Vol. 554: 81–97, 2016
doi: 10.3354/meps11814

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published July 28

Seawater acidification more than warming presents a challenge for two Antarctic macroalgal-associated amphipods

Julie B. Schram[1,2,*], Kathryn M. Schoenrock[1], James B. McClintock[1], Charles D. Amsler[1], Robert A. Angus[1]

[1]Department of Biology, University of Alabama at Birmingham, Birmingham, Alabama 35294-1170, USA

[2]*Present address:* Oregon Institute of Marine Biology, University of Oregon, Charleston, Oregon 97420, USA

ABSTRACT: Elevated atmospheric pCO_2 concentrations are triggering seawater pH reductions and seawater temperature increases along the western Antarctic Peninsula (WAP). These factors in combination have the potential to influence organisms in an antagonistic, additive, or synergistic manner. The amphipods *Gondogeneia antarctica* and *Paradexamine fissicauda* represent prominent members of macroalgal-associated mesograzer assemblages of the WAP. Our primary objective was to investigate amphipod behavioral and physiological responses to reduced seawater pH and elevated temperature to evaluate potential cascading ecological impacts. For 90 d, amphipods were exposed to combinations of seawater conditions based on present ambient (pH 8.0, 1.5°C) and predicted end-of-century conditions (pH 7.6, 3.5°C). We recorded survival, molt frequency, and macroalgal consumption rates as well as change in wet mass and proximate body composition (protein and lipid). Survival for both species declined significantly at reduced pH and co-varied with molt frequency. Consumption rates in *G. antarctica* were significantly higher at reduced pH and there was an additive pH–temperature effect on consumption rates in *P. fissicauda*. Body mass was reduced for *G. antarctica* at elevated temperature, but there was no significant effect of pH or temperature on body mass in *P. fissicauda*. Exposure to the pH or temperature levels tested did not induce significant changes in whole body biochemical composition of *G. antarctica*, but exposure to elevated temperature resulted in a significant increase in whole body protein content of *P. fissicauda.* Our study indicates that while elevated temperature causes sub-lethal impacts on both species of amphipods, reduced pH causes significant mortality.

KEY WORDS: Western Antarctic Peninsula · Crustacean · Survival · Growth · Molt frequency · Consumption rates · Climate change

INTRODUCTION

As a result of global climate change, today's oceans are warmer and more acidic than at any time since the beginning of the industrial revolution (IPCC 2014). This long-term trend could have irreversible impacts on a variety of marine invertebrates, including ecologically and economically important members of the Crustacea (reviewed by Dissanayake 2014, Gattuso et al. 2015). As a result of an approximate 40% increase (278 to 400 ppm) in atmospheric CO_2 concentrations, the average ocean pH has decreased by 0.1 pH units since the 1750s (IPCC 2014, Gattuso et al. 2015). This CO_2-induced reduction in ocean pH, a process known as ocean acidification (Caldeira & Wickett 2003) is linked to increased atmospheric and oceanic temperatures also resulting from elevated atmospheric pCO_2.

Previous investigations of the potential effects of CO_2-reduced pH have revealed a variety of re-

*Corresponding author: jschram@uoregon.edu

Publisher: Inter-Research · www.int-res.com

sponses in crustaceans, including reductions in prey sensing and foraging behaviors in crabs (de la Haye et al. 2011, Dodd et al. 2015), increased calcification in the basal plates of barnacles (McDonald et al. 2009), extracellular acid–base balance (Spicer et al. 2007, Dissanayake et al. 2010), amphipod feeding preferences and survival (Poore et al. 2013), and seasonal alternations in amphipod abundance near natural CO_2 vents (Ricevuto et al. 2012). For instance, when compared to the effects of reduced salinity, reduced pH influenced early development time of the amphipod *Echinogammarus marinus* but did not significantly influence oxygen uptake rates (Egilsdottir et al. 2009). However, in this instance reduced salinity influenced amphipod development more than exposure to CO_2-acidified seawater. Osmoregulation and metabolic responses of amphipods exposed to elevated temperature and pCO_2 varied at the level of the individual (Calosi et al. 2013), indicating that individual variation to local seawater physicochemical fluctuations may influence species level responses to climate change (Stillman & Paganini 2015). Those species exposed to more highly variable physical and chemical environments may be more resilient to seawater changes predicted to occur by 2100 (IPCC 2014, Gattuso et al. 2015, Stillman & Paganini 2015). To date, most studies of crustaceans have focused on species living in highly variable temperate intertidal or coastal subtidal zones, which may influence species-specific responses (reviewed by Whiteley 2011, Dissanayake 2014). Studies on the effects of ocean acidification on polar organisms have focused largely on pelagic crustaceans such as krill. These studies have revealed increased sensitivity of eggs and early developmental stages of krill exposed to elevated pCO_2 (Kawaguchi et al. 2011, 2013) and metabolic shifts in adult krill (Saba et al. 2012).

There are few geographic regions in which the impacts of climate change are more dramatic than in the Southern Ocean, and particularly the region along the western Antarctic Peninsula (WAP) (Ducklow et al. 2013, Turner et al. 2014). Here, changing sea ice dynamics are increasing the potential for absorption of atmospheric CO_2 and heat (Turner et al. 2013), resulting in shifting terrestrial and marine landscapes (Kaiser et al. 2013, Constable et al. 2014). As a result, some models developed for high latitudes predict that the waters around Antarctica will be undersaturated with respect to aragonite and calcite ($\Omega \leq 1$) several decades sooner than temperate and tropical regions (McNeil & Matear 2008). Shoaling of aragonite and calcite horizons coupled with elevated pCO_2 may increase the cost of maintaining or producing calcified structures in marine Antarctic organisms (reviewed by Fabry et al. 2009).

The majority of studies examining the potential impacts of reduced pH on Antarctic marine invertebrates have focused on model macroinvertebrate species such as the krill *Euphausia superba* (see citations above), the sea urchin *Sterechinus neumayeri* (e.g. Ericson et al. 2010, Kapsenberg & Hofmann 2014, Sewell et al. 2014, Suckling et al. 2014, 2015, Collard et al. 2015), and the pteropod *Limacina helicina antarctica* (Bednaršek et al. 2012, Seibel et al. 2012). Studies on other Antarctic species' responses to combinations of ocean acidification and elevated temperature have included encrusting or fleshy macroalgae (Schoenrock et al. 2015, 2016), bivalves (Cummings et al. 2011), benthic gastropods (Schram et al. 2014, 2016b), and notothenoid fish (Enzor et al. 2013). To date, no Antarctic-based studies have investigated responses of species that comprise highly mobile crustacean mesograzers, an ecologically important assemblage of marine invertebrates (Huang et al. 2007).

