure tank (Figure S1-1)); **significantly different from control (p<0.05).

Development. When MDT of both generations were analyzed together a significant interaction between generation and CO_2 exposure was observed ($F_{3,16}$=3.33, p=0.046), indicating a change in MDT response to CO_2 exposure between the P- and F_1 generations. A significant overall effect of CO_2 exposure was observed on median development time (MDT) of the parental generation P ($F_{3,8}$=6.22, p=0.017) (Figure 3A). More specifically, MDT was significantly higher for both the medium (p=0.016) and high CO_2 exposures compared to control (p=0.022). Compared to the MDT of control C5 copepodites (34.3 days), MDT of C5 exposed to medium and high CO_2 was delayed by 2.5 (7.3 %) and 3.8 (11.2 %) days, respectively. In contrast, CO_2 exposure had no significant effect on the MDT of pre-feeding nauplii stages N1 and N2 ($F_{3,8}$=0.13, p=0.939). Overall the MDT of the F_1 generation was also affected by CO_2 exposure ($F_{3,8}$=5.42, p=0.025) although only the high CO_2 treatment significantly increased MDT compared to control (p=0.038) (Figure 3B). Compared to the MDT of control C4 copepodites (28.0 days), MDT of

C4 exposed to high CO_2 was delayed 8.9 days (31.8 %). As for the P generation copepods, no significant deviation from normal development was observed for the pre-feeding stages N1 and N2 of the F_1 generation ($F_{3,8}$=2.7, p=0.12).

Fecundity. F_1 generation females given algae *ad libitum* while exposed to low, medium or high CO_2 concentration showed egg production rates (EPR) of 49±20, 31±23 and 22±20 eggs day^{-1}, respectively, which were not significantly different from that of the control, 42±20 ($F_{3,26}$=1.866, p=0.162) (Figure 3C). In contrast, F_1 females submitted to a restricted feeding regime exhibited a significant suppressive effect of CO_2 exposure on egg production ($F_{3,27}$=7.047, p=0.001) (Figure 3D). The EPR of females exposed to the high CO_2 (9±12 eggs day^{-1}) was significantly reduced compared to the control (28±12 eggs day^{-1}) (p=0.02) and the medium CO_2 treatment (33±12 eggs day^{-1}) (p=0.013). The EPR of females exposed to low CO_2 was 31±12 eggs day^{-1}.

Fertility. The overall hatching success of all generation eggs was 68±10 % (Figure S1-4 in SI). The CO_2 exposure history of the parent F_1 adults had no significant influence on the hatching success of their offspring, F_2 ($F_{3,28}$=1.159, p=0.343). Likewise, the seawater pCO_2 condition experienced during hatching had no effect on hatching success ($F_{3,28}$=0.036, p=0.991) and there was no interaction between the seawater pCO_2 condition during egg production and hatching ($F_{9,28}$=0.327, p=0.959) (Figure S1-5 in SI).

DISCUSSION

The present study is the first to report multigenerational effects of elevated seawater pCO_2 in the *Calanus*-genus under limited food-availability. Furthermore, the present study is the first to report on ecological relevant responses, such as vital rates (ontogenetic development, somatic growth, fecundity), in a trans-generational context, and to link these responses to energy constraints induced by higher maintenance costs, due to elevation in energy demanding processes involved in compensatory responses such as such as acid-base regulation, in high pCO_2-environments by considering metabolic- and feeding rates, together with scope for growth estimations.

Morphometric characters. Morphometric characters such as dry weight and body length showed a pCO_2 dependent reduction in sub-adult copepodites from the F_1 generation (Figure 2A, B). A near significant negative relationship between lipid content and pCO_2 (p= 0.05) also suggests that in addition to being smaller, the animals were also leaner under elevated pCO_2 conditions (Figure 2C). In contrast, no effect on these morphometric characters was observed among sub-adults in a medium-term study where *C. finmarchicus* was exposed for up to 7300 µatm during *ad libitum* conditions [22], or in another medium term study with *Calanus glacialis* and *Calanus hyperboreus* were also food was provided in excess[23]. Thus, food provisioning seems to offset negative effects of elevated pCO_2 in copepods, as reported for mussels[49, 50], suggesting increased energy demands in the exposed animals.

