Seawater pH Predicted for the Year 2100 Affects the Metabolic Response to Feeding in Copepodites of the Arctic Copepod *Calanus glacialis*

Peter Thor¹*, Allison Bailey¹, Claudia Halsband², Ella Guscelli³, Elena Gorokhova⁴, Agneta Fransson¹

¹ Norwegian Polar Institute, Fram Centre, Tromsø, Norway, ² Akvaplan-niva, Fram Centre, Tromsø, Norway, ³ University of Florence, Florence, Italy, ⁴ Department of Environmental Science and Analytical Chemistry, Stockholm University, Stockholm, Sweden

* peter.thor@npolar.no

Abstract

Widespread ocean acidification (OA) is transforming the chemistry of the global ocean, and the Arctic is recognised as a region where the earliest and strongest impacts of OA are expected. In the present study, metabolic effects of OA and its interaction with food availability was investigated in *Calanus glacialis* from the Kongsfjord, West Spitsbergen. We measured metabolic rates and RNA/DNA ratios (an indicator of biosynthesis) concurrently in fed and unfed individuals of copepodite stages CII-CIII and CV subjected to two different pH levels representative of present day and the “business as usual” IPCC scenario (RCP8.5) prediction for the year 2100. The copepods responded more strongly to changes in food level than to decreasing pH, both with respect to metabolic rate and RNA/DNA ratio. However, significant interactions between effects of pH and food level showed that effects of pH and food level act in synergy in copepodites of *C. glacialis*. While metabolic rates in copepodites stage CII-CIII increased by 78% as a response to food under present day conditions (high pH), the increase was 195% in CII-CIIIs kept at low pH—a 2.5 times greater increase. This interaction was absent for RNA/DNA, so the increase in metabolic rates were clearly not a reaction to changing biosynthesis at low pH *per se* but rather a reaction to increased metabolic costs per unit of biosynthesis. Interestingly, we did not observe this difference in costs of growth in stage CV. A 2.5 times increase in metabolic costs of growth will leave the copepodites with much less energy for growth. This may infer significant changes to the *C. glacialis* population during future OA.

Introduction

Widespread ocean acidification (OA) is transforming the chemistry of the global ocean, and the Arctic is recognised as the region where the earliest and strongest impacts of OA are expected [1–3]. Sea ice melt has low hydrogen-ion (H⁺) buffering capacity and increasing ice
melt makes Arctic waters increasingly susceptible to OA [3]. Additionally, increasing Atlantic water inflow carries large amounts of anthropogenic CO$_2$ to the Arctic Ocean [4]. Arctic organisms are therefore the first to face the effects of OA and will continue to experience stronger OA in the future [3]. Contrary to cold adapted eurythermal animals, true Polar species show low energetic costs for maintenance at low temperatures. But such low costs also results in a lower capacity for acid-base regulation [5]. Consequently, true Arctic species may be less capable of countering OA. Moreover, Arctic communities are characterized by simpler food webs both in terms of number of trophic levels and diversity on each trophic level. Effects of environmental change on predator-prey interactions are often buffered by niche sharing at both the predator and the prey level [6]. If any particular species is severely impacted, another will be able to take its place. Such buffering is lessened in simple food webs characterised by a few keystone species and fewer predator-prey interaction points [6].

*Calanus glacialis* constitutes a keystone species in the Arctic Ocean and adjacent seas [7–9]. Along the continental shelf this species dominates in terms of biomass, may exert significant grazing pressure on the microplankton community, and is a very important prey item for many Arctic fish species, baleen whales, and marine birds [10–12]. As a consequence, much attention has been given *C. glacialis* and its possible future during Arctic change. Previous studies have shown that while naupliar development and growth may not be significantly affected at OA levels down to pH$_T$ 7.47 [13], unchanged development was upheld by increasing physiological buffering at decreasing pH: gene expression was significantly altered in groups of genes coding for such important functions as DNA repair and transcription (Bailey et al. submitted). It seems that pH stress is countered by altered gene expression patterns to maintain unchanged developmental rates. Also, Lewis and colleagues [14] have reported increased naupliar mortality at pH$_T$ 7.8 of nauplii caught from under the ice in the high Canadian Arctic. Unfortunately, mortality rates were not measured in the Bailey et al. [13] study. Hatching of eggs also seems somewhat affected. Weydmann et al. [15] found that short-term exposure of mothers (7–9 d) at very low pH (pH$_{NBS}$ 6.9) reduced egg hatching success significantly, whereas incubation at levels more closely mimicking predictions for the year 2100 (pH$_{NBS}$ 7.6) did not show any effects. Our recent studies show significant effects on both ingestion rate and metabolic rate (Thor et al. submitted). We found significantly increasing metabolic rates and decreasing ingestion rates with decreasing pH in copepodite stage IV (CIV) but interestingly not in CV. Hildebrandt et al. [16] also found no effects on ingestion rates in CV at pH$_T$ 7.21. Also, long term incubations of females have shown no effects at pH$_T$ 7.24 on metabolic rate, gonad maturation rate, or mortality [17]. This suggests that responses to OA may vary among developmental stages in *C. glacialis*.

