



FEATURE ARTICLE

Combined effects of ocean acidification and elevated temperature on feeding, growth, and physiological processes of Antarctic krill *Euphausia superba*

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ABSTRACT: Antarctic krill Euphausia superba is a key species in the Southern Ocean, where its habitat is projected to undergo continued warming and increases in pCO₂. Experiments during 2 summer field seasons at Palmer Station, Antarctica, investigated the independent and interactive effects of elevated temperature and pCO2 (decreased pH) on feeding, growth, acid-base physiology, metabolic rate, and survival of adult Antarctic krill. Ingestion and clearance rates of chlorophyll were depressed under low pH (7.7) compared to ambient pH (8.1) after a 48 h acclimation period, but this difference disappeared after a 21 d acclimation. Growth rates were negligible and frequently negative, but were significantly more negative at high (3°C, -0.03 mm d^{-1}) compared to ambient temperature (0°C, -0.01 mm d⁻¹) with no effect of pH. Modest elevations in tissue total CO₂ and tissue pH were apparent at low pH but were short-lived. Metabolic rate increased with temperature but was suppressed at low pH in smaller but not larger krill. Although effects of elevated temperature and/or decreased pH were mostly sublethal, mortality was higher at high temperature/low pH (58%) compared to ambient temperature/pH or ambient temperature/low pH (>90%). This study identified 3 dominant patterns: (1) shorter-term effects were primarily pH-dependent; (2) krill compensated for lower pH relatively quickly; and (3) longer-term effects on krill growth and survival were strongly driven by temperature with little to no pH effect.

KEY WORDS: Antarctic krill \cdot Ocean acidification \cdot Ocean warming \cdot Feeding \cdot Growth \cdot Physiology \cdot Metabolism \cdot Survival

Antarctic krill *Euphausia superba*, a regional keystone species, inhabit a rapidly warming and acidifying Southern Ocean.

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1. INTRODUCTION

Antarctic krill *Euphausia superba* is a key species in Antarctic food webs (Everson 2000, Atkinson et al. 2004). Over the past 90 yr, historical hotspots of postlarval krill abundance have shifted southward from the Southwest Atlantic Sector to the West Antarctic Peninsula (WAP) shelf system (Atkinson et al. 2019). These southward shifts, along with sharp declines in juvenile abundance and recruitment since the 1970s, are attributed to increasing temperatures and winds as well as reductions in sea ice (Atkinson et al. 2019).

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Continued environmental warming is likely to exacerbate both the decline in krill biomass (Klein et al. 2018) and the southward shift in their distribution.

In addition to continued warming, the Southern Ocean is likely to be one of the first areas affected by ocean acidification. Earlier models projected that undersaturation with respect to aragonite would occur in the Southern Ocean by the end of this century (Orr et al. 2005, McNeil & Matear 2008). However, a recent study detected much shallower aragonite saturation horizons, indicating that this process is occurring faster than previously projected (Negrete-García et al. 2019). Combined, the projected increases in temperature (+1.1–5.1°C) and decreases in pH (up to 0.4 units) for the Antarctic region (RCP8.5; IPCC 2013) may pose a risk to krill.

Antarctic krill inhabit a narrow temperature range south of the Antarctic convergence (-1.8 to 5.5° C) (McWhinnie & Marciniak 1964). Across that range, the metabolic temperature sensitivity of routine metabolism (E=0.74; equivalent to a Q_{10} near 2.8; Tarling 2020) is within the range reported for diverse marine species (Deutsch et al. 2020). Thus, the metabolic rate at the warmer end of this species' range is nearly double that at the colder end. Warmer temperatures also reduce the interval between molting events (intermolt period, IMP) and decrease growth rates for this species (Poleck & Denys 1982, Buchholz 1991, Atkinson et al. 2006, Brown et al. 2010).

Negative effects of elevated partial pressure of carbon dioxide (pCO₂) in seawater on Antarctic krill have been observed in early life stages and associated with decreased hatching success and irregular embryonic development (Kawaguchi et al. 2011, 2013). Long-term elevations in energetic costs in response to increased warming and/or pCO2 may eventually impact growth and reproduction (e.g. Wickins 1984, Kurihara et al. 2008, Cooper et al. 2017, McLean et al. 2018). One study of adult E. superba found that rates of chlorophyll ingestion and nutrient excretion were 1.5- to 3-fold higher under elevated pCO₂, which could result from, among other possible explanations, increased energetic costs of maintaining internal acid-base equilibria under these conditions (Saba et al. 2012). When provided with a constant supply of food, adult E. superba survival, growth, maturation, lipid biochemistry, and metabolism were resilient during 1 yr of experimental exposure to near-future ocean acidification conditions (Ericson et al. 2018, 2019). These results highlight the importance of food availability to survival and success in a changing ocean (Saba et al. 2012,

Seibel et al. 2012, Sperfeld et al. 2017, Ericson et al. 2019) and suggest that krill may have difficulty meeting increased demands for energy in time periods (e.g. winter) or locations (e.g. northern WAP) with low food availability.

Elevated pCO₂ can also impact marine invertebrates by disturbing their acid-base physiology, resulting in hypercapnia (CO₂-induced acidification of body fluids; reviewed for crustaceans by Whiteley 2011). Some organisms suppress metabolism when seawater pH is low (Hand 1998, Langenbuch & Pörtner 2004, Rosa & Seibel 2008, Maas et al. 2012, Seibel et al. 2012). However, this suppression is typically a response to natural CO2 variability that correlates with periods of resource limitation (e.g. overwintering, low tides, daily migrations into deep hypoxic zones). Alternatively, organisms may compensate for changes in seawater pH by shifting acid-base and ion equilibria to new steady-state values (reviewed by Baumann 2019, Melzner et al. 2020); such compensation often incurs an energetic cost (Hu et al. 2011, Heuer & Grosell 2016). Furthermore, physiological oxygen transport systems may be compromised (Pörtner 1990, Pörtner et al. 2004), making them less effective at extracting oxygen (O₂) and requiring organisms to process more water to obtain sufficient oxygen to meet their metabolic demands. Bridges et al. (1983) found high pH-sensitivity of hemocyanin in E. superba at -1.5°C, suggesting that without pH compensation, elevated CO₂ could reduce oxygen transport capacity in these animals. However, Birk et al. (2018) found that extracellular pH is typically fully compensated, and the effect of CO₂ on oxygen-binding affinity is unlikely to produce measurable effects on oxygen consumption rates, even in animals with highly pH-sensitive blood, such as squids and the krill in the present

Although the effects of elevated temperature and high $p\mathrm{CO}_2$ on Antarctic krill feeding, growth, and physiology have been reviewed (e.g. Flores et al. 2012) or studied separately, their combined impact has not yet been investigated. The combination of multiple stressors may exacerbate responses to changing ocean conditions through antagonistic, additive, or synergistic effects (Breitburg et al. 2015, Kroeker et al. 2017) as evidenced by a meta-analysis of marine taxa that found greater sensitivity to ocean acidification at higher temperatures (Kroeker et al. 2013). The present study explores the independent and interactive effects of elevated temperature and $p\mathrm{CO}_2$ on adult E. superba. Specifically, we investigated shorter-term (hours) and longer-term (weeks)

effects of these climate-related variables on Antarctic krill feeding rates, growth, acid-base status, metabolism, and survival during 2 field seasons at Palmer Station, Antarctica.

2. MATERIALS AND METHODS

Multiple experiments were conducted during 2 field seasons, austral summers 2013/2014 and 2014/2015 (hereafter season 2014 and 2015, respectively). These experiments, and their target treatments, are described below and summarized in Table 1.