Shallow marine benthic communities of the hard substrata of the WAP are dominated primarily by brown macroalgae, including *Desmarestia menziesii*, *D. anceps,* and *Himantothallus grandifolius* along with their associated mesoinvertebrate assemblages (Wiencke & Amsler 2012). The most abundant mesoinvertebrates of these macroalga-associated assemblages are amphipods (Huang et al. 2007), which can occur in densities estimated as high as 300 000 ind. m^{-2} of benthos in solid stands of *D. menziesii* (Amsler et al. 2008). Associations between crustacean mesograzers and macroalgae can be complex. For example, in many of these macroalgal-associated assemblages, amphipods prefer to associate with species that are chemically defended against macrograzer and mesograzer consumption (Zamzow et al. 2010, Amsler et al. 2014). Despite these close macroalgal associations, the majority of amphipods do not directly consume their macroalgal hosts, but rather seek shelter on their hosts for defense against predation (Zamzow et al. 2010) while feeding on epiphytic diatoms and filamentous epiphytes (Aumack et al. 2011a,b). By removing the epiphytes, these mesograzers serve a critical role in preventing fouling, which if left unchecked can compete with host macroalgae for light or nutrients (van Montfrans et al. 1984, Hughes et al. 2004).

Gondogeneia antarctica is an abundant, omnivorous gammarid amphipod associated with the common brown- and red-macroalga species along the WAP (Huang et al. 2007, Aumack et al. 2011a). As a habitat generalist, *G. antarctica* does not associate with a single preferred macroalgal host, but is found

in greater densities on those macroalgae that are chemically defended (Huang et al. 2007). Previous studies have indicated that *G. antarctica*, an endemic species along the WAP, is more tolerant of seasonal changes in temperature than other subtidal amphipods. Accordingly, individuals may be metabolically equipped to survive the elevated temperatures that occur during the austral summer (Doyle et al. 2012, Gomes et al. 2013). Another endemic gammarid amphipod, *Paradexamine fissicauda*, also occurs commonly along the WAP (Crame 2014). Subsequent analyses have found that *P. fissicauda* consumed its macroalgal host and sequestered the alga's deterrent metabolites in its own tissues in order to deter fish predation (Amsler et al. 2013). In contrast to *G. antarctica*, this species is larger, less active (authors' pers. obs.), and exhibits both habitat and feeding specialization, living in association with the chemically defended red macroalga *Plocamium cartilagineum* (Amsler et al. 2013).

At the individual level, organismal metabolism can be defined as the thermodynamically constrained uptake of energy and material from the environment and the subsequent conversion to biomass; therefore it determines the demands that an organism places on its environment for resources (Brown et al. 2004). Temperature influences metabolism and metabolic rates by driving the rates of biochemical reactions involved in the conversion of energy to biomass (Gillooly et al. 2001). The metabolic theory of ecology proposes that organismal metabolic rates fundamentally govern many patterns that influence ecology via mortality rate, life span, biomass production rates, etc. which are fundamentally related to body size and temperature (Brown et al. 2004). Additionally, the metabolic theory of ecology predicts that metabolic demands will drive other biologically relevant rates, including consumption and growth (Brown et al. 2004). By documenting changes in factors such as mortality, consumption, or growth rates following exposure to reduced seawater pH in conjunction with elevated temperature it is possible to learn more about the role of these combined abiotic factors in regulating basal metabolic rates, which potentially have far-reaching ecological effects.

The overall objective of the present study was to evaluate the prospective effects of combinations of near-future reduced seawater pH (pH 7.6) and elevated temperature (3.5°C) predicted to occur by 2100 (IPCC 2014) and present ambient conditions (pH 8.0, 1.5°C) on 2 common, sympatric benthic species of amphipod endemic to the WAP with contrasting habitat and feeding patterns. We hypothesized that reduced seawater pH and elevated temperature would have greater negative impacts on the specialist *P. fissicauda* compared to the more generalist *G. antarctica* due to the presumed greater increase in physiological and metabolic demands associated with the larger adult body size of *P. fissicauda*. We anticipated that reduced pH and elevated temperature would also influence net metabolism, effectively increasing net energetic demands as amphipods maintained homeostasis in a warmer environment enriched with hydrogen ions. To test our hypotheses, we evaluated the survival, molt frequency, consumption rates, growth, and biochemical composition (soluble protein and nonpolar lipid) of both amphipod species over a 90 d exposure period.

MATERIALS AND METHODS

Amphipod collection

Macroalgae and their associated amphipods were collected in March 2013 using SCUBA at depths ranging from 5 to 30 m within 3.5 km of Palmer Station (64° 46′ S, 64° 03′ W), operated by the US Antarctic Program. In the coastal region within 3.5 km of Palmer Station, the temperature and pH conditions do not vary greatly with depth. For example, a maximum change in temperature by depth of ≤1°C has been reported (Tortell et al. 2014). Targeted macroalgae were gently removed from the substrate and then carefully enveloped in fine mesh collection bags (mesh size <0.5 mm) to retain associated mobile mesograzers (see Huang et al. 2007). To collect the amphipod *Gondogeneia antarctica* we targeted collections of the brown macroalga *Desmarestia menziesii* (Huang et al. 2007). To collect the amphipod *Paradexamine fissicauda*, we targeted collections of *Plocamium cartilagineum* (Amsler et al. 2013). Macroalgae and their associated mesograzers were transported to Palmer Station submerged in 19 l buckets of seawater and transferred to 3800 l flow-through seawater tables. Macroalgae were repeatedly rinsed within their respective collection bag under flow-through seawater to dislodge amphipods. Amphipods were then sorted for similar sized adults of *G. antarctica* and *P. fissicauda*. Individuals of each species were placed in separate 4 l plastic Nalgene® bottles, each equipped with a window (ca. 10 cm^2) of fine-mesh (1 mm^2) screening to allow for free circulation of ambient seawater while floating in a flow-through seawater table. Each flow-through bottle contained either individuals of *G. antarctica* or *P. fis-*

sicauda and finely branched brown (*D. menziesii* or *D. anceps*) or red (*P. cartilagineum*) macroalgal thalli to provide structure that mimicked their preferred habitats (Zamzow et al. 2010). Amphipods were maintained on an ad libitum diet of the highly palatable red macroalgae *Palmaria decipiens* for ~10 d prior to the initiation of the experiment.