The above-mentioned experimental studies have been conducted using satiating food levels and no studies have considered OA effects at realistic levels of prey availability. Copepods experience vast changes, both temporal and spatial, in prey availability, including starvation, and their metabolism varies accordingly [18–20]. Consequently, any reaction to environmental stress such as OA would be overlaid metabolic variation due to varying ingestion rate, which may outweigh the stress reaction itself. However, OA could interact with energy intake and create more complex responses to OA itself. For instance, such interaction may result from compensatory feeding to meet increased energetic demands or impaired gut absorption under OA stress [21]. In the study presented here, we investigated the metabolic effect of OA and its interaction with food availability in *C. glacialis*. We measured metabolic rate and RNA/DNA ratio (an indicator of biosynthesis [22]) concurrently in fed and unfed individuals subjected to two different pH levels representative of present day and the “business as usual” IPCC scenario (RCP8.5) prediction for the year 2100 [23].
Methods

Collection of copepods

*Calanus glacialis* were caught by oblique tows of a 200 μm WP2 plankton net with a closed cod end in the Kongsfjord, Svalbard (79.0°N, 11.7°E) during July 2014. No specific authorisation was needed for collecting copepods and no endangered species were involved. On deck, the content of the cod end was diluted in 25 L seawater produced from water collected at 80 m. Another study conducted at the time of sampling showed a salinity of approximately 34.9, temperature of 5.5 °C, pH$_\text{T}$ of 8.17 and $p\text{CO}_2$ of 295 μatm in this water regime [24]. Copepods were then transported to a cold room (5 °C) at the nearby Kings Bay Marine Laboratory (Ny-Ålesund, Svalbard). *Calanus glacialis* copepodes stages II-III, and V (hereafter CII-III, and CV) were selected under the stereoscope using cut off plastic Pasteur pipettes keeping all vessels on ice to avoid high temperatures. Copepodites were identified by number of pleopods and abdominal segments. They were distinguished from *C. hyperboreus* and *C. finnarchicus* copepodites on the basis of size [9], the presence of red pigmentation in the antennules, a characteristic distinguishing *C. glacialis* from *C. finnarchicus* [25], and the lack of lateral spikes on the distal prosome segment, a characteristic of *C. hyperboreus*.

Copepod incubations

We conducted one experiment with CII-IIIIs and two experiments with CVs. For each experiment, copepods were incubated for 7 days in four different treatments: 1) high pH/no food 2) high pH/high food, 3) low pH/no food, and 4) low pH/high food. Batches of incubation water were prepared from 0.3 μm filtered sea water ($fsw$). Water for the high pH treatments was used unchanged whereas water for the low pH treatments was mixed to the target acidity with small volumes of $fsw$ acidified to ca. pH 5.5 by bubbling with CO$_2$ (Mapcon CO$_2$, Yara Praxair, Tromsø, Norway). For food, the cryptophyte *Rhodomonas baltica* were obtained from cultures at the Marine Research Institute’s Austevoll Research Station (Norway) and cultured in $f/2$ algal growth medium (Bigelow National for Marine Algae and Microbiota) at exponential growth rates. The growth medium was prepared from 0.3 μm filtered seawater ($fsw$). Cultures were maintained at 18 °C and a light:dark cycle of 14h:10h. Algae were added to the batch water for a final concentration of 20 000 cells mL$^{-1}$ (equivalent to 800 μgC L$^{-1}$ considering a cell carbon content of ca. 40 pgC cell$^{-1}$ [26]), a concentration sufficient to ensure maximum and invariant rates of ingestion and metabolism at the far end of the copepod functional response [18]. Any bias from variations in algal concentrations during incubations were therefore avoided. Algal concentrations were measured in the incubation batches by cell counts in a 1 mL flat counting chamber under the microscope at 10x magnification.

Three replicate 620 mL bottles were prepared with water from each batch. For each replicate, 10 individuals were pipetted into the bottle using cut off plastic Pasteur pipettes. All bottles were closed making sure no bubbles were present and placed on a slowly rotating plankton wheel (0.5 rpm) at 5 °C in dim light. Every other day approximately 500 mL water was removed from each bottle by reverse filtering. A length of pipe fitted with a 200 μm screen at the bottom was inserted into the bottle. A piece of tubing was inserted 4/5 into this pipe and the water was siphoned off. Newly prepared incubation water was then carefully filled into the bottle through the pipe. Samples for total alkalinity ($A_T$) and total dissolved inorganic carbon ($C_T$) were taken from the incubation water batches at every water change, fixed with HgCl$_2$ (60 μL to 250 mL sample) and stored cold and dark for later analysis.
Water carbon chemistry determination

Water samples were analyzed for $A_T$, $C_T$, and salinity at the Institute of Marine Research, Tromsø, Norway following the methods described in Dickson et al. [27]. Briefly, $C_T$ was determined using gas extraction of acidified samples followed by coulometric titration and photometric detection using a Versatile Instrument for the Determination of Titration carbonate (VINDTA 3C, Marianda, Germany). $A_T$ was determined in the water samples from potentiometric analyses from one sample, was within ±1 μmol kg$^{-1}$. Routine analyses of Certified Reference Materials (CRM, provided by A. G. Dickson, Scripps Institution of Oceanography, USA) ensured the accuracy of the measurements, which was better than ±1 μmol kg$^{-1}$ and ±2 μmol kg$^{-1}$ for $C_T$ and $A_T$, respectively. Salinity was measured by a conductivity meter (WTW Cond 330i, Germany) with the precision and accuracy of ±0.05.

$C_T$, $A_T$, salinity, and temperature were used for each sample as input parameters in the CO2SYS software [28] to calculate total hydrogen-ion scale pH ($pH_T$) and partial pressure of CO$_2$ using the HSO$_4^-$ dissociation constant from Dickson [29], and the carbonate system dissociation constants ($K_1$ and $K_2$) estimated by Mehrbach et al. [30], modified by Dickson and Millero [31].

