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Effects of elevated pCO_2 on the survival, growth, and moulting of the Pacific krill species, *Euphausia pacifica*

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While ocean acidification (OA) is expected to have wide-ranging negative effects on marine species, organisms currently living in variable pH environments that expose them intermittently to pH values approaching those predicted for the future, may be better adapted to tolerate prolonged exposure to high pCO_2 levels caused by OA. Seasonal upwelling brings low pH water to the surface along the Pacific Coast of North America. In Monterey Bay, California *Euphausia pacifica*, a key species supporting a diverse multi-trophic-level ecosystem, currently experiences broad pCO_2 and pH ranges due to both diel vertical migrations and seasonal upwelling. We determined tolerances of *E. pacifica* to prolonged exposure to pH levels predicted for 2100 by maintaining adults at two pCO_2 levels (380 and 1200 μatm) for 2 months. Rates of survival and moulting were the same at both pCO_2 levels. High pCO_2 slowed growth in all size classes. In additional experiments to determine pCO_2 threshold levels above which *E. pacifica* is adversely affected, survival was not affected down to pH 6.96 (6050 μatm), but declined rapidly at pH 6.92 (7228 μatm) and lower, with 100% mortality within 10 d at pH 6.89.

Keywords: growth, krill, moulting, ocean acidification, survival, threshold.

Introduction

Ocean acidification (OA) is the decline in ocean pH and carbonate ion concentration caused by the uptake of atmospheric CO_2 by ocean surface waters (Orr *et al.*, 2005; Fabry *et al.*, 2008; Doney *et al.*, 2009). Many biological investigations of OA show adverse effects on marine organisms, including reduced growth, lower calcification rates, reduced fertilization success, and abnormal larval development (Kurihara *et al.*, 2004; Kleypas *et al.*, 2006; Andersson *et al.*, 2011; Ross *et al.*, 2011). However, these effects vary across taxa and habitats (Kroeker *et al.*, 2010), and some studies show positive or no effects of OA (Ries *et al.*, 2009; Kroeker *et al.*, 2010; Wittmann and Pörtner, 2013).

A quickly expanding area of research is the role of natural environmental variation in determining the tolerances of organisms to future OA levels. Near shore habitats around Monterey Bay, California experience highly variable pH and pCO_2 levels caused by seasonal wind-driven upwelling that brings deep, CO_2-rich waters to the surface (Feely *et al.*, 2008). These habitats can fluctuate by ~0.35 pH units over several days (Hofmann *et al.*, 2011). Organisms living in such upwelling regions may already be well adapted to future OA, due to histories of repeated exposure to lower pH water for much of the year (Thomsen *et al.*, 2010; Lewis *et al.*, 2013). Conversely, these organisms may already be living near their limits of tolerance (Yu *et al.*, 2011), and may face increasing pH stress in the future, since OA is likely to increase the frequency, duration, and intensity of low pH conditions (Feely *et al.*, 2008; Hauri *et al.*, 2009; Gaylord *et al.*, 2011; Barton *et al.*, 2012).

As a group, crustaceans have mixed responses to OA, but overall, they seem more tolerant of OA than many other taxa (Kroeker *et al.*, 2010, 2013; Wittmann and Pörtner, 2013). At ecologically relevant elevated pCO_2 levels, crustaceans may experience declines in egg production, hatching success, growth, and survival and/or changes in mineral content of structures (Kurihara *et al.*, 2008; Arnold *et al.*, 2009; Findlay *et al.*, 2009; Long *et al.*, 2013; Lewis *et al.*, 2013; Cripps *et al.*, 2014; Sperfeld *et al.*, 2014; Zheng *et al.*, 2015), although many crustacean species show no negative effects

at these levels (Arnold *et al.*, 2009; McConville *et al.*, 2013). Within-taxon differences in responses to increased pCO_2 are often attributed to differences in metabolic rates, habitat variation, and/or life stages (Pane and Barry, 2007; Kroeker *et al.*, 2013; Cripps *et al.*, 2014).

Euphausiids, commonly known as krill, are pelagic marine crustaceans with global distributions (Mauchline and Fisher, 1969). Krill form ecologically and economically important links between primary producers (phytoplankton) and many forage and commercial fish species (e.g. salmon, herring) that feed directly on krill, as well as to the seabirds and great whales at the top of these food chains. Despite their importance in marine foodwebs, responses of krill to OA remain largely unknown. Previous studies have focused primarily on the Antarctic krill, *Euphausia superba*, in which embryonic development and larval behaviour were unaffected at pCO_2 of 1000 μatm, but eggs completely failed to hatch at 2000 μatm (Kawaguchi *et al.*, 2011, 2013). Adult *E. superba* respond to elevated pCO_2 by increasing feeding rates and excretion of nutrients, both of which suggest OA increases metabolic rates (Saba *et al.*, 2012). The only northern hemisphere species studied to date is *Nyctiphanes couchii*, which lives in the northern Atlantic and is abundant in the North Sea and Celtic Sea. Elevated pCO_2 reduced survival and increased the proportion of deaths associated with moulting in sub-adult *N. couchii*, but did not affect intermoult period or growth rate (Sperfeld *et al.*, 2014).