Scope for growth. Respiration measurements revealed a linear pCO_2-dependent increase in the weight-specific metabolism of sub-adult C5 copepodites of the F_1 generation (Figure 2D). A similar response has been observed in marine invertebrates with a limited capacity to regulate

extracellular pH in response to elevated seawater pCO_2,[51-53] attributed to increased costs due to elevation in energy demanding acid-base regulatory processes from trying to maintain a normal extracellular and intracellular pH[49]. Reports of metabolic responses to elevated seawater pCO_2 in copepods are few and they provide a complex picture: While increased metabolism was observed in field-collected adults of the calanoid copepod *Centropages tenuiremis* when exposed to 1000 µatm CO_2 for up 72h[54], no apparent change was seen in field collected pre-adults and adults of *C. glacialis* and *C. hyperboreus* that developed for up to 86 days at 3000 µatm CO_2[23]. These apparently contradictory results could be explained by a form of epigenetic effect where the metabolic rate is pre-determined by environmental conditions experienced in an earlier developmental stage or by parents (i.e. maternal effects). Alternatively, the elevated metabolism in our study could result from a directional selection towards high routine metabolism phenotypes. To gain a picture of how the energy status of the animals is affected by elevated seawater pCO_2, feeding rates were also measured on the F_1 generation sub adults. The measurements revealed that the higher energy demand observed in response to elevated pCO_2 was not compensated by increased feeding (Fig. 2E), indicating a reduction of the animals scope for growth with increasing pCO_2 exposure (Fig. 2F). Scope for growth is an energy budget approach[47] that has been successfully applied to assess status and explain responses of organisms to various stressors, and was recently used to investigate the response in feeding echinoderm larvae to elevated pCO_2[55]. As in the present study, feeding rates remained constant in spite of increased metabolism in the larvae, pointing towards a drop in the scope for growth with increasing seawater pCO_2.

Development. Effects of elevated seawater pCO_2 on ontogenetic development rate differed between the P- and the F_1 generation of the present study. At 2080 µatm CO_2 the MDT was

significantly reduced in the P generation, while no reduction was apparent in the next generation among the F_1 animals. However, at 3080 µatm CO_2 the reduced development rate prevailed throughout both generations (Figure 3A, B). Normalization of development rate observed at 2080 µatm CO_2 suggests that calanoid copepods may possess considerable adaptive capacity through phenotypic plasticity and/or adaptive selection[26] to counteract the potentially negative impact of ocean acidification scenarios predicted for the year 2300. This is one of the first reports of CO_2-induced delay in development rate in a copepod (but see the referral to a similar response in *Acartia tonsa* in Dupont and Thorndyke[25]), and also one of the first clear examples of how the response in terms of developmental rate may change between generations under OA-conditions. Our results suggest that *Calanus* species may possess a considerable adaptive capacity (phenotypic plasticity and/ or adaptive selection) to counteract potential negative impacts from pCO_2 concentrations that could be reached within year 2300 (i.e.≤ 2000 µatm). The *C. finmarchicus* used in the present study had been in culture for about 30 generations and may therefore have lost some of their natural genetic variability. It can therefore be argued that the adaptive potential observed herein represent a conservative estimate of that of wild type copepod populations.

Although feeding rate was not measured in the medium-term study on *C. glacials* and *C. hyperboreus* copepodites by Hildebrandt et al.[23], it is noteworthy that the normal growth and development rate they observed at high pCO_2 were consistent with the normal metabolism the animals displayed. The reduction in development rate observed at the two highest pCO_2-treatments (2080 and 3080 µatm) in our study is consistent with the increased metabolism and reduced scope for growth observed in the F_1 generation sub-adults (Figure 2D-F), and suggest a slower development due to energy constraints. The same increase in metabolic rate, while

406 feeding rate remained constant, also caused a reduced scope for growth in planktotrophic sea
407 urchin larvae at elevated pCO_2 (1000 µatm)[55], and was linked to energy-budget constraints
408 inflicted by a higher pCO_2[25, 49, 54, 55]. A similar CO_2-induced reduction in energy budgets has also
409 been linked to reduced calcification in mussels[49, 50] and gill performance of fish[56].

410 In echinoderms, pre-feeding stages have been found to develop normally[57-59], or even faster in
411 the lecitotrophic sea star larvae *Crossaster papposus*[60], and developmental delay is only
412 observed at increased pCO_2 in the later planktotrophic stages of sea urchins[61]. The different
413 response may reflect that pre-feeding larvae are fueled by endogenous energy reserves and may
414 therefore not necessarily experience the energy limitation that exogenous feeding stages face
415 under elevated pCO_2 (see e.g. Gianguzza et al.[62]). Our study confirm this pattern since a normal
416 development rate in pre-feeding nauplii stages (nauplii I & II) was observed at elevated pCO_2
417 (Fig.3A, B), and thus support that negative effects of elevated pCO_2 are due to energy
418 constraints.

419 **Fecundity.** The EPR observed in the present study was comparable to that of wild *C.*
420 *finmarchicus* populations in periods when food is limited[63]. Fecundity of *C. finmarchicus* is
421 known to be dependent of food abundance both in the field[64, 65] and laboratory[37]. The observed
422 increase in daily egg production from 23 to 40 eggs female^{-1} when food availability was
423 increased from restricted to *ad libitum* was therefore expected (Figure 3C, D).