Metabolic rate measurements

For estimates of specific metabolic rate ($MR$), oxygen consumption rates ($\dot{M}O_2$) were measured on individual CVs or groups of 3–5 CII-CIIIs. Water was siphoned off the bottles as above and copepods gently poured into Petri dishes kept on ice. While sorting copepods, under the stereoscope, water was continuously replenished from the corresponding incubation bottle. Copepods were pipetted into 1.6 mL vials fitted with fluorescent O$_2$ reactive foil discs (PSt3 spots, PreSens, Regensburg, Germany) and vials filled with water from the corresponding incubation bottle. Vials were then sealed with Teflon caps and O$_2$ concentrations were measured at 0, 3, and 6 h using an optode O$_2$ system (Fibox 3, PreSens, Regensburg, Germany). $\dot{M}O_2$ (nmol O$_2$ ind$^{-1}$ d$^{-1}$) was calculated by subtracting the average O$_2$ depletion rate measured in five controls 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 two-minute 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 were 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 and measurement of prosome length. Finally, copepods were pipetted into 1.5 mL centrifuge tubes (Eppendorf, Hamburg, Germany) and 1 mL RNA$\textit{later}$ (Qiagen, Hilden, Germany) was added for preservation and later nucleic acid analysis.

Copepod prosome lengths were measured 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)$^{3.57}$ [32]. $\dot{M}O_2$ (nmol O$_2$ ind$^{-1}$ h$^{-1}$) was converted to carbon weight specific $MR$ (μgC μgC$^{-1}$ d$^{-1}$) by dividing by body mass (μg ind$^{-1}$), multiplying by a respiratory coefficient of 0.97 mol C mol O$_2^{-1}$ [33], multiplying by the mole weight of C (0.012 μg nmol$^{-1}$), and multiplying by 24 h d$^{-1}$.

Nucleic acid analysis

Gut DNA was quantified in CV’s by staining with PicoGreen (Invitrogen, Carlsbad, CA, USA) according to Gorokhova [34]. Using a sharp needle, a pair of ultrafine forceps and a dissecting
microscope, we excised guts of the CV individuals. From each copepod, the gut and the
degutted body were transferred into separate 1.5 mL centrifuge tubes containing 50 μl and
300 μl extraction buffer (1% sarcosyl in TE buffer), respectively. For the analysis of the earlier
developmental stages, intact individuals were placed in 100 μl extraction buffer. Body RNA
and DNA were quantified with RiboGreen (Invitrogen, Carlsbad, CA, USA) according to Gor-
okhova and Kyle [35], with some modifications, as follows: All samples were homogenized for
30 s and subjected to a repeated (×3) sequence of ultrasound (30 s) and ice bath (1 min). Subse-
quently, samples were allowed to shake for 2 h at room temperature. Total DNA in the gut
samples was determined using 15 μl of sample, 85 μl of TE buffer without dye and 100 μl of TE
buffer containing PicoGreen diluted 1:200. All solutions were combined in a solid white Costar
96-well microplate (Corning, Corning, NY, USA), incubated at room temperature for 5 min,
and fluorescence was read in a FLUOstar Optima (BMG Labtechnologies, Ortenberg, Ger-
many) at 485 nm excitation and 520 nm emission. Fluorescence of samples designated for
RNA/DNA ratio analysis was measured using the same equipment and black solid flat-bottom
microplates (Greiner Bio One, Kremsmünster, Austria). Plates were scanned with 0.2 s well
measurement time and 10 measurements per well two times firstly after incubation at room
temperature for 5 min with 70 μL RiboGreen well⁻¹ and secondly after digestion with 5 μl
RNAase well⁻¹ at 37˚C for 30 min. On each analytical occasion, wells containing samples,
nucleic acid standards, and negative controls were measured concomitantly. Mean standard
curve slope ratio (mDNA/mRNA), determined according to Caldarone et al. [36], was 1.58.

Statistical analysis

Water temperature and pCO₂ were compared among experiments and pH treatments by per-
mutational analysis of variance (PERMANOVA) on similarity matrices assembled using
Euclidian distances in Primer 6+ [37] using the design: experiment + pH + experiment x pH.

Separately for each experiment, metabolic rates, RNA/DNA ratios, and gut DNA content
were also compared among treatments by PERMANOVA on similarity matrices assembled
using Euclidian distances. Two to four samples were measured from each replicate bottle, and
we therefore applied a nested design: pH + food level + pH x food level + bottle(pH x food
level) with pH and food level as fixed factors and bottle as a random factor nested within pH
and food level.

All PERMANOVA tests very followed by PERMDISP tests to verify the assumption of
homogeneity of multivariate dispersions.

Results

Average water chemistry during incubations is shown in Table 1. There were no differences in
temperature among experiments or pH treatments (2-factor PERMANOVA, experiments:
Pseudo F₃,₃₇ = 0.88, P = 0.40; treatments: Pseudo-F₁,₃₇ = 0.074, P = 0.89). pH₇ was significantly
different between pH treatments (2-factor PERMANOVA: Pseudo F₁,₁₉ = 349, P < 0.001), and
not significantly different among experiments, except for the low pH in CV experiment 1,
which was significantly different from the low pH treatments of all other experiments (2-factor
PERMANOVA: pair-wise tests P < 0.05).

In CII–CIII and CV experiment 1, metabolic rate was significantly higher in individuals
offered food compared to the un-fed (3-factor PERMANOVA, CII–IIIs: Pseudo F₁,₁₉ = 24.1,
P < 0.001; CVs Exp 1: Pseudo-F₁,₈ = 18.15, P = 0.007; Tables 2 and 3; Figs 1 and 2), whereas
CVs in experiment 2 showed no difference in metabolic rate between food levels (3-factor
PERMANOVA: Pseudo-F₁,₈ = 0.0013, P = 0.97; Fig 2). pH did not show any main effect on
metabolic rate in either CII–IIIs or CVs, but in CII–IIIs, pH interacted significantly with food
level (3-factor PERMANOVA: Pseudo-F_{1,19} = 5.57, P = 0.020; Table 2). While metabolic rates in CII-CIIIs increased by 78% as a response to food in the high pH treatment, the increase was 195% in the low pH treatment (Fig 1).