Euphausia pacifica is a dominant krill species in the northeast Pacific, with a North American range extending from Southern California to Alaska (Brinton, 1962). *Euphausia pacifica* is abundant year-round in Monterey Bay, California, where it is associated with the deep waters of the submarine Monterey Canyon. Major population increases of *E. pacifica* occur following upwelling in late spring and early summer (Brinton, 1976). These dense aggregations of *E. pacifica* support large populations of resident and migratory seabirds (Ainley *et al.*, 1996), pinnipeds and cetaceans, including the largest ocean predator (blue whale), that are attracted to Monterey Bay in summer to feed (Croll *et al.*, 2005).

Adult *E. pacifica* typically undergo diel vertical migrations, often from hundreds of meters depth during the day to the surface at night where they feed (Brinton, 1976; Bollens *et al.*, 1992; Marinovic, and Mangel, 1999). Off the Pacific coast of North America, where strong summer winds induce upwelling of cold, high pCO_2, and low-pH water, vertical migration of *E. pacifica* exposes them daily to pH ranges from ~8.1 at the surface to 7.6 at depth (Feely *et al.*, 2008). While little is known about how *E. pacifica* will be affected by increased pCO_2, juveniles in one study tolerated pH 7.54 for 7 d (pH was lowered by addition of acid, not increased pCO_2) (Yamada, and Ikeda, 1999).

We assessed the effects of continuous exposure of adult *E. pacifica* to high pCO_2/low pH by measuring survival, growth, and moulting frequency during a 2-month incubation experiment. The results led to additional survival experiments, across a broad pH range that was designed to constrain pCO_2 values for the critical thresholds of pH tolerance.

Material and methods

Collections

We collected krill after sunset (when krill had migrated to the surface), ~13 kilometres offshore in Monterey Bay by taking oblique tows from ~30 m depth to the surface using a 1-m diameter plankton net (500 μm mesh) with a 1 l, non-filtering codend. Krill were sorted on-board ship into groups of 8–10 individuals that were placed in 750 ml jars and kept on ice in a cooler for transport back to the Long Marine Laboratory of the University of California, Santa Cruz. Once at the laboratory, krill were maintained individually in 750 ml jars of filtered seawater in darkness in a water table at 9°C for 1 week before the experiments began. Each krill was fed to excess daily by adding 10 ml of a diatom culture (*Thalassiosira*; $3–5 \times 10^5$ cells ml^{-1}) to each 750 ml jar; this was supplemented every 4–5 d with newly hatched brine shrimp (*Artemia*) nauplii. This feeding regime was continued throughout the experiment. Krill were maintained at ambient pCO_2 before the start of the experiment.

Seawater preparation

Mixtures of air and CO_2 with the desired pCO_2 were prepared by adding a small amount of certified pure CO_2 gas (Prax-Air) to a 30 l steel cylinder, then adding compressed air until the desired concentration was reached. pCO_2 content was monitored with a CO_2 analyser (Quibit Systems S151). New gas mixtures were blended twice a week throughout the study. The seawater supply at the Long Marine Laboratory is taken from Monterey Bay, passed through sand filters to header tanks, then gravity-fed to individual laboratories. We then filtered the water to 0.2 μm, and stored it in 20 l carboys. The appropriate pCO_2 gas mixture was bubbled into the carboys for 4 d through gel membrane bubblers, until the water equilibrated with the gas, and pH was at the desired level. Experimental seawater was prepared weekly.

Growth experiment

Experimental design

We measured effects of pCO_2 on krill survival, intermoult period, and growth in a single factor, two-treatment experiment. The two pCO_2 levels were selected to represent recent atmospheric levels ($pCO_2 = 380$ ppm, $pH_T = 8.01$) and a level above the IPCC scenario RCP8.5 or "high emissions scenario" for 2100 ($pCO_2 = 1200$ ppm, $pH_T = 7.60$) (Stocker *et al.*, 2013; Edenhofer *et al.*, 2014). Both treatments were housed in a single water table maintained at 9 ± 1°C by recirculating the water through an inline chiller (Aqua Logic Delta Star). 9°C is representative of conditions in Monterey Bay at 200 m depth and within the temperature range of maximum growth efficiency for *E. pacifica* (Iguchi and Ikeda, 1995; Pennington and Chavez, 2000). The table held ten 20 l glass aquaria (five per treatment). Each aquarium was sealed with a gas-tight plexiglas lid, and contained four polycarbonate jars standing in seawater. Each jar held one adult krill in ~750 ml of seawater (20 individuals per treatment).