424 A 3.2-fold reduction in EPR was observed under restricted food availability at 3080 µatm pCO_2
425 but not at lower CO_2 concentrations (Figure 3D). Adverse reproductive effects have been
426 reported previously for copepods exposed to ≤ 2000 µatm pCO_2[7, 20, 66] and other marine
427 invertebrates[26, 67]. However, for *Calanus* species detrimental effect on reproduction has to date

428 only been observed at seawater pCO_2 ≥7000 µatm[17-20], and similar robustness in reproduction
429 have been observed for other copepods[28, 68, 69]. Our findings support the theory that calanoid egg
430 production is relatively robust against elevated pCO_2 conditions predicted for the year 2300.
431 However, contradictory reports warrant further research.

432 The reported suppressive effect of 3080 µatm pCO_2 on EPR disappeared at *ad libitum* food
433 provision (Figure 3) indicating increased energetic cost for the maintenance of homeostasis
434 under high pCO_2. This finding is consistent with the observed reduction in scope for growth of
435 sub-adult copepodites (Figure 4C) and suggests that the animals may be able to increase their
436 scope for growth by compensatory feeding when food is provided *ad libitum*. Together with
437 previous observations on calcification and growth in blue mussel adults[49] and juveniles[50], this is
438 one of the first examples of how increased food provision can ameliorate negative effects of
439 elevated pCO_2, and supports the hypothesis that many of the chronic effects of exposure to
440 elevated pCO_2 could be linked to a reduced energy status inflicted by elevated costs from the
441 elevation in energy demanding processes required to maintain normal intracellular- and
442 extracellular pH.

443

444 **Fertility.** Exposure to seawater pCO_2 ≤ 3080 µatm had no significant effect on hatching success.
445 This is consistent with previous reports of impaired calanoid fertility at ≥ 8000 µatm CO_2[17-19, 70].
446 Preconditioning for two entire generations had no effect on hatching success in the present study
447 (Figure S1-4 and S1-5), consistent with findings in previous studies on copepods[28, 68], barnacles[71]
448 and echinoderms[72-74]. Absence of significant effects when the CO_2 concentration changed
449 between the egg production- and hatching conditions (transfer experiment) suggests that carry-

over effects (e.g. maternal effects) may be of minor importance for hatching success when *C. finmarchicus* is long-term exposed to elevated CO_2 during food limitation. However, decreasing water pH by 0.4 units (~1000 µatm CO_2) between egg production and hatching of a field collected *Acartia* species caused hatching success to respond first negatively, then positively and finally neutrally after one, three and five days of pre-acclimation, respectively[66]. The authors suggested that any negative carry-over effect of parental CO_2-exposure could have been neutralized by a gradual improvement of their nutritional status due to the optimal food provision in the laboratory.

From an ecological perspective, the results from the present study carry relevance to the predicted decline in biological productivity and reduced phytoplankton concentration (averaged by 25% in the North Sea) expected in the future (see Gröger et al.[30]). This trans-generational study indicates that the physiological tipping point for *C. finmarchicus* lies somewhere between 2080 and 3080 µatm pCO_2, and thus confirms that this key species seems to be quite robust even against direct negative effects of the most pessimistic ocean acidification scenarios that are predicted for the year 2300[22]. However, the observed pCO_2-dependent increase in weight specific oxygen consumption rate may have potential implications for food web trophodynamics. Since *C. finmarchicus* in one of the major contributors to the transfer of energy from primary producers to higher tropic levels[13], an increased energy expenditure through respiration could potentiate the negative consequences from expected future primary production reductions[30], with important implications for the overall productivity of the northern Atlantic ecosystem.

To summarize, vital rates (ontogenetic development, somatic growth, fecundity and hatching success) were investigated in relation to metabolic and feeding rates in *Calanus* cohorts exposed to different ocean acidification scenarios for two consecutive generations under a food-limited

473 situation. A significant delay in development rate among the P generation animals exposed to
474 2080 µatm CO_2, but not in the following F_1 generation animals under the same conditions,
475 indicates that *C. finmarchicus* can adjust to the new situation after transgenerational acclimation
476 and is resilient against negative direct effects of even the most pessimistic scenario predictions
477 for the coming centuries, even under limited food conditions. Here we show that exposure to
478 elevated seawater pCO_2 cause a dose dependent increase in the weight-specific routine
479 metabolism of the animals, which is not compensated by corresponding increase in feeding,
480 resulting in a reduction of the scope for growth. However, this reduction in scope for growth
481 seems only to cause detrimental effects on development and egg production rate at pCO_2 levels
482 higher than expected in the future (i.e. 3080 µatm). Because of the important role of *C.*
483 *finmarchicus* in energy transfer from phytoplankton to higher tropic levels, the increased
484 metabolic cost associated with a more acid ocean could imply that energy transfer from lower
485 tropic levels could be reduced, with potential repercussions for the overall productivity of the
486 northern Atlantic ecosystem.