RNA/DNA ratios were significantly different between CII-IIIs offered food and the un-fed (3-factor PERMANOVA: Pseudo-F_{1,19} = 12.3, P = 0.004; Fig 3). This was also true for CVs in experiment 1 (3-factor PERMANOVA: Pseudo-F_{1,8} = 63.3, P = 0.001; Fig 4), whereas food level had no effect in CV experiment 2 (3-factor PERMANOVA: Pseudo-F_{1,8} = 0.47, P = 0.5272; Fig 4). There were no significant main effect of pH on RNA/DNA ratios in any of the experiments, and, contrary to metabolic rates, there were no interaction effects of pH and food (3-factor PERMANOVAs: P > 0.05).

Gut DNA content was significantly different between fed and un-fed CVs in both experiments (3-factor PERMANOVAs: Pseudo-P < 0.05; Fig 5) but there were no effects, main nor interactions, of pH (3-factor PERMANOVAs: Pseudo-P > 0.05). However, in experiment 2 the increase in gut DNA content when offered food was only 27% of that in experiment 1 (Fig 5).

All data are available as S1 Table.

Table 1. Means ± standard deviations of temperature (T), salinity (S), total alkalinity (A_T), total dissolved inorganic carbon (C_T), total hydrogen ion scale pH (pH_T), and CO_2 partial pressure (pCO_2) during incubations.

| Treatment | T °C | S µmol Kg^{-1} | A_T µmol Kg^{-1} | C_T µmol Kg^{-1} | pH_T | pCO_2 µatm |
|-----------|------|----------------|-----------------|-----------------|------|------------|
| CII-III High pH | 4.41±0.23 | 34.68±0.13 | 2306±7 | 2136±6 | 8.110±0.026 | 335±22 |
| Low pH | 4.49±0.18 | 34.67±0.24 | 2303±11 | 2265±7 | 7.726±0.031 | 881±63 |
| CV exp1 High pH | 4.94±0.23 | 34.77±0.13 | 2312±8 | 2152±12 | 8.082±0.023 | 361±21 |
| Low pH | 4.94±0.23 | 34.89±0.19 | 2312±16 | 2295±11 | 7.653±0.055 | 1060±136 |
| CV exp2 High pH | 5.03±0.63 | 34.70±0.17 | 2307±10 | 2138±4 | 8.103±0.028 | 341±25 |
| Low pH | 5.18±0.91 | 34.60±0.24 | 2305±10 | 2265±7 | 7.731±0.029 | 871±58 |

doi:10.1371/journal.pone.0168735.t001

Table 2. Calanus glacialis CII-III. Result of the PERMANOVA on metabolic rates.

| Source | df | SS 10^{-3} | MS 10^{-3} | Pseudo-F | P  |
|--------|----|-------------|-------------|-----------|----|
| pH     | 1  | 1.48        | 1.48        | 2.73      | 0.115 |
| Food   | 1  | 1.32        | 1.32        | 24.1      | <0.001|
| pH x Food | 1  | 3.03        | 3.03        | 5.57      | 0.020 |
| Bottle(pH x Food) | 19  | 1.08        | 5.67        | 1.66      | 0.232 |
| Residuals | 12 | 4.10        | 3.42        |           |       |
| Total  | 34 | 3.33        |            |           |       |

doi:10.1371/journal.pone.0168735.t002

Table 3. Calanus glacialis CV experiment 1. Result of the PERMANOVA on metabolic rates.

| Source | df | SS 10^{-6} | MS 10^{-4} | Pseudo-F | P  |
|--------|----|-------------|-------------|-----------|----|
| pH     | 1  | 3.51        | 6.30        | 0.001     | 0.967 |
| Food   | 1  | 6.30        | 6.30        | 18.1      | 0.007 |
| pH x Food | 1  | 4.91        | 4.91        | 0.141     | 0.731 |
| Bottle(pH x Food) | 8   | 2.79        | 3.49        | 1.056     | 0.422 |
| Residuals | 19  | 6.28        | 3.30        |           |       |
| Total  | 30 | 1.57        |            |           |       |

doi:10.1371/journal.pone.0168735.t003
Fig 1. Carbon weight specific metabolic rates (means ± standard deviations) of *Calanus glacialis* copepodite stage II-III.

doi:10.1371/journal.pone.0168735.g001

Fig 2. Carbon weight specific metabolic rates (means ± standard deviations) of *Calanus glacialis* copepodite stage V, experiment 1 (A) and experiment 2 (B).

doi:10.1371/journal.pone.0168735.g002

Fig 3. RNA/DNA ratios (means ± standard deviations) in *Calanus glacialis* copepodite stage II-III.

doi:10.1371/journal.pone.0168735.g003
Discussion

The copepods responded more strongly to the difference in food level than to the difference in pH, both with respect to metabolic rate and RNA/DNA ratio. Copepods show strong functional responses, and significant increases in both metabolic rate and RNA/DNA ratio with prey concentration are not surprising [38–40]. Such observations may provoke the conclusion that future OA effects will be masked by much stronger variations caused by natural temporal and spatial variability of prey concentrations. Our results show, however, that such conclusions are premature, and that effects of pH and food level are not mutually exclusive. We found significant interactions between pH and food level. While metabolic rates in CII-CIIIs were 78% higher in fed than unfed individuals under present day conditions (high pH), the increase was 195% in CII-CIIIs kept at low pH—a 2.5 times greater increase. This interaction was absent for RNA/DNA, so the differences in metabolic rates were clearly not a reaction to changing biosynthesis at low pH per se but rather a reaction to increased metabolic costs per unit of biosynthesis. Interestingly, we did not observe this difference in the CVs. In experiment
metabolic rates increased by 56% and 58%, in high and low pH respectively, while in experiment 2 we did not observe any increase at all possibly because the copepods fed at very low rates (as indicated by only a minor increase in gut DNA in individuals offered food). Conclusively, the results show that future OA may change the metabolic costs of biosynthesis in *C. glacialis* and that this change may be stage dependent.

