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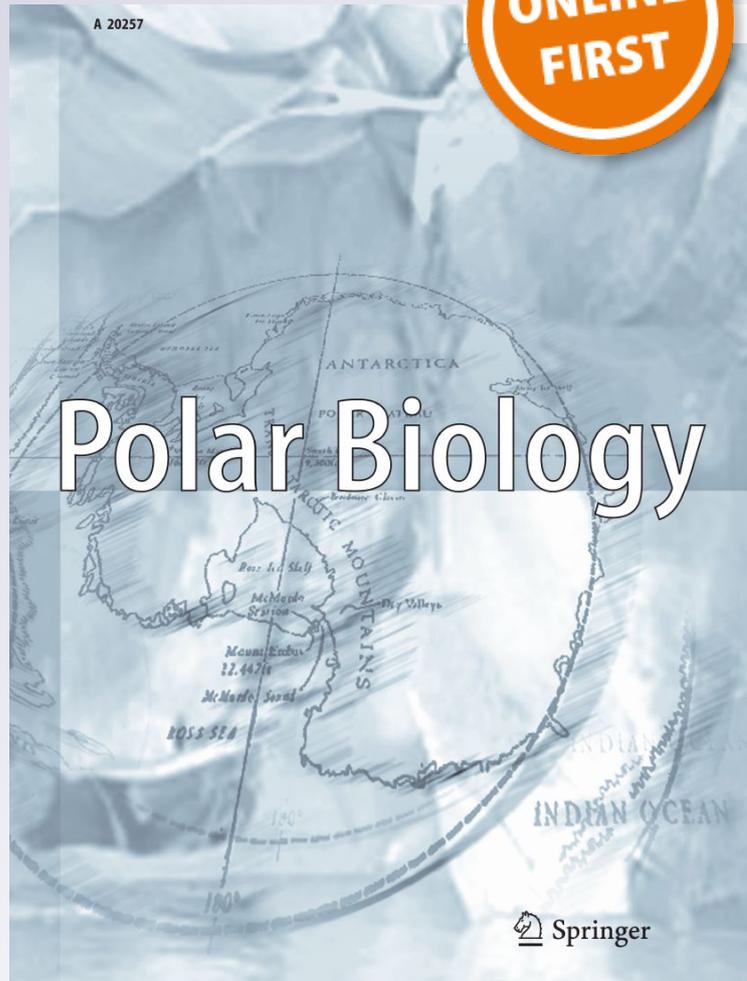
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Overwintering individuals of the Arctic krill *Thysanoessa inermis* appear tolerant to short-term exposure to low pH conditions

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Abstract Areas of the Arctic Ocean are already experiencing seasonal variation in low pH/elevated $p\text{CO}_2$ and are predicted to be the most affected by future ocean acidification (OA). Krill play a fundamental ecological role within Arctic ecosystems, serving as a vital link in the transfer of energy from phytoplankton to higher trophic levels. However, little is known of the chemical habitat occupied by Arctic invertebrate species, and of their responses to changes in seawater pH. Therefore, understanding krill's responses to low pH conditions has important implications for the prediction of how Arctic marine communities may respond to future ocean change. Here, we present natural seawater carbonate chemistry conditions found in the late polar winter (April) in Kongsfjord, Svalbard (79°North) as well as the response of the Arctic krill, *Thysanoessa inermis*, exposed to a range of low pH conditions. Standard metabolic rate (measured as oxygen consumption) and energy metabolism markers

(incl. adenosine triphosphate (ATP) and L-lactate) of *T. inermis* were examined. We show that after a 7 days experiment with *T. inermis*, no significant effects of low pH on MO_2 , ATP and L-lactate were observed. Additionally, we report carbonate chemistry from within Kongsfjord, which showed that the more stratified inner fjord had lower total alkalinity, higher dissolved inorganic carbon, $p\text{CO}_2$ and lower pH than the well-mixed outer fjord. Consequently, our results suggest that overwintering individuals of *T. inermis* may possess sufficient ability to tolerate short-term low pH conditions due to their migratory behaviour, which exposes *T. inermis* to the naturally varying carbonate chemistry observed within Kongsfjord, potentially allowing *T. inermis* to tolerate future OA scenarios.

Keywords *Euphausiacea* · Arctic Ocean · Kongsfjord · Ocean acidification · Ocean change · Crustaceans

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Introduction

Specific ocean regions have been highlighted as high priority areas for research, as these are predicted to experience a widespread undersaturation of CaCO_3 , low pH and elevated $p\text{CO}_2$ by mid-twenty-first century (Fabry et al. 2008; Steinacher et al. 2009). One such area of concern is the Arctic Ocean, where the largest change in pH (0.3–0.5 units) is expected to occur (Steinacher et al. 2009) and seasonal undersaturation of aragonite (Ω aragonite = <0.7–1) with subsequent low pH and high $p\text{CO}_2$ has been documented (Bates et al. 2009). Shelf regions of the Arctic are susceptible to changes in oceanic and atmospheric conditions, typically through the variation in Atlantic water intrusion and glacial meltwater (Cottier

et al. 2005). Fjords are considered the link between ocean and land via cross-shelf exchange with fjord dynamics seen to actively respond to variation in these conditions. Thus, the properties of water masses in Arctic fjords, especially along the west coast of Svalbard make the area a particularly good indicator of change (Cottier et al. 2005). The Arctic fjord of Kongsfjord in West Svalbard (Norway) is a region that experiences seasonal variations in dominant water masses (Cottier et al. 2005). The fjord is influenced by Arctic and Atlantic currents, while receiving large amounts of freshwater from melting glaciers in the summer (Hop et al. 2002; Cottier et al. 2005; Buchholz et al. 2010). This combination of different water masses creates seasonal gradients of temperature, salinity and density both vertically and horizontally throughout the fjord (Weslawski et al. 2000; Hop et al. 2002; Cottier et al. 2005).