Seawater chemistry

Daily seawater pH and temperature measurements were made using a solid-state pH_T probe (resolution of 0.01; Durafet ISFET pH probe, Honeywell) and a Digi-Sense® ThermoLogR Thermister (resolution of 0.01°C; Cole-Parmer) (Table 1). All pH probes were calibrated at ambient temperature to ensure accuracy for cold measurement temperatures using certified Tris buffer (Dickson et al. 2007) provided by A. Dickson at Scripps Institute of Oceanography, University of California San Diego. Twice weekly, we determined seawater pH spectrophotometrically and performed total alkalinity (TA) titrations, described in detail by Schram et al. (2015b) and summarized here. Seawater samples for analysis were siphoned directly from each replicate into thoroughly seawater-rinsed 300 ml borosilicate bottles, which were then sealed with a ground glass stopper with no headspace to eliminate gas exchange and stored in a cool, dark location for a maximum of 4 h prior to analysis, at which time any unanalyzed samples were fixed with 0.10 ml of a 50% saturated mercuric chloride solution to halt biological activity. With rare exceptions, samples were analyzed within 10 to 30 min of collection. An ultraviolet-visible spectrophotometry Spectrometer Lambda 40P (Perkin Elmer) with an internal temperature-controlled cell plumbed to a water bath (Digital One RTE 17 Chiller Recirculating Water Bath, Neslab, Thermo Scientific) was used to determine seawater pH on the total hydrogen scale (pH_T) following addition of the pH sensitive indicator dye *m*-cresol purple (SOP 6b; Dickson et al. 2007). Through approximately weekly analysis of replicate seawater samples and monthly analysis of certified reference material (CRM) provided by A. Dickson at Scripps Institute of Oceanography, we determined an instrument and technician precision of 0.03 pH units (mean SD, N = 11). A manual T50 open cell titrator equipped with a pH probe (Model DGi115-SC, Mettler-Toledo) was used to determine seawater TA by open cell potentiometric titration (outlined in SOP 3b of Dickson et al. 2007). Each seawater sample was siphoned into a 250 ml jacketed beaker plumbed to a small water bath (Digital One RTE 17 Chiller Recirculating Water Bath, Neslab) to maintain constant sample temperature throughout seawater titration (10°C). Titrant volumes were recorded in real time using Mettler-Toledo LabX® software and used to calculate seawater TA (Dickson et al. 2007). Replicate analyses of individual seawater (SW) samples and CRMs yielded an instrument and technician precision of 4.02 µmol kg^{-1} SW (mean SD, N = 17).

At the time of seawater sample collections for spectrophotometric pH (pH_T) and TA measurements, temperature and salinity were also recorded for carbonate chemistry calculations. Seawater sample salinity was determined with either a Model 3200 conductivity instrument (YSI) with conductivity cell (3253 Model B, YSI) with precision of ±0.1 ppt or refractometer (1.000 to 1.070 specific gravity, Model A366ATC, Vista) with precision of ±1 ppt. We used CO_2 constants from Roy et al. (1993) and a $KHSO_4$ acidity constant from Dickson (1990) for carbonate chemistry calculations (Table 2) in CO2calc software (Robbins et al. 2010).

Experimental setup

In each of the 4 pH–temperature treatments, amphipods were haphazardly placed into 1 of 12 replicates (15 × 15 × 13 cm square opaque plastic containers with clear acrylic lids). Each replicate was partitioned into 4 compartments using fine mesh plastic screen. One of the 4 compartments was designated for individuals of the amphipod *G. antarctica* and another for individuals of the amphipod *P. fissicauda*; each of these 2 compartments was provisioned with a 10 cm tall, plastic, finely branched macroalga analog to simulate their preferred habitat without introducing an unmonitored food source. The remaining 2 compartments in each replicate were allocated to maintaining a branch of brown macroalgae, either *D. menziesii* or *D. anceps*, for a separate concurrent experi-

Table 1. Daily mean (±1 SD) pH (total scale, pH_T) and temperature measurements for ambient conditions and treatments representing present day (pH 8.0, 1.5°C) and near-future (pH 7.6, 3.5°C) experimental conditions for the 90 d exposure period (March to May 2013)

	in situ	Ambient	Reduced pH	Elevated temp	Reduced pH–elevated temp
pH_T	8.02 ± 0.04	8.07 ± 0.09	7.59 ± 0.16	8.05 ± 0.05	7.58 ± 0.25
Temp (°C)	0.29 ± 0.79	1.51 ± 0.27	1.49 ± 0.27	3.52 ± 0.35	3.52 ± 0.30

Table 2. Seawater chemistry parameters (mean ±1 SD) for experimental combinations of present day (pH 8.0, 1.5°C) and near-future (pH 7.6, 3.5°C) experimental conditions, calculated from spectrophotometric pH, total alkalinity (TA), temperature, and salinity. Carbonate chemistry parameters (±SD) of partial pressure of carbon dioxide (pCO_2; µatm), dissolved inorganic carbon (DIC) and saturation states of aragonite (Ω_{arg}) and calcite (Ω_{cal}) were calculated using the aforementioned data and CO2calc software. All seawater chemistry measurements are available on the Antarctic Master Directory (www.usap-data.org/entry/NSF-ANT10-41022)

	in situ	Ambient	Reduced pH	Elevated temp	Reduced pH–elevated temp
pH_T	8.10 ± 0.03	8.05 ± 0.14	7.55 ± 0.18	8.05 ± 0.10	7.55 ± 0.17
TA (µmol kg^{-1} SW)	2283 ± 36	2268 ± 60	2292 ± 55	2271 ± 56	2294 ± 36
Temp (°C)	0.91 ± 0.72	1.45 ± 0.30	1.46 ± 0.57	3.19 ± 0.78	3.32 ± 0.98
Salinity (ppt)	35.6 ± 0.6	35.8 ± 0.78	35.7 ± 0.63	35.8 ± 0.6	35.7 ± 0.56
pCO_2 (µatm)	327 ± 28	409 ± 290	1407 ± 583	393 ± 127	1433 ± 531
DIC (µmol kg^{-1} SW)	2132 ± 43	2133 ± 71	2316 ± 82	2125 ± 54	2311 ± 67
Ω_{arg}	1.67 ± 0.12	1.55 ± 0.32	0.57 ± 0.25	1.66 ± 0.29	0.61 ± 0.26
Ω_{cal}	2.66 ± 0.19	2.46 ± 0.51	0.91 ± 0.40	2.63 ± 0.47	0.96 ± 0.41

ment (Schoenrock et al. 2015). The presence of both amphipods and macroalgal thalli in separate compartments of the same replicates provided a natural diurnal balance of photosynthesis and respiration (12 h light:12 h dark photoperiod sustained over the course of the 90 d experiment) while preventing trophic interactions.