Previous studies have shown that environmental stress can change copepods' metabolic costs of biosynthesis. In copepods, the increase in metabolism during feeding (i.e the specific dynamic action; [41]) is caused partly by an increased energetic demand of overcoming fluid drag during the generation of feeding currents [42], but to a larger extent by increased costs of growth associated with protein turnover during digestion, assimilation, and biosynthesis [18, 43]. Accordingly, copepods experiencing prey of poor nutritional quality exhibit significantly lowered specific dynamic action [44]. Maintenance of cellular acid/base balance, the process most likely to be affected by decreasing external pH [45], would normally be assigned a maintenance role, and should as such be unrelated to the costs of growth [46, 47]. Nevertheless, environmental stress may induce significant energetic re-allocation so that resources are moved from anabolic processes involved in growth to accommodate increased maintenance requirements [46, 48] as has been demonstrated in mussels reacting to extreme temperatures [49].

Another calanoid species, *Pseudocalanus acuspes*, have shown interactions between food level and pH similar to the ones found here for *C. glacialis* [50]; While a population in the Kongsfjord showed no change due to pH$_T$, a population in the boreal Gullmarsfjord, Swedish west coast, showed both main effects of pH and interacting effects with food concentration on ingestion rate and metabolic rate [50]. In this study the magnitude of the specific dynamic action (measured as the slope of the linear relation between rates of ingestion and metabolism) more than doubled in individuals from the boreal population subjected to OA ranging from pH$_T$ 7.95 to pH$_T$ 7.47. Costs of growth have also been shown to increase during salinity stress. Studies by Calliari et al. [51] have shown significant increases in the ratio between metabolic rate and egg production during salinity stress in the boreal copepod species *Acartia clausi*. OA has been shown to inflict increased protein damage connected to decreased extracellular pH in other crustaceans such as the Norway lobster, *Nephrops norvegicus* [52]. Should such damage also occur in copepods, it could be responsible for increased specific dynamic action because increased damage would lead to increased protein repair, which in turn would incur increased energetic expenses of protein synthesis.

We did not observe any main effects of OA on the metabolism in either developmental stage. Other calanoid copepods have shown significant direct metabolic effects of OA in the range tested here. Metabolic rate increased significantly from pH$_{NBS}$ 8.18 to 7.83 in *Centropages tenuiremis* (no developmental stage indicated) and so did rates in *Pseudocalanus acuspes* females from pH$_T$ 8.06 to 7.75 [53, 54]. In *Acartia grani* females, metabolic rates doubled from pH$_T$ 8.06 to pH$_T$ 7.66 (although low replication rendered the difference non-significant) whereas no clear effect was observed in female *A. clausi* exposed to pH$_T$ 8.03 and pH$_T$ 7.83 in a combined OA and temperature experiment [55, 56]. The lack of response of *C. glacialis* CVs in our study have been shown to last during longer term incubations (62 d) of *C. glacialis* CVs and *C. hyperboreus* CVs and females [17]. Similarly, CVs of the sibling species *C. finmarchicus* do not respond to OA in the range tested here. While metabolic rates were found to increase linearly in a very wide range of OA from pH$_T$ 8.02 to pH$_T$ 7.16 in culture reared CVs of this species [57], this change would not be sufficient to create any significant difference by the OA scenario tested in the present study (pH$_T$ 8.10–7.70). Accordingly, wild caught *C. finmarchicus* CVs did not show any difference between pH$_T$ 7.92 and pH$_T$ 7.51 in a later study [58]. To our knowledge, no previous studies have investigated metabolic OA effects in *Calanus* CII-CIIIs.
While the increase in metabolism during feeding was affected by OA in the CII-CIIIs, we did not observe this in the CVs. *Calanus* exhibit many stage specific differences in their metabolism and in this respect the CV stage stands out. While somatic growth is the main purpose for early copepodite stages, metabolism is reconfigured to target lipid storage in CVs for the preparation for hibernation. Hence, the lipid mass of CVs approaching hibernation is ten-fold higher than of CIVs [59]. During hibernation, *C. glacialis* CVs may experience extracellular pH approaching 5.5, a possible result of metabolic depression [60]. It is therefore quite conceivable that mechanisms to handle low pH could be activated in this particular stage as part of the general physiological re-organisation to accommodate hibernation. This would render CVs particularly unresponsive to ambient pH. Furthermore, it would be evolutionarily beneficial to avoid the activation of such, possibly costly, mechanisms in earlier developmental stages where they would not be needed.

Even when restricted to specific developmental stages, increased costs of growth may induce reduced life time fitness and thus influence population development [46]. As a rough estimate ca. 70% of assimilated energy is allocated to growth in copepods (0.70 net growth efficiency) [18], with the remainder (30%) used to cover costs of growth by respiration. When *C. glacialis* CII-CIIIs experience a 2.5 times higher increase in metabolic costs of growth during OA, 75% (2.5 x 30%) of assimilated energy is lost by respiration with only 25% left for growth. Such diminished growth rates in the early copepodite stages will obviously have significant effects on the development of any copepod population. Certainly, changes in spring production can influence summer biomass. Long-term sampling series in the North Sea have shown that years with low juvenile growth during spring results in lower summer biomass than years with higher juvenile growth [61]. Similar variations have also been observed in the sub-Arctic Pacific *Neocalanus plumchrus* population. This population experiences significant inter-decadal variations in summer biomass [62]. While both differences in the timing of spawning and differences in larval mortality was hypothesised as the origin of these variations, a third hypothesis put forward was that variations in copepodite growth rate influenced the timing of peak biomass among years.