2.1. Collection and husbandry

In each season, krill were collected near Palmer Station (Anvers Island, Antarctica) during a Long-Term Ecological Research cruise on the ARSV 'Laurence M. Gould' using a 2×2 m square frame net with 1000 µm mesh and a non-filtering codend. Krill were placed in insulated coolers filled with filtered surface seawater and immediately transported to Palmer Station, either via the ARSV 'Laurence M. Gould' or small vessels, where they were quickly transferred to holding tanks in the Palmer Station aguarium facility. The time between collection and transport to the station and placement in the tanks was typically <1 h. Krill were placed into either rectangular (675 l) or round (1900 l) tanks. All tanks were supplied with flow-through unfiltered seawater sourced from Arthur Harbor and circulated at a rate that replaced the volume of the tank at least 3 times per day. The unfiltered seawater allowed krill to feed ad libitum on the natural prey assemblage. Due to the noted lack of natural krill schooling behavior when artificial lighting was on in the aquarium room, no artificial lighting was used in the aquarium room or environmental chambers throughout the holding period and experimental incubations. Windows in the aquarium facility admitted ambient light, so krill were exposed to the seasonal day/night

cycle and displayed schooling behavior in the holding tanks at these ambient light levels. Ambient temperature and pH of the seawater feeding the tanks were selected as our experimental ambient values.

2.2. Design and summary of experiments

Two krill feeding experiments and 1 metabolic rate experiment were conducted during 2014 (Table 1). These experiments comprised 3 target treatments: (1) ambient temperature and pH (0°C/pH = 8.1; hereafter referred to as 'ambient temperature/pH'); (2) ambient temperature/low pH (0°C/pH = 7.7), a pH

Table 1. Summary of Antarctic krill experiments, target treatments, and actual conditions measured during 2 field seasons at Palmer Station, Antarctica. Target treatments in **bold** represent ambient conditions (temperature and/or pH).

ND: not determined

Field season	Experiment target treatment	Temperature (°C) (mean ± SE)	pH $(mean \pm SD)$	Initial chl <i>a</i> (µg l ⁻¹) (mean ± SD)
2014	Feeding Expt 1 0°C , pH = 8.1 0°C , pH = 7.7 3°C , pH = 7.7	$n = 60$ 0.3 ± 0.32 0.3 ± 0.32 3.8 ± 0.13	$n = 14$ 8.29 ± 0.07 7.60 ± 0.12 7.50 ± 0.13	$n = 2$ 1.70 ± 0.02 1.30 ± 0.13 1.41 ± 0.23
2014	Feeding Expt 2 0°C, pH = 8.1 0°C, pH = 7.7 3°C, pH = 7.7	$n = 60$ 0.3 ± 0.32 0.3 ± 0.32 3.8 ± 0.13	$n = 14$ 8.04 ± 0.02 7.68 ± 0.04 7.66 ± 0.06	$n = 2$ 1.46 ± 0.01 1.62 ± 0.35 0.39 ± 0.09
2014	Metabolic rate 0°C, pH = 8.1 0°C, pH = 7.7 3°C, pH = 7.7	$n = 60$ 0.2 ± 0.21 0.2 ± 0.21 3.7 ± 0.10	n = 6 8.27 ± 0.17 7.73 ± 0.06 7.73 ± 0.06	ND ND ND
2014	Survival 1 0°C, pH = 8.1 0°C, pH = 7.7 3°C, pH = 7.7	$n = 4$ 0.0 ± 0.01 0.0 ± 0.01 3.6 ± 0.03	n = 20 8.13 ± 0.17 7.71 ± 0.14 7.66 ± 0.14	ND ND ND
2014	Survival 2 0°C , pH = 8.1 0°C , pH = 7.7 3°C , pH = 7.7	n = 4 0.0 ± 0.01 0.0 ± 0.01 3.6 ± 0.03	n = 45 8.20 ± 0.13 7.71 ± 0.15 7.52 ± 0.28	ND ND ND
2015	Growth and survival ^a 0°C, pH = 8.0 ^a 0°C, pH = 7.5 0°C, pH = 7.1 3°C, pH = 8.0 ^a 3°C, pH = 7.5	$n = 60$ 0.7 ± 0.02 0.7 ± 0.02 0.7 ± 0.02 3.5 ± 0.02 3.5 ± 0.02	$n = 18$ 7.99 ± 0.02 7.52 ± 0.06 7.22 ± 0.07 7.99 ± 0.02 7.52 ± 0.06	ND ND ND ND ND
2015	3°C, pH = 7.1 Acid-base physiology 0°C, pH = 8.1 0°C, pH = 7.7 3°C, pH = 8.1 3°C, pH = 7.7 ent conditions included	3.5 ± 0.02 $n = 58$ 0.1 ± 0.07 0.1 ± 0.07 3.6 ± 0.12 3.6 ± 0.12 in survival and	7.22 ± 0.07 n = 39 7.90 ± 0.13 7.64 ± 0.08 7.90 ± 0.13 7.64 ± 0.08	ND ND ND ND ND

value selected as a drop of up to 0.4 units projected under RCP8.5 (IPCC 2013); and (3) high temperature/low pH (3°C/pH = 7.7), temperature selected as the mean projected increase in temperature under RCP8.5 (Bopp et al. 2013, IPCC 2013) (Table 1). Unfortunately, due to logistical constraints during the 2014 field season, specifically the lack of a fourth vessel to equilibrate seawater to treatment conditions, we were unable to conduct the feeding and metabolic rate experiments as a fully crossed factorial design that included a fourth experimental treatment (high temperature (3°C)/ambient pH).

One growth experiment and 4 acid–base physiology experiments were conducted during 2015 (Table 1). The growth experiment comprised 6 target treatments: ambient and high temperature (0 and 3°C, respectively) with 3 pH targets at each temperature: ambient (8.0), 7.5, and 7.1 (Table 1). The acid–base experiments comprised 4 treatments: (1) ambient temperature/pH (0°C/pH = 8.1); (2) ambient temperature/low pH (0°C/pH = 7.7); (3) high temperature/ambient pH (3°C/pH = 8.1); and (4) high temperature/low pH (3°C/pH = 7.7).

Seawater for incubations was equilibrated to each experimental temperature (0 and 3°C) using recirculating systems consisting of an 800 l cylindrical carboy, chiller (Delta Star), and temperature controller. Target temperatures were maintained during incubations in environmental chambers set to either 0 or 3°C. Jars filled with filtered seawater were placed in each environmental chamber and used to monitor temperature throughout each field season using either daily checks with a standard laboratory thermometer (2014) or HOBO loggers (2015). For elevated pCO₂ treatments, pure CO₂ gas was injected into the tank via a peristaltic pump fed directly into the inflow of an aquarium pump (Petco, King 160 Powerhead) following methods modified from Jokiel et al. (2014). Target pH was determined using a standard curve of treatment water pH with peristaltic pump rate.

Salinity was measured with a benchtop conductivity meter (YSI 3100) calibrated daily with a conductivity standard (50 000 µS cm⁻¹; Ricca Chemical). Seawater pH was determined spectrophotometrically using the indicator dye thymol blue on a Shimadzu spectrophotometer (Zhang & Byrne 1996, Dickson et al. 2007). Total alkalinity (TA) was determined from 100 ml subsamples with an open-cell, potentiometric titration of seawater (Metrohm 888 Titrando) with 0.1 M HCl following the potential of a pH electrode (Dickson et al. 2007). Alkalinity data were processed using Tiamo software (version 2.3). Measurements of pH and TA were quality controlled

using certified reference materials from Andrew Dickson, UCSD Scripps Institute of Oceanography. Water samples collected from each incubation bottle during the feeding experiments were filtered through 200 µm mesh onto individual GF/F filters, wrapped in foil, frozen, and analyzed for chlorophyll *a* (chl *a*) on a Turner Designs model 10 fluorometer (Parsons et al. 1984).