The seawater in each aquarium, and in each jar within the aquarium, was pre-equilibrated to the desired pCO_2 level, and the treatment pCO_2–air mixture was pumped slowly through the lid of each aquarium to maintain the desired pCO_2 level in the headspace of each aquarium. The top of each jar was open to the aquarium headspace to maintain the pCO_2. Water in every jar was changed every 2 d. The krill were maintained in darkness, except for a few minutes each day while they were checked for moults under low light. pH and temperature were measured daily in each jar with an Oakton WD-35613 hand-held pH meter. Water samples (100 ml) were taken from each aquarium every 7 d, poisoned with mercuric chloride, and stored for subsequent total dissolved carbon (C_T) and alkalinity (A_T) analyses. The experiment ran for 57 d, from 21 August through 16 October 2012. One krill died from trauma and was excluded from all analyses.

Survival, intermoult period, and growth

Krill were checked daily for moulting and mortality by transferring each animal and its treatment water into a white plastic tub, searching for moulted exoskeletons, then returning the krill and water to the jar. All moults were collected and measured immediately using a Wild M3 dissecting microscope outfitted with a Canon Rebel 2Ti DSLR camera, and the images were analysed using ImageJ software (NIH). Growth was determined from the difference in telson length of consecutive moults, measured from the middle of the rounded rise at the anterior end to the posterior tip of the telson (spines excluded). The telson is well preserved in moults, and its length is proportional to total body length (Shaw *et al.*, 2010). Between moults, euphausiids can grow larger, maintain size, or shrink (often in response to stressful conditions) (Marinovic and Mangel, 1999), so size is not a continuous monotonic-positive progression. Growth rate during the experiment was calculated for each individual by fitting a regression line to repeated telson measurements against number of days since start of the experiment. Rates are reported as change in telson length per day (mm d^{-1}).

For comparison with published growth data, absolute growth rates (mm d^{-1}) were calculated by converting telson length into total body length using the equation from Shaw *et al.* (2010) for *E. pacifica:*

$$\text{Total length (mm)} = (4.937 \times \text{telson length (mm)}) - 0.4142$$

pCO_2 threshold experiments

The upper thresholds of tolerance of *E. pacifica* to high pCO_2/low pH were determined in experiments using methods similar to those in the growth experiment. The main differences were that treatment levels were determined by pH instead of pCO_2 because the CO_2 monitor was not rated to test gases above 2000 ppm. Experimental water was prepared by bubbling very high pCO_2 gas into seawater in plastic carboys then diluting with ambient pCO_2 seawater until the desired pH was achieved. Each experimental jar was filled with pre-equilibrated water and sealed with a gas-tight lid to prevent CO_2 off-gassing because no additional gas was pumped into the headspace. Jars were placed directly into the water table, which was maintained at 13°C. While this experiment was conducted at a temperature slightly higher than the growth experiment, it is still within the range of temperatures experienced by *E. pacifica* in surface waters and at the upper limit of maximum growth efficiency for *E. pacifca* (maximum growth efficiency peaks at 11°C) (Iguchi and Ikeda, 1995; National Data Buoy Center, 2015). Each jar was checked every morning for mortality, and the water in the jars was replaced every 2 d.

Two thresholds were defined: (i) days until 50% of animals were dead and (ii) days until all animals were dead. Eight pH treatments from pH 7.87 to 6.84 were tested, with eight krill per treatment, and the experiment ran for 16 d. pH and temperature were measured daily in each jar (with the krill remaining alive) using an Oakton WD-35613 hand-held pH meter, and 100 ml discrete water samples were taken from representative jars approximately every 4 d for complete C_T and A_T analyses and to calibrate the hand-held pH meter readings.

Chemical analyses

Water samples were collected and poisoned with mercuric chloride following standard practices (Dickson *et al.*, 2007) before being stored. They were analysed for total inorganic carbon (C_T) using a CM5011 carbon coulometer (UIC, Inc.) and total alkalinity (A_T) using an automated open cell titration procedure. Instruments were calibrated using certified seawater standards (Batch 118) from Andrew Dickson's laboratory at the Scripps Institution of Oceanography. pH, pCO_2, and aragonite saturation state (Ω_{arag}) were calculated with CO2sys software (Pierrot *et al.*, 2006) using the C_T and A_T data and CO_2 disassociation constants from (Mehrbach *et al.*, 1973), refitted by Dickson and Millero (1987). pH is expressed in total scale (pH_T). Daily pH meter measurements were calibrated against the CO2sys calculated carbonate data.

