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ORIGINAL PAPER

Metabolic responses of the North Pacific krill, *Euphausia pacifica*, to short- and long-term pCO_2 exposure

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Abstract While ocean acidification is likely to have major effects on many marine organisms, those species that regularly experience variable pCO_2 environments may be more tolerant of future predicted changes in ocean chemistry. *Euphausia pacifica* is an abundant krill species along the Pacific coast of North America and one that regularly experiences varying pCO_2 levels during seasonal upwelling, as well as during daily vertical migrations to depth where pCO_2 is higher. Krill were collected from Monterey Bay, California (36.8°N, 121.9°W), and experiments were performed from June to August 2014 and maintained at two pCO_2 levels (400 and 1200 µatm). Three metabolic responses (oxygen consumption, ingestion rate, and nutrient excretion rates) of *E. pacifica* were measured. Oxygen consumption declined by 31 % in the first 24 h following exposure to high pCO_2 and remained low after 21 days. Oxygen consumption at low pCO_2 was low for the first 12 h, increased by 34 % at 24 h, but returned to initial values by 21 days. After 3 weeks of continuous exposure, oxygen consumption rates were 32 % lower in the high pCO_2 group. Ingestion and ammonium excretion rates were both significantly lower in the high pCO_2 group after 24-h exposure, but not after 7 or 21 days. There was no effect of pCO_2 on phosphate excretion. Taken together, these results indicate that *E. pacifica* has a lower metabolic rate during both short-term (24 h) and longer-term (21 days) exposure to high pCO_2. Such metabolic depression may explain previously reported declines in growth of *E. pacifica* exposed to high pCO_2.

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Introduction

Absorption of atmospheric CO_2 into the world's oceans is causing declines in ocean pH and carbonate ion concentration, a phenomenon known as ocean acidification (OA) (Caldeira and Wickett 2003, 2005; Orr et al. 2005; Fabry et al. 2008; Doney et al. 2009; Feely et al. 2009). After declining by 0.1 unit since the pre-industrial era, ocean pH is projected to decline another 0.2 to 0.3 units over the next century (Caldeira and Wickett 2003, 2005; Solomon 2007; Feely et al. 2009; Stocker et al. 2013; Edenhofer et al. 2014), a rate unprecedented in recent history (Doney and Schimel 2007). These projected changes to ocean chemistry are likely to alter the survival, distribution, growth, reproduction, gene expression, and behavior of many marine organisms (Kleypas et al. 2006; Dupont et al. 2008; Fabry et al. 2008; Kurihara 2008; Ross et al. 2011). While known effects of OA on marine organisms are overwhelmingly negative, this generalization may be biased by the focus of OA research on calcifying organisms. Responses to OA are known to differ among taxa, populations, habitats, and life stages (Pane and Barry 2007; Kroeker et al. 2010; Lewis et al. 2013; Kroeker et al. 2013; Cripps et al. 2014), and these variable responses make predicting long-term ecosystem effects of OA difficult, without having a broader understanding of what traits or organisms are more likely to cope well with future OA. Recent evidence suggests that

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organisms living in variable pCO_2 habitats are more tolerant of pCO_2 levels under future OA scenarios (Maas et al. 2012; Pespeni et al. 2013; Lewis et al. 2013), possibly due to physiological adaptations that buffer changes in external pCO_2 (Seibel 2003).

Many marine organisms maintain narrow internal pH and ion concentrations that are optimal for their basic biological and physiological functions, and most eliminate excess CO_2 via diffusion gradients across their external cell membranes from higher pCO_2 inside their bodies to lower external pCO_2. As environmental pCO_2 increases, the slope of this gradient declines and elimination of CO_2 is reduced, leading to lower extra- and intra-cellular pH (i.e., acidosis) that may be countered either by passive buffering (e.g., bicarbonate buffering system or respiratory proteins) or by active transport of ions across membranes (Heisler 1986; Seibel 2003). Complete compensation (maintaining pH at pre-disturbance levels) may be limited by the necessity of also maintaining ionic homeostasis for biological functions (Cameron and Wood 1985; Cameron and Iwama 1987; Whiteley et al. 2001).

Disturbances in the acid–base balance have varied metabolic and physiological effects, and inability to compensate for pH disturbances may lead to metabolic depression, disrupted enzyme functioning, and altered gene expression (Seibel 2003; Pörtner et al. 2005). Low pH of extracellular fluids reduces the affinity of respiratory proteins for oxygen, may limit oxygen supply to body tissues, and can lower metabolic activity (Seibel 2003). Conversely, organisms with efficient compensation for acidosis may incur high metabolic costs linked to the continual, ATP-intensive active pumping of ions, which diverts energy away from other important processes such as growth or reproduction (Wood et al. 2008; Deigweiher et al. 2010; Whiteley 2011; Stumpp et al. 2012). An increased ventilation rate in response to reduced oxygen transport is likely to increase metabolic costs (Pörtner et al. 2004). Increasing feeding rates is one way to compensate for elevated metabolic expenditures that may enhance tolerance of changing pCO_2 (Holcomb et al. 2010; Saba et al. 2012), but organisms may simultaneously become more susceptible to fluctuations in food availability. Long-term consequences of these metabolic changes may include altered growth, development rates, feeding or swimming behavior, or reproduction (Henry and Wheatly 1992; Seibel 2003; Pörtner et al. 2004, 2005; Kurihara et al. 2007; Miles et al. 2007; Widdicombe and Spicer 2008; Hu et al. 2014).

Different metabolic responses in similar OA experiments have been attributed to prior exposure to different natural pCO_2 regimes, iono-regulatory abilities, and respiratory pigment (Seibel 2003; Pörtner et al. 2004, 2005; Maas et al. 2012). Organisms that experience natural fluctuations in pCO_2 during seasonal upwelling, or during diel vertical migrations to deeper water, may also be well adapted to OA due to their frequent and prolonged exposure to lower pH waters (Seibel 2003; Thomsen and Melzner 2010; Lewis et al. 2013).

Along the California coast, seasonal upwelling brings cold, low pH/high pCO_2 waters to the surface, exposing many planktonic and pelagic organisms to substantial variation in pCO_2. One of these is the Pacific krill, *Euphausia pacifica*, a small planktonic marine crustacean widely distributed throughout the north-eastern Pacific Ocean (Brinton 1962). As the most abundant krill species in Monterey Bay, California, it is an ecologically and economically important link between primary producers (phytoplankton) and higher trophic levels. *E. pacifica* makes daily vertical migrations to depths of 250 meters during the day (and sometimes to 500 m) in the Monterey submarine canyon, returning to the surface at night to feed (Brinton 1976; Bollens et al. 1992; Marinovic and Mangel 1999). At these depths, it is exposed daily to lower oxygen (as low as 20 % saturation)(Tremblay 2014), lower pH (approximately 7.60), and higher pCO_2 (1200 µatm) than at the surface (Feely et al. 2008).