Krill lengths and wet weights were determined from fresh animals and are reported in the following units throughout this paper. Lengths are expressed as total length (TL, tip of rostrum to tip of telson) in millimeters (mm). Krill TL was calculated from the uropod length (UL) using the following equation generated from our measurements at Palmer Station:

$$TL (mm) = 4.0677 \times UL (mm) + 12.703$$
 (1)

Krill were weighed on a Mettler Toledo XS205 DualRange balance, and units are expressed as wet weight in milligrams (mg). Length—wet weight conversion from the Palmer data was:

Wet weight (mg) =
$$38.315 \times TL \text{ (mm)} - 1058.4 \text{ (R}^2 = 0.95)$$
 (2)

2.3. Feeding experiments

Two replicate feeding experiments were conducted in 2014, differing in the amount of time krill were acclimated to treatment conditions prior to the start of each 24 h experiment. Krill used in Experiment 1 (Feeding Expt 1; 24–25 January) and Expt 2 (Feeding Expt 2; 24-25 February) were exposed to treatment conditions for 48 h and 21 d, respectively, in 19 l plastic buckets with airtight lids at densities of 19 krill per bucket. During acclimation, water was changed every 24 h, whereby 80% of the water was siphoned out and replaced to minimize excretory and respiratory effects of the animals on treatment conditions. A subset of the siphoned water was used for salinity, pH, and total alkalinity measurements every other day. Due to differences in acclimation time between the 2 experiments, krill were larger in Expt 2 (see Section 3.1).

After acclimation, experimental incubations were conducted using 4 l wide-mouth polycarbonate bottles. Each of the 3 treatments in each experiment comprised 14 bottles filled with the appropriate equilibrated seawater. Two bottles per treatment served as T_0 controls (no krill added) and were used for an initial suite of samples. Two bottles served as $T_{\rm final}$ (24 h) controls (no krill added), and 1 krill was added to each of the remaining 10 bottles per treat-

ment ($T_{\rm final}$ treatments). The $T_{\rm final}$ bottles were capped to maintain target $p{\rm CO}_2/p{\rm H}$ and incubated at the appropriate temperature (0°C for ambient, 3°C for high temperature) for approximately 24 h before collecting end-point samples. Water samples were collected at T_0 and $T_{\rm final}$ for pH, total alkalinity, and fluorometric chl a. Clearance and chl a ingestion rates of krill were calculated according to Marin et al. (1986), and expressed as ml ind. $^{-1}$ h $^{-1}$ and $\mu{\rm g}$ chlorophyll ind. $^{-1}$ d $^{-1}$, respectively.

2.4. Growth experiment

Growth data are from 1 long-term experiment conducted in 2015 (Table 1). Krill for this experiment were collected on 1 February by scientists aboard the ARSV 'Laurence M. Gould' and transferred to Palmer Station as previously described. Krill were held in large tanks in the aquarium facility (details in Section 2.1) at ambient temperature and pH (0°C/pH = 8.0) for 1 wk prior to the start of the experiment.

This experiment examined krill growth in relation to temperature and pH over the course of 1 molt cycle (i.e. the krill had to molt twice in order to assess the effect of treatment conditions on growth). To determine the starting point of the molt cycle, adult krill of comparable size were incubated individually in 4 l

bottles of ambient seawater in the 0°C environmental chamber until they molted. Water was changed as previously described and bottles were checked daily for molts. Consistent color in the hepatopancreas indicated that krill were feeding on the natural prey assemblage in the ambient seawater.

When an animal molted in an ambient bottle (the 'initial molt'), indicating the start of a new molt cycle, the individual was transferred to 1 of the 6 treatments comprising this experiment (Tables 1 & 2). For the high temperature treatments, krill were transferred to ambient temperature treatment water and placed in the 3°C environmental room to avoid temperature shock. The date of transfer is the 'start date' and represents the start of exposure to experimental treatment conditions. Start dates span a period of 21 d (Table 2) due to the timing of initial molts. Animals were assigned to treatment groups such that the number in each group was similar at all times, resulting in a range of incubation times for individual krill (1-24 d) with a mean of 13 d (Table 2). Each treatment ultimately comprised 26 individuals. Krill remained in treatment water from their start date until the end of the experiment. Incubation jars remained in environmental chambers except for a brief period each day to change the water and check for molts. Salinity, pH, and total alkalinity were measured frequently for each treatment.

Table 2. Summary information for Antarctic krill growth experiment. Data comprise initial molts at ambient temperature/pH (0° C/pH = 8.0) and second molts from all 6 treatments (see Table 1). Growth rate and intermolt period (IMP) could not be determined for the 0° C treatments due to the low number of krill that molted twice, so data for ambient temperature/pH in this study are from initial molts only.

—: not applicable

Temp (°C)	рН	First animal start	Last animal start	Mean (range) no. of days incubated	No. molted twice	No. dead	IMP (d)	Mean (range) total length (mm)	Mean (range) growth rate (mm d ⁻¹)	% negative growth rates	% zero growth rates	% positive growth rates
Initial	Initial molts (Holding period in environmental room at ambient conditions)											
0	8.0	9 Feb	3 Mar	11.4 (1–23)	-	-	23	42.74	-0.01 (-0.15 to +0.04)	56 (n = 85)	22 (n = 34)	22 (n = 34)
Secon	Second molts (Treatment conditions)											
0	8.0	11 Feb	4 Mar	12 (1–22)	1	0	-	42.74 (37.55–47.62)	-	_	-	_
0	7.5	11 Feb	3 Mar	12 (3–22)	1	0	-	42.06 (37.17–46.14)	-	-	_	-
0	7.1	11 Feb	3 Mar	13 (4–22)	0	2	-	41.60 (36.50–45.90)	_	-	-	-
3	8.0	11 Feb	2 Mar	14 (4–23)	7	6	16	40.55 (36.37–46.48)	-0.05 (-0.08 to -0.02)	100 (n = 7)	0	0
3	7.5	11 Feb	2 Mar	14 (5–23)	11	1	17	41.30 (36.64–46.49)	-0.03 (-0.09 to +0.10)	73 (n = 8)	9 (n = 1)	$ \begin{array}{c} 18 \\ (n = 2) \end{array} $
3	7.1	11 Feb	3 Mar	15 (5–24)	11	1	18	40.97 (37.44–46.34)	-0.03 (-0.07 to +0.03)	73 (n = 8)	18 (n = 2)	9 (n = 1)

The uropods of the molts were measured after each experiment check, and converted to TL using Eq. (1). At the end of the experiment, each krill was weighed, measured (body length, TL, uropod length, telson length), and life history stage (female, male) was recorded along with notes on the condition of the animal. Animals that died during the experiment were processed as above at the time of their death. All measurements were made by 1 author (C.T.S.) to minimize observer bias.

Growth and IMP of euphausiids are commonly measured following the instantaneous growth rate (IGR) protocol (Quetin & Ross 1991, Nicol et al. 1992, Atkinson et al. 2006, Kawaguchi et al. 2006, Tarling et al. 2006, Shaw et al. 2010 and references therein). This method utilizes short-term incubations (typically 4-5 d for Euphausia superba) of freshly collected krill, where IMP is estimated based on the proportion of animals that molt during the experiment, and growth rate (mm d⁻¹) is calculated by dividing the growth increment (change in length pre-molt to post-molt) by the IMP. IMP may also be measured directly in longterm incubations that maintain krill in captivity over the course of 2 or more molt cycles (Pinchuk & Hopcroft 2007), although this method is less commonly used due to the experimental effort and potential confounding factors from long-term captivity. In the present study, neither method of estimating IMP was applicable to both initial and second molts. To use direct measurement for initial molters would require a second molt under ambient conditions, which was not available because individuals were transferred to a treatment after their initial molt. To use the IGR method for second molters would require that incubations of all individuals had begun simultaneously, whereas they actually spanned a period of 21 d. As such, IMP for initial molters at ambient temperature/ pH was estimated following the IGR method and based on the number of molts during the first 4 d of the incubation. For 3°C treatments, IMP was determined directly from the number of days between initial and second molts of krill that molted twice.

2.5. Survival

Survival was analyzed as an adjunct to long-term incubation experiments. As experimental protocols often necessitated sacrificing animals at intermediate time points, only 3 experiments were suitable for survival analysis: the 2015 growth experiment (results presented here) and 2 additional experiments from 2014. Other than survival, measurements from these

2014 experiments are not reported in the present study due to too few molts during growth experiments or lack of inter-comparability due to differences in experimental temperatures or other metrics. Survival analysis comprised the following target treatments: (1) ambient temperature/pH (0°C /pH = 8.1, n = 52); (2) ambient temperature/low pH (0°C, pH = 7.7, n = 52); (3) high temperature/ambient pH (3°C, pH = 8.0, n = 20); and (4) high temperature/low pH (3°C, pH = 7.7, n = 52). Survivorship was recorded daily over incubation periods of 9–24 d.