487

488 **ASSOCIATED CONTENT**

489 **Supporting information**

490 Supplementary Table S1-1 contains water parameters (pH_{Tot}, temperature and algae concentration) from;
491 1) tanks were F_1 generation eggs were collected, 2) egg laying cups under restricted- and *ad libitum*
492 feeding conditions, 3) water used during egg incubation experiment. Figure S1-1 shows a flow chart of
493 the exposure system. Figure S1-2 and S1-3 show the pCO_2 and algae concentrations during the
494 experiment. Figure S1-4 and S1-5 show the hatching success observed in F_2 generation eggs.

495

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Notes

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Supporting Information

Multigenerational exposure to ocean acidification during food limitation reveals consequences for copepod scope for growth and vital rates

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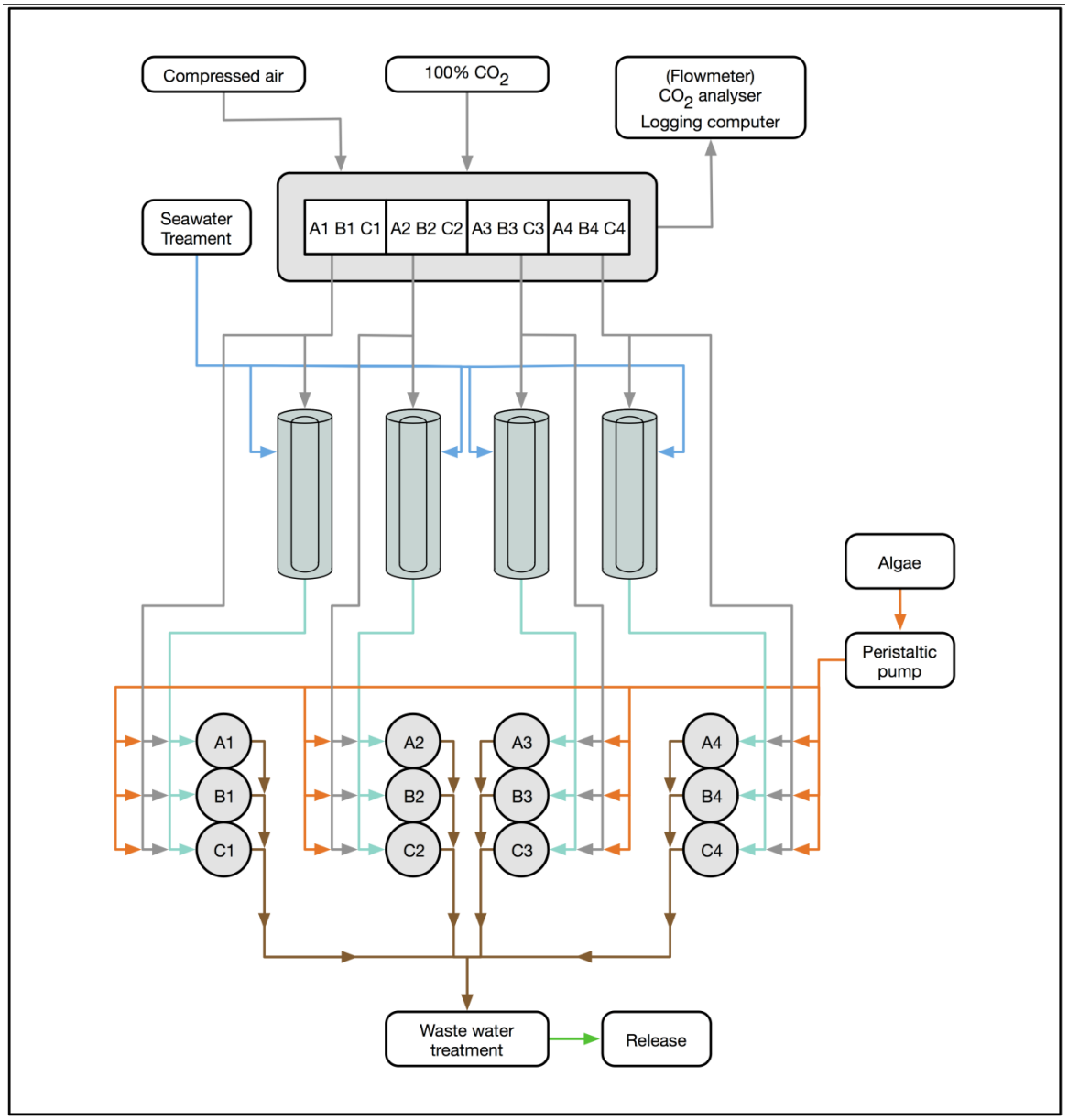