Many crustaceans are more tolerant of OA conditions than other phyla (Pane and Barry 2007; Kroeker et al. 2010), and many characteristics of *E. pacifica* (metabolic activity, variable environment) suggest that this species should have strong acid–base regulation. We demonstrated previously that molting and survival of *E. pacifica* from Monterey Bay did not change when exposed to elevated pCO_2 (1200 µatm), but that growth rates declined (Cooper et al. 2016). While changes in growth rates may well be linked to changing metabolic rates, it remains unknown whether *E. pacifica*'s metabolism is stimulated in response to pH compensation demands (possibly requiring increased feeding rates), is suppressed by uncompensated pH disturbances (possibly reducing feeding rates), or remains unaffected by pH changes. In this paper, we describe two experiments that both examine initial metabolic responses of *E. pacifica* to short-term (first 24 h) exposure to elevated pCO_2 and then compare them with long-term responses after acclimation during 21-day exposure. The first experiment measured oxygen consumption, and the second measured rates of ingestion and nutrient excretion.

Materials and methods

Collections

Euphausia pacifica were collected after sunset, when they had migrated to the surface ~13 km offshore in Monterey Bay. We used a 1-m-diameter plankton net (500-µm mesh) with a solid non-filtering codend (1 L volume) to take

oblique tows from ~30 m depth to the surface. Krill were divided on board ship into groups of 8–10 individuals that were placed in 750-mL jars of seawater and kept on ice in a cooler for transport (~2 h) back to the Long Marine Laboratory of the University of California, Santa Cruz. At the laboratory, healthy krill were sorted into 4-L glass jars, with 6–8 krill per jar and placed in an 11 ± 1 °C recirculating water table maintained by an inline chiller (Aqua Logic Delta Star). They were kept in darkness, except for a few minutes each day when the jars were checked for mortality under low light. Krill were fed daily with 25 mL of a phytoplankton mixture of *Thalassiosira, Isochrysis* and *Rhodomonas*, and water in the jars was changed every second day. All krill were acclimated to laboratory conditions for a minimum of 1 week prior to experimentation.

Experimental design

Both experiments used two pCO_2 treatments: one representing recent "ambient" atmospheric CO_2 levels (pCO_2 = 400 µatm, pH_T = 8.01) and the other exceeding the IPCC RCP8.5 or "high emissions" scenario for 2100 (pCO_2 = 1200 ppm, pH_T = 7.60) (Stocker et al. 2013; Edenhofer et al. 2014). These pCO_2 levels are also similar to those *E. pacifica* already experiences during its diel migration between the surface and at depth, respectively. In Experiment 1, the "Respiration Experiment," oxygen consumption of individual krill was measured after short-term (0, 12, and 24 h) and long-term (21 days) exposure to the experimental pCO_2 treatments. In Experiment 2, the "Nutrition Experiment," ingestion and nutrient excretion rates of individual krill were measured after exposure to the same two pCO_2 conditions for 1, 7, or 21 days.

In both experiments, krill were exposed to the experimental treatments by maintaining groups of 6–10 individuals in 4-L glass jars containing pre-treated water at the desired pCO_2 and then continuously supplying small amounts of air + CO_2 gas mixtures to maintain a slight positive pressure and a constant pCO_2. Before taking measurements, krill were starved for over 12 h and then transferred to individual chambers containing water with the same pCO_2 as in their treatment jars. Different individuals were used for each treatment, and each individual was measured on only one day.

Carbonate chemistry

Water preparation

Gases 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 and then diluting it with bursts of compressed air while measuring the CO_2 content with a CO_2 analyzer (Quibit Systems S151) until the desired concentration was reached. New gas mixtures were blended twice a week for the duration of the study. Seawater taken from Monterey Bay via the flow-through seawater system at the Long Marine Laboratory was filtered to 0.2 µm and stored in 20-L carboys. The appropriate CO_2 mixtures were then bubbled into the carboys for 4 days using gel membrane bubblers, until the seawater equilibrated with the gas mixture and seawater pH reached the desired level. Experimental seawater was prepared weekly.

Water analyses

In both experiments, pH and temperature were measured daily with an Oakton WD-35613 handheld pH meter. pH is expressed as total scale (pH_T). Water samples were taken from the experimental chambers of all animals during each of the trials and every 5–8 days from the holding jars of the long-term groups (7 or 21 days). Water was analyzed 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. Water samples of 40 mL were used for each analysis. Instruments were calibrated using certified seawater standards (Batch 135) from Andrew Dickson's laboratory at the Scripps Institution of Oceanography. pH, pCO_2, and aragonite saturation state (Ω_{arag}) were calculated with CO_2Sys software (Pierrot et al. 2006) using the C_T and A_T data and CO_2 disassociation constants from Mehrbach (1973) refitted by Dickson and Millero (1987). Daily pH meter readings were calibrated against the full carbonate calculated values and reported as $pH_{T\ lab}$ and $pH_{Tcalculated}$, respectively; $pH_{Tcalculated}$ is used in all statistical tests.

Experiment 1: Respiration

Oxygen consumption was measured by closed-chamber respirometry after placing individual krill in 25-mL scintillation vials fitted with optical oxygen sensors (Sensor Spots O2, Presens, Regensburg, Germany) and a magnetic stir bar (separated from the krill by fine mesh). Vials (9 at a time) were placed in a circulating water bath above a magnetic stir plate. After acclimating for 30 min, the vials were flushed with seawater of the appropriate pCO_2 and then sealed. Oxygen concentration (µmol O_2 L^{-1} and % saturation) in each vial was measured every 10 min by placing the fiber-optic reader to the sensor (Fitbox4 transmitter, Presens, Regensburg, Germany). One to two vials without krill were used as controls in each run. Each trial lasted 2–3 h, and oxygen saturation never dropped below 75 %. Oxygen consumption rates were calculated as the slope of the declining oxygen concentration inside each vial over the course of the experiment.

For the short-term exposure group, O_2 consumption rate was measured 3 times for each of 13 individuals, first before any exposure to pCO_2 (hour 0) and then 12 and 24 h following initiation of pCO_2 exposure. For the long-term exposure group, 27 krill were maintained at the experimental conditions for 21 days, and then, O_2 consumption was measured once for each krill. After measurement, each krill was rinsed, frozen, and later freeze-dried and weighed. The effects of pCO_2 and exposure time on oxygen consumption in the short-term group were analyzed in a repeated measures multivariate ANOVA (MANOVA) which is robust to violations of sphericity assumptions. Mean oxygen consumption rates of the long-term groups were compared with a 2-sample *t* test.