Statistical analyses

All statistical analyses used the software JMP PRO 12. All chemical data are reported as mean ± 1 standard deviation (s.d.). In the growth experiment, mean values for all chemical parameters were calculated per aquaria and used to determine the mean for each treatment group.

Total survival over the course of the experiment was first analysed in a nested proportional hazards model (aquaria nested within pCO_2 treatment). Since there was no significant effect of aquaria on survival (Wald test, $p = 0.9973$), the aquarium term was dropped from the analyses and individual krill were used as replicates to increase the power of subsequent analyses.

Individuals with fewer than 3 moults were excluded from intermoult period and growth analyses. Consecutive intermoult periods were recorded for each individual krill, and the effect of duration of the experiment (days) on intermoult period was tested in a one-way ANOVA. Because there was no effect of duration ($F_{3,222} = 0.9589$, $p = 0.4124$), the mean intermoult period was calculated for each individual and a nested one-way ANOVA was run (aquaria nested within treatment) to compare mean intermoult periods in the two pCO_2 treatments.

Growth rates were estimated by regressing telson size on day of the experiment, with aquaria nested within pCO_2 treatment to account for any aquaria level effects. A quantile regression was used to determine whether growth rate, or the effect of pCO_2 on growth, differed along a distribution of sizes in the krill population. Quantile regression estimates the relationship between dependent and predictor variables in different quantiles of the response variable, and not just the mean value as in least-squares regression. Because linear growth rates may change with body size, mean values may mask some of the variation in responses; quantile regression techniques allow more detailed examination of the variation in responses across sizes and assessment of different responses (e.g. maximum, average, or minimum). Growth rates of krill in the 90th, 50th, and 10th size quantiles were compared.

For the pCO_2 threshold experiments, survival was expressed as number of days until (i) 50% mortality and (ii) 100% mortality. Kaplan–Meier survival curves were plotted as the daily proportion alive against number of days from start of the experiment in a stepped format. A log-rank test (also known as Mantel-Cox) was used to test for differences in the survival curves of the pH treatments. The log-rank test compares the observed number of deaths and the expected number of deaths, with the null hypothesis that the expected number of deaths is the same between groups.

Results

Growth experiment

Chemical data

Table 1 summarizes water conditions in the growth experiment. In both treatments, temperature and salinity remained constant within the

Table 1. Carbonate chemistry parameters (mean ± 1 s.d.) of seawater in krill growth experiment.

Parameter	Low pCO_2	High pCO_2	*p*-value
pH_T	8.06 ± 0.05	7.60 ± 0.06	<0.0001
pCO_2 µatm	395 ± 58	1289 ± 179	<0.0001
A_T µmol kg^{-1}	2244 ± 9	2255 ± 8	0.06
C_T µmol kg^{-1}	2068 ± 16	2238 ± 12	<0.001
Ω_{arag}	1.94 ± 0.17	0.77 ± 0.09	<0.001
Temp (°C)	8.9 ± 0.2	8.9 ± 0.2	1
Salinity (ppt)	33 ± 0.5	33 ± 0.5	1
N	5	5	

Data were compared with two-sample *t*-tests. Mean values ± s.d. of pCO_2 and aragonite saturation state (Ω_{arag}) calculated using CO2sys with measured input variables total alkalinity (A_T), total dissolved inorganic carbon (C_T), salinity, and temperature. Temperature was measured directly daily using hand-held meter.

Table 2. Survival, intermoult period (mean ± s.d.), and estimated telson growth rates (quantile ± s.e.) of *E. pacifica* in the growth experiment.

Treatment group	Low pCO_2	High pCO_2	*p*-value
Survival (%)	70.0	78.9	0.3688
Intermoult period (days)	6.2 ± 0.4	6.1 ± 0.2	0.2232
Telson growth rate (mm d^{-1})			
10th Quantile	0.009 ± 0.0013	0.006 ± 0.0009	<0.001
50th Quantile	0.009 ± 0.0023	0.005 ± 0.0016	0.6
90th Quantile	0.001 ± 0.0020	−0.005 ± 0.0013	<0.001

limits of instrumental precision throughout the experiment. Mean pCO_2 in the high pCO_2 treatment was 3.3 times greater than in the low pCO_2 treatment (1289 µatm vs. 395 µatm; $t = 10.64$, d.f. = 8 $p < 0.0001$), and this maintained a difference between treatments of 0.46 pH_T units (7.60 vs. 8.06; $t = -13.90$ d.f. = 8, $p < 0.0001$). Total inorganic carbon was higher in the high pCO_2 treatment ($C_T = 2238$ vs. 2068 µmol kg^{-1}; $t = 18.95$, d.f. = 8, $p < 0.001$) while aragonite saturation state was lower ($\Omega_{arag} = 0.77$ vs. 1.94, $t = -13.32$, d.f. = 8, $p < 0.001$). Total alkalinity was similar in both treatments ($A_T = 2255$ vs. 2244 µmol kg^{-1}; $t = 2.19$, d.f. = 8, $p = 0.06$).