Despite the fact that Kongsfjord has been the site of many ocean acidification (OA) laboratory and mesocosm investigations (Findlay et al. 2010; Lischka and Riebesell 2012; Niehoff et al. 2013; Riebesell et al. 2013), there are limited studies that combine observations of natural conditions in seawater chemistry within the fjord, particularly $p\text{CO}_2$ and pH, and relate these to an organism's response to natural variation in pH/ $p\text{CO}_2$ and future conditions (Fabry et al. 2009; Comeau et al. 2012; Aguilera et al. 2013; Lewis et al. 2013). As Kongsfjord experiences variations in water mass properties, animals within the pelagic realm are more likely to experience a range of seawater conditions (Hop et al. 2002; Buchholz et al. 2010; Comeau et al. 2012). In fact, pH at depth (200–300 m) in Kongsfjord has been recorded to range between 8.13 and 7.68, fluctuating over a monthly period (Lischka and Riebesell 2012). Additionally, the vast majority of Arctic low pH/elevated $p\text{CO}_2$ studies have been carried out in summer, and therefore April (polar spring) OA studies using overwintering organisms in the Arctic are rare. Overwintering organisms may be particularly sensitive to environmental changes, as low food availability may increase their sensitivity to stress (Comeau et al. 2012; Lischka and Riebesell 2012; Lewis et al. 2013).

Krill are one of the most abundant first order consumers in Arctic ecosystems (Falk-Petersen et al. 2000; Hop et al. 2002). As a dominant member of the zooplankton community, krill play a vital role in the transfer of energy between primary producers and higher trophic levels (Hop et al. 2002). High lipid content and abundance make krill an important prey item for fish, sea birds and marine mammals in the Arctic (Hop et al. 2002; Dahl et al. 2003). In addition to their role in the Arctic food web, euphausiid species have been used as indicators of advection and warming in Kongsfjord and are considered good indicators of change due to their mid-trophic level position (Buchholz et al. 2010). Therefore, understanding krill responses to OA

is essential for predicting the future of Arctic ecosystems. The Kongsfjord zooplankton including krill, experience variations in seawater chemistry on a daily and seasonal basis due to changes in water mass dominance and migratory behaviour (Weslawski et al. 2000; Buchholz et al. 2010; Agersted et al. 2011). Large aggregations of krill, possibly due to hydrological forces such as estuarine circulation patterns, have been found in Kongsfjord at the glacier fronts during Arctic summer, June–August (Weslawski et al. 1994, 2000; Hop et al. 2002). Here, meltwater can significantly lower the pH of the seawater as a result of dilution (Azetsu-Scott et al. 2010).

In general, crustaceans should be more tolerant to ocean acidification due to the fact that they inhabit areas with fluctuating environmental conditions; however, to date physiological studies have shown that polar species may struggle to compensate for changes set by low pH (Whiteley 2011; Thor and Dupont 2015; Bailey et al. 2017). Due to the potential tolerance level of crustaceans, it is necessary to understand organism behaviour, life history and ecology in relation to the environmental conditions in which they live to assess possible sensitivity in a changing Arctic ecosystem. Zooplankton, in particular those with migratory behaviours, may have evolved to withstand predicted Arctic conditions based on their exposure to a range of $p\text{CO}_2$ /pH conditions on a daily basis (Lewis et al. 2013), however, very few studies address both the natural and predicted future pH conditions when looking at organism response.

Previous works have suggested that species and populations living in elevated $p\text{CO}_2$ habitats (e.g. deep-sea, CO_2 vents, upwelling zones) are more tolerant to elevated $p\text{CO}_2$ conditions ($>900 \mu\text{atm}$) than their counterparts living in habitats with lower $p\text{CO}_2$ (Maas et al. 2012; Calosi et al. 2013b; Pespenti et al. 2013). In particular, deep-sea copepods from the subarctic North Pacific were found to have a higher tolerance to mortality in high $p\text{CO}_2$ conditions than shallow living sub-tropical copepods (Watanabe et al. 2006). Vertically migrating Arctic copepods have been shown to experience a range of $p\text{CO}_2$ conditions ($>140 \mu\text{atm}$) as they make daily movements, with a minimum $p\text{CO}_2$ of $240 \mu\text{atm}$ in the surface waters and maximum $p\text{CO}_2$ ($564.2 \mu\text{atm}$) at depth (Lewis et al. 2013). Due to this movement and exposure to varying $p\text{CO}_2$ conditions, elevated $p\text{CO}_2$ (700 and $1000 \mu\text{atm}$) had no significant effect on the mortality of adults of the copepods *Calanus glacialis* and *Calanus hyperboreus* in the high Canadian Arctic. In contrast, surface water dwelling adult copepods of *Oithona similis* experienced significant increases in mortality due to elevated $p\text{CO}_2$ as they are exposed to a smaller range of $p\text{CO}_2$ conditions ($<75 \mu\text{atm}$) and vertical migrations are minimal in this species (Lewis et al. 2013).

As a pelagic species that exhibits migratory behaviour, Arctic krill *Thysanoessa inermis*, is one of the most important zooplankton within Kongsfjord (Hop et al. 2006) and has a life span of three to four years in the Arctic with spawning taking place just after the start of the spring bloom (Falk-Petersen et al. 2000). Due to shortages of food availability in the winter months, krill have adapted to store large amounts of lipids as wax esters and triacylglycerols, taking advantage of the short intense periods of primary productivity to rapidly increase in weight from March to May (Sargent and Falk-Petersen 1981; Falk-Petersen et al. 2000). The large lipid reserves are enough to sustain body function in *T. inermis* throughout the winter with no food intake, with lipid stores reserved for either spring growth or reproduction (Sargent and Falk-Petersen 1981).

In spite of being an integral part of Arctic ecosystems, very little is known about krill responses to low pH/elevated $p\text{CO}_2$ conditions with most studies centred on Antarctic and Northern Atlantic krill species. Moreover, most krill investigations related to OA have focused on egg hatching, development and mortality. A study on the physiological responses of the Antarctic krill, *Euphausia superba*, to elevated $p\text{CO}_2$ showed an increase in ingestion rates, nutrient release rates and metabolic enzyme activity at 750 μatm (Saba et al. 2012). Kawaguchi et al. (2013) demonstrated that *E. superba* hatching rates were significantly affected at 1250 and 1500 μatm of $p\text{CO}_2$ and no hatching occurred at 1750 and 2000 μatm $p\text{CO}_2$. In addition, development of *E. superba* was shown to be severely inhibited before gastrulation at 2000 μatm , though the krill appear to be able to develop normally up to 1000 μatm , possibly as the result of adaptation to low pH/elevated $p\text{CO}_2$ conditions found in the natural environment (Kawaguchi et al. 2011).