Experiment 2: Nutrition

Feeding and nutrient excretion by individual krill were measured over a 24-h period for each exposure duration (1, 7, or 21 days). For each duration, 31 polycarbonate chambers (750 mL) were prepared containing seawater pre-equilibrated to the desired pCO_2 levels. Phytoplankton (50 mL of a *Thalassiosira* culture) were added to each chamber and mixed well. Five chambers were sampled immediately to count initial phytoplankton cell concentrations, and 26 chambers were used in the trials. Individual krill were placed in 10 of the 13 chambers at each pCO_2 level, and the other 3 chambers were phytoplankton-only controls. All chambers had gas-tight caps, were placed in the water table at 11 °C, and were kept in darkness. Chambers were inverted manually every 2 h to maintain even suspension of phytoplankton. After 24 h, the pH was measured and discrete water samples were taken for phytoplankton cell counts (200 mL), carbonate chemistry (C_T and A_T; 40 mL each), and nutrient analyses (NH_4^+ and PO_4^{3-}; 20 mL each). The krill were then rinsed, frozen, and later dried and weighed on a microbalance.

Ingestion rates of phytoplankton

Phytoplankton cell concentrations in each chamber were counted in 200 mL water samples preserved with 5 % Lugol's solution. Three 1 mL subsamples were placed on a Sedgewick rafter, and 3 replicate transects of >100 cells were counted for each 1 mL subsample. Ingestion rates (I) were calculated from the change in phytoplankton cell concentrations over 24 h using an equation from Conover (1978):

$$I = (C_0 - C_t + [C]e^{(k \times t)} - 1)/t.$$

where C_0 is initial cell concentration (cells mL^{-1}); C_t is cell concentration (cells mL^{-1}) at time t; k is the algal population growth coefficient calculated as $\frac{\ln (C_t - C_0)}{t}$; g′ is the grazing coefficient for krill calculated as $\frac{-\ln \frac{C_t}{C_0}}{t}$; t is the duration of incubation in hours; and [C] is the mean cell concentration (cells mL^{-1}) calculated as $\frac{C_0 \times \left(1 - e^{\left(-g' \times t\right)}\right)}{t \times g'}$.

Because calculated ingestion rates account for phytoplankton growth (k) by cell division, k is calculated from the control jars containing only phytoplankton. This means negative ingestion rates are possible if phytoplankton growth accelerates in experimental jars and exceeds krill ingestion rates, due to the phytoplankton taking up nutrients excreted by krill (Lehman 1980).

Nutrient excretion rates

Seawater samples (20 mL) for nutrient analysis were filtered and frozen at the end of each trial. NH_4^+ and PO_4^{3-} were analyzed on a Lachat Quickchem flow injection analyzer (FIA +800 series), using the single end point method which assumes beginning nutrient levels are equivalent, since all water was taken from the same source and filtered at the same time. Apparent excretion rates (E) of ammonium and phosphate were calculated from Ikeda's equation (Harris et al. 2000) $E = [(C_{t'} - C_0) - (C_t - C_0)] \times \frac{(V_c - V_z)}{t \times N}$ which simplifies to

$$E = (C_{t'} - C_t) \times \frac{(V_c - V_z)}{t \times N}$$

where C_0 is the concentration of nutrients at the start of incubation; C_t and $C_{t'}$ are the concentrations of nutrients in control and experimental containers, respectively, at the end of the incubation; V_c is the volume of experimental bottles; V_z is the volume of krill (V_z is calculated from wet weight of krill assuming 1 mL = 1 g wet weight); *t* is the duration of incubation in hours; and N is the number of krill per chamber ($n = 1$).

This rate represents the apparent excretion rate, and the gross excretion rate equals the apparent excretion rate plus the nutrient uptake rate by phytoplankton. Because phytoplankton uptake rate is not measured directly, differences in phytoplankton uptake rates between the control and experimental containers, due to the presence of krill, can lead to underestimation of gross excretion rates (and even give negative apparent excretion rates) (Takahashi and Ikeda 1975).

All statistical analyses for these data were conducted using JMP Pro v. 12.0.

Results

The mass of krill used in the experiments ranged from 0.987 to 6.729 mgdw, with a mean value of 3.204 ± 1.398

Table 1 Water chemistry during respiration experiment

Exposure		Low CO_2		High CO_2	
		n		*n*	
Initial	Salinity	7	33.4 ± 0.2	9	33.4 ± 0.2
	Temp °C	11	11.1 ± 0.7	13	11.1 ± 0.6
	$pH_{T\ lab}$	11	7.96 ± 0.13	13	7.60 ± 0.02
	$pH_{T\ calculated}$	7	8.02 ± 0.10	9	7.66 ± 0.10
	pCO_2 µatm	7	434.6 ± 133	9	1078.9 ± 288
	A_T µmol kg^{-1}	7	2231.1 ± 29	9	2239.9 ± 14
	C_T µmol kg^{-1}	7	2060.3 ± 19	9	2195.4 ± 38
	Ω_{arag}	7	1.94 ± 0.38	9	0.92 ± 0.18
Long term	Salinity	13	33.4 ± 0.2	13	33.4 ± 0.2
	Temp °C	143	10.2 ± 0.5	119	10.1 ± 0.6
	$pH_{T\ lab}$	143	8.02 ± 0.10	119	7.60 ± 0.13
	$pH_{T\ calculated}$	13	8.02 ± 0.08	13	7.67 ± 0.10
	pCO_2 µatm	13	430.8 ± 97	13	1027.2 ± 253
	A_T µmol kg^{-1}	13	2259.5 ± 15	13	2257.4 ± 12
	C_T µmol kg^{-1}	13	2092.6 ± 37	13	2209.0 ± 28
	Ω_{arag}	13	1.90 ± 0.26	13	0.95 ± 0.22

Mean chemical properties (mean ± SD) of water in low and high pCO_2 treatments during short-term (initial 24 h) and long-term (21 days) exposures. Temperature and $pH_{T\ lab}$ were measured daily; total alkalinity (A_T) and total inorganic carbon (C_T) were measured in discrete water samples collected from experimental chambers for all respiration trials, and every 5–8 days from maintenance jars during the long-term exposure. All other parameters ($pH_{calculated}$, pCO_2, and aragonite saturation state (Ω_{arag}) were calculated from A_T and C_T using CO_2Sys Software. $pH_{calculated}$ is used in all statistical analyses

mgdw (mean ± SD, $n = 71$). There was no difference in krill mass between pCO_2 treatments (2-sample t test, t (68.6) = 0.29, $p = 0.776$).