Survival

Ten of the 39 individual krill died during the experiment (six in low pCO_2 and four in high pCO_2), giving survival of 70 and 79%, respectively, but the difference was not significant (Table 2; Wald test, $p = 0.3688$).

Moulting

The intermoult period (time between moults) of individual krill did not change during the experiment (ANOVA, $F_{3,322} = 0.9589$, $p = 0.4124$). Krill moulted on average every 6 d in both treatments, and the rate was not affected by pCO_2 level (Table 2; $F_{8,9} = 1.451$, $p = 0.2232$).

Growth

Mean initial telson length of krill was 2.46 ± 0.47 mm (average of both CO_2 treatments; range = 1.81–3.42 mm), which is equivalent to 11.74 ± 2.34 mm (range 8.51–16.48 mm) total body length calculated using Shaw's equation (Shaw *et al.*, 2010). Initial calculated body sizes for both CO_2 treatments are provided in Table 3. The sizes of krill in the experiment are associated with both juvenile and adult stages (Brinton *et al.*, 1999). There were no differences in initial sizes

Table 3. Initial body length (mm) of krill.

	Initial body size (mm)	
Size class	Low CO_2	High CO_2
Minimum	8.72	8.51
10th quantile	8.85	9.16
50th quantile	11.20	11.14
Mean ± 1 s.d.	11.57 ± 2.32	11.84 ± 2.37
90th quantile	14.97	15.94
Maximum	16.49	16.29

Body length (mm) calculated using equation from Shaw *et al.* (2010) for regression of total length on telson length.

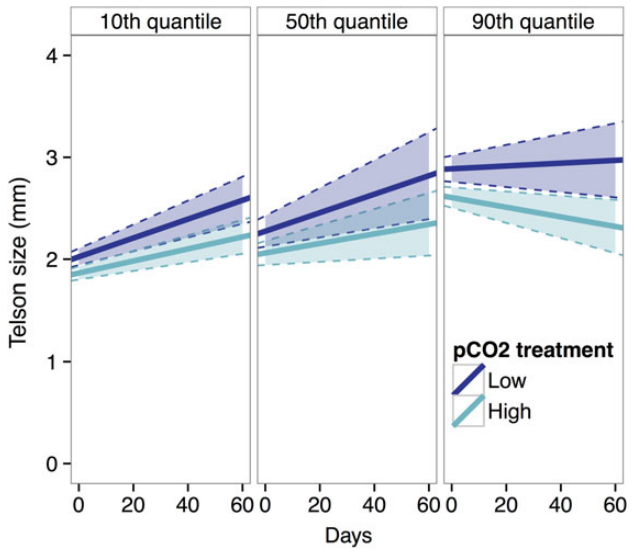

Figure 1. Fitted growth rates of 10th, 50th, and 90th size quantiles of *E. pacifica* in high and low pCO_2 treatments. Solid lines represent estimated growth rates; shaded areas are 95% confidence intervals. Intercepts and slopes are fitted using estimated parameters from quantile regression. This figure is available in black and white in print and in colour at *ICES Journal of Marine Science* online.

between the pCO_2 treatments (ANOVA, $F_{1,9} = 0.197$, $p = 0.6646$). Growth between moults varied among individuals and many individuals both grew and shrank at different times during the 57-d experiment. Overall, telson growth (average of both pCO_2 treatments) was fastest in smaller (10th percentile) krill (0.008 mm d^{-1}) compared with 0.006 mm d^{-1} in the 50th percentile krill, while the largest (90th percentile) krill declined in length over the course of the experiment (−0.002 mm d^{-1}).

Krill in all size classes at low pCO_2 grew faster than at high pCO_2 (Figure 1, Table 2). Smaller krill (10th percentile) grew faster in the low pCO_2 treatment (0.009 mm d^{-1}) than in the high pCO_2 treatment (0.006 mm d^{-1}; $p < 0.001$). The trend was similar in the 50th size quantile, but growth rates in this size class were more variable (low pCO_2: 0.009 mm d^{-1} vs. high pCO_2: 0.005 mm d^{-1}; $p = 0.06$). For the largest krill (90th percentile), those at high pCO_2 shrank (−0.005 mm d^{-1}), while krill in the low pCO_2 treatment had slow but positive growth (0.001 mm d^{-1}; $p < 0.001$).

pCO_2 threshold experiments

Water chemistry

Water conditions during the threshold experiment are summarized in Table 4. Mean pH for the eight treatments ranged from 7.87 to 6.84.