Figure S1-1. A schematic flow chart of the exposure system used to expose *C. finmarchicus* to four different levels of CO_2 during two consecutive generations. A custom built gas mixer produced four different mixtures of CO_2-enriched air which were distributed to a primary and secondary system for gas-water equilibration. The primary system consisted of four double cylinders (columns filled with grey), one for each CO_2 concentration, where a counter current system facilitated the dissolution of the gas in the water (incoming blue lines). The equilibrated water (turquoise lines) were distributed from the primary system to twelve 90L exposure tanks, with three replicate tanks (A-C) for each of four exposure levels (1-

4). A secondary air-gas equilibration system, with adjustable gas flow, provided stability and a means to fine tune the CO_2-level within each individual tank. A peristaltic pump continually distributed algae suspension (orange lines) to each tank.

Table S1-1. Water and algae parameters measured; (1) in 50 L polyethylene tanks during collection of F_1-generation eggs; (2) in egg-laying cups during the first egg laying experiment (ad libitum feeding); (3) in egg-laying cups during second experiment (restricted feeding); (4) in water used for incubation of F_2 eggs to determine the hatching success. For easy comparison, the mean pH_{Tot} in the exposure tank (Target pH_{Tot}) are also listed. Listed values represent means ± SD and number of replicate measurements is indicated between brackets.

Experiment	Treatment	Target pH_{Tot}	Measured pH_{Tot}	Temperature (°C)	Algae level (µg C L^{-1})
Collection of F_1-generaton eggs	Control	8.02 ± 0.02	8.05 ± 0.02 (3)	9.7 ± 0.7 (3)	364 ± 32 (3)
	Low CO_2	7.65 ± 0.04	7.68 ± 0.02 (3)	9.6 ± 0.4 (3)	361 ± 47 (3)
	Medium CO_2	7.38 ± 0.03	7.45 ± 0.04 (3)	9.6 ± 0.5 (3)	383 ± 71 (3)
	High CO_2	7.13 ± 0.02	7.26 ± 0.02 (3)	9.5 ± 0.4 (3)	377 ± 59 (3)
First egg laying experiment	Control	8.02 ± 0.02	8.02 ± 0.04 (3)	9.4 ± 0.1 (3)	1087 ± 237 (5)
	Low CO_2	7.65 ± 0.04	7.56 ± 0.02 (3)	9.2 ± 0.0 (3)	"
	Medium CO_2	7.38 ± 0.03	7.37 ± 0.01 (3)	9.2 ± 0.1 (3)	"
	High CO_2	7.13 ± 0.02	7.12 ± 0.01 (3)	9.2 ± 0.1 (3)	"
Second egg laying experiment	Control	8.02 ± 0.02	8.02 ± 0.03 (5)	9.4 ± 0.1 (3)	120 ± 41 (5)
	Low CO_2	7.65 ± 0.04	7.68 ± 0.01 (3)	9.3 ± 0.0 (3)	110 ± 93 (5)
	Medium CO_2	7.38 ± 0.03	7.58 ± 0.06 (6)	9.6 ± 0.1 (3)	143 ± 29 (5)
	High CO_2	7.13 ± 0.02	7.47 ± 0.04 (4)	9.6 ± 0.3 (3)	95 ± 30 (5)
Egg incubation experiment	Control	8.02 ± 0.02	8.02 (1)	N.D.	N.D.
	Low CO_2	7.65 ± 0.04	7.72 (1)	"	"
	Medium CO_2	7.38 ± 0.03	7.53 (1)	"	"
	High CO_2	7.13 ± 0.02	7.27 (1)	"	"