The study presented here accentuate the elusiveness of biological OA effects. Only a few studies have investigated the possibility of interactions of OA effects with food level, but if the relationship found in the present study is common to many species, it makes predictions of general OA responses exceedingly difficult. Moreover, our study shows that OA effects vary not only among taxa, but also among different developmental stages (See also the work by Dupont et al. [63]). This warrant a warning against premature conclusions that specific species are tolerant to OA based on single measurements of specific processes such as fecundity of adults or effects on specific developmental stages without considering effects in different environments (such as e.g. food level) and through the entire ontogenesis.

**Supporting Information**

**S1 Table. Raw data from all three experiments.** Metabolic rates (μgC μgC⁻¹ d⁻¹) and RNA/DNA ratios of CII-IIIIs and CVs and gut DNA content (μgDNA ind⁻¹) of CVs at the 4 treatments high pH/No food, high pH/Food, low pH/No food, and low pH/Food. (XLSX)

**Acknowledgments**

We would like to thank the administrative and technical staff at the Sverdrup Station, Ny-Ålesund and the Kings Bay Marine Lab, Ny-Ålesund for their invaluable support during experiments.
Author Contributions

Conceptualization: PT AB CH E. Guscelli.
Data curation: PT AB CH E. Guscelli.
Formal analysis: PT AB.
Funding acquisition: PT CH.
Investigation: PT AB CH E. Guscelli E. Gorokhova AF.
Methodology: PT AB CH E. Guscelli.
Project administration: PT AB.
Resources: PT AB CH E. Guscelli E. Gorokhova AF.
Supervision: PT.
Validation: PT AB CH E. Guscelli E. Gorokhova AF.
Visualization: PT AB CH E. Guscelli E. Gorokhova AF.
Writing – original draft: PT.
Writing – review & editing: PT AB CH E. Guscelli E. Gorokhova AF.

References

1. Fabry VJ, McClintock JB, Mathis JT, Grebmeier JM. Ocean acidification at high latitudes: The bellweather. Oceanography. 2009; 22(4): 160–71.
2. Steinacher M, Joos F, Frölicher TL, Plattner GK, Doney SC. Imminent ocean acidification in the Arctic projected with the NCAR global coupled carbon cycle-climate model. Biogeosciences. 2009; 6(4): 515–33.
3. AMAP. AMAP Assessment 2013: Arctic Ocean acidification. Oslo: Arctic Monitoring and Assessment Programme; 2013.
4. Fransson A, Chierici M, Anderson LG, Bussmann I, Kattner G, Peter Jones E, et al. The importance of shelf processes for the modification of chemical constituents in the waters of the Eurasian Arctic Ocean: implication for carbon fluxes. Cont Shelf Res. 2001; 21(3): 225–42.
5. Portner H-O. Oxygen- and capacity-limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems. J Exp Biol. 2010; 213(6): 881–93. doi: 10.1242/jeb.037523 PMID: 20190113
6. Finke DL, Denno RF. Predator diversity dampens trophic cascades. Nature. 2004; 429(6990): 407–10. doi: 10.1038/nature02554 PMID: 15164061
7. Mumm N, Auel H, Hanssen H, Hirche HJ. Breaking the ice: large-scale distribution of mesozooplankton after a decade of Arctic and transpolar cruises. Polar Biol. 1998; 20: 189–97.
8. Weydmann A, Coelho NC, Serrão EA, Burzyński A, Pearson GA. Pan-Arctic population of the keystone copepod Calanus glacialis. Polar Biol. 2016: 1–8.
9. Arkmværn G, Daase M, Eiane K. Dynamics of coexisting Calanus finmarchicus, Calanus glacialis and Calanus hyperboreus populations in a high-Arctic fjord. Polar Biol. 2005; 28(7): 528–38.
10. Lowry L. Foods and feeding ecology. In: Montague JJCowles CJ, editors. Bowhead whales. Lawrence, KS: Society of Marine Mammalogy, Allen Press.; 1993.
11. Karnovsky NJ, Kwaśniewski S, Węsławski JM, Walkusz W, Beszczyńska-Möller A. Foraging behavior of little auks in a heterogeneous environment. Mar Ecol Prog Ser. 2003; 253: 289–303.
12. Hop H, Gjøsåetet H. Polar cod (Boreogadus saida) and capelin (Mallotus villosus) as key species in marine food webs of the Arctic and the Barents Sea. Mar Biol Res. 2013; 9(9): 878–94.
13. Bailey A, Thor P, Brownman HI, Fields DM, Runge J, Vermont A, et al. Early life stages of the Arctic copepod Calanus glacialis are unaffected by increased seawater pCO2. ICES J Mar Sci. 2016.
14. Lewis CN, Brown KA, Edwards LA, Cooper G, Findlay HS. Sensitivity to ocean acidification parallels natural pCO2 gradients experienced by Arctic copepods under winter sea ice. Proc Natl Acad Sci U S A. 2013; 110(51): E4960–E7. doi: 10.1073/pnas.1315162110 PMID: 24297880

15. Weydmann A, Sereide JE, Kwasniewski S, Widdicombe S. Influence of CO2-induced acidification on the reproduction of a key Arctic copepod Calanus glacialis. J Exp Mar Biol Ecol. 2012; 428: 39–42.