A physiological and biochemical approach is necessary to further our understanding of organism response to environmental change (Pörtner et al. 1999; Somero 2002). Evidence of physiological tolerance to low pH/elevated $p\text{CO}_2$ based on exposure to environmental gradients has been observed in oxygen minimum zones. Shelled pteropods are considered to be particularly sensitive to OA due to their aragonite shells. However, metabolic rates and ammonia excretion, as indicators of physiological response, were measured in pteropod species after exposure to low pH/elevated $p\text{CO}_2$ (1000 μatm) (Maas et al. 2012). *Hyalocylis striata*, *Clio pyramidata*, *Cavolinia longirostris* and *Creseis virgule* migrate naturally into oxygen minimum zones with high $p\text{CO}_2$ and showed no effect of low pH/elevated $p\text{CO}_2$ (Maas et al. 2012). Conversely, low pH/elevated $p\text{CO}_2$ and temperature negatively affected whole organism and cellular physiology of *Littorina littorea* when considering complex responses to environmental change such as metabolic rates, adenylate

energy nucleotide concentrations and end-product metabolite concentrations (Melatunan et al. 2011).

This study aims to investigate whole organism and cellular physiological responses to exposure to low pH/elevated $p\text{CO}_2$ of overwintering individuals of an understudied, yet ecologically important Arctic krill species from a fjord environment that would be expected to have naturally variable carbonate chemistry. There has been no investigation to date, where an integrated whole and cellular organism level approach (i.e. the characterization of metabolic rates in addition to cellular aerobic and anaerobic metabolite accumulation) has been used to examine Arctic krill under low pH/elevated $p\text{CO}_2$ conditions. By investigating overwintering *T. inermis*' short-term biological responses to low pH/elevated $p\text{CO}_2$ conditions we hypothesise that krill may be able to withstand short-term changes in pH due to their migratory behaviour and pre-exposure to a range of pH conditions. This study provides insight into the future of krill in Arctic ecosystems during a potentially vulnerable stage of their life history.

Methods

Study area and field work

Kongsfjord is located on the west coast of Spitsbergen, Svalbard, Norway 79°N, 12°E (Fig. 1). It is an open Arctic fjord that is approximately 30 km long and 10 km wide, with depths in some areas reaching >300 m. Krill were collected from the centremost area of Kongsfjord (78°56'963N, 12°02'358E) on April 22, 2014 using the Kings Bay boat, *Tiesten*. Mesopelagic trawls were conducted for 30 min using a 200- μm WP2 zooplankton net, travelling an average speed of 1.5 knots. The net was

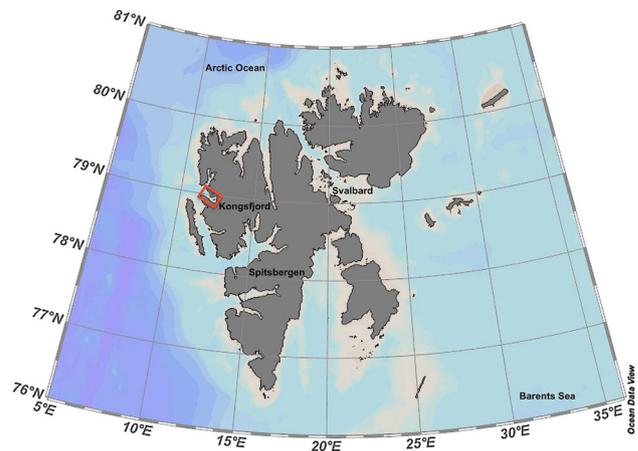


Fig. 1 The red box highlights the location of Kongsfjord on the west coast of Spitsbergen, Svalbard, Norway 79°N, 12°E. Map was created using Ocean Data View 4.6.2. (Color figure online)

trawled horizontally in depths ranging from 60 to 200 m. Krill were collected at depth (1.6 ± 0.03 °C), carefully and quickly removed from the net then transferred to sealed buckets containing seawater. Once back in Ny-Ålesund, the krill were transferred to a holding tank for one day to acclimatise to the laboratory setting then distributed randomly to the experimental tanks, where they were left for another day in ambient conditions (temperature 3.0 ± 0.2 °C, pH_{total} 8.03 ± 0.005 , dissolved oxygen $105.7 \pm 0.3\%$, salinity 35 ± 0.0) before CO_2 bubbling was started. The water in both the holding and experimental tanks was continuously pumped into the laboratory from the middle of Kongsfjord at 80 m depth. During this time, a sub-sample of the krill was taken for identification purposes. Krill were identified as adult individuals of *T. inermis* (3.1–61.3 mg WW), as abdominal spines were present, according to Boden et al. (1955), Nemoto (1966), Mauchline (1980) and Kathman et al. (1986). Water samples were collected on board the Kings Bay boat, *Tiesten*, on April 25th, 2014 at five stations throughout the fjord (Online Resource 1) for determining the natural conditions that the krill were experiencing at the time of the experiment. Conductivity, temperature and depth were recorded using a SAIV A/S CTD (Model SD204, Bergen, Norway) to create a profile of the water column at each station. 10-L Niskin bottles were lowered to depths ranging from the surface to 300 m (Online Resource 1) for water sample collection for total alkalinity (TA) and dissolved inorganic carbon (DIC) measurements. Water samples were stored in 50-mL glass bottles and treated with 20 μL of mercuric chloride (HgCl_2) for preservation for future analysis following standard protocols of Dickson et al. (2007).

Ocean acidification experiment

The seven-day laboratory experiment used a range of pH (four) conditions as suggested by Dupont and Pörtner (2013), similar to the approach used by Christen et al. (2013) to cover both present and future levels of seawater pH and $p\text{CO}_2$ in order to acquire a greater predictive ability on pH-dependent responses. The chosen range also follows future scenarios predicted for the Arctic Ocean as a decrease by 0.3–0.5 pH units could occur over the next century (Caldeira and Wickett 2003). The target pH levels (total scale, pH_{total} , calculated in CO2SYS version 2.1, Lewis and Wallace 1998) for the experiment were a control pH_{total} of 8.00 as this was the ambient pH of the fjord water that was pumped into the Kings Bay Laboratory; and target treatment levels of 7.75, 7.65 and 7.35 (equivalent to $p\text{CO}_2$ levels of 750, 1000 and 2000 μatm , respectively), mimicking both fjord conditions and future scenarios. The second lowest target pH of 7.65 (1000 μatm $p\text{CO}_2$) is reflective of winter conditions within Kongsfjord (Lischka