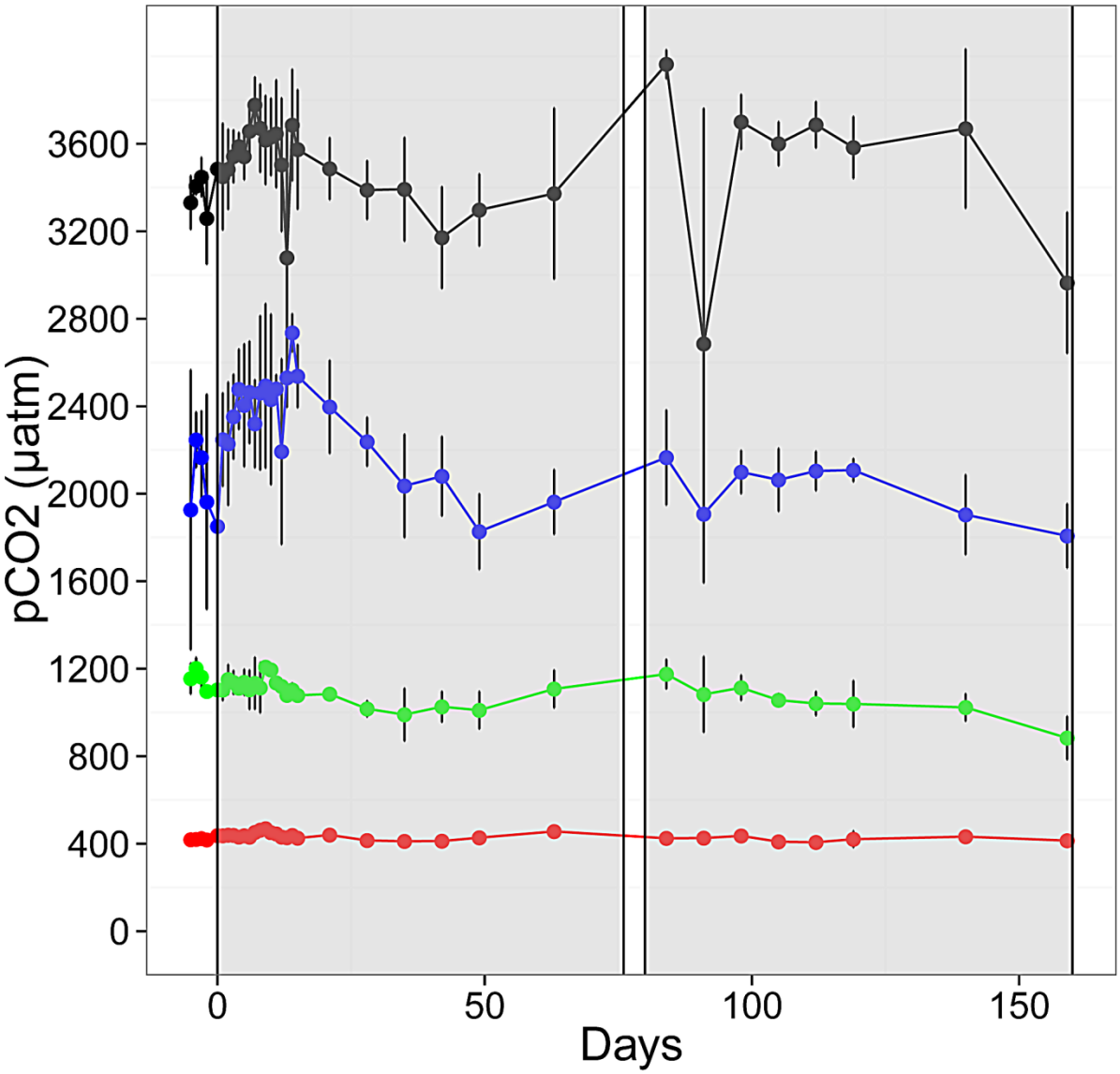

Figure S1-2. Development in pCO_2 in the control (red), low- (green), medium- (blue), and high CO_2-treatments (black) during the experiment (mean±SD, n=3). The grey areas indicate the duration of the P (left) and F_1 generation (right).