Experiment 1: Respiration

Water chemistry: Chemical data for water in the Respiration experiment are summarized in Table 1. Partial pressures of CO_2 in the high pCO_2 treatment waters were 1079 ± 288 µatm (short term; mean ± SD, $n = 9$) and 1027 ± 253 µatm (long term; $n = 13$), both slightly lower than the target of 1200 µatm but still approximately 2.5 times the pCO_2 in the low pCO_2 treatments (435 ± 133 and 431 ± 97 µatm; $n = 7$ and 13, respectively). This maintained differences of 0.36 and 0.35 pH units between the two treatments within the short-term (2-sample t test, t (12.79) = −6.99, $p < 0.001$) and long-term (2-sample t test, t (22.42) = −9.60, $p < 0.0001$) treatments. Within each pCO_2 level, there was no significant difference in pH of the water used for short-term and long-term exposures (2-sample t tests, high pCO_2 treatment: t (17.62) = 0.50, $p = 0.63$; low pCO_2 treatment: t (9.74) = 0.09, $p = 0.93$).

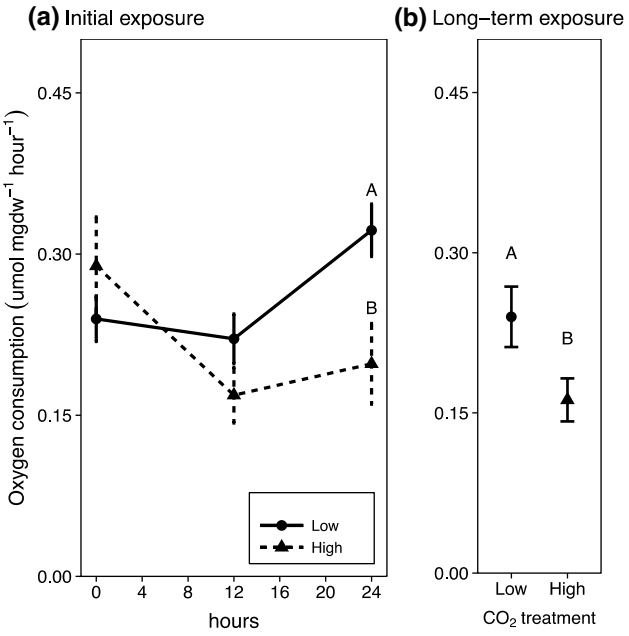

Fig. 1 Oxygen consumption by *Euphausia pacifica* in respiration experiment. Oxygen consumption rates (mean ± SE) of individual krill **a** during the initial 24 h and **b** after 21 days of exposure to low or high pCO_2. Units are µmol oxygen normalized for dry body weight of krill ($mgdw^{-1}$) per hour. *Letters* indicate significant differences in two-sample t tests (Tukey HSD, $p < 0.05$)

Oxygen consumption: Oxygen consumption rates ranged from 0.162 to 0.322 µmol O_2 $mgdw^{-1}$ h^{-1} (Fig. 1). In the initial exposure group, mean oxygen consumption rates for individuals exposed to high pCO_2 declined by 42 % over the first 12 h (from 0.289 ± 0.13 to 0.168 ± 0.07 µmol $mgdw^{-1}$ h^{-1}; mean ± SD, $n = 9$) and remained low (31 % lower than initial) after 24 h (0.198 ± 0.11 µmol $mgdw^{-1}$ h^{-1}; $n = 9$). Consumption by individuals in the low pCO_2 treatment did not change in the first 12 h (from 0.239 ± 0.05 to 0.221 ± 0.05 µmol $mgdw^{-1}$ h^{-1}; $n = 5$), but then increased by 46 % after 24 h (to 0.322 ± 0.05 µmol $mgdw^{-1}$ h^{-1}; $n = 5$). The two groups differed in their responses to pCO_2 treatment over time (significant MANOVA interaction term, F (2, 9) = 7.3317, $p = 0.013$; Fig. 1a), and mean consumption rates in the two treatments were significantly different by hour 24 (2-sample t test: t (9.90) = 2.68, $p = 0.03$). After 21 days, oxygen consumption by individuals in the high pCO_2 treatment (0.162 ± 0.07 µmol $mgdw^{-1}$ h^{-1}; mean ± 1 SD, $n = 12$) was essentially the same as after 12–24 h and was 32 % lower than consumption by low pCO_2 individuals after 21 days (0.240 ± 0.109 µmol $mgdw^{-1}$ h^{-1}; $n = 15$; 2-sample t test, t (22.3) = −2.19, $p = 0.04$; Fig. 1b). Oxygen consumption by low pCO_2 krill after 21 days was essentially the same as during the first 12 h of day 1.

Table 2 Water chemistry during nutrition experiment

Exposure		Low CO_2 *n*		High CO_2 *n*	
1 Day	Salinity	11	33.4 ± 0.2	9	33.4 ± 0.2
	Temp °C	32	11.6 ± 0.5	27	11.7 ± 0.4
	$pH_{T\ lab}$	32	8.14 ± 0.07	27	7.53 ± 0.10
	$pH_{T\ calculated}$	11	7.97 ± 0.07	9	7.52 ± 0.27
	pCO_2 µatm	11	487.4 ± 92	9	1732.8 ± 835
	A_T µmol kg^{-1}	11	2277.0 ± 18	9	2282.0 ± 13
	C_T µmol kg^{-1}	11	2118.8 ± 22	9	2275.8 ± 80
	Ω_{arag}	11	1.84 ± 0.3	9	0.83 ± 0.6
7 Days	Salinity	6	33.4 ± 0.2	13	33.4 ± 0.2
	Temp °C	59	10.8 ± 0.6	42	10.9 ± 0.5
	$pH_{T\ lab}$	59	8.11 ± 0.06	42	7.62 ± 0.07
	$pH_{T\ calculated}$	6	7.98 ± 0.08	13	7.67 ± 0.07
	pCO_2 µatm	6	485 ± 112	13	1042.4 ± 173
	A_T µmol kg^{-1}	6	2272.6 ± 10	13	2265.7 ± 13
	C_T µmol kg^{-1}	6	2119.3 ± 35	13	2219.0 ± 18
	Ω_{arag}	6	1.79 ± 0.30	13	0.93 ± 0.16
21 Days	Salinity	16	33.4 ± 0.2	16	33.4 ± 0.2
	Temp °C	155	10.2 ± 0.5	131	10.3 ± 0.6
	$pH_{T\ lab}$	155	8.00 ± 0.11	131	7.61 ± 0.09
	$pH_{T\ calculated}$	16	7.97 ± 0.12	16	7.66 ± 0.10
	pCO_2 µatm	16	503.0 ± 179	16	1063.4 ± 260
	A_T µmol kg^{-1}	16	2265.7 ± 20	16	2258.9 ± 21
	C_T µmol kg^{-1}	16	2114.7 ± 58	16	2214.2 ± 31
	Ω_{arag}	16	1.77 ± 0.37	16	0.93 ± 0.21