and Riebesell 2012), while the lowest pH treatment of 7.35 was chosen as a future value not presently observed within Kongsfjord, in order to test *T. inermis*' response to low pH beyond what they are currently exposed to. However, note that the measured values were slightly different (7.96, 7.70, 7.65 and 7.28) from the target pH values and we used the measured means in further discussion and analysis. Pure CO_2 was bubbled into header tanks and regulated by pH controllers (Aqua Digital pH 201, Precise Instruments, J & K Aquatics Ltd, North Petherton, UK). Each header tank fed water via black gas impermeable tubing into three replicate 5-L containers with each replicate housing 30 adult krill. *Thysanoessa inermis* is a known herbivore within Kongsfjord (Falk-Petersen et al. 2000) and diatom *Thalassiosira weissflogii* has been used as a food source in laboratory settings in previous experiments (Pinchuk and Hopcroft 2006; Dalpadado et al. 2008; Agersted et al. 2011). In the evening, krill were fed approximately 1000 cells mL^{-1} (16.7 μL per container) of Instant Algae Diatoms, *T. weissflogii* (Batch #14053 CCMP 1051/TW sp.) to mimic the amount of food available in the fjord at the time of the experiment (AWIPEV Underwater Observatory, https://cosyna-nodes.shinyapps.io/svl_ferrybox/). Krill were also consistently kept in the dark to mimic natural fjord conditions until data collection were carried out. Temperature, salinity, dissolved oxygen and pH were recorded using a hand-held probe (SevenGo Pro, Mettler-Toledo, Columbus, OH, USA) daily in the header tanks and calibrated every other day. Water samples for alkalinity (TA and DIC) were taken from the replicate tanks on the third, sixth and seventh day to limit the number of times tank lids were opened. The water samples were then treated with 20 μL of mercuric chloride (HgCl_2) to preserve for future analysis. pH was converted to total scale from pH measured on the NBS scale using CO2SYS (version 2.1, Lewis and Wallace 1998) so as to be compared to fjord pH_{total} that was calculated based on TA and DIC analysis.

Seawater chemistry

Seawater samples collected from the laboratory experiments were analysed for total alkalinity. Total alkalinity was measured by Hydrochloric (0.08 M) acid-titration using a seawater gran titrator (AS-ALK2, Apollo Sci-Tech Inc., Bogart, GA, USA) and a pH bench top metre (ORION 3 STAR, Thermo Fisher Scientific Inc., Waltham, MA, USA). Total alkalinity was measured in the seawater samples in duplicates of 12 mL. Water samples collected from Kongsfjord were analysed for both TA and DIC. Dissolved inorganic carbon was measured using a DIC analyser and CO_2 detector (AS-C3 and a LI-COR LI-7000 $\text{CO}_2/\text{H}_2\text{O}$ Analyzer, Apollo Sci-Tech Inc., Bogart, GA, USA). For both TA and DIC, Certified Reference Materials

(Dickinson Laboratory, University of California, Batch 137) were used to assess precision. Once values for TA and DIC were recorded, CO2SYS (Lewis and Wallace 1998) version 2.1 was used to calculate the values of $p\text{CO}_2$ for the laboratory samples along with $p\text{CO}_2$ and pH for the fjord seawater samples. The constants used for CO2SYS were from Mehrbach et al. (1973) (refitted by Dickson and Millero 1987). Water column profiles of temperature and salinity in Kongsfjord were constructed using SAIV A/S CTD (Model SD204, Bergen, Norway) data along with measured TA, DIC and calculated pH, $p\text{CO}_2$ in Ocean Data View (Version 4.6.2).

Determination of standard metabolic rate

Oxygen consumption rates (MO_2) of *T. inermis* were determined at the end of the 7-d exposure period and used as a proxy for standard metabolic rate, following the methods by Melatunan et al. (2011) and Donohue et al. (2012). Due to the small size of the krill, and in order to carry out individual tests, blacked-out screw cap micro-centrifuge tubes (1.5 mL) were used as respirometry chambers. Centrifuge tubes have been previously used as a gas tight (O_2) chamber over a 48-h period (Terai et al. 2002). Each tube was filled with double filtered (pore size 0.4 μm) water, to reduce the amount of background respiration within the chambers, taken from each individual krill's designated treatment to maintain the same pH level. Each filled chamber, while fully submerged, was swabbed with a cotton bud to remove any trapped air bubbles before the krill were placed into the chamber. Krill individuals were gently inserted into the micro-centrifuge tubes using a modified pipette that was cut to make the opening large enough for the krill, and then the tubes were quickly sealed. All these operations were undertaken under water. Once closed, the chambers were placed in a continuous-flow water bath on top of a magnetic stirrer plate. Each chamber contained a magnetic flea (0.5 mL) under a fine plastic mesh (0.5 mL) held within each cap of the tube to ensure appropriate mixing of the water, in order to maintain conditions homogeneous within the chamber. The amount of seawater in each chamber was calculated, taking into account the volume of the stirrer, mesh and individual krill using volume displacement. Each MO_2 trial (five in total) had 12 krill individuals, one from each container, and three blank chambers to measure background respiration. Oxygen concentration in the chamber ($\mu\text{mol L}^{-1}$) was measured approximately every 4 min during the 15 min incubation period, following a 10 min resting period to allow krill to recover from being inserted into the respirometry chambers. The length of incubation was determined by preliminary tests such that the krill did not experience hypoxic conditions (<80% saturation) so as to not cause undue stress (Storch et al. 2009). O_2 measurements

were recorded using an O_2 probe with a non-invasive fibre optic cable (Fibox 4 PSt 3, Pre Sens, Regensburg, Germany) that was placed on top of a prefixed oxygen sensor dot (Sensor Spots, Pre Sens) within each chamber. MO_2 was calculated using the delta of the O_2 level at the beginning and at the end of the incubation trial, minus the background respiration from the blanks. After each trial, krill were removed from the chambers, gently blotted then rapidly weighed; the cephalothorax and abdomen were separated, and individually frozen with liquid nitrogen. The abdomen was preserved for future biochemical assays. The krill were stored in Eppendorf tubes at -80°C in the Kings Bay Marine Lab freezer until the samples were shipped on dry ice to Plymouth University where they were stored again at -80°C until biochemical analyses were carried out.