2.6. Acid-base physiology experiments

Two shorter-term (12–48 h) and 2 longer-term (7–14 d) experiments were conducted to assess the effects of treatment conditions on acid–base balance. Krill were held in 1 of 4 treatments (as described in Section 2.2; Table 1) and acid–base parameters measured at time = 0, 1, 6, 12, 24, and 48 h, and 7 and 14 d. The 7 and 14 d time points were removed from further analysis due to an insufficient number of measurements. Incubations were conducted in 19 l plastic buckets with airtight lids. Buckets were filled with treatment water, stocked at a density of 19 krill per bucket, and placed in environmental chambers set to each experimental temperature (ambient at 0°C or high at 3°C). Water was changed every 24 h to maintain water quality.

At each time point, blood was collected from n =5-10 krill using a 20-gauge hypodermic needle for pH and lactate measurements. Blood was collected in gastight Hamilton syringes and transferred to Eppendorf tubes for immediate pH measurement. pH was measured in the absence of air. Total time required for collection from multiple krill for each pH and lactate measurement was ~5-10 min. Blood samples were pooled to measure: (1) blood pH using a temperaturecontrolled pH microelectrode (Microelectrodes) (n = 2pooled samples per treatment per time point; calibrated using certified reference materials); and (2) blood lactate with a handheld lactate analyzer (Roche Accutrend) (n = 2 pooled samples per treatment per time point). Abdominal segments were excised and flash frozen for tissue analysis. For determination of tissue total CO₂ (TCO₂) and tissue pH, tissue from each individual was weighed and homogenized in 500 µl homogenate buffer containing 150 mmol potassium fluoride and 5 mmol nitrilotriacetic acid (Pörtner et al. 1990). Homogenate (75 µl) was injected into a Corning 965 Total CO₂ analyzer to determine TCO₂ $(n = 4-5 \text{ ind. treatment}^{-1} \text{ at each time point})$. The remaining homogenate was used to measure tissue pH $(n = 5-10 \text{ ind. treatment}^{-1} \text{ at each time point})$ using a pH microelectrode (as above) calibrated to a pH 7.11 standard. Tissue lactate $(n = 3-5 \text{ ind. treatment}^{-1} \text{ at each time point})$ was measured by homogenizing tissue at 1:1 in deionized water spiked with 2 mM lactate, using 3 ml glass homogenizers on ice. Supernatant was then measured for lactate as above.

2.7. Metabolic rate experiment

Metabolic rate was measured using end-point respirometry. Krill were held under treatment conditions (n = 40 ind. treatment⁻¹, Table 1) for 24 h in 19 l plastic buckets. Biological oxygen demand (BOD) bottles (300 ml) were then filled with 2 µm filtered treatment water, and 1 krill was placed into each bottle. Each treatment included 1 control bottle (containing treatment water but no krill) to correct for residual microbial respiration. All bottles were incubated in the dark for 6 h in a water bath in an environmental chamber held at the experimental temperature (ambient at 0°C or high at 3°C). At the end of the incubation period, dissolved oxygen in each bottle was measured using an oxygen electrode (Strathkelvin Instruments). Respiration rates (in μ mol O₂ g⁻¹ h⁻¹) were calculated as the difference between the dissolved oxygen concentration in the control bottle (no krill) and the final dissolved oxygen concentration in each treatment bottle standardized to the body mass in wet weight of each individual krill. Oxygen never declined below $75\,\%$ saturation, which is well above the critical pO_2 for the species (Torres et al. 1994).

2.8. Statistics

Significance among treatment conditions in the feeding and growth experiments was determined using the 'Statistics and machine learning toolbox' in Matlab (version 2019a). Clearance and ingestion rate data were tested for normal distributions (Shapiro-Wilk test), normalized using rank transformation, and compared within and among experiments and treatments using multi-way ANOVA and Tukey-Kramer post hoc tests (p < 0.05). Due to the non-normal distribution and variable sample sizes, comparisons of growth rates among treatments required a non-parametric Kruskal-Wallis test (p < 0.05), and a Dunn post hoc test with no adjustment (p < 0.05).

For the survival, acid-base physiology, and metabolic rate analyses, there were no significant differences in survival among experiments within each experimental treatment (log-rank test); therefore, data from all 3 experiments were combined for analysis. Curves of combined survival data were compared using the log-rank test for statistical significance in Graphpad Prism 7.0. Differences were considered significant at p < 0.05. Acid-base parameters were log transformed and analyzed as a 3-way factorial design using PROC GLIMMIX in SAS 9.4 with temperature, CO₂, and time as fixed effects. Fixed effects were specified as classification variables using levelization through the CLASS statement in SAS and the generalized linear model fit by the method of maximum likelihood. The Kenwood-Rogers method of degrees of freedom was used along with an autoregressive covariance structure (TYPE = AR[1]) that best fit the time series. The 'SLICE' option within GLIMMIX was used to discern differences among treatment groups within each time point. Time point t = 0 was eliminated from statistical analysis due to a lack of measurements for all 4 treatment groups. The slope of the relationship between metabolic rate and body mass was compared among treatment groups using ANCOVA in SAS 9.4.

3. RESULTS

Experimental temperature and pH measured during all experiments, and initial chl a concentrations in the 2 feeding experiments, are summarized in Table 1. In addition, mean \pm SD pH values measured at water changes during the 21 d acclimation period for Feeding Expt 2 were 8.24 ± 0.13 , 7.78 ± 0.05 , and 7.74 ± 0.03 for the ambient temperature/pH, ambient temperature/low pH, and high temperature/low pH conditions, respectively (n = 11 per treatment condition). Raw data collected during the experimental incubations and water changes, including additional carbonate chemistry data, are available from the Biological & Chemical Oceanography Data Management Office (BCO-DMO) data repository: https://www.bco-dmo.org/project/721363.

3.1. Feeding experiments

Krill in Expt 1 had a mean weight of 210 mg and mean \pm SD length of 33.1 \pm 1.3 mm (n = 30). Krill in Expt 2 were larger due to growth during the longer acclimation period (21 d), with a mean weight of 447 mg and mean length of 39.3 \pm 2.0 mm (n = 30).

Clearance and ingestion rates were significantly lower under low pH compared to ambient when acclimated to experimental treatments for 48 h (Expt 1, Fig. 1; ANOVA, Tukey-Kramer post hoc, p < 0.0001). However, there was no difference between

the ambient temperature/low pH and the high temperature/low pH treatments (Fig. 1, Expt 1). In Expt 2, where krill were acclimated to treatment conditions for 21 d, there were no differences in clearance and ingestion rates between ambient temperature/ pH and ambient temperature/low pH treatments. However, rates were lower in the high temperature/low pH treatment (Fig. 1; ANOVA, Tukey-Kramer post hoc, p < 0.0001). Both clearance and ingestion rates in ambient temperature/pH conditions were lower in Expt 2 compared to Expt 1 (ANOVA, Tukey-Kramer post hoc, p < 0.0001). The mean clearance rates in ambient temperature/pH conditions for Expt 1 and Expt 2 were 370 and 196 ml ind.⁻¹ h⁻¹, respectively. The mean ingestion rates in Expts 1 and 2 were 15.1 and 6.9 µg chlorophyll ind.⁻¹ d.⁻¹, respectively.

The initial concentration of chl a in the high temperature/low pH treatment in Expt 2 (mean = $0.39 \mu g l^{-1}$, Table 1) was ~75% lower than those concentrations measured in all other treatments in both Expts 1 and 2 (means $1.30-1.70 \, \mu g \, l^{-1}$). Additionally, differential changes in chlorophyll over time (T_0 to T_{final}) occurred in the controls (no krill added). These changes were minimal in the ambient temperature/pH condition (+7 and -7% in Expts 1 and 2, respectively), but were highly negative under ambient temperature/low pH (-66 and -37 % in Expts 1 and 2, respectively) and high temperature/low pH (-34 and -32 % in Expts 1 and 2, respectively).