Mean chemical properties (mean ± SD) of water in low and high pCO_2 treatments. Temperature and $pH_{T\ lab}$ were measured daily. Total alkalinity (A_T) and total inorganic carbon (C_T) were measured in discrete water samples collected from experimental chambers on days of ingestion/excretion trials and every 5–8 days from maintenance jars. All other parameters ($pH_{calculated}$, pCO_2, and aragonite saturation state (Ω_{arag}) were calculated from A_T and C_T using CO_2Sys Software. $pH_{calculated}$ is used in all statistical analyses

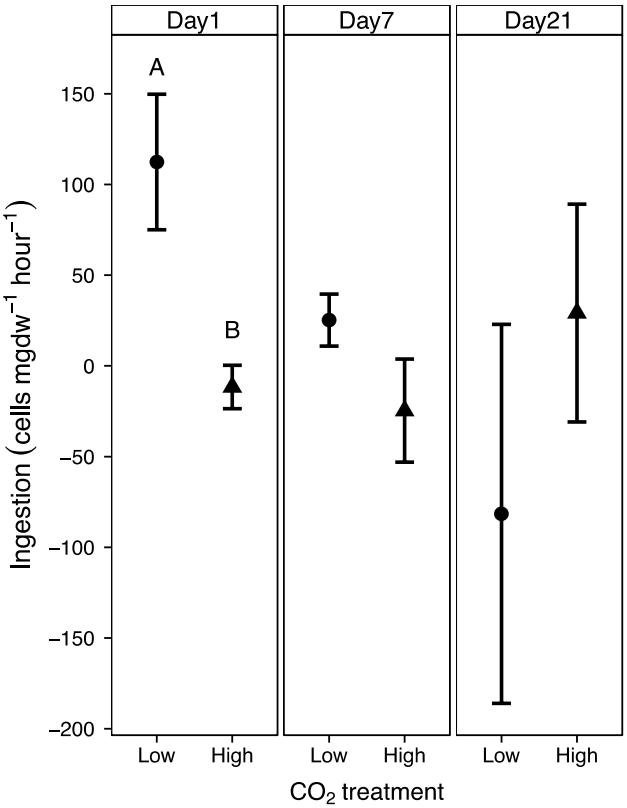

Fig. 2 Ingestion rates of *Euphausia pacifica* in nutrition experiment. Ingestion rate (mean ± SE) of phytoplankton normalized for dry body weight of each animal (cells phytoplankton $mgdw^{-1}$ h^{-1}) after 1-, 7-, and 21-day exposure to high and low pCO_2. $n = 10$ for each group. *Letters* indicate significant differences in an ANOVA of days 1 and 7, after excluding day 21 data ($p < 0.05$)

Experiment 2: Nutrition

Water chemistry

In the low pCO_2 treatment, both pCO_2 (485–503 µatm) and $pH_{T\ calculated}$ (7.97–8.11) of the water were almost constant over the 21 days of the experiment (Table 2). However, in the high pCO_2 treatment, pCO_2 was significantly higher on day 1 (1733 ± 835 µatm; mean ± SD, $n = 9$) than on day 7 (1042 ± 173 µatm; $n = 13$) or day 21 (1063 ± 260 µatm; $n = 16$; ANOVA, F (2, 30) = 6.0451, $p = 0.007$; Tukey HSD (day 1 vs day 7: t (28) = 2.67, $p = 0.03$, day 1 vs day 21: t (28) = 3.27, $p = 0.008$). These differences in pCO_2 resulted in a lower pH (7.52 ± 0.10; mean + SD; $n = 9$) on day 1 than on day 7 (7.67 ± 0.07; $n = 13$) and day 21 (7.67 ± 0.10; $n = 16$). pCO_2 was very similar on day 7 and day 21 (Tukey HSD, t (28) = −0.09, $p = 0.996$). Including the elevated value on day 1, mean pCO_2 in the high CO_2 treatment (1042–1732 µatm) was always 2–3.5 times greater than in the low CO_2 treatment (485–503 µatm) throughout the Nutrition experiment (Table 2), and pH differed by 0.3–0.5 pH units between the treatment groups throughout the experiment.

Ingestion rates

Mean ingestion rates of phytoplankton ranged from a high of 112 ± 118 to a low of −81.6 ± 330 cells $mgdw^{-1}$ h^{-1} across the three durations (mean ± SD, $n = 10$ per group; Fig. 2). Negative ingestion rates imply that the phytoplankton culture divided at a faster rate than the krill ingestion rate. While neither pCO_2 [2-way ANOVA, F (1, 61) = 0.2475, $p = 0.6208$] nor duration [F (2, 61) = 1.0912, $p = 0.3429$; Table 3a] affected ingestion rates, there was a strong, though not significant, interaction (ANOVA, F (2, 61) = 2.60, $p = 0.08$).

Table 3 Ingestion rates of phytoplankton by *Euphausia pacifica*

Source	DF	SS	F	P
3a. Three exposure durations				
Exposure	2	60668.93	1.0912	0.3429
pCO_2	1	6881.44	0.2475	0.6208
pCO_2 × exposure	2	144461.05	2.5982	0.0834
Error	56	1556808.8		
Total	61	1769455.1	1.5298	0.1954
3b. Two exposure durations (excluding day 21 data)				
Exposure	1	26299.584	3.9983	0.0527
pCO_2	1	79286.410	12.0539	0.0013*
pCO_2 × exposure	1	14414.707	2.1915	0.1470
Error	38	249951.49		
Total	41	366938.15	5.9285	0.0020*

Summary tables for 2-way ANOVAs comparing different durations of exposure at two pCO_2 levels. **a.** Three exposure durations (1, 7, and 21 days). **b.** Two exposure durations (1 and 7 days)

* $p < 0.05$

Ingestion rates were much more variable on day 21 than on days 1 and 7 (Fig. 2), and declines in phytoplankton cell counts in control jars without krill on day 21 suggest the phytoplankton culture may have become less healthy. Therefore, we repeated the analyses excluding day 21 from the model (Table 3b). With day 21 excluded, ingestion rates on both day 1 and day 7 were higher in the low pCO_2 treatment (ANOVA, F (1, 41) = 12.05 $p = 0.0013$, Table 3b), and ingestion rates were marginally lower on day 7 than on day 1 (ANOVA, F (1, 41) = 3.9983, $p = 0.053$; Table 3b). In post hoc comparisons, the ingestion rate on day 1 was significantly lower at high pCO_2 than at low pCO_2 (post hoc Tukey HSD, t (38) = −3.42, $p = 0.008$), but there was no significant difference between pCO_2 treatments on day 7 (Fig. 2, post hoc Tukey HSD, t (38) = −1.44, $p = 0.481$).