Biochemical assays

The abdominal muscles of experimental krill were used for the biochemical assays. The tissue samples were weighed then prepared using 12 parts of 0.9 M perchloric acid to one part tissue sample. After the acid was introduced, the sample was sonicated (Misonix Microson Ultrasonic Cell Disruptor XL 2000, Qsonica LLC, Newtown, CT, USA) for 10 s. The sample solution was then centrifuged (Centrifuge 5418, Eppendorf AG, Hamburg, Germany) in a controlled temperature room (4°C) for 10 min at 14,000 rpm after which the supernatant was removed and three parts of potassium carbonate (K_2CO_3) to one part of the tissue sample was added. The supernatant and K_2CO_3 solution was again centrifuged for 10 min. The supernatant was removed, placed into a new Eppendorf tube and then stored at -80°C until biochemical analysis was conducted.

ATP concentration was determined using a commercial luciferase reagent kit (BioThema, Handen, Sweden, ATP Kit SL, 144-041). This reagent is a sustained light reagent, where certain concentrations of luciferase and luciferin will lead to an output of light in the presence of ATP, where the rate of light output is proportional to the concentration of ATP present. Derived from the kit instruction sheet, the method uses an internal standard as the rate of light output is dependent on the enzymatic activity of the luciferase which can be affected by several factors in ATP extracts like phosphate (Lundin 2000). Luminescence was measured using a luminometer (Pi-102, Hygiene LLC, Camarillo, CA, USA) using the slope of the reaction where the presence and absence of the internal ATP standard was used to determine ATP concentration.

L-lactate concentration was determined using a commercial kit (Trinity Biotech PLC, Bray, Co Wicklow, Ireland) in a 96-well plate format, using a plate reader (Versa Max Microplate, Molecular Devices Corp., Sunnyvale,

Table 1 Values (Mean \pm SD) for laboratory seawater chemistry per target pH treatment: pH (NBS scale), temperature ($^{\circ}$ C), salinity and total alkalinity (TA) were measured

Target pH	<i>N</i>	Measured pH (NBS)	pH _{total}	Temperature ($^{\circ}$ C)	Salinity	Total alkalinity (μ mol kg ⁻¹)	pCO ₂ (μ atm)
8.12	9	8.06 \pm 0.06 ^a	7.96 \pm 0.06 ^a	4.4 \pm 0.2	34.81 \pm 0.0	2386.5 \pm 14.5 ^a	488.4 \pm 82.2 ^a
7.85	9	7.79 \pm 0.06 ^b	7.70 \pm 0.07 ^b	4.6 \pm 0.3	34.81 \pm 0.0	2391.4 \pm 18.4 ^a	1010.5 \pm 219.6 ^b
7.75	9	7.75 \pm 0.10 ^b	7.65 \pm 0.10 ^b	4.5 \pm 0.3	34.81 \pm 0.0	2390.8 \pm 13.7 ^a	1049.4 \pm 282.8 ^b
7.45	9	7.38 \pm 0.06 ^c	7.28 \pm 0.06 ^c	4.5 \pm 0.1	34.81 \pm 0.0	2386.2 \pm 13.0 ^a	2647.2 \pm 455.7 ^c

pH_{total} and pCO₂ values were calculated using CO2SYS

Superscripts represent differences among pH treatments based on a fitted line regression and a post hoc Tukey test ($\alpha = 0.05$): ^{a,b,c} $p = 0.000$

CA, USA). Concentrations of L-lactate were determined using a standard curve. Absorbance was read at 540 nm.

Statistical analysis

Lactate data was transformed using log₁₀ to meet the assumptions of normality of distribution and homogeneity of variances while all other parameters (MO₂ and ATP) met this assumption without transformation. Fitted line regressions were used to investigate the consistency of laboratory seawater chemistry. First, a general linear model (GLM) was run for each biological parameter against pH treatment as a fixed factor, tank as a random nested variable within a specific pH treatment and body mass as a covariate to ascertain whether our replicate tanks per treatment had any significant effect on the selected parameters. Tank had no significant effect on krill biology (GLM ANOVA, MO₂: $F(8,25) = 1.72$, $p = 0.144$; ATP: $F(8,26) = 1.21$, $p = 0.333$; L-lactate: $F(8,15) = 0.29$, $p = 0.958$), and thus this term was removed from subsequent analyses. To account for the difference in krill body mass between treatments, an individual sample approach was used (see Bennett 1987; Calosi et al. 2013c). A GLM was run for each biological parameter (MO₂, ATP and L-lactate) with pH/pCO₂ treatment as a fixed factor and body mass as a covariate. After which the residuals, the remaining variability not explained by body mass, from the previous analysis were used to investigate the effect of seawater chemistry (pH/pCO₂) on the biological parameter using a GLM again, as suggested by Bennett (1987). All statistical analysis was conducted using Minitab 17.

Results

Seawater chemistry

Laboratory conditions

Laboratory seawater pH conditions were comparable to the target pH treatment values originally set and were distinct across treatments (Fitted line regression, $F(1,46) = 309.53$,

$p = 0.000$; Table 1). Total alkalinity (TA) measurements from the laboratory samples were consistent across all pH treatments (Fitted line regression $F(1,46) = 0.02$, $p = 0.883$; Table 1).

Kongsfjord conditions

On average, fjord seawater was cooler and slightly fresher ($T < -0.56$ $^{\circ}$ C; $S < 34.81$) in the inner fjord, and warmer and more saline ($T > 1.72$ $^{\circ}$ C; $S > 35.13$) in the outer fjord. While the inner fjord waters were more stratified, with temperature (Fig. 2a) and salinity (Fig. 2b) both increasing with depth; the outer fjord was well mixed, with temperature and salinity remaining stable throughout the water column. Total alkalinity (TA) (Fig. 3a) was lowest (< 2248.9 μ mol kg⁻¹) at 30 m in the inner fjord, while the