To maintain targeted temperatures, replicates were partially submerged in 1 of 4 acrylic rectangular recirculating water tables (length × width × depth: 113 × 58 × 13 cm). Each water table was plumbed individually to 1 of 4 Digital Water Bath Recirculators (temperature control precision: ±0.01°C; 1186D, VWR Scientific), which circulated a 30% glycol solution through the water baths at target temperatures. To facilitate sufficient circulation and ensure temperature stability, 8 additional submersible water pumps were distributed around each water table. Replicates were randomly assigned to positions within each water table to avoid introducing confounding artefacts due to spatial arrangement. Target pH levels were maintained with an automated AT-Control (Aqua Medic) pH regulation system, which monitored and adjusted CO_2 levels based on continuous real time pH measurements. The seawater in each reduced pH replicate was bubbled with ambient air–CO_2 gas combinations mixed using a Multi-tube Gas Proportioning Rotameter (Omega Engineering). The rotameter was plumbed to a Super Luft SL-65 high-pressure aquarium air pump (Coralife) and a CO_2 gas cylinder containing pure CO_2. The resultant air–CO_2 gas combinations were bubbled as needed to replicates to maintain an appropriate CO_2 enrichment to adjust seawater to the target pH level. Seawater in each replicate was partially exchanged (~½ replicate volume) every other day to maintain water quality.

We utilized a 2 × 2 factorial experimental design to investigate the combined effects of reduced seawater pH and elevated temperature with 4 treatment groups (n = 12 for each treatment) with 16 *G. antarctica* and 10 *P. fissicauda* randomly assigned to each replicate at the beginning of the experimental period (numbers were chosen to reach target mass of samples). The experimental pH–temperature levels were determined based on current annual ambient seawater pH and temperature conditions at Palmer Station (Schram et al. 2015b) and those predicted for 2100. Four pH–temperature treatment combinations were included: current mean ambient (pH 8.0, 1.5°C), reduced pH (pH 7.6, 1.5°C), elevated temperature (pH 8.0, 3.5°C), and combined reduced pH–elevated temperature (pH 7.6, 3.5°C). Based on previous studies of feeding preferences in Antarctic amphipods (Amsler et al. 2005, Amsler et al. 2013), both species used in the present study were fed *P. decipiens* and its associated epiphytic diatoms. Amphipods in each replicate were provisioned with an ad libitum diet of 5 mm diameter disks of fresh *P. decipiens* thallus tissue cut with a cork borer. Care was taken to prevent overfeeding and to remove feces daily to ensure healthy water quality and stable experimental seawater carbonate chemistry. Amphipods were maintained in the experimental treatments for a 90 d period during the late austral summer and autumn (March to May 2013).

Amphipod survival and molt frequency

To assess amphipod survivorship and molt frequency during the 90 d period, we surveyed each replicate during daily fecal pellet removal. If dead am-

phipods or whole body molts were present, they were immediately removed from replicates and recorded.

Amphipod consumption rates

Beginning on Day 68 of the 90 d experimental period, every 2 d over a 10 d period we recorded consumption rates for *G. antarctica* (n = 12, 9, 12, and 9 replicates for ambient, reduced pH, elevated temperature, and reduced pH–elevated temperature treatments, respectively) and *P. fissicauda* (n = 12, 8, 12, and 5 replicates for ambient, reduced pH, elevated temperature, and reduced pH-elevated temperature treatments, respectively). The variation in numbers of replicates available for consumption rate assays in both amphipod species was the result of the differential survival in temperature–pH treatments. To assess consumption rates of *G. antarctica* and *P. fissicauda*, the 5 mm diameter disks of the red alga *P. decipiens* were weighed before and after each feeding period on a top-loading balance (±0.01 g; Ohaus). Wet disks were blotted for 10 s on absorbent lab tissue to remove excess moisture. We also included an autogenic control in our experimental design, with disks held under identical conditions without amphipods to account for changes in disk mass not related to herbivory. Experimental food disks were presented to both species of amphipods and paired autogenic control disks were added to each replicate at the initiation of each consumption rate experiment. Paired food disks were subsequently monitored and removed before all of the algal tissue of experimental disks was consumed by amphipods, and re-weighed. Consumption rates (µg h^{-1} amphipod^{-1}) were calculated based on the amount of macroalgal tissue consumed and changes in paired autogenic control disk mass (see Schram et al. 2015a).

Mean amphipod mass

Collective wet weight of the amphipods in each replicate for *G. antarctica* (n = 16) and *P. fissicauda* (n = 10) were recorded prior to the experiment. Following the exposure period, *G. antarctica* (n = 12, 7, 12, and 7 replicates for ambient, reduced pH, elevated temperature, and reduced pH–elevated temperature treatments, respectively) and *P. fissicauda* (n = 12, 6, 11, and 5 replicates for ambient, reduced pH, elevated temperature, and reduced pH–elevated temperature treatments, respectively) were reweighed to determine change in wet weight. Differential mortality across replicates at the end of the experiment left unequal numbers of individuals for each species with which to assess mean amphipod mass. Accordingly, we calculated the mean change in wet mass per amphipod. Following the 90 d exposure period, amphipods from each replicate were re-weighed and we calculated the change (in mg) of mean wet mass (M):

$$M = \frac{\text{group wet mass}}{\text{no. of amphipods}} \quad (1)$$

The initial wet mass (M_i) was recorded before placement into replicates and immediately following the experimental exposure period (M_f). Amphipods for each replicate were weighed wet as a group to reduce individual handling stress during the initial measurement. Amphipod cohorts were gently placed on a paper towel for ~10 s with sufficient space between cohorts to absorb excess seawater and then transferred to a top-loading microbalance (±0.01 mg; XP26, Mettler-Toledo) and weighed. We were careful to ensure uniformity in handling and measurement of wet weights, but it should be noted that it is possible that the aggregate weights for replicates with more amphipods at the end of the experiment may have been artificially inflated by the additional water weight. To account for this potential bias, we calculated the percent change (%Δ) in wet weight per amphipod as the difference between the final mean wet weight per amphipod and initial mean wet weight per amphipod divided by the initial wet weights:

$$\%\Delta M = \frac{M_f - M_i}{M_i} \quad (2)$$

Proximate whole body composition

After assessing the percent change in wet mass, amphipods remaining in each replicate were pooled by replicate and frozen at −80°C for later whole-body tissue analysis of soluble protein and total nonpolar lipid content. Pooled individuals of *G. antarctica* ($N_{protein}$ = 12, 7, 12, and 7 and N_{lipid} = 4, 2, 4, and 1 for ambient, reduced pH, elevated temperature, and reduced pH–elevated temperature treatments, respectively) and *P. fissicauda* ($N_{protein}$ = 12, 6, 10, and 6 and N_{lipid} = 12, 4,11, and 3 for ambient, reduced pH, elevated temperature, and reduced pH–elevated temperature treatments, respectively) were lyophilized and then ground to a fine homogenous powder using a ceramic mortar and pestle. The variation in numbers of replicates available for tissue analysis in both

amphipod species was the result of the differential survival in temperature–pH treatments. Powdered tissue for proximate body composition analyses were weighed to the nearest 0.1 mg on a top-loading balance (Mettler AM 100, Mettler-Toledo International). For soluble protein analysis, pooled lyophilized amphipod tissues were extracted in 5 ml of 1 M NaOH for 24 h. Following extraction, 5 µl of each protein extract was added to 250 µl of Bradford Dye Reagent (Bio-Rad) and analyzed in triplicate on a microplate spectrophotometer (BioTek® Instruments; Bradford 1976). The absorbance of each sample was measured at 595 nm and plotted against a standard curve developed from bovine serum albumin. Protein concentrations were then standardized to the dry weight of each individual in order to calculate µg protein mg^{-1} dry tissue.

Nonpolar lipids were determined for lyophilized amphipod tissues using a modified Folch et al. (1957) technique. Pooled lyophilized amphipod tissues were weighed to the nearest 0.01 g, heated in 25 ml of a 2:1 chloroform:methanol solution, and then placed in a water bath at 60°C for 30 min. Following heating, solvent solutions were filtered through Whatman 541 filter paper and then 4 ml of 0.9% NaCl was added to the filtrate. Solutions were then shaken for 5 min and then placed in a centrifuge for 30 min followed by removal of the upper water–methanol phase. The remaining solvent solution was evaporated at 50°C in a water bath. The lipids were then transferred to preweighed shell vials and the remaining solvent evaporated. Lipid content was determined gravimetrically as the percent mass of the amount of dry whole body tissue extracted. We graphed nonpolar lipid content (% dry weight; calculated as in Eq. 2) as a function of soluble protein content (% dry weight; calculated as in Eq. 2) to synthesize how changes in each component contributed to the proximate composition of whole-body tissues of amphipods exposed to the 4 pH–temperature treatments.

Statistics

Prior to statistical comparisons between the pH–temperature treatments, all data were tested for normality using the Shapiro-Wilks test. In cases where data were not normally distributed, we performed an additional Grubb's test to determine the presence of potential outliers which, if present, were eliminated from subsequent analyses. In no case were more than 2 outliers removed from any dataset. The cutoff for statistical significance was set at $p \leq 0.05$. Amphipod survival rates over the 90 d exposure period among pH–temperature treatments were compared using a Cox's proportional hazard (CPH) model with multiple effects and multiple levels in JMP® v.12.0.1 (SAS Institute). The CPH model is a semi-parametric regression model that relates the risk of a hazard (death) to explanatory variables (e.g. pH or temperature). The CPH model evaluated hazard risk associated with the explanatory variables pH (8.0 vs. 7.6), temperature (1.5 vs. 3.5°C), species (*G. antarctica* vs. *P. fissicauda*) and all variable interactions (e.g. pH × temperature × species). The CPH models generated imposed no assumptions on the mortality rate variability over time. A 'significant' treatment effect indicated that the proportion of mortality associated with the explanatory variable differed from expected with >95% confidence. To compare the CPH model fit with subsequent inclusion of pH, temperature, species, or any pH–temperature–species interaction, likelihood ratio tests were performed. To determine the proportion of mortality attributable to treatment variables (pH 7.6, 3.5°C) compared to controls (pH 8.0, 1.5°C), relative hazard ratios were calculated. Hazard ratios express the relative hazard risk of being a part of group *a* versus group *b*, where values >1 indicate an elevated relative risk for group *a* compared to group *b* and values <1 indicating reduced relative risk of belonging to group *a* versus group *b*. In addition to CHP models, Kaplan-Meier survival curves for both amphipods were generated (see Fig. 1). The Kaplan-Meier product-limit method was selected because survival curves could be generated from incomplete (right-censored) data (i.e. data for which exact survival time was unknown due to study termination).

To investigate the possible association between mortality and molting, we used the Tukey's test for additivity (Tukey 1949, Johnson & Graybill 1972) to determine whether amphipod survival curves and plots of molt frequency were approximately parallel. If the interaction effect between mortality and molt frequency was non-significant, it indicated that increases or decreases in mortality were co-occurring with similar changes in molt frequency. Macroalgal disk consumption rates were rank transformed and analyzed using a 2-way repeated measures analysis of variance (RMANOVA) in SYSTAT® (Systat Software). The influence of pH and temperature on the change in mean wet mass and the proximate composition of whole body tissues (protein and lipid) were analyzed using 2-way ANOVAs in JMP®. The significance level was set at $\alpha \leq 0.05$ for all data analysis in the present study.