16. Hildebrandt N, Sartoris FJ, Schulz KG, Riebesell U, Niehoff B. Ocean acidification does not alter grazing in the calanoid copepods Calanus finmarchicus and Calanus glacialis. ICES J Mar Sci. 2016; 73(3): 927–36.

17. Hildebrandt N, Niehoff B, Sartoris FJ. Long-term effects of elevated CO2 and temperature on the Arctic calanoid copepods Calanus glacialis and C. hyperboreus. Mar Pollut Bull. 2014; 80(1–2): 59–70. doi: 10.1016/j.marpolbul.2014.01.050 PMID: 24529340

18. Kierboe T, Mehlenberg F, Hamburger K. Bioenergetics of the planktonic copepod Acartia tonsa: relation between feeding, egg production and respiration, and composition of specific dynamic action. Mar Ecol Prog Ser. 1985; 26: 85–97.

19. Thor P, Cervetto G, Besiktepe S, Ribera-Mayacas E, Tang KW, Dam HG. Influence of two different green algal diets on specific dynamic action and incorporation of carbon into biochemical fractions in the copepod Acartia tonsa. J Plankton Res. 2002; 24: 293–300.

20. Vidal J. Physioecology of zooplankton. III. Effects of phytoplankton concentration, temperature, and body size on the metabolic rate of Calanus pacificus. Mar Biol. 1980; 56: 195–202.

21. Stumpf M, Hu MY, Melzner F, Gutowska MA, Dorey N, Himmerkus N, et al. Acidified seawater impacts sea urchin larval pH regulatory systems relevant for calcification. Proc Natl Acad Sci U S A. 2012; 109(44): 18192–7. doi: 10.1073/pnas.1209174109 PMID: 23077257

22. Dahlhoff EP. Biochemical indicators of stress and metabolism: Applications for marine ecological studies. Annu Rev Physiol. 2004; 66(1): 183–207.

23. Collins M, Knutti R, Arblaster J, Dufresne J-L, Fichefet T, Friedlingstein P, et al. Long-term Climate Change: Projections, Commitments and Irreversibility. In: Stocker TF, Qin D, Plattner G-K, Tignor M, Allen SK, Boschung J, et al., editors. The Physical Science Basis Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change: Cambridge University Press; 2014.

24. Fransson A, Chierici M, Hop H, Findlay HS, Kristiansen S, Wold A. Late winter-to-summer change in ocean acidification state in Kongsfjorden, with implications for calcifying organisms. Polar Biol. 2016; 1–17.

25. Nielsen TG, Kjellerup S, Smolina I, Hoarau G, Lindeque P. Live discrimination of Calanus glacialis and C. finmarchicus females: can we trust phenological differences? Mar Biol. 2014; 161(6): 1299–306.

26. Strathmann RR. Estimating the organic carbon content of phytoplankton from cell volume or plasma volume. Limnol Oceanogr. 1967; 12: 411–8.

27. Dickson AG, Sabine CL, Christian JR. Guide to best practices for ocean CO2 measurements: PICES; 2007.

28. Pierrot D, Lewis E, Wallace DWR. MS Excel program developed for CO2 system calculations. ORNL/CDIAC-105a. Oak Ridge, Tennessee: Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, US Department of Energy; 2006.

29. Dickson AG. Standard potential of the reaction: AgCl(s) + 12H2(g) = Ag(s) + HCl(aq), and the standard acidity constant of the ion HSO4− in synthetic sea water from 273.15 to 318.15 K. The Journal of Chemical Thermodynamics. 1990; 22(2): 113–27.

30. Mehrbach C, Culberson CH, Hawley JE, Pytkowicz RM. Measurement of the apparent dissociation constant of carbonic acid in seawater at atmospheric pressure. Limnol Oceanogr. 1973; 18(6): 897–907.

31. Dickson AG, Millero FJ. A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. Deep Sea Research Part A Oceanographic Research Papers. 1987; 34(10): 1733–43.

32. Madsen SD, Nielsen TG, Hansen BW. Annual population development and production by Calanus finmarchicus, C. glacialis and C. hyperboreus in Disko Bay, western Greenland. Mar Biol. 2001; 139(1): 75–93.

33. Omori M, Ikeda T. Methods in marine zooplankton ecology. New York: Wiley; 1984.

34. Gorokhova E. Toxic cyanobacteria Nodularia spumigena in the diet of Baltic mysids: Evidence from molecular diet analysis. Harmful Algae. 2009; 8(2): 264–72.