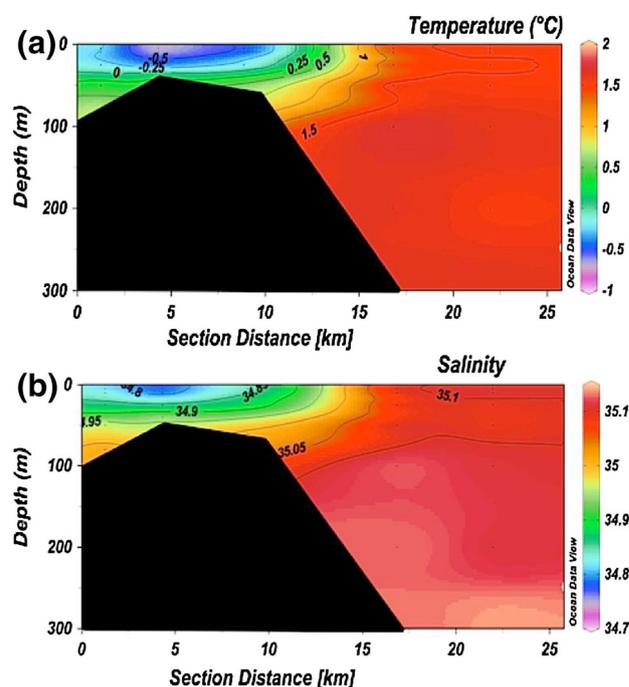


Fig. 2 Kongsfjord water column profiles for all five sampling stations: **a** Temperature ($^{\circ}$ C), **b** Salinity. Water column figures were created using Ocean Data View 4.6.2. (Color figure online)

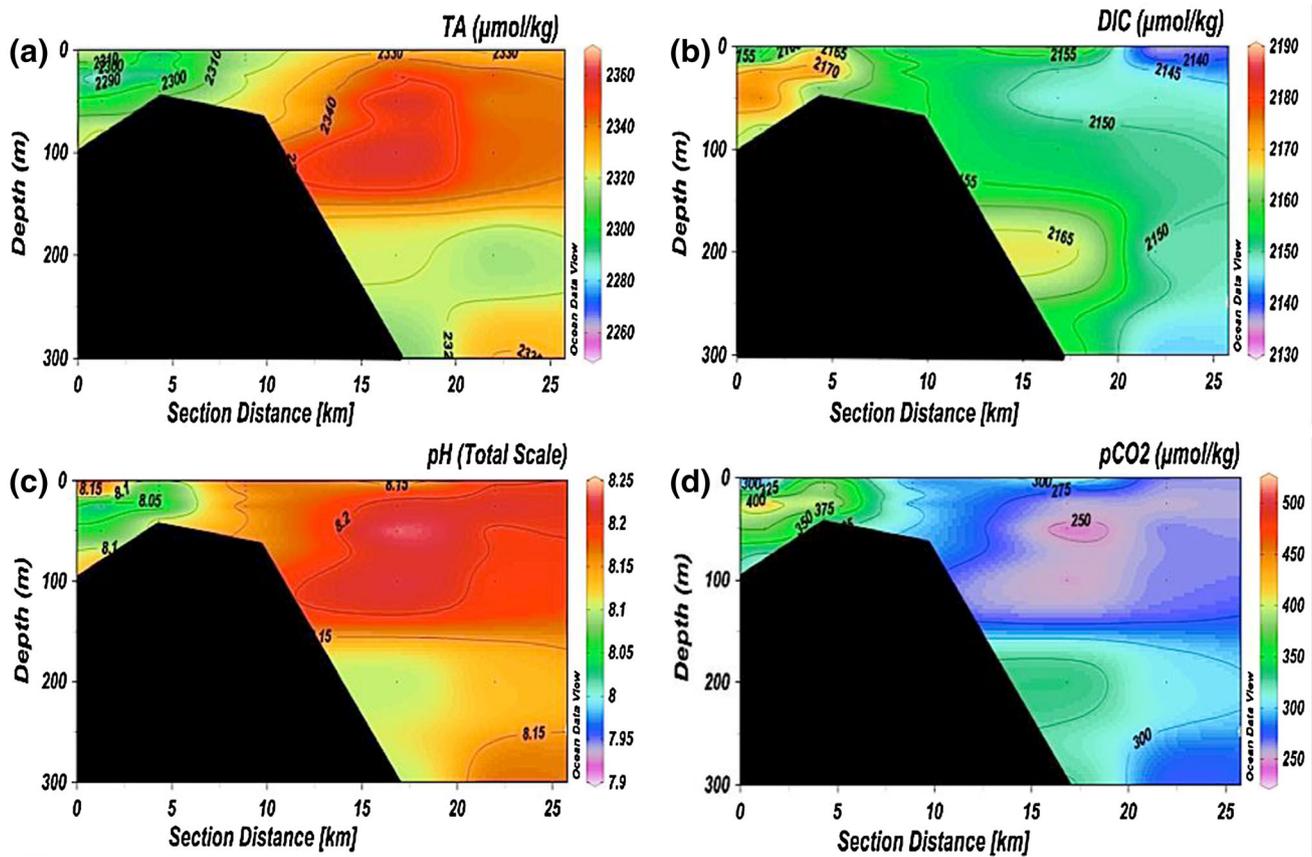


Fig. 3 Kongsfjord water column profiles for all five sampling stations: **a** Total Alkalinity ($\mu\text{mol kg}^{-1}$), **b** Dissolved Inorganic Carbon ($\mu\text{mol kg}^{-1}$), **c** calculated pH_{total} , **d** calculated $p\text{CO}_2$ (μatm). CO2SYS calculations were performed using constants from

Mehrbach et al. (1973) refit by Dickson and Millero (1987). Water column figures were created using Ocean Data View 4.6.2. (Color figure online)

Table 2 Values (mean \pm SD, (N)) for the biological parameters measured in the Arctic krill *Thysanoessa inermis* at the four different pH treatments

pH_{total}	O_2 ($\mu\text{mol h}^{-1} \text{g}^{-1} \text{WW}$)	O_2 ($\mu\text{mol h}^{-1} \text{g}^{-1} \text{DW}^{\text{a}}$)	ATP ($\mu\text{mol g}^{-1}$)	Lactate (mmol L^{-1})	Body mass (g)
7.96	6.9 ± 4.8 (8)	27.4 ± 19.3 (8)	0.052 ± 0.037 (8)	1.084 ± 0.276 (8)	0.009 ± 0.003 (8)
7.70	4.6 ± 2.6 (12)	18.2 ± 10.6 (12)	0.060 ± 0.041 (12)	0.810 ± 0.485 (7)	0.019 ± 0.020 (12)
7.65	4.1 ± 2.7 (10)	16.2 ± 10.9 (10)	0.037 ± 0.026 (10)	0.708 ± 0.192 (7)	0.010 ± 0.003 (10)
7.28	4.9 ± 5.1 (8)	19.4 ± 20.3 (8)	0.052 ± 0.039 (8)	0.763 ± 0.673 (6)	0.020 ± 0.018 (8)