3.2. Growth experiment

Krill in the growth experiment were of similar sizes (mean \pm SD length = 39.4 ± 1.5 mm, mean weight = 451 mg;

n=181; Table 2). There were too few second molts (n=3 total) in the 3 ambient temperature treatments (0°C) to determine separate growth rates or IMPs for these treatments (Table 2). However, growth rates from the initial molts serve as a dataset for ambient

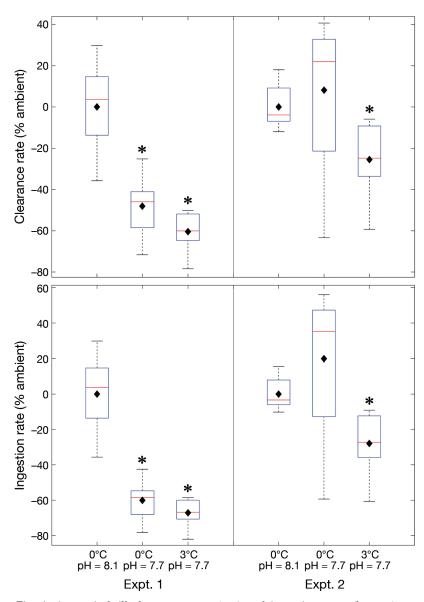


Fig. 1. Antarctic krill clearance rates (top) and ingestion rates (bottom) on chlorophyll a expressed as % ambient during Expts 1 and 2. Temperature = 0°C and pH = 8.1 represents ambient conditions. The mean clearance rates in ambient temperature and pH conditions for Expts 1 and 2 were 370 and 196 ml ind. $^{-1}$ h $^{-1}$, and the mean ingestion rates in ambient temperature and pH conditions were 15.1 and 6.9 µg chlorophyll ind. $^{-1}$ d $^{-1}$, respectively. These means were set to zero here, and the 2 other treatments in each experiment (ambient temperature/low pH, 0°C/pH = 7.7; high temperature/low pH, 3°C/pH = 7.7) are expressed as % ambient rates. The box limits extend from the lower to upper quartiles, with a line at the median and a diamond at the mean. Whiskers show the range of the data. Significantly lower clearance or ingestion values within an experiment are denoted by * (multi-way ANOVA, Tukey-Kramer post hoc tests, p < 0.0001)

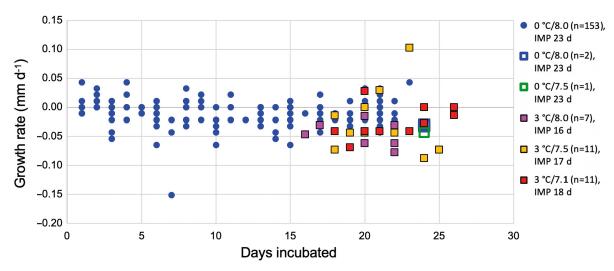


Fig. 2. Growth rates of individual Antarctic krill shown by number of days incubated prior to molting. Blue circles represent growth rates from initial molts (ambient temperature/pH). Squares represent growth after exposure to treatment conditions over 1 molt cycle. Key shows treatment conditions, number of data points, and intermolt period (IMP) for each treatment. Note that axes do not intersect at zero

temperature/pH since these rates were obtained under the same conditions as the ambient temperature/pH treatment (0°C, pH = 8.0). Thus, data from this experiment comprise growth rates and IMPs from initial molts at ambient temperature/pH and from second molts at high temperature treatments for pH = 8.0 (ambient), 7.5, and 7.1 (Table 2).

Because IMP is strongly tied to temperature (e.g. Poleck & Denys 1982, Buchholz 1991, Atkinson et al. 2006, Kawaguchi et al. 2006, Tarling et al. 2006, Wiedenmann et al. 2008, Brown et al. 2010), most of

the krill that molted twice during the experiment were from the high temperature treatments. Using initial molts to represent ambient temperature/pH conditions, the total number of molts was n = 153, with an IMP of 23 d. In the high temperature treatments, krill exposed to ambient pH had n = 7molts and IMP of 16 d, krill in pH = 7.5had n = 11 molts and IMP of 17 d, and krill in pH = 7.1 had n = 11 molts and IMP of 18 d. The IMP was significantly shorter at high temperature (mean among the 3 pH treatments = 17 d) compared to ambient (23 d) but was unaffected by pH (Fig. 2). The number of days incubated and TLs of krill (average and range) were similar among treatments (Table 2).

Individual krill growth rates were negligible, frequently negative, and

generally ranged between 0 and -0.05 mm d⁻¹ (Fig. 2). Growth rates at ambient temperature/pH were significantly higher than in the 3 high temperature treatments, but there were no significant differences among the 3 pH treatments at high temperature (Fig. 3; Kruskal-Wallis, Dunn post hoc tests, p < 0.05). At ambient temperature, the average growth rate was -0.01 mm d⁻¹. This rate was the same whether averaging all initial growth rates (n = 153) or just the initial growth rates of the krill that subsequently molted again in one of the treatments (n =

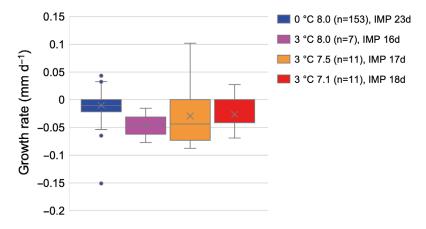


Fig. 3. Growth rates of Antarctic krill from initial molts under ambient conditions (0°C, pH 8.0) and from second molts in high temperature (3°C) treatments. The color key shows treatment conditions, number of data points, and intermolt period (IMP) for each treatment. The box limits extend from the lower to upper quartiles, with a line at the median and an X at the mean. Median = lower quartile in the high temperature/pH = 7.1 treatment. Whiskers show the range of the data. Blue dots represent outliers present in the ambient temperature/pH treatment

32). Average growth rate at high temperature (n = 29 total among all treatments) was -0.03 mm d⁻¹. The growth rates of the few individuals (n = 3 total) that molted twice in the ambient temperature/low pH (pH = 7.5 or 7.1) treatments were similar to growth rates in the high temperature treatments (Fig. 2). The overall average growth rate for all individuals that molted twice, in ambient and high temperature treatments combined, was -0.04 mm d⁻¹.

3.3. Survival

Krill analyzed for survival had a mean \pm SD weight of 618 \pm 208 mg and mean length of 40.88 \pm 2.5 mm. Survival ranged from 58 to 98%, with significant differences among treatment groups (p = 0.001; Fig. 4). There was no difference in survival between krill held at ambient temperature/pH or ambient temperature/low pH (90 and 98%, respectively). However, mortality was higher at high temperature/low pH (58%) than at ambient temperature/pH (p = 0.02). Additionally, there was an indication (p = 0.08) of slightly higher mortality at high temperature/ambient pH (67%) than at ambient temperature/pH, due solely to temperature. However, only 1 of the 3 trials analyzed for survival included a high temperature/ ambient pH treatment group (2015 growth experiment), resulting in a smaller sample size (n = 20) than the other groups (n = 52 each). Further, in the 2015 growth experiment, anomalous mortality (n = 6)occurred in the high temperature/ambient pH treat-

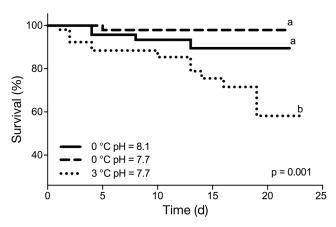


Fig. 4. Survival of Antarctic krill at various levels of experimental temperature and pH. Survival curves with different letters are significantly different (p < 0.05). Ambient temperature and pH conditions are $0^{\circ}\text{C/pH} = 8.1$. Survival was also assessed in individuals at $3^{\circ}\text{C/pH} = 8.0$. Due to differences in sample size, this group is not included here but all comparisons can be found in Section 3

ment. The low mortality at high temperature in other treatments (Table 2) suggests that this mortality was unrelated to treatment conditions (discussed further in Section 4.2). Therefore, although the trend aligns with what might be expected, results for survival at high temperature/ambient pH are preliminary, and this group was omitted from Fig. 4.