35. Gorokhova E, Kyle M. Analysis of nucleic acids in Daphnia: development of methods and ontogenetic variations in RNA-DNA content. J Plankton Res. 2002; 24(5): 511–22.
36. Caldarone EM, Clemmensen CM, Berdalet E, Miller TJ, Folkvord A, Holt GJ, et al. Intercalibration of four spectrotirolurometric protocols for measuring RNA/DNA ratios in larval and juvenile fish. Limnology and Oceanography: Methods. 2006; 4(5): 153–63.
37. Anderson MJ. A new method for non-parametric multivariate analysis of variance. Austral Ecol. 2001; 26(1): 32–46.
38. Dam HG, Lopes RM. Omnivory in the calanoid copepod *Temora longicornis*: feeding, egg production and egg hatching rates. J Exp Mar Biol Ecol. 2003; 292(2): 119–37.
39. Hansen B, Tande KS, Berggreen U. On the trophic fate of *Phaeocystis pouchetii* (Hariot). III. Functional responses in grazing demonstrated in juvenile stages of *Calanus finmarchicus* (Copepoda) fed diatoms and *Phaeocystis*. J Plankton Res. 1990; 12(6): 1173–87.
40. Wendt I, Thor P. Influence of prey species and concentration on egg production efficiency and hatching success in Dana (Copepoda, Calanoida). Crustaceana. 2015; 88(6): 675–87.
41. Jobling M. Towards an explanation of specific dynamic action. J Fish Biol. 1983; 23: 549–56.
42. Svetlichny LS, Hubareva ES. The energetics of *Calanus euxinus*: locomotion, filtration of food and specific dynamic action. J Plankton Res. 2005; 27(7): 671–82.
43. Thor P. Relationship between Specific Dynamic Action and protein deposition in calanoid copepods. J Exp Mar Biol Ecol. 2000; 24: 171–82.
44. Thor P. Specific dynamic action and carbon incorporation in *Calanus finmarchicus* copepodites and females. J Exp Mar Biol Ecol. 2002; 272: 159–69.
45. Zhang D, Li S, Wang G, Guo D, Xing K, Zhang S. Biochemical responses of the copepod *Centropages tenuiremis* to CO2-driven acidified seawater. Water, Science, and technology. 2012; 65: 30–7.
46. Calow P, Sibly RM. A Physiological Basis of Population Processes: Ecotoxicological Implications. Funct Ecol. 1990; 4(3): 283–8.
47. Pan T-CF, Applebaum SL, Manahan DT. Experimental ocean acidification alters the allocation of metabolic energy. Proc Natl Acad Sci U S A. 2015.
48. Sokolova IM, Frederich M, Bagwe R, Lannig G, Sukhotin AA. Energy homeostasis as an integrative tool for assessing limits of environmental stress tolerance in aquatic invertebrates. Mar Environ Res. 2012; 79: 1–15. doi: 10.1016/j.marenvres.2012.04.003 PMID: 22622075
49. Petes LE, Menge BA, Murphy GD. Environmental stress decreases survival, growth, and reproduction in New Zealand mussels. J Exp Mar Biol Ecol. 2007; 351(1–2): 83–91.
50. Thor P, Oliva EO. Ocean acidification elicits different energetic responses in an Arctic and a boreal population of the copepod *Pseudocalanus acuspes*. Mar Biol. 2015; 162: 799–807.
51. Calliari D, Andersen CM, Thor P, Gorokhova E, Tiselius P. Salinity modulates the energy balance and reproductive success of co-occurring copepods *Acartia tonsa* and *A. clausi* in different ways. Mar Ecol Prog Ser. 2006; 312: 177–88.
52. Hernroth B, Sköld HN, Wiklander K, Jutfelt F, Baden S. Simulated climate change causes immune suppression and protein damage in the crustacean *Nephrops norvegicus*. Fish Shellfish Immunol. 2012; 33(5): 1095–101. doi: 10.1016/j.fsi.2012.08.011 PMID: 22974540
53. Thor P, Dupont S. Transgenerational effects alleviate severe fecundity loss during ocean acidification in a ubiquitous planktonic copepod. Glob Change Biol. 2015;
54. Li W, Gao K. A marine secondary producer respires and feeds more in a high CO2 ocean. Mar Pollut Bull. 2012; 64(4): 699–703. doi: 10.1016/j.marpollbul.2012.01.033 PMID: 22364924
55. Isari S, Zervoudaki S, Saiz E, Pelejero C, Peters J. Copepod vital rates under CO2-induced acidification: a calanoid species and a cyclopoid species under short-term exposures. J Plankton Res. 2015.
56. Zervoudaki S, Frangoulis C, Giannoudi E, Krasakopoulou E. Effects of low pH and raised temperature on egg production, hatching and metabolic rates of a Mediterranean copepod species (*Acartia clausi*) under oligotrophic conditions. Mediterr Mar Sci. 2015; 16(4): 48–56. doi: 10.1080/11280320.2015.103156
57. Pedersen SA, Hakedal OJ, Salaberry I, Tagliati A, Gustavsson LM, Jenssen BM, et al. Multigenerational exposure to ocean acidification during food limitation reveals consequences for copepod scope for growth and vital rates. Environ Sci Technol. 2014; 48(20): 12275–84. Epub 2014/09/17. doi: 10.1021//es501581 PMID: 25225957
58. Runge JA, Fields DM, Thompson CRS, Shema SD, Bjelland RM, Durfl CMF, et al. End of the century CO2 concentrations do not have a negative effect on vital rates of *Calanus finmarchicus*, an ecologically critical planktonic species in North Atlantic ecosystems. ICES J Mar Sci. 2016; 73(3): 937–60.
59. Scott LC, Kwasniewski S, Falk-Petersen S, Sargent RJ. Lipids and life strategies of *Calanus finmarchicus*, *Calanus glacialis* and *Calanus hyperboreus* in late autumn, Kongfjorden, Svalbard. Polar Biol. 2000; 23(7): 510–6.
60. Freese D, Niehoff B, Søreide JE, Sartoris FJ. Seasonal patterns in extracellular ion concentrations and pH of the Arctic copepod *Calanus glacialis*. Limnol Oceanogr. 2015; 60(6): 2121–9.

61. Clark RA, Frid CLJ, Nicholas KR. Long-term, predation-based control of a central-west North Sea zooplankton community. ICES J Mar Sci. 2003; 60(2): 187–97.

62. Mackas DL, Goldblatt R, Lewis AG. Interdecadal variation in developmental timing of Neocalanus plumchrus populations at Ocean Station P in the subarctic North Pacific. Can J Fish Aquat Sci. 1998; 55(8): 1878–93.

63. Dupont S, Dorey N, Thorndyke M. What meta-analysis can tell us about vulnerability of marine biodiversity to ocean acidification? Estuarine, Coastal and Shelf Science. 2010; 89(2): 182–5.