Nutrient excretion rates

Mean apparent excretion rates for ammonium ranged from −0.16 ± 0.10 to 0.20 ± 0.30 μg NH_4^+ $mgdw^{-1}$ h^{-1} (mean ± SD, $n = 10$ per group; Fig. 3a) and for phosphate from 0.05 ± 0.28 to 0.39 ± 0.87 μg PO_4^{3-} $mgdw^{-1}$ h^{-1} ($n = 10$ per group on days 1 and 7, $n = 5$ per group on day 21; Fig. 3b). Negative excretion rates imply that phytoplankton uptake of nutrients was greater in the experimental containers (with krill) than in the control containers (no krill), causing underestimation of the true excretion

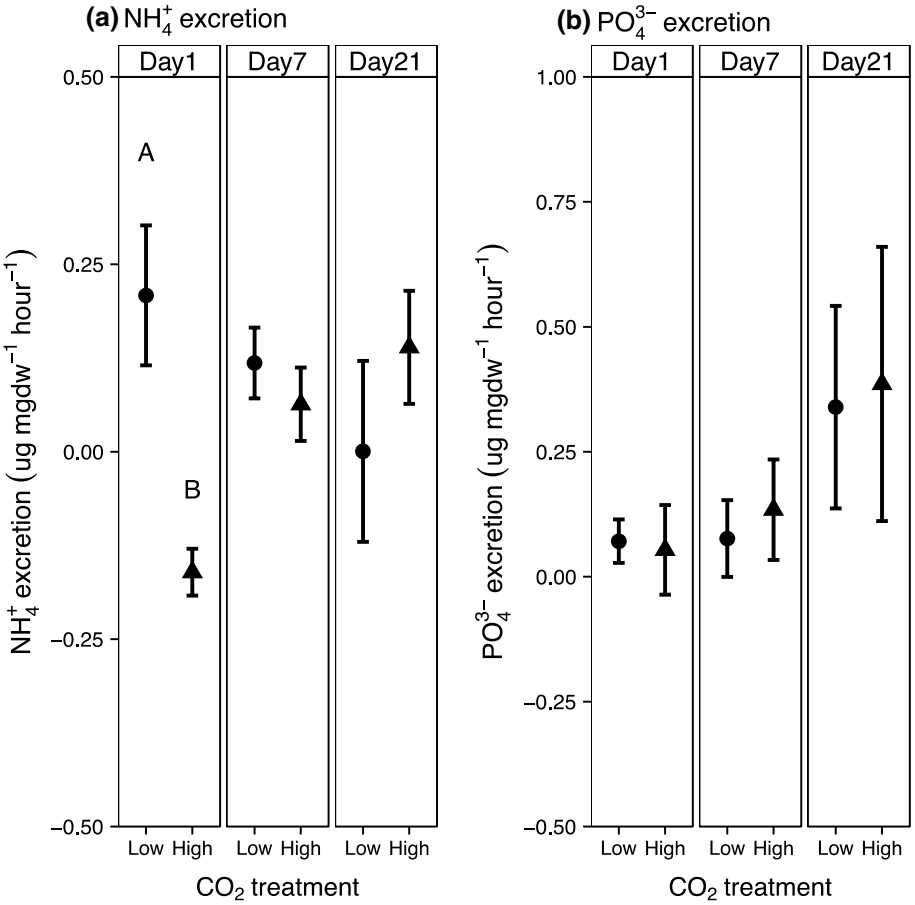

Fig. 3 Nutrient excretion rates of *Euphausia pacifica* in nutrition experiment. Apparent excretion rates (mean ± SE) of individual krill, normalized for dry body weight, after 1-, 7-, and 21-day exposure to high and low pCO_2 of **a** ammonium and **b** phosphate. $n = 10$ for each group on days 1 and 7, and $n = 5$ for each group on day 21. Letters indicate significant differences (Tukey HSD, $p < 0.05$)

Table 4 ANOVA of NH_4^+ excretion rates in nutrition experiment

Source	DF	SS	F	P
4a. Three exposure durations				
Exposure	2	0.04493881	0.4588	0.6352
pCO_2	1	0.09479403	1.9355	0.1715
pCO_2 × exposure	2	0.46807027	4.7786	0.0135*
Error	42	2.0569624		
Total	47	2.8415162	3.2039	0.0154*
4b. Two exposure durations (excluding day 21 data)				
Exposure	1	0.04364074	1.1713	0.2865
pCO_2	1	0.43807150	11.7579	0.0016*
pCO_2 × exposure	1	0.23992143	6.4395	0.0158*
Error	35	1.3040185		
Total	38	2.0456617	6.6353	0.0011*

Summary tables for 2-way ANOVAs comparing different durations of exposure at two pCO_2 levels. **a.** Three exposure durations (1, 7, and 21 days). **b.** Two exposure durations (1 and 7 days)

* $p < 0.05$

rate. The effects of pCO_2 and duration of exposure were evaluated in separate 2-way ANOVAs for ammonium and phosphate excretion (Table 4), after deleting four extreme NH_4^+ values (each >3 standard deviations from the mean) to improve normality and homogeneity of variances. The interaction between exposure and pCO_2 for NH_4^+ excretion was significant (ANOVA, F (2, 47) = 4.7786, $p = 0.0135$, Table 4a; Fig. 3a), but not for either factor alone. NH_4^+ excretion was lower at high pCO_2 during the first 24 h of exposure (Tukey HSD, t (42) = −3.73, $p = 0.007$), but not when exposed for longer periods of time (Tukey HSD, day 7: t (42) = −0.54, $p = 0.994$ and day 21: t (42) = 0.93, $p = 0.9353$, Table 4a, Fig. 3a). Although NH_4^+ excretion rates were more variable after 21-day exposure, results of analyses were similar when day 21 was excluded from the analyses (Table 4b). Neither pCO_2 (ANOVA, F (1, 51) = 0.0605, $p = 0.8067$) nor exposure (ANOVA, F (2, 51) = 2.0203, $p = 0.1442$) significantly affected phosphate (PO_4^{3-}) excretion (Table 5a, Fig. 3b). Phosphate excretion was also much more variable after 21 days than 1 or 7 days, but excluding day 21 from the model did not alter the results (Table 5b).