3.4. Acid-base physiology experiments

Krill analyzed in these experiments had a mean ± SD weight of 803 ± 154 mg (equivalent length 48 mm). Full acid-base variables and statistical analyses are reported in Table S1 in the Supplement at www.intres.com/articles/suppl/m665p001_supp.pdf. Tissue TCO2 was highly variable both within and across treatments and time, ranging from a mean of 6.41 ± $3.08 \text{ to } 18.84 \pm 3.94 \text{ mmol kg}^{-1}$ (Fig. 5A). Environmental pCO₂ had a significant effect on tissue TCO₂, which was higher under low compared to ambient pH (Fig. 6B). Time also had a significant effect on TCO₂, which generally increased through 12 h in all treatments, then declined by 48 h (Fig. 6C). Tissue pH ranged from a mean \pm SD of 7.14 \pm 0.03 to 7.30 \pm 0.08 (Fig. 5B) and showed an effect of time similar to that of TCO₂, with higher tissue pH at 6 h compared to shorter and longer exposures regardless of treatment (Fig. 6F). Tissue pH was also higher under low compared to ambient pH in krill held at high temperature, although this acid-base disturbance was transient with a return to baseline by 24 h (Fig. 5B). Tissue lactate was low, ranging from 0.0 ± 0.00 to $1.6 \pm$ 1.82 mmol kg⁻¹ (Table S1), and exhibited no change with temperature, pH, or time.

Blood pH ranged from 8.11 ± 0.16 to 8.21 ± 0.04 (Fig. 5C). There was no effect of temperature or pH on blood pH. Blood lactate ranged from 0.00 to 4.5 ± 1.70 mmol l⁻¹ (Fig. 5D) and declined from a high at 1–6 h to a low at 24–48 h (Fig. 6L).

3.5. Metabolic rate experiment

Metabolic rate was determined across 2 size ranges of adult krill, designated as smaller (116–294 mg, 31–35 mm) and larger (490–953 mg, 40–52 mm). Mass-specific oxygen consumption rates per gram wet weight (MO₂; µmol O₂ g⁻¹ h⁻¹) declined significantly with body mass (M) in each treatment according to $MO_2 = aM^b$, where a is a normalization constant and b is the scaling coefficient describing the slope of the relationship (at ambient temperature/

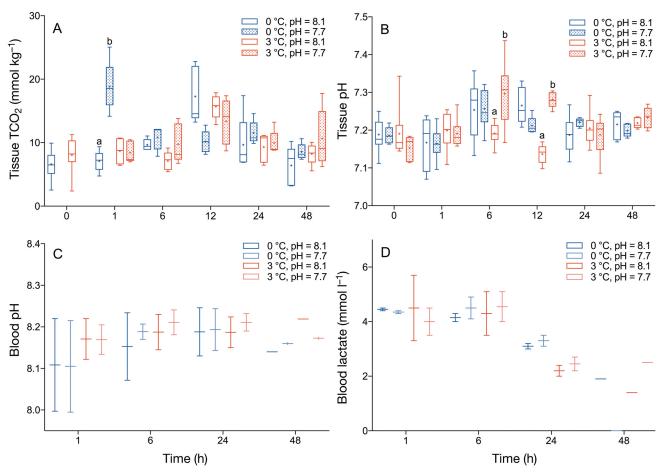


Fig. 5. Acid-base physiology of Antarctic krill at various levels of experimental temperature and pH. Included are (A) tissue total CO_2 (TCO_2 ; mmol kg^{-1}), (B) tissue pH, (C) blood pH, and (D) blood lactate (mmol l^{-1}) by treatment group and time. Ambient temperature and pH conditions are 0° C/pH = 8.1. (A,B) The box limits extend from the lower to upper quartiles, with a line at the median and a + symbol at the mean. Whiskers show the range of the data. (C,D) Whiskers show the range of the data with a line at the median. Significant differences between groups within a time point and temperature treatment are denoted by different letters (p < 0.05)

pH, $MO_2 = 2.8923M^{-0.308}$; at ambient temperature/ low pH, $MO_2 = 2.9221M^{-0.125}$; and at high temperature/low pH, $MO_2 = 3.5036M^{-0.407}$). The scaling coefficients were significantly different for each treatment (Fig. 7; ANCOVA, p < 0.0001). At ambient temperature, larger krill exhibited similar oxygen consumption rates regardless of pH treatment (mean \pm SD = 3.2 \pm 0.6 μ mol O₂ g⁻¹ h⁻¹). However, rates diverged in smaller krill, with higher rates at ambient pH (5.0 \pm 1.5 μ mol O₂ g⁻¹ h⁻¹) compared to low pH $(3.7 \pm 1.0 \mu mol O_2 g^{-1} h^{-1})$. At high temperature, metabolic rates were higher across the full size range $(4.2 \pm 0.8 \text{ and } 7.2 \pm 2.4 \text{ } \mu\text{mol O}_2 \text{ g}^{-1} \text{ h}^{-1} \text{ in larger and}$ smaller krill, respectively), and the scaling coefficient was significantly higher compared to either of the ambient temperature treatments, indicating a greater sensitivity of metabolic rate to body mass (Fig. 7). In the low pH treatments, the effect of tem-

perature appeared greater for smaller, compared to larger, krill.

4. DISCUSSION

This study, which is the first to examine Antarctic krill feeding, growth, and physiological responses under combined warming and ocean acidification conditions, supports 3 dominant conclusions. (1) High $p\text{CO}_2$ had a slight effect on feeding rates, metabolic rates, and acid–base balance in the short term, with greater effects on smaller-sized krill at high temperature. (2) Krill have the ability to compensate for lower pH within hours to days, as observed in the feeding and acid–base experiments. (3) Longer-term effects on krill growth and survival were strongly driven by temperature, with little to no effect from reduced pH.

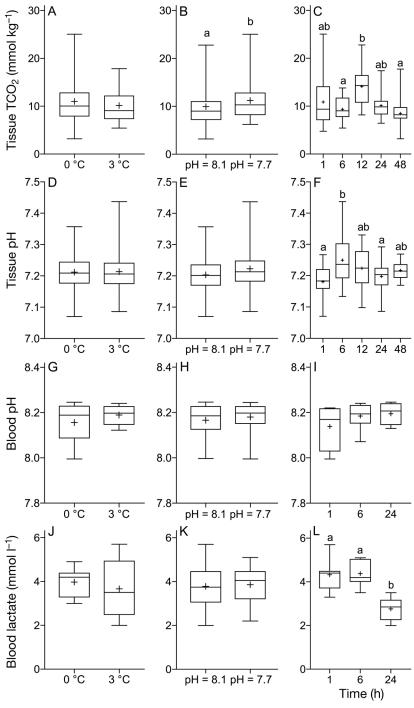


Fig. 6. Acid–base physiology of Antarctic krill with main effects of temperature, pH, or time. Included are (A–C) tissue total CO_2 (TCO₂, mmol kg⁻¹), (D–F) tissue pH, (G–I) blood pH, and (J–L) blood lactate (mmol l⁻¹). Ambient temperature and pH conditions are 0°C/pH = 8.1. Box plot definitions as in Fig. 5

4.1. Feeding processes

Ingestion rates in the present study (mean = $436 \mu g$ C krill⁻¹ d⁻¹; using carbon:chlorophyll conversion of 63:1; Ducklow et al. 1993, Bernard et al. 2012) were

mostly within the range of ingestion rates previously reported for Euphausia superba in the summer (50-445 µg C krill⁻¹ d⁻¹, Perissinotto et al. 1997; 129-447 µg C krill⁻¹ d⁻¹, Bernard et al. 2012). Ingestion rates above and below those rates reported in previous studies occurred under ambient temperature/pH conditions in Expt 1 $(610-1231 \mu g C krill^{-1} d^{-1}; mean =$ 948 µg C krill⁻¹ d⁻¹) and in the high temperature/low pH treatment in Expt 2 (0-74 µg C krill⁻¹ d^{-1} ; mean = 22 μg C krill⁻¹ d⁻¹; see below), respectively. Despite initial chlorophyll concentrations nearly 50% lower in the present study than those concentrations observed by Saba et al. (2012), ingestion rates were higher overall in the present study compared to the former (Saba et al. 2012; 0-154 µg C $krill^{-1} d^{-1}$, mean = 51 µg C $krill^{-1} d^{-1}$). Phytoplankton community composition was not analyzed in the present study; however, despite the lower chlorophyll concentration, it is possible that the food quality (i.e. phytoplankton species and cell size) in the present study was more favorable compared to Saba et al. (2012), when phytoplankton composition was dominated by small cryptophytes that krill cannot feed on efficiently (Quetin & Ross 1985). The krill in the present study were collected farther north in the WAP (near Palmer Station) and were smaller adults (Expts 1 and 2 combined mean length = 36.2 mm) compared to krill in the study by Saba et al. (2012; mean = 46 mm), so differences in feeding rates between these studies could result from differences in size, maturity stage, or even physiological state.