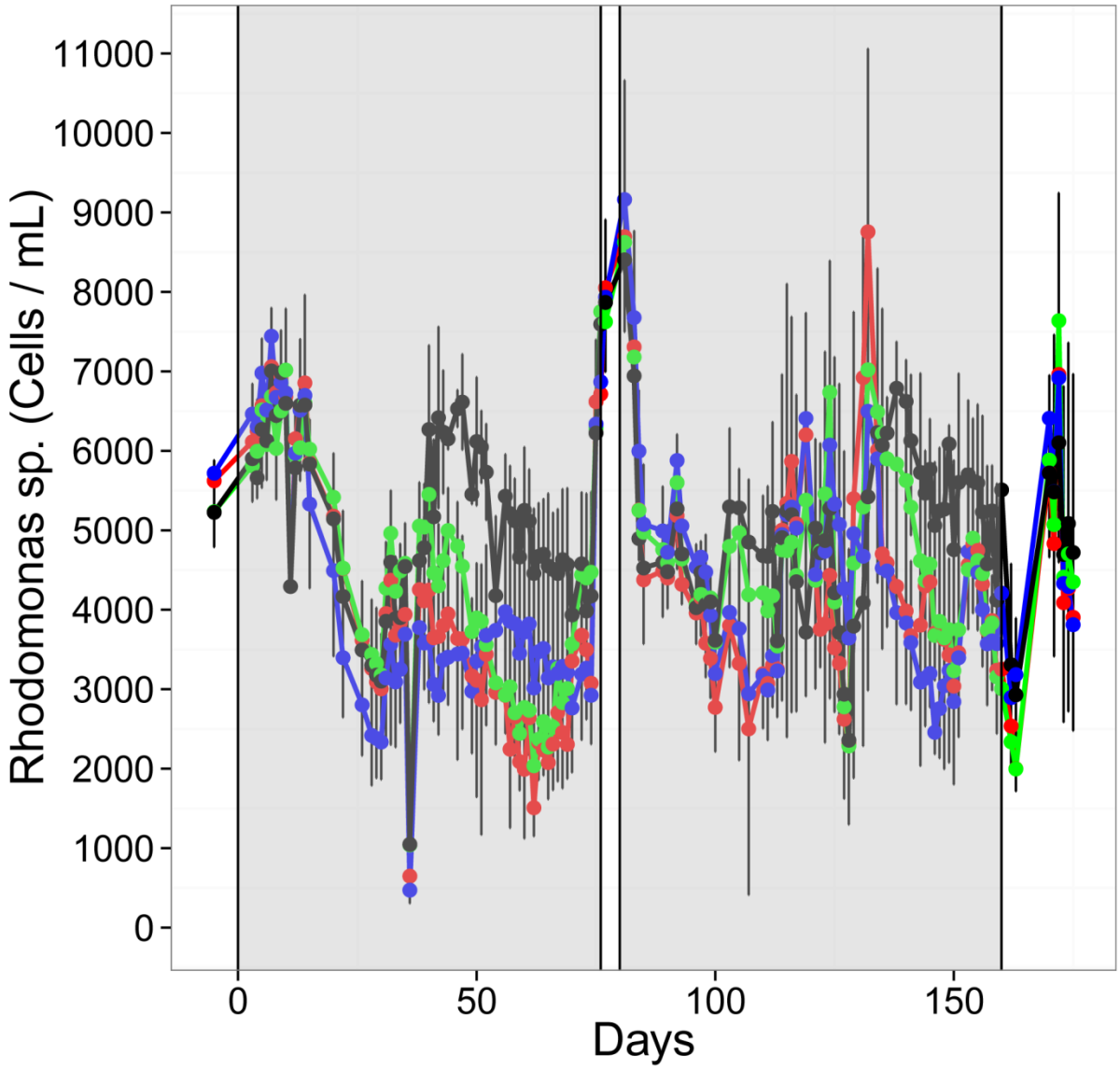

Figure S1-3. Development of algal concentration in the control (red), low- (green), medium- (blue), and high CO_2-treatments (black) during the experiment (mean±SD, n=3). The grey areas indicate the duration of the P (left) and F_1 generation (right).

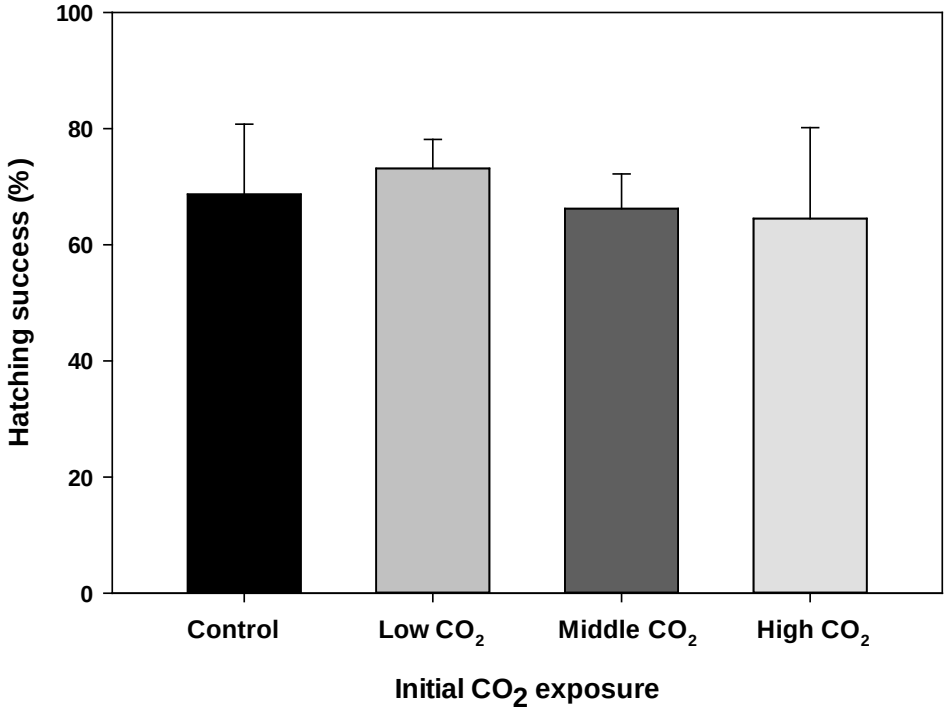

Figure S1-4. Hatching success of F_2 generation eggs when incubated nominally to either 390 (control), 1080 (low), 2080 (medium) or 3080 (high) µatm CO_2, regardless of the exposure conditions of their F_1 progenitors during egg production (mean± SD; n=3 (n=2 in high CO_2 treatment)).