RESULTS

Amphipod survival

Amphipod survivorship over the 90 d exposure period varied significantly among the 4 pH–temperature treatment groups for both *Gondogeneia antarctica* (CPH model, χ^2 = 120.7, df = 3, p < 0.001) and *Paradexamine fissicauda* (CPH model, χ^2 = 257.0, df = 3, p < 0.001). The sharpest declines in survival (Fig. 1) were observed following the initial 20 to 30 d of exposure for both amphipod species, with the greatest mortality in the 2 reduced pH treatments. Amphipod survivorship in the reduced pH treatments was significantly lower than survivorship in the ambient pH treatments (Fig. 1, Table 3). The combined mean survival (± combined SD) in treatments exposed to ambient pH, including the ambient (n = 12) and elevated temperature (n = 12) treatments at the end of the 90 d experimental period for *G. antarctica* was 86 ± 13% (n = 24). In contrast, there was a combined mean amphipod survival of 51 ± 29% (n = 23) for amphipods exposed to reduced pH, including the reduced pH (n = 11) and reduced pH–elevated temperature (n = 12) treatments. This resulted in an overall 35% decrease in survival for *G. antarctica* (Fig. 1a). Mortality of *G. antarctica* within each given treatment occurred across all replicates. No replicates in either the ambient and elevated temperature treatments experienced ≥50% mortality, but we documented ≥50% mortality of *G. antarctica* in 2 replicates in the reduced pH treatment and 3 replicates in the combined reduced pH–elevated temperature treatment. High mortality in both species did not occur in all of the same replicates nor did they occur at the same rate within a given replicate. The mean (±SD) percent survival for *P. fissicauda* in treatments exposed to ambient pH, including the ambient (n = 12) and elevated temperature (n = 12) treatments, was 88 ± 12% (combined n = 24). The combined survival for *P. fissicauda* (n = 14) in the treatments exposed to reduced pH, including the reduced pH (n = 9) and reduced pH–elevated temperature (n = 5) treatments was 23 ± 24%. The difference in mean percent survival for *P. fissicauda* between pH treatments was 65% (Fig. 1b). Mortality of *P. fissicauda* within each treatment occurred across all replicates. No replicates in the ambient and elevated temperature treatments experienced ≥50% mortality. In contrast, we documented ≥50% mortality in 4 replicates in the reduced pH treatment and in 10 of the 12 replicates in the combined reduced pH–elevated temperature treatment. There was no signifi-

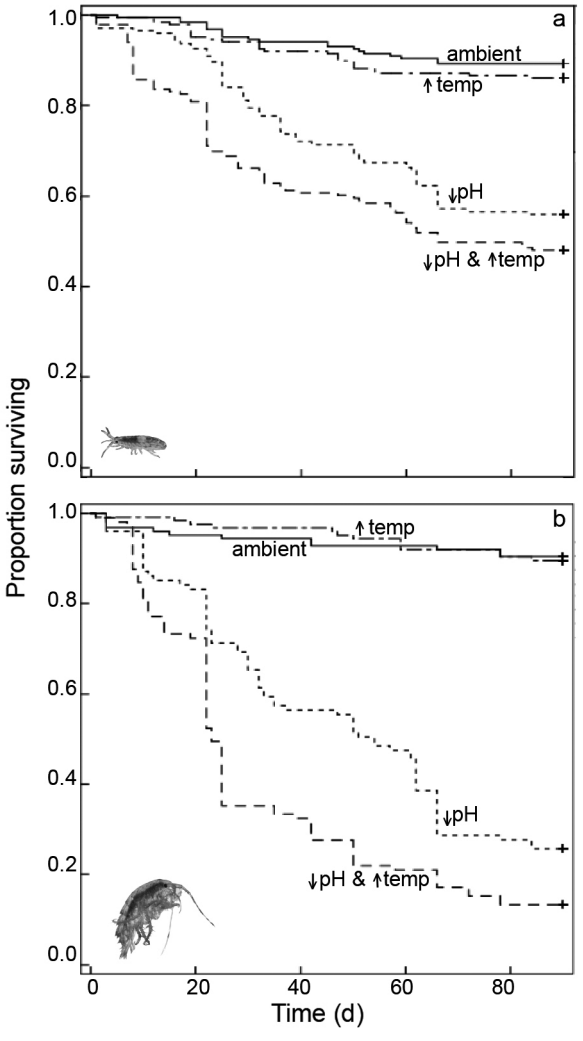

Fig. 1. Kaplan-Meier survival curves. Survival trajectories were generated for both (a) *Gondogeneia antarctica* and (b) *Paradexamine fissicauda* maintained in combinations of present day (pH 8.0, 1.5°C) and near-future (pH 7.6, 3.5°C) experimental conditions for the 90 d exposure period (March to May 2013)

Table 3. Amphipod survival. Cox's proportional hazard models were used to compare the hazard (risk of mortality) based on exposure to decreased pH and elevated temperature. df: degrees of freedom; p: probability level. **Bold** lettering indicates tests showing significant treatment effects

Source	Likelihood ratio test		
	χ^2	df	p
Temp	4.7	1	**0.03**
pH	352.5	1	**<0.001**
Temp × pH	0.7	1	0.39
Species	4.9	1	**0.02**
Temp × species	0.003	1	0.96
pH × species	15.2	1	**<0.001**
Temp × pH × species	0.5	1	0.47

cant temperature or interactive pH–temperature effect on the survivorship of *G. antarctica* or *P. fissicauda* (Table 3).

The calculated hazard ratios indicated that significantly greater risk was associated with both reduced pH and elevated temperature. The hazard ratios for ambient and reduced pH (lower, upper 95% confidence interval) were 0.12 (0.09, 0.15) and 8.44 (6.51, 11.51), respectively, indicating a significantly greater relative risk of mortality at reduced pH (χ^2 = 352.5, p < 0.001). The hazard ratios for ambient and elevated temperature were 0.74 (0.57, 0.97) and 1.34 (1.03, 1.76), respectively, indicating a significantly greater relative risk of mortality at elevated temperature (χ^2 = 4.69, p = 0.03). The hazard ratios associated with a species comparison of *G. antarctica* and *P. fissicauda* were 0.73 (0.56, 0.96) and 1.36 (1.04, 1.77), respectively, indicating a significantly greater relative risk of mortality for *P. fissicauda* compared to *G. antarctica* (χ^2 = 4.9, p = 0.02).