A recent review found that ingestion rates of arthropods (including krill) were highly variable in response to ocean acidification conditions (Cle-

ments & Darrow 2018). Indeed, even *E. superba* ingestion rates with respect to pH from the present study differ from those rates reported by Saba et al. (2012). Ingestion rates in the present study were suppressed at ambient temperature/low pH (Expt 1) and

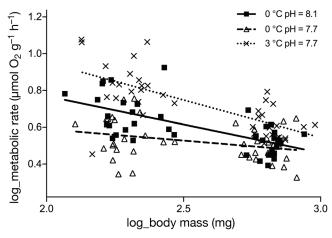


Fig. 7. Mass-specific metabolic rate per gram wet weight (log transformed, $\mu mol~O_2~g^{-1}~h^{-1}$) with body mass (log transformed, mg) in smaller (116–294 mg) and larger (490–953 mg) adult Antarctic krill at various levels of experimental temperature and pH. Ambient temperature and pH conditions are 0°C/pH = 8.1. Slopes between treatment groups were significantly different (p < 0.0001)

in the high temperature/low pH treatment (Expts 1 and 2) compared to ingestion rates at ambient temperature/pH, whereas ingestion rates increased under low pH in Saba et al. (2012). In the former study, ingestion rates were only significantly affected by pH in gravid females, suggesting that sex, reproductive condition, and/or maturity state affect how krill respond to the combined effects of elevated temperature and pCO₂. Furthermore, the differences relative to acclimation times between the 2 studies (no acclimation in Saba et al. 2012 vs. 48 h in our Expt 1) suggest that even a short exposure to treatment conditions is sufficient for krill to acclimate to changes in seawater chemistry and/or temperature, yielding a vastly different response. In the present study, the response of krill acclimated for 48 h was consistent with metabolic suppression, similar to that observed in the littoral mysid Praunus flexuosus and the Antarctic pteropod Limacina helicina after prolonged exposure (Seibel et al. 2012, Sperfeld et al. 2017).

Differences in pH effects on feeding rates (both ingestion and clearance rates) between Expts 1 and 2 could be a function of krill size and/or acclimation time. Feeding rates (Expt 1) were affected by pH only in the smaller krill size range (30–36 mm, 100–300 mg). Krill in feeding Expt 2 were larger, which is consistent with the lack of pCO_2 effects observed in larger krill in the acid–base, growth, and metabolic experiments. Therefore, larger (non-gravid) adults may have the capacity for physiological resilience with increasing environmental pCO_2 . The present

study also demonstrates that krill may continue acclimating to changes in pH over the course of 21 d, as indicated by a smaller effect of pH on feeding rates in krill in Expt 2 (21 d acclimation) compared to Expt 1 (48 h acclimation). A similar effect of elevated pCO_2 on ingestion rates also occurred in North Pacific krill $E.\ pacifica$ over a 21 d acclimation period (Cooper et al. 2016).

Feeding rates in Expt 2, however, were still significantly lower in the high temperature/low pH treatment. A confounding factor was the low initial concentration of chl a in the high temperature/low pH treatment in Expt 2 (mean = $0.39 \mu g l^{-1}$), which may have been too low to stimulate krill feeding behavior (Price et al. 1988). Why the initial chl a concentration was low in this treatment is unknown, as all treatments were prepared following the same protocols. Although this confounding factor precludes a meaningful comparison of the effects of combined high temperature/low pH between these 2 experiments, our results suggest that warming and/or acidification may have indirectly impacted krill feeding behavior in the present study by reducing phytoplankton biomass. Comparison of chl a concentrations between T₀ and T_{final} in the controls showed steep declines over time in the ambient temperature/low pH and high temperature/low pH treatments in both Expts 1 and 2. However, we were unable to determine if this decrease in chl a was due to reduced phytoplankton growth, a change in community composition, microzooplankton grazing, and/or other factors. Phytoplankton exhibit significant heterogeneity in their responses to ocean acidification (reviewed by Hancock et al. 2020), but most studies on Antarctic phytoplankton species or communities show negligible differences in chlorophyll concentrations between ambient and high pCO_2 levels near 1000 ppm (e.g. Davidson et al. 2016, Deppeler et al. 2018, Westwood et al. 2018). However, changes in Antarctic phytoplankton community composition have been reported in response to elevated pCO_2 (e.g. Hoppe et al. 2013, Davidson et al. 2016). Additionally, interactive effects of warming and ocean acidification favored the Antarctic diatom Pseudo-nitzschia subcurvata over the prymnesiophyte Phaeocystis antarctica (Zhu et al. 2017).

Additionally, microzooplankton grazing activity was possibly enhanced under elevated temperature and/or pCO_2 . Although few studies have assessed microzooplankton grazing rates under these conditions, a species of tintinnid ciliate and a heterotrophic dinoflagellate increased ingestion rates of the phytoplankton prey item $Emiliania\ huxleyi$ under elevated

pCO₂ (Olson et al. 2018), and Ross Sea microzooplankton abundance increased by 43% after a 1 wk incubation at 4°C (Rose et al. 2009). An increase in microzooplankton abundance in our low pH treatments would have provided an alternative food source for krill during our feeding experiments. Clearance and ingestion rates on ciliate-carbon were not significantly different between pH treatments and were much lower relative to feeding rates on chlorophyll reported by Saba et al. (2012), but the independent or combined effect of temperature was not tested in that study. Additional investigation is required to address potential mechanisms of changing phytoplankton biomass under warming and ocean acidification conditions, either directly through changes in growth rates or community composition or indirectly through variations in microzooplankton grazing.

4.2. Growth processes and survival

The effects of temperature (Poleck & Denys 1982, Buchholz 1991, Atkinson et al. 2006, Tarling et al. 2006, Brown et al. 2010) and ocean acidification (Sperfeld et al. 2014, 2017, Cooper et al. 2017, Ericson et al. 2018, Opstad et al. 2018) on molting and growth of E. superba or similar species have been independently studied, but to our knowledge no studies have addressed their combined effects. Results from the present study suggest that temperature, rather than ocean acidification, was a dominant driver of krill growth processes, although further study is needed to verify this finding. The warmer temperature resulted in a shorter IMP and lower growth rates, with no effect from pH. As a result of the strong link between IMP and temperature, most of the krill that molted twice during the experiment were from the high temperature treatments. Previous studies found a similar decrease in post-larval krill growth rates at higher temperatures: Atkinson et al. (2006) at temperatures >1°C and Brown et al. (2010) at both 1 and 3°C. The IMP values determined by Brown et al. (2010) were 24 d at 1°C and 19 d at 3°C, similar to IMP values in the present study (23 d at 0°C and 17 d at 3°C). Also similar to the present study, the IMP of the north Atlantic euphausiid Nyctiphanes couchii did not change under elevated pCO₂ (Sperfeld et al. 2014), although juvenile Praunus flexuosus (mysid) showed an increased IMP with increasing pCO_2 (Sperfeld et al. 2017). Although pCO₂ reduced growth rates in other crustaceans (Wickins 1984, Kurihara et al.