Table 4. Carbonate chemistry of experimental seawater in threshold experiments.

Mean pH_T	pCO_2 (μatm)	A_T (μmol kg^{-1})	C_T (μmol kg^{-1})	Ω_{arag}	Run
7.87 ± 0.04	712 ± 200	2257 ± 48	2138 ± 43	1.53 ± 0.46	1
7.82 ± 0.09	938 ± 800	2289 ± 58	2154 ± 98	1.80 ± 1.31	2
7.05 ± 0.05	5364 ± 2343	2295 ± 44	2479 ± 89	0.3 ± 0.23	1
7.02 ± 0.06	4349 ± 677	2289 ± 42	2429 ± 30	0.29 ± 0.04	2
6.96 ± 0.06	6052 ± 1256	2254 ± 39	2467 ± 33	0.21 ± 0.05	2
6.92 ± 0.04	7228 ± 2022	2326 ± 9	2588 ± 85	0.19 ± 0.06	1
6.89 ± 0.02	9906 ± 4720	2249 ± 37	2606 ± 152	0.17 ± 0.13	2
6.84 ± 0.02	8889 ± 340	2300 ± 13	2631 ± 3	0.15 ± 0.01	1

Mean values ± 1 s.d. ($n = 3–6$) of pCO_2 and aragonite saturation state (Ω_{arag}) calculated using CO2sys with measured input variables total alkalinity (A_T), total dissolved inorganic carbon (C_T), salinity (33.5 + 0.5 ppt), and temperature (14.0 ± 0.8°C). Mean values of pH_T and temperature ($n = 26–118$) were measured directly daily using hand-held meter, then corrected against calculated pH using C_T and A_T parameters in CO2sys. Correction factor applied was 2.367 + (0.6856 × pH meter value), $R^2 = 0.80$, $p < 0.0001$, $n = 35$. The experiment was performed as two consecutive runs (four pH levels at a time), indicated by run number.

Table 5. Survival of *E. pacifica* in the pCO_2 threshold experiments.

pH_T	Days until 50% dead	Days until 100% dead	Survival (%)
7.87	>16	>16	66.7
7.82	>16	>16	62.5
7.04	>16	>16	75
7.02	>16	>16	75
6.96	8	>16	50
6.92	6	10	0
6.89	6	6	0
6.84	3	6	0

Total alkalinity (A_T) was independent of pH (ANOVA; $F_{7,34} = 2.02$; $p = 0.0895$), but aragonite saturation (Ω_{arag}) ($F_{7,34} = 8.19$; $p < 0.0001$) declined significantly with declining pH. Total inorganic carbon (C_T) ($F_{7,34} = 21.44$; $p < 0.0001$) and pCO_2 ($F_{7,34} = 9.30$; $p < 0.0001$) both increased with declining pH.

Survival

The median survival thresholds (days until 50% died) for the four pH treatments ≥7.02 were >16 d (Table 5). This contrasts with the lowest three pH treatments (pH 6.92, 6.89, and 6.84) in which median thresholds were either 6 or 3 d, and all animals were dead by day 10. The intermediate treatment (pH 6.96) had a median threshold of 8 d, but the four remaining krill were still alive at the end of the experiment (16 d).

There were significant differences in the survival curves of the 8 pH groups (log-rank test $\chi^2 = 48.5$, d.f. = 7, $p < 0.001$; Figure 2). The survival curves of the four highest pH groups did not differ significantly from each other ($\chi^2 = 2.09$, d.f. = 3, $p = 0.55$), and they did not differ from the intermediate (pH 6.96) group ($\chi^2 = 4.66$, df = 4, $p = 0.32$). Survival curves in the lowest three pH levels also did not differ significantly from each other ($\chi^2 = 4.86$, df = 2, $p = 0.09$), but they were all significantly lower than at the intermediate level (pH 6.96) ($\chi^2 = 10.57$, df = 3, $p = 0.01$). Taken together, these results suggest a significant decline in survival occurs between pH 6.96 and 6.92, with mortality increasing rapidly below pH 6.96.