Table 5 ANOVA of PO_4^{3-} excretion rates in nutrition experiment

Source	DF	SS	F	P
5a. Three exposure durations				
Exposure	2	0.64188299	2.0203	0.1442
pCO_2	1	0.00961612	0.0605	0.8067
pCO_2 × exposure	2	0.01594535	0.0502	0.9511
Error	46	7.3074353		
Total	51	7.9744804	0.8398	0.5285
5b. Two exposure durations (excluding day 21 data)				
Exposure	1	0.01919054	0.2746	0.6033
pCO_2	1	0.00423889	0.0606	0.8068
pCO_2 × exposure	1	0.01472580	0.2107	0.6488
Error	38	2.6559225		
Total	41	2.6948750	0.1858	0.9054

Summary tables for 2-way ANOVAs comparing different durations of exposure at two pCO_2 levels. **a.** Three exposure durations (1, 7, and 21 days). **b.** Two exposure durations (1 and 7 days)

Discussion

Metabolic activity of the diurnally vertically migrating krill species *E. pacifica* was reduced at elevated pCO_2 and included declines in oxygen consumption, food ingestion, and NH_4^+ excretion rates. This metabolic suppression occurred within the first 24 h of exposure to high pCO_2 conditions and was maintained during the 3-week exposure period.

Respiration experiment

Declines of approximately 30–40 % in oxygen consumption rates in *E. pacifica* when exposed to elevated pCO_2 reflect reduced aerobic metabolic rates in response to both acute (24 h) and longer-term (3 weeks) exposure to high pCO_2. The decline in metabolism may indicate that *E. pacifica* is unable to completely compensate for the disturbance of pH in intra- and/or extracellular fluids (Pörtner et al. 2005). Although *E. pacifica* has many traits that indicate it should be a strong acid–base regulator (e.g., crustacean, variable environment, metabolically active) (Wheatly and Henry 1992; Melzner et al. 2009), its ability to compensate for acidosis may be limited by the simultaneous need to maintain ionic homeostasis (Cameron and Wood 1985; Cameron and Iwama 1987; Whiteley et al. 2001). Declines of 11–30 % in oxygen consumption have been reported for other crustacean species exposed to high pCO_2, including some that naturally experience variable pH environments (Dissanayake and Ishimatsu 2011; Carter et al. 2013), although responses vary by life stage and between broods (Carter et al. 2013). In contrast, other crustaceans experience either increases or no changes in metabolism under high pCO_2 (Saba et al. 2012; Zervoudaki et al. 2013; Isari et al. 2015; Li et al. 2015; Thor and Dupont 2015).

The metabolic suppression seen in our experimental manipulations of pCO_2 may be derived from the usual diel metabolic changes in *E. pacifica* during its daily forays to depth. In species with diel vertical migrations, metabolic suppression of energetically costly processes (such as protein synthesis or ion transport) may be an adaptation for

short-term tolerance of the extreme conditions they experience daily at depth (e.g., hypoxia, low temperatures, and high pCO_2) and not an inability to regulate their acid–base balance (Guppy and Withers 1999; Seibel 2003, 2011). The jumbo squid, *Dosidicus gigas*, which migrates to the oxygen minimum zone, has similarly reduced oxygen consumption (up to 31 %) at elevated pCO_2, a response that was attributed to the extreme pH sensitivity of their respiratory pigments (Rosa and Seibel 2008).

Several other diel migrators do not experience metabolic suppression in response to experimentally elevated pCO_2, although those experiments have been limited to 24-h durations (Maas et al. 2012; Saba et al. 2012). While the metabolic rates of pteropods undergoing daily vertical migrations were not affected by elevated pCO_2 in the laboratory, both oxygen consumption and ammonium excretion declined in non-migrating species (Maas et al. 2012). Metabolic rates in the Antarctic krill, *Euphasia superba*, increased under high pCO_2, and this was attributed to the acceleration of iono-regulatory processes to compensate for altered pH, although metabolism was measured by metabolic enzyme activity, ingestion, and nutrient excretion and not directly by oxygen consumption (Saba et al. 2012). Differing responses among diel vertical migrating species to elevated pCO_2 may be due to differences in their buffering capabilities, acid–base regulation, or respiratory pigment sensitivity. In our experiments, metabolic suppression persisted in *E. pacifica* over the 3 weeks of exposure to elevated pCO_2, but because studies of other diel migrators have only examined the first 24 h of exposure, it is unknown whether their initial responses are maintained over longer exposure times.

Nutrition experiment

Rates of food ingestion and ammonium excretion were lower in the high pCO_2 treatment on day 1, with a similar (although not significant) trend on day 7. While there were no differences in phosphate excretion rates across pCO_2 or exposure treatments, the overall results of the Nutrition experiment add further support to our conclusion from the Respiration experiment that metabolism declines in *E. pacifica* at elevated pCO_2 levels.

Our results differ from those reported for some other crustaceans. Several studies of copepods found that elevated pCO_2 had no effect on the grazing rates of the studied copepod species, suggesting no increase in energy demands due to maintaining acid–base balance (Isari et al. 2015; Hildebrandt et al. 2015; Li et al. 2015). Conversely, the copepod, *Centropages tenuiremis*, had higher feeding rates (Li and Gao 2012). The closest taxonomic comparison to *E. pacifica*, the Antarctic krill, *E. superba*, exhibited higher rates of feeding and ammonium and phosphate excretion after 24-h exposure to elevated pCO_2 (Saba et al. 2012). These studies hypothesized that increased ingestion and excretion corresponded to increased metabolic costs associated with ramping up acid–base regulation to maintain intra- or extracellular pH (Li and Gao 2012; Saba et al. 2012).

Further work examining acid–base compensation of both krill species, *E. pacifica* and *E. superba*, under elevated pCO_2 might identify differences in their buffering capabilities and possibly explain their different metabolic responses. Although the two krill species are taxonomically similar and both undergo vertical migrations exposing them to variable pCO_2, they differ greatly in body size and habitat (e.g., temperature, seasonal patterns). Evidence supporting phylogenetic patterns in acid–base balance capabilities and ion regulation that could determine sensitivity to OA is still limited, and habitat or developmental stage may be more important in determining OA sensitivity (Widdicombe and Spicer 2008).

Unlike the oxygen consumption rates, ingestion and NH_4^+ excretion rates varied with duration of exposure, and differences between pCO_2 treatments were not significant on day 7 or day 21. The high pCO_2 treatment was higher (1733 µatm) during day 1 of the Nutrition experiment than on day 7 (1042 µatm) and day 21 (1063 µatm), and higher than pCO_2 at all three time periods of the Respiration experiment (1027–1078 µatm). While elevated pCO_2 in the Respiration experiment caused significant declines in oxygen consumption, it is possible that a higher pCO_2 level (such as that on day 1 of the Nutrition experiment) is needed to detect significant differences in ingestion and excretion rates.