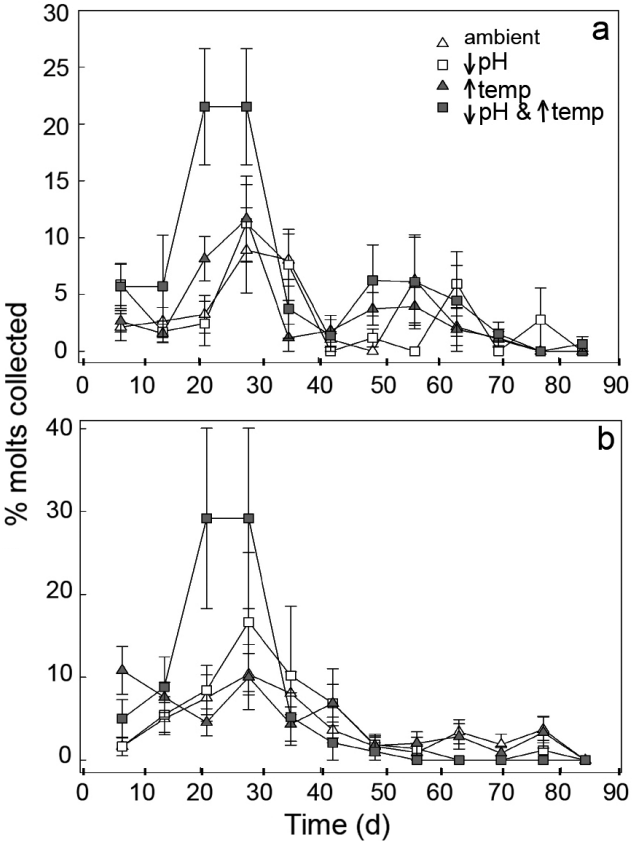

Fig. 2. Amphipod molt frequency. Percentage molts collected daily and presented weekly for (a) *Gondogeneia antarctica* and (b) *Paradexamine fissicauda* over the 90 d exposure period maintained in combinations of present day (pH 8.0, 1.5°C) and near-future (pH 7.6, 3.5°C) experimental conditions. Symbols represent weekly mean (±1 SE) percentages of molts

Molt frequency

Molt frequency tended to be low over the 90 d exposure period, with no new molts collected in treatments for approximately the last 49 d for both *G. antarctica* and *P. fissicauda* (Fig. 2). Molting activity was elevated above 5% for *G. antarctica* during the first 14 to 35 d and then again in the decreased pH and elevated temperature treatment during 49 to 56 d (Fig. 2a). Otherwise, throughout the remainder of the experimental period the percentage of molting in *G. antarctica* remained below 5%. For each of the treatments, there were no significant interactions between survival and molt frequency in *G. antarctica*, indicating that the time trends for mortality and molting are approximately parallel (Table 4, Figs. 1 & 2). For *P. fissicauda*, the percentage of amphipods molting remained above 5% for the first 10 to 42 d, after which the percentage of molting in *P. fissicauda* decreased to almost zero for the balance of the experimental period (Fig. 2b). Consistent with the results for *G. antarctica,* there were no significant interactions between survival and molt frequency, indicating that changes in these 2 measurements were co-occurring (Table 4).

Amphipod consumption rates

Consumption rates in *G. antarctica* of macroalgal disks were significantly influenced by both temperature and pH when initiated on Day 68 of the experiment (Fig. 3a, Table 5). There was a significant effect of time over the 10 d series of feeding trials, with the majority of consumption rates tending to increase over time (Fig. 3a, Table 5). When maintained at the

Table 4. Comparison of survival and molt frequency for *Gondogeneia antarctica* and *Paradexamine fissicauda*. We compared the mortality each week to the number of amphipod molts collected using Tukey's test for additivity. df: degrees of freedom; MS: mean square; *F*: *F*-ratio; p: probability level

Experimental treatment	df	MS	*F*	p
G. antarctica				
Ambient	1	4.83	2.85	0.12
Reduced pH	1	4.83	1.13	0.31
Elevated temp	1	1.99	1.34	0.28
Reduced pH–elevated temp	1	1.51	0.69	0.43
P. fissicauda				
Ambient	1	2.04	0.91	0.36
Reduced pH	1	1.97	0.63	0.45
Elevated temp	1	22.09	3.98	0.07
Reduced pH–elevated temp	1	21.13	3.53	0.09

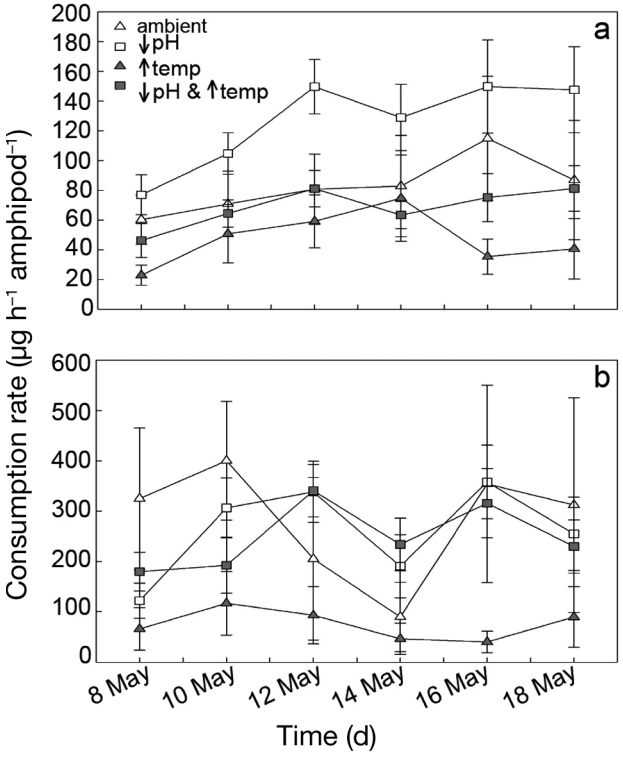

Fig. 3. Amphipod consumption rates. The mean (±1 SE) consumption rates measured every other day over a 10 d period (8 to 18 May 2013) in (a) *Gondogeneia antarctica* (n = 12, 9, 12, 9 for ambient, reduced pH, elevated temperature, and reduced pH–elevated temperature treatments, respectively) and (b) *Paradexamine fissicauda* (n = 12, 8, 12, 5 for ambient, reduced pH, elevated temperature, and reduced pH–elevated temperature treatments, respectively). Amphipods were maintained in combinations of present-day (pH 8.0, 1.5°C) and near-future (pH 7.6, 3.5°C) experimental conditions

same temperature, either ambient or elevated, amphipod consumption rates in the reduced pH treatments were significantly higher than those in ambient pH treatments (Fig. 3a, Table 5). The consumption rates in *G. antarctica* were significantly greater in the ambient temperature treatments (1.5°C; Table 5), and the highest consumption rates were consistently observed in the reduced pH treatments (Fig. 3a). The *G. antarctica* held in the elevated temperature treatments, regardless of pH, had lower consumption rates than those maintained in ambient temperature treatments, with the lowest consumption rates observed in the elevated temperature treatment (pH 8.0, 3.5°C) (Fig. 3a, Table 5). There were no significant interaction effects between reduced pH, elevated temperature, or time (Table 5).