^a Dry weight was assumed to be 25% of the wet weight as per Saborowski et al. (2002)

outer fjord was divided with an area of high TA ($>2341.0 \mu\text{mol kg}^{-1}$) from the surface down to 150 m, after which the TA decreased. Dissolved inorganic carbon (DIC) (Fig. 3b) was highest ($>2172.4 \mu\text{mol kg}^{-1}$) in an area between 10–80 m in the inner fjord, while the outer fjord was more stratified but had overall lower DIC. pH was lowest ($\text{pH}_{\text{total}} < 8.0$) between 10 and 80 m in the inner fjord, while the outer fjord was distinctly divided, with highest pH ($\text{pH}_{\text{total}} > 8.2$) found from the surface to 150 m, after which pH decreased with depth (Fig. 3c). $p\text{CO}_2$ was highest ($>404.9 \mu\text{atm}$) at 30 m in the inner fjord

with more stratified waters, while the outer fjord had two distinct areas where $p\text{CO}_2$ was lowest ($<268.6 \mu\text{atm}$) down to 150 m, then increased with depth (Fig. 3d).

Krill physiological responses

Seawater pH had no significant effect on the residual of the biological parameters *versus* individuals' body mass, i.e. the remaining unexplained variability in the biological parameter after accounting for body mass, of MO_2 , ATP and \log_{10} -lactate (Tables 2, 3). Krill survival averaged

Table 3 Summary of the statistical results for the general linear models of the residual of the biological parameters versus individuals body mass, i.e. the remaining unexplained variability in the biological parameter after accounting for body mass

Biological parameter	df	Adj. MS	F	p
Residual MO ₂	3	0.000013	0.02	0.995
Residual ATP	3	0.000000	1.13	0.352
Residual Log-Lactate	3	0.02836	0.68	0.573

Residual MO₂, ATP and Log₁₀-Lactate of *Thysanoessa inermis* tested against pH as a fixed factor

df degrees of freedom, Adj. MS adjusted mean of squares, F F ratio, p probability level

87.8, 90, 81.1, and 87.8% on day 3 and 62.2, 60, 63.3 and 57.8% on day 7 for pH treatments 8.06, 7.79, 7.65 and 7.38, respectively.

Discussion

To our knowledge, this study is the first to examine the short-term biological responses of overwintering Arctic krill to ocean acidification (OA) in relation to natural conditions found in Arctic fjord seawater chemistry. Overall, we found no significant physiological impacts of OA on overwintering individuals of *T. inermis* from the Arctic fjord of Kongsfjord.

Global change has the potential to impact Kongsfjord in a number of ways. In the outer fjord there will be a large influence from changing oceanographic conditions, such as an increased penetration of warmer, more saline Atlantic water (Willis et al. 2006); while the inner fjord could be exposed to increased river run-off and melt from the large tidal glaciers (Svendsen et al. 2002). Similar to previous studies carried out in April (Cottier et al. 2005; Willis et al. 2006), the presence of a warmer, more saline, well-mixed water column throughout most of Kongsfjord, with a stratified water column of colder fresher water in the inner fjord, indicates a large influence of Atlantic water (AW), or modified-Atlantic water (MAW) within the fjord. The stratified inner fjord could be the remains of trapped Arctic water as well as an input of fresh meltwater.

The carbonate chemistry data presented here are comparable to previously reported results from within Kongsfjord. Total alkalinity (TA) measured between 200 and 300 m depth has been reported to range from 2295 to 2334 $\mu\text{mol kg}^{-1}$. Additionally, pH recorded at this depth ranged from 8.13 to 7.68, whereas $p\text{CO}_2$ ranged from a low of 309–979 μatm (Lischka and Riebesell 2012). This data is also comparable to those from the MAW and AW masses in the Fram Strait, located between Greenland and Svalbard, for TA (2297 ± 5 and $2325 \pm 7 \mu\text{mol kg}^{-1}$,

respectively) and dissolved inorganic carbon (DIC: 2148 ± 5 and $2120 \pm 20 \mu\text{mol kg}^{-1}$, respectively) (Anderson et al. 1998; Jeansson et al. 2011). Total alkalinity was lowest at the stations near the glacial front, and highest in the outer fjord, suggesting a freshwater dilution of TA. In contrast, DIC was highest near the glacier front likely because of remineralisation of organic matter releasing CO₂ and thus increasing DIC, as a result of movements of glaciers or icebergs stirring up organic matter (Feely et al. 2010). The benthic organic matter in Kongsfjord is regulated singularly by zooplankton grazing (Hop et al. 2002). CO₂ released during respiratory remineralisation causes a decrease in pH (Shadwick et al. 2013), which is evidenced here in the inner fjord with an area of lower pH and higher $p\text{CO}_2$. Changes in water mass dominance, Arctic versus Atlantic, are a usual occurrence in Kongsfjord and are most likely to influence the pelagic system (Hop et al. 2002). Zooplankton like *T. inermis* are advected to the glacial front where they are exposed to fresh meltwater and subsequent low pH (Hop et al. 2002) and shifts in zooplankton community composition have been linked to water mass advection in Kongsfjord (Willis et al. 2006).

With respect to OA, an organisms' habitat and consequent exposure to a range of $p\text{CO}_2$ conditions has been shown to lead to a greater tolerance to such stress (Watanabe et al. 2006; Maas et al. 2012; Calosi et al. 2013a; Lewis et al. 2013; Pespeni et al. 2013; Lucey et al. 2015). Specifically, this has also been observed in crustaceans that are regularly exposed to variable environmental conditions through behaviour and life history characteristics (Watanabe et al. 2006; Lewis et al. 2013), as well as physiological adaptation (Turner et al. 2016). In detail, deep-living copepods from the subarctic North Pacific were found to be more tolerant to high $p\text{CO}_2$ than their sub-tropical counterparts, which could be attributed to variable $p\text{CO}_2$ conditions in the subarctic ocean (Watanabe et al. 2006). Adult *Calanus* spp. in the high Canadian Arctic exposed to a range of $p\text{CO}_2$ conditions during daily vertical migrations were less sensitive to high $p\text{CO}_2$ conditions than surface water dwelling *O. similis* (Lewis et al. 2013). Our work further corroborates this, as we show that low pH does not significantly affect *T. inermis*' physiology when considering individuals' metabolic rates and metabolite concentrations. This tolerance to low pH could be due to either phenotypic plasticity or adaptation to the naturally variable pH found within the fjord.