Alternatively, this trend of lower oxygen consumption, but similar or elevated ammonium excretion in the high pCO_2 treatment at days 7 and 21, would result in a lower O/N ratio. A lower O/N ratio indicates a switch from lipid- to protein-based metabolism, a mechanism used by some organisms to increase net acid extrusion and maintain ion homeostasis under acidified conditions (Harris et al. 2000; Stumpp et al. 2012). Our methodology limits the ability to directly compare O/N ratios of the treatment groups in this study, and more work is needed to determine whether *E. pacifica* alters its metabolic substrate in response to OA exposure. If these findings are substantiated, it would further suggest that although *E. pacifica* may be adapted to short-term CO_2 exposure, longer-duration exposure could alter their physiology and fitness.

Interactions between ingestion and nutrient excretion processes, and subsequently with phytoplankton growth rates, may mask some of the effects of pCO_2 on ingestion and excretion rates. Because phytoplankton can divide and take up nutrients even in the dark (Vaulot and Chisholm 1987), the equations used to calculate ingestion rates

include terms for phytoplankton growth rates that use data from control containers without krill. However, excretion by krill in the experimental containers may have contributed additional nutrients that caused the phytoplankton to divide faster in containers with krill, leading to underestimates of true ingestion rates (Roman and Rublee 1980), including negative ingestion rates. Both ammonium and phosphate excretion rates were variable, with negative excretion rates recorded in several instances, and these data are consistent with increased nutrient uptake by phytoplankton leading to underestimation of the true krill excretion rates (Takahashi and Ikeda 1975).

The density of the phytoplankton culture varied between days 1, 7, and 21 and negative growth rates by day 21 in the algal controls indicate that the food culture was declining quantitatively (and probably qualitatively) by the end of the experiment. This decline in the phytoplankton culture and/or the effects of 3 weeks in the experimental conditions on krill health or behavior may have also contributed to the highly variable ingestion and nutrient excretion rates seen on day 21. Therefore, ingestion rates at day 21, and comparisons of day 21 with days 1 and 7, must be interpreted with caution, due to possible independent effects of culture density (or quality) on ingestion rates. However, comparisons between the low and high pCO_2 groups on days 1 and 7 appear to be unaffected by food condition and are still valid, and the overall declines in ingestion rates and NH_4^+ release at elevated pCO_2 are consistent with the declining oxygen consumption. All of these trends may be indicative of lowered energy demands and protein synthesis associated with metabolic suppression (Guppy and Withers 1999; Langenbuch and Pörtner 2002; Seibel 2011).

Under future OA levels, moderate metabolic suppression may be adaptive, facilitating persistence in low pH habitats (Enright 1977; Guppy and Withers 1999; Calosi et al. 2013), but such a strategy also comes with trade-offs. Specifically, the direct link between protein synthesis and growth and reproduction means that metabolic suppression cannot be a long-term adaptive strategy without having negative effects on the fitness of individuals or species (Guppy and Withers 1999; Seibel 2003; Langenbuch and Pörtner 2004; Pörtner et al. 2004; Melzner et al. 2009; Seibel 2011; Hu et al. 2014). Slower growth of *E. pacifica* over a 2-month period at pCO_2 levels of 1200 µatm probably reflects the metabolic suppression demonstrated in this study (Cooper et al. 2016). While direct effects of pCO_2 on krill reproduction are unknown, krill size may affect fecundity via the smaller brood size associated with reduced carapace size (Gómez-Gutiérrez et al. 2006).

While *E. pacifica* and other diel vertical migrators are likely to have several adaptations (including metabolic suppression) for short exposures to higher pCO_2 encountered routinely during their daily migrations to depth, the ability to increase metabolic rates at night, when exposed to lower surface pCO_2, is also adaptive. As increasing OA leads to higher pCO_2 levels in both surface and deeper ocean waters, *E. pacifica* will be exposed to higher pCO_2 values throughout its entire diel migration. As a result, metabolic activity may become suppressed across its entire depth range and throughout the 24-h day, not just at depth as it does currently, with subsequent effects on growth and/or reproduction of *E. pacifica*. At its current depth range of 250–500 m, ocean pCO_2 is expected to approach 3500 µatm by the year 2100 (Brewer and Peltzer 2009). These pCO_2 levels are much higher than those in the experiments reported here, and such increases are likely to have additional physiological effects on *E. pacifica* that may narrow the available depth range by making the deep ocean uninhabitable, with other ecological ramifications such as prolonging exposure to daytime predators near the surface. Because *E. pacifica* is the most abundant primary consumer near the base of many coastal food webs of the Eastern Pacific, any OA-induced declines in *E. pacifica* populations caused by adverse effects on reproduction, growth, or altered predation rates are likely to affect numerous other species at many higher trophic levels. In addition, elevated pCO_2 will be combined with simultaneous declines in oxygen concentration and rising temperatures, especially at depth (Stramma et al. 2008; Diaz and Rosenberg 2008; Brewer and Peltzer 2009). The synergistic effects of high pCO_2 and low pO_2 on *E. pacifica* are currently unknown. As in other krill studies, our experiments maintained oxygen levels and temperature equivalent to current surface conditions.

Although organisms inhabiting variable pH environments seem to be more tolerant of chronic OA than those from static pH environments (Maas et al. 2012; Pespeni et al. 2013; Lewis et al. 2013), their growth, development, and metabolism are still affected by OA conditions, but not necessarily in the same ways (Rosa and Seibel 2008; Thomsen and Melzner 2010; Kawaguchi et al. 2011; Stumpp et al. 2012; Saba et al. 2012; Kawaguchi et al. 2013; Cooper et al. 2016). Similar organisms (e.g., other diel vertical migrators or other krill species) may have very different metabolic responses to elevated pCO_2, suggesting that they have different mechanisms for dealing with changing environmental pH (Rosa and Seibel 2008; Maas et al. 2012; Saba et al. 2012). In particular, understanding how these organisms differ in regulating their acid–base balances or in the effects of pCO_2 on respiratory pigments will provide greater insights into the drivers of different metabolic responses among animals from similar environments or taxa. Both increased and decreased metabolic activity can have negative effects on the short-term fitness of individual organisms, whether through suppression of protein synthesis or reallocation of energy budgets, but the long-term consequences for populations and communities,

as well as for individuals, remain unknown. Identifying which individual traits confer an ability to acclimate or adapt to elevated pCO_2 and determining how they are distributed among the species in a community are both necessary precursors for understanding the ecological consequences of OA for marine ecosystems.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. This article does not contain any studies with human participants performed by any of the authors.

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