2008, McLean et al. 2018) and the krill species E. pacifica (Cooper et al. 2017), the present study and previous studies found no significant impact of pCO_2 on growth in krill (adult Antarctic krill during a 1 yr perturbation experiment in Ericson et al. 2018; North Atlantic krill in Sperfeld et al. 2014, Opstad et al. 2018). For E. superba, IMP and growth are so strongly affected by temperature that assessing the combined effect of temperature and pH would likely require much longer incubations than were feasible during this field season.

There is a great deal of complexity inherent in determining growth rate and IMP in krill. Both are likely influenced by factors in addition to temperature (e.g. time in captivity, food, container size, life history stage, etc.), but these causal relationships could not be distinguished in the present study due to time limitations and small sample size. Although negative growth suggests suboptimal conditions, adult krill are known to shrink in relation to season, sex, maturity stage, food quantity and quality, time in captivity, and container size (Siegel 1986, Price et al. 1988, Nicol 2000, Ross et al. 2000, Kawaguchi et al. 2006, Meyer 2012, Meyer & Teschke 2016, Tarling et al. 2016). Kawaguchi et al. (2006) found that growth rates decreased over the course of the austral summer season and were close to zero or negative by April. Thus, there may have been a seasonal effect on growth rates in the present study, which took place from February to early March. In addition, previous work suggests that in captivity, krill growth rates decrease (Kawaguchi et al. 2006) and processes such as feeding, growth, and respiration do not occur at natural rates (Buchholz 1991, Quetin et al. 1994, Tarling et al. 2006). Container size may also impact krill behavior, particularly feeding behavior. Although krill grew larger in 19 l buckets during the 21 d acclimation period of the 2014 feeding experiment presented here, another feeding study using smaller containers (5 l), similar to the size used in the growth experiment (4 l), found that krill tended to bump into the sides or swim around the circumference of the bottle without feeding (Price et al. 1988). There is also increasing evidence that growth and IMP differ between females and males (Buchholz et al. 1996, Atkinson et al. 2006, Tarling et al. 2006, Brown et al. 2010). Tarling et al. (2006) suggested that the IMP of males was likely to be 50% longer than that of females of similar size, particularly at temperatures above 2°C. Thus, the sex ratio in an experiment may influence average growth and IMP, an effect that may be exacerbated when comparing temperatures below and above 2°C. Future experimental designs should consider these complex aspects of the life history of *E. superba*.

There was an indication of decreased survival at high temperature (58% at high temperature/low pH and 67% at high temperature/ambient pH) compared to ambient (90% at ambient temperature/pH and 98% at ambient temperature/low pH); however, additional studies are needed to verify this finding. Further, the slightly lower growth rate in the high temperature/ambient pH treatment in the 2015 growth experiment is likely a result of the small sample size (n = 7), which was affected by the anomalously high mortality (n = 6) in this treatment group compared to the low mortality in the high temperature/pH = 7.5 and high temperature/pH = 7.1 treatments (n = 1 per treatment). A previous long-term study of E. superba found that ~75% of mortality occurred in relation to ecdysis (Poleck & Denys 1982). Therefore, mortality in the 2015 growth experiment may not have been directly caused by treatment conditions. Instead, mortality was likely related to post-molt condition issues not apparent in the appearance or behavior of the krill, and the occurrence of these individuals in the high temperature/ ambient pH treatment could have been a mere coincidence. The likelihood that elevated pCO2 enhanced survival seems low, as mortality was low in all other treatments in the present study, and increased survival at elevated pCO2 has not been observed in other published ocean acidification studies focused on multiple krill species (Sperfeld et al. 2014, Cooper et al. 2017, Ericson et al. 2018, Opstad et al. 2018). The present study provides an enticing first look at the combined effects of temperature and pH on growth, IMP, and survival, but a full understanding will require further studies with larger sample sizes.

4.3. Physiological processes

High pCO_2 resulted in slight increases in both tissue TCO_2 and tissue pH; however, these disturbances were moderate and transient with a return to baseline by 24 h. There was also a tendency for tissue TCO_2 , tissue pH, and blood pH to increase with time over the first 12 h regardless of treatment. This trend appears related to a substantial lactic acidosis that may have been caused by handling stress and resolved within 24 to 48 h of exposure.

Metabolic rates increased with temperature. A precise temperature coefficient could not be determined due to the different scaling coefficients between the 2 temperatures. Under low pH conditions, the high temperature (3°C) treatment scaled with body mass with a greater slope relative to the ambient temperature (0°C) treatment under low pH conditions (Fig. 7). At the smaller end of the size range, metabolic rates were 48% lower at ambient temperature/low pH compared to those rates at high temperature/low pH. This difference declined to 24% at the larger end of the size range. These values are equivalent to a Q_{10} of 8.33 and 2.75 at the smaller and larger end of the size range, respectively. By comparing our results to literature findings, we infer that pH likely had no effect on metabolic rates of larger krill at 3°C because the metabolic scaling coefficient at 3°C and low pH and/or the temperature effect at large size and low pH reported in the present study were within the range previously reported for euphausiids (Ikeda 2013) and for E. superba specifically (Tarling 2020) at ambient pH. Additionally, the rates measured at 3°C and low pH in the present study are within the range previously reported at similar temperatures (2-5°C) at ambient pH (rates compiled by Meyer & Teschke 2016, Tarling 2020).

At the colder temperature (0°C), high pCO_2 dramatically reduced the scaling coefficient, suggesting a pronounced metabolic suppression amongst smaller animals. This suppression likely contributed to the large temperature effect observed in small krill held at low pH. Metabolic suppression is typically an evolved response to resource limitation, sometimes triggered by pH or pCO2 (Seibel & Fabry 2003, Melzner et al. 2020). For krill, resources may be limited during over-winter periods spent in deeper waters where pO_2 is reduced and pCO_2 is elevated (McNeil et al. 2010, Meyer 2012). Although we did not find any prolonged intra- or extracellular acid-base disturbances that could explain the metabolic suppression, acid-base parameters were only measured in larger krill, for which metabolism was unaffected. If a sizable extracellular (blood) acid-base disturbance in smaller krill occurred, blood-oxygen binding affinity would have also been impacted due to the large Bohr coefficient (reported by Bridges et al. 1983), which could cause oxygen limitation and lead to the reduced metabolic rates evident in the smaller size range. Given the reduction in metabolic rate in smaller krill and the absence of any substantial acid-base disturbance in larger krill, costs of ion transport and acid-base regulation may not be a significant factor for krill. This species apparently possesses substantial buffering and acid-base regulatory capacity (Ericson et al. 2018).

5. CONCLUSIONS

Future ocean condition scenarios include a combination of warming temperatures, increasing ocean acidification, and possible additional stressors such as decreasing dissolved oxygen levels. Our study examined the combined effects of ocean acidification and warming temperature and found that shorterterm effects were primarily due to decreased environmental pH, and krill were able to acclimate to these conditions in a matter of hours to days. However, krill size was a factor in acclimation potential, with preliminary evidence that smaller adults may be more susceptible to immediate negative impacts with ocean acidification. Longer-term effects were primarily due to elevated temperature, and krill may need to compensate for these impacts through efficient energetic partitioning or higher consumption of prey. Therefore, food intake and availability should be considered when determining how krill will respond to environmental change.

How krill populations will tolerate future climate change in the Antarctic remains an open question. High variability across studies and among individual animals prompts the need for additional research with an improved experimental approach to maximize sample size, prolong exposure to treatment conditions, and incorporate details including food quantity and quality as well as life history stage. Laboratory studies must also account for differences between conditions in captivity and the open ocean, particularly container size and differences in the prey field. Future studies would benefit from determining threshold food concentrations. Additional multi-stressor experiments with prolonged exposure periods would further elucidate the underlying physiological mechanisms of ocean acidification- and temperature- induced responses, potential costs of increased energetic demand, potential acclimation of krill to ocean acidification and warming, and associated feedbacks on the ecosystem and biogeochemical cycles.

Data availability. Data presented in this paper are available at: https://www.bco-dmo.org/project/721363.

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