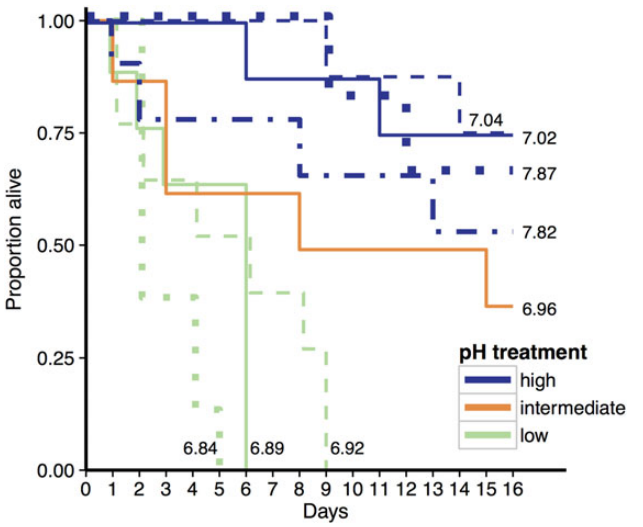

Figure 2. Kaplan–Meier survival curves for *E. pacifica* in the pCO_2 threshold experiments. Data are proportions remaining alive each day in each pH treatment. Line shades indicate the pH groupings described in the text and each curve is labelled with the specific pH treatment. pH treatments within each group did not differ statistically. The high and intermediate pH groups did not differ from each other, but both the high and intermediate pH groups were statistically different from the low group ($p = 0.01$). This figure is available in black and white in print and in colour at *ICES Journal of Marine Science* online.

Discussion

In the growth experiment, a pCO_2 level of 1200 μatm had no effect on either survival or moulting frequency. This pCO_2 level corresponds to the IPCC "high emissions" scenario for 2100 and is also the upper pCO_2 level currently experienced by *E. pacifica* during their diel migrations. These findings differ from those reported for the Atlantic krill, *Nyctiphanes couchii*, which had greater mortality at lower pCO_2 levels (800 and 1100 μatm), although its' highest mortality was at a pCO_2 of 1700 μatm (Sperfeld *et al.*, 2014), a level considerably higher than we used in our growth study.

Sperfeld *et al.* (2014) also found increased pCO_2 had no effect on moult rates in *N. couchii* in a 34 d experiment; this is consistent with our results over 57 d, and supports a more general hypothesis that the moulting process in krill may be unaffected by pCO_2, at least for short-term exposures of up to 60 d. The average intermoult period in our study (6 d) was within the range reported elsewhere for *E. pacifica* (4–10 d) (Pinchuk and Hopcroft, 2007). Although intermoult period in our growth experiment was not affected by increased pCO_2, intermoult period is known to vary in response to other environmental conditions, including temperature and food availability (Fowler *et al.*, 1971; Buchholz, 1991; Chang and Mykles, 2011). Because intermoult period is physiologically constrained to some degree (Fowler *et al.*, 1971; Marinovic and Mangel, 1999), hormonal control of the timing of moulting may outweigh environmental cues related to changing pH. It is also

possible that hormones respond only to internal osmotic conditions within the organism, and if these are highly regulated, hormones may not be sensitive to environmental pH or pCO_2.

Many individuals both grew and shrank at different times during the growth experiment, and since similarly variable growth has been reported previously for *E. pacifica* (Marinovic and Mangel, 1999; Shaw *et al.*, 2010), this variation probably was not an artefact of our experimental system nor a response to the experimental manipulations. Another general trend in the growth experiment was the faster growth rates in smaller krill (10th and 50th quantiles) that declined with increasing body size. This is not uncommon, as linear growth often slows with increased body size (Labat and Cuzin-Roudy, 1996; Atkinson *et al.*, 2006). The larger krill (90th quantile) had negative net growth rates, possibly due to diversion of energy from growth to lipid accumulation, or to sexual maturation and egg production by mature females (Feinberg *et al.*, 2007; Pinchuk and Coyle, 2008; Shaw *et al.*, 2010). After converting telson lengths into total body lengths, the whole body growth rates across both pH treatments in the growth experiment ranged from -0.02 to 0.04 mm d^{-1}. This is within the range of published rates ($0.01-0.03$ mm d^{-1}) for other laboratory and instantaneous growth rate studies (Shaw *et al.*, 2010).

Krill in all size classes had lower growth rates in the elevated pCO_2 treatment than in the low pCO_2 treatment, although the difference was statistically stronger in the 10th and 90th quantiles. Reduced growth under elevated pCO_2 has been linked in some organisms to metabolic changes associated with higher energetic costs of acid–base regulation that divert energy away from other physiological processes including growth (Wood *et al.*, 2008; Deigweiher *et al.*, 2010; Whiteley, 2011; Stumpp *et al.*, 2012). At higher pCO_2 levels, the metabolic rate of the Antarctic krill (*E. superba*) rises (as measured by increased food consumption and nutrient excretion), which suggests that maintaining normal functioning at high pCO_2 requires additional food to meet increased metabolic demands (Saba *et al.*, 2012). If food is limited, or if krill cannot maintain feeding rates high enough to compensate for increased energetic costs of acid–base regulation, growth may be the first process to be affected.