Metabolic activity for *T. inermis* reported in our study are comparable to mean respiration rates reported for *T. inermis* collected in Hornsund (Svalbard, Norway) and incubated at similar temperatures (4 °C) (Huenerlage and Buchholz 2015). In addition, *T. inermis* metabolic activity is similar but slightly lower than those previously reported for the krill *Meganyctiphanes norvegica* ($19.9\text{--}92.9 \mu\text{mol O}_2 \text{g}^{-1} \text{h}^{-1}$

DW) at ambient pH and comparable temperatures (Mayzaud 1973; Sameoto 1976; Båmstedt 1979; Hirche 1984; Saborowski et al. 2002). *Thysanoessa inermis* metabolic rate might be expected to be slightly lower than that of *M. norvegica* due to interspecific differences as well as geographic location, *T. inermis* is found living in overall colder habitats (Clarke and Peck 1991; Clarke 1998). In addition, total lipid percentages for *M. norvegica*, span 20–50% of their dry mass in the Fram Strait with *T. inermis* in Kongsfjord within that range but slightly lower at 21–42% dry mass (Falk-Petersen et al. 2000). The similar metabolic rate compared to other studies suggests that the krill in our experiments were not unduly stressed by handling prior to incubation or the relatively short-term incubation we employed in our study. Mean metabolic rate was comparable across all pH treatments, indicating that krill exposed to low pH for a short time period (7 days) were able to maintain metabolic rates comparable to those previously reported for animals in ambient pH seawater. Daily and seasonal variability (AWIPEV Underwater Observatory (only monitors surface waters, node located at 11 m depth), https://cosyna-nodes.shinyapps.io/svl_ferry_box/) of fjord carbonate chemistry in combination with the migratory behaviour of *T. inermis* could provide them with a pre-exposure that has given the species an advantage to cope with changes in environmental pH. The ability to maintain metabolic rates at low pH (7.95, 7.80, 7.61) has been observed in other species, including the Arctic copepod *Pseudocalanus acuspes* from Kongsfjord, although the combination of low pH and prey concentration affected metabolic rates significantly (Thor and Oliva 2015). Additionally, exposure to elevated $p\text{CO}_2$ over a 2 month period had no detrimental effects on the oxygen consumption rate of early life stages of the Arctic copepod, *C. glacialis* (Bailey et al. 2017). The ability to maintain metabolic rates at low pH has been observed in non-Arctic species, like the deep-sea pteropods of the Pacific, which migrate into elevated $p\text{CO}_2$ oxygen minimum zones (Maas et al. 2012).

The ATP concentrations observed here were lower than values previously reported for *M. norvegica* (Skjoldal and Båmstedt 1977; Ventura 2006). This difference could be due to interspecific differences, as well as differences in methodology and the timing of our sampling: i.e. we sampled krill prior to the onset of the spring bloom, as herbivorous species these krill will reach peak ATP levels during the spring bloom (Skjoldal and Båmstedt 1977). Importantly, the mean ATP concentrations reported here show that there was very little energy commitment being made by *T. inermis* during this time, potentially an indication of their overwintering state.

Like metabolic rate, mean ATP concentration and mean L-lactate L-lactate concentration were also consistent across pH treatments, indicating that the krill are able to maintain aerobic metabolism and that energy metabolism

was not compromised at different pH levels, i.e. maintenance of metabolic rates came at no apparent energetic cost as there was no observable differences in ATP concentration or evidence supporting an increase in anaerobic metabolism. In contrast, Antarctic krill, *E. superba*, exposed to elevated $p\text{CO}_2$ (672 μatm) conditions for just 24 h, showed an increase in nutrient release rates and metabolic activity that are associated with the maintenance of internal acid–base equilibrium (Saba et al. 2012). One explanation for these different responses is the different length of experimental exposure between our study (7 days) and that of Saba et al. (2012) (24 h). The metabolic response, and subsequent increased ingestion found by Saba et al. (2012) could plausibly be that responses recorded following a short-term exposure (several hours) to low pH/elevated $p\text{CO}_2$ are not maintained over a longer period of exposure (i.e. several days as tested here or weeks to months), as shown by Sperfeld et al. (2014) and Suckling et al. (2015). Long-term metabolic rate adjustments in response to low pH and increased temperature were observed in the Antarctic sea urchin, *Sterechinus neumayeri*, where adults took 6–8 months to acclimatise to experimental conditions but showed no measurable effect of low pH and increased temperature on metabolic rates after this period (Suckling et al. 2015). Indeed Sperfeld et al. (2014) exposed *Nyctiphanes couchii*, a Northern Atlantic krill species, to elevated CO_2 conditions for 5 weeks, and found no consistent detrimental impacts of near future elevated $p\text{CO}_2$ (<1100 μatm) on growth or their exoskeleton, although survival decreased and the frequency of moult-related deaths increased above 1100 μatm .

Furthermore, it is also important to consider that the susceptibility to OA may be associated with differences in lifestyle, life history stage, as well as the ability to compensate for changes in the environment (Whiteley 2011). For instance, krill embryonic development and larvae were found to become impacted by $p\text{CO}_2$ elevated above 1000 μatm (Kawaguchi et al. 2011, 2013), and gravid females were found to be more sensitive to elevated CO_2 than non-gravid krill (Saba et al. 2012), while the sub-adults from Sperfeld et al. (2014) and adults in this study suggest that these stages are potentially more tolerant to elevated CO_2 .

Our findings suggest that exposure to natural gradients in seawater chemistry (pH, $p\text{CO}_2$) has resulted in the ability to tolerate at least short-term exposure to low pH in overwintering individuals of *T. inermis*. Nonetheless, limited food availability during the winter months along with a potential demand for more food to compensate for the negative effects of low pH could still represent a challenge for Arctic krill in the future. Furthermore, warming, along with acidification, poses a serious threat to Arctic ecosystems, and hence future work should also include *T.*

inermis's response to multiple stressors. Future OA studies at high latitudes should consider conducting long-term exposure to low pH/elevated $p\text{CO}_2$ (Rodríguez-Romero et al. 2015; Thor and Dupont 2015; Suckling et al. 2015; Lucey et al. 2016). However, logistics and a short field season might present a problem in conducting longer-term experiments.

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