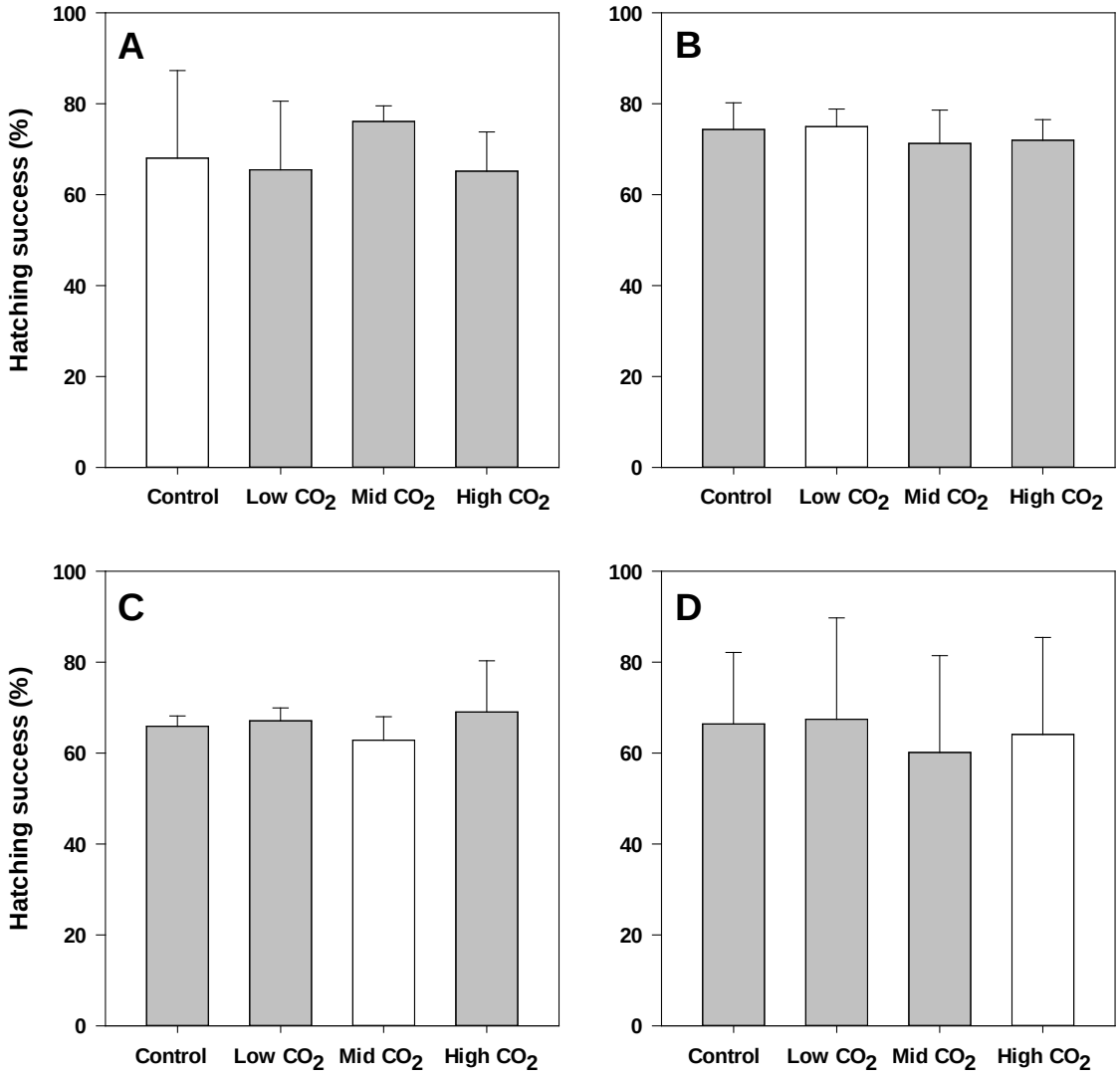

Figure S1-5. The hatching success of F_2 generation eggs developed and spawned at control (A), low- (B), medium- (C) and high CO_2 treatment (D), and hatched under different pCO_2 conditions: control, low-, medium and high CO_2. The white bar in each sub-figure indicates where the pCO_2 condition during egg production and hatching were identical. Bars show the calculated mean hatching success, whiskers SD; n=3 replicates, except for high CO_2 exposure for which n = 2 replicates. Due to no egg production, vials from one of the high CO_2 exposure tanks could not be included. For the same reason, the number of sub-replicate vials included from another of the high CO_2 tanks had to be reduced from six to four.