Alternatively, survival time in many vertically migrating species exposed to low pO_2/high pCO_2 at depth is extended by metabolic suppression achieved by shutting down such energy-demanding processes as protein synthesis (Guppy and Withers, 1999; Seibel and Walsh, 2003). While temporary metabolic suppression may be an advantageous short-term strategy, long-term suppression by OA can lead to reduced growth or reproduction, and may become detrimental not only to individuals but also for population-level processes (Guppy and Withers, 1999; Seibel and Walsh, 2003). The effects of pCO_2 on reproduction in *E. pacifica* are unknown, but metabolic changes (e.g. reduced metabolism or diversion of energy to acid–base regulation) that lower growth in *E. pacifica* could also affect their reproduction. Because larger females often have larger broods, possibly due to increased carapace volume available for ovaries (Gómez-Gutiérrez *et al.*, 2006), lower growth rates may also affect krill fecundity via smaller brood sizes.

The pCO_2 threshold experiments indicate that there is a critical threshold between pH 6.96 and pH 6.92 (pCO_2 equivalent to 6050 and 7200 μatm) below which *E. pacifica* experiences rapid mortality. At pH 6.96 and above, survival was similar to that in the highest pCO_2 levels that krill experience naturally (~1200 μatm). This suggests that *E. pacifica* survival is unlikely to decline as a direct response to future OA levels, since even at their deepest diel migrations (~500 m), models predict pCO_2 will not exceed 3500 μatm by the year 2100 (Brewer and Peltzer, 2009). In our experiments, *E. pacifica* survived short-term exposure to pCO_2 of 6000 μatm, nearly twice the maximum predicted level.

While mortality rates did not differ at intermediate pCO_2 levels, we did see some behavioural changes, including periods of inactivity, abnormal swimming, and periods lying on their backs. This suggests that, although the critical threshold for mortality is near pH 6.92, non-lethal physiological effects are present at higher pH levels. It is known that some organisms are less able to buffer changes in pCO_2 when exposed simultaneously to hypoxic conditions (Pane and Barry, 2007), and since pO_2 also declines with depth, increasing hypoxia with depth may increase the vulnerability of *E. pacifica* to additional OA stresses. In our experiments, pO_2 was always equivalent to that of surface water in equilibrium with the atmosphere; therefore, we cannot predict how relatively hypoxic conditions at their migration depths may affect *E. pacfica*'s tolerance of elevated pCO_2.

While responses of larval *E. pacifica* to pCO_2 remain unknown, larvae of this species do remain near the surface until late larval or early juvenile stages, so early life stages are not exposed to the daily variations in pH and temperature experienced by adults during vertical migrations. Perhaps early developmental stages may be more susceptible to harm from increasing OA than adults. In the Antarctic *E. superba*, embryonic development and larval behaviour were unaffected at pCO_2 of 1000 μatm, but eggs completely failed to hatch at 2000 μatm (Kawaguchi *et al.*, 2011, 2013).

Because organisms living where natural variation in pCO_2/pH is common tend to have broader tolerances of fluctuations in pCO_2/pH, perhaps the natural (e.g. pre-industrial) regimes of pCO_2 variation experienced by organisms may determine their sensitivity to future changes in ocean chemistry, and hence be a good predictor of likely responses to OA (Hofmann *et al.*, 2011; Lewis *et al.*, 2013). This may be because organisms living in more variable environments are more likely to have evolved well-developed acid–base regulation systems for active maintenance of internal osmotic and ionic balances, and these systems may enable them to buffer changes in pH due to OA (Seibel and Walsh, 2003; Pane and Barry, 2007).

Since *E. pacifica* regularly experiences a range of pCO_2 values during its diel migration, it probably has well-developed acid–base regulation systems, and these may enhance its survival when exposed to elevated pCO_2 for long periods, and also during extreme though short-term pH declines. Whether *E. pacifica* uses increased acid–base regulation or metabolic suppression to tolerate exposures to low pH, the reduced growth rates in our experiments suggest that even organisms likely to be well adapted for acid–base regulation under high pCO_2 may be adversely affected by OA. Consequently, the central role of *E. pacifica* in coastal ecosystems may be affected indirectly by elevated pCO_2 acting on growth or other processes (e.g. reproduction) or on early life stages (eggs, larvae). Because *E. pacifica* inhabits an ecosystem likely to see increased frequency and intensity of acidic events (Feely *et al.*, 2008), understanding its responses to high pCO_2 is critical for understanding the ecosystem-wide effects of OA.

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