In contrast to *G. antarctica*, there was a significant pH–temperature interaction effect on consumption rates in *P. fissicauda* (Table 5). Consumption rates

Table 5. Amphipod algal consumption rates. The consumption rates (µg h^{-1} $amphipod^{-1}$) for *Gondogeneia antarctica* and *Paradexamine fissicauda* were measured every other day over a 10 d period (8 to 18 May 2013) and later rank-transformed and analyzed using a 2-way repeated measures analysis of variance (RMANOVA). df: degrees of freedom; MS: mean square; *F*: *F*-ratio; p: probability level. **Bold** lettering indicates tests showing significant treatment effects

Source	df	MS	*F*	p
G. antarctica				
Temp	1	8.31	5.61	**0.02**
pH	1	18.91	12.77	**0.001**
Temp × pH	1	0.36	0.24	0.63
Time	5	0.58	3.44	**0.005**
Time × temp	5	0.15	0.87	0.50
Time × pH	5	0.15	0.90	0.50
Time × temp × pH	5	0.13	0.75	0.60
P. fissicauda				
Temp	1	7.37	7.40	**0.01**
pH	1	12.88	12.95	**0.001**
Temp × pH	1	12.51	12.57	**0.001**
Time	5	1.51	1.72	0.13
Time × temp	5	0.58	0.65	0.66
Time × pH	5	1.18	1.35	0.25
Time × temp × pH	5	0.57	0.65	0.67

were highly variable among individuals when maintained at ambient temperature, with neither pH treatment having consistently higher or lower consumption rates in individuals at this temperature (Fig. 3b). In the elevated temperature treatment, the consumption rates for individuals maintained at reduced pH tended to be higher than those maintained at ambient pH (Fig. 3b). However, there was no direct significant effect of reduced pH, elevated temperature, or time on consumption rates in *P. fissicauda* (Table 5).

Mean amphipod mass

Regardless of pH treatment, when *G. antarctica* was exposed to elevated temperature, individuals exhibited a significant decrease in mean wet mass after the 90 d exposure period. The greatest decline in wet mass was for individuals held in the reduced pH–elevated temperature treatment (Fig. 4a, Table 6). There was a significant temperature effect on the change in mean wet mass in *G. antarctica*, but no significant pH or pH–temperature interaction (Table 6). In contrast to *G. antarctica*, there was no significant effect of temperature or pH on the change in mean wet mass of *P. fissicauda* over the exposure period. The small numbers of surviving individuals at the

Table 6. Growth and proximate body composition of *Gondogeneia antarctica* and *Paradexamine fissicauda*. Changes in growth (%Δ mass), percent protein, and percent lipid were analyzed using a 2-way ANOVA following the 90 d exposure period. df: degrees of freedom; MS: mean square; *F*: *F*-ratio; p: probability level. **Bold** lettering indicates tests showing significant treatment effects

Trait	Source	df	MS	*F*	P
G. antarctica					
%Δ mass	Temp	1	1541.50	7.42	**0.01**
	pH	1	0.01	0.00	1.00
	Temp × pH	1	63.99	0.31	0.58
% protein	Temp	1	15.97	0.84	0.37
	pH	1	43.38	2.27	0.14
	Temp × pH	1	0.30	0.16	0.90
P. fissicauda					
%Δ mass	Temp	1	1044.71	3.37	0.08
	pH	1	471.60	1.52	0.23
	Temp × pH	1	877.66	2.83	0.10
% protein	Temp	1	59.43	6.81	**0.01**
	pH	1	4.41	0.50	0.48
	Temp × pH	1	4.26	0.49	0.49
% lipid	Temp	1	0.03	0.08	0.78
	pH	1	0.08	0.18	0.68
	Temp × pH	1	0.08	0.19	0.67

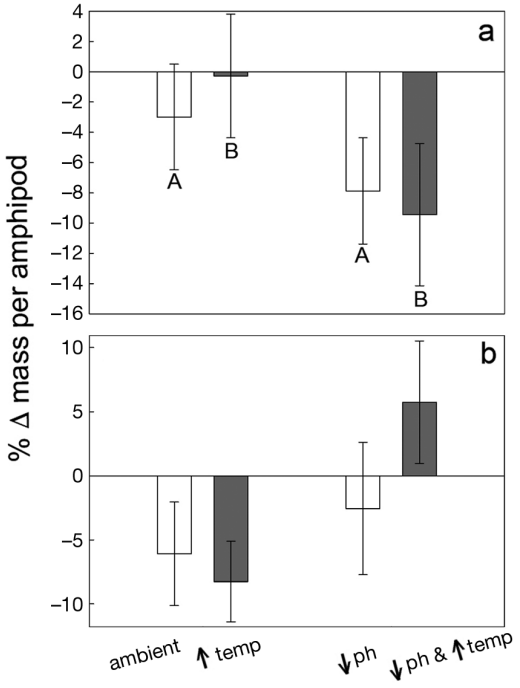

Fig. 4. Amphipod net growth. Calculated mean (±1 SE) percent change in mean wet mass for (a) *Gondogeneia antarctica* (n = 12, 7, 12, 7 for ambient, reduced pH, elevated temperature, and reduced pH–elevated temperature treatments, respectively) and (b) *Paradexamine fissicauda* (n = 12, 6, 11, 5 for ambient, reduced pH, elevated temperature, and reduced pH–elevated temperature treatments, respectively). Amphipods were maintained in combinations of present-day (pH 8.0, 1.5°C) and near-future (pH 7.6, 3.5°C) experimental conditions

end of the experiment in the reduced pH–elevated temperature treatment demonstrated a slight but not significant increase in mean wet mass in contrast to individuals held in other pH–temperature treatments, which had lost wet mass over the 90 d exposure period (Fig. 4b).

Proximate body composition

Whole body tissues of *G. antarctica* exhibited no significant temperature or pH changes in percent protein content over the 90 d exposure period (Table 6). Percent lipid content of the whole body tissues of *G. antarctica* maintained in the reduced pH–elevated temperature treatment tended to be higher than those observed in all other treatments (Fig. 5a). However, due to the lack of tissue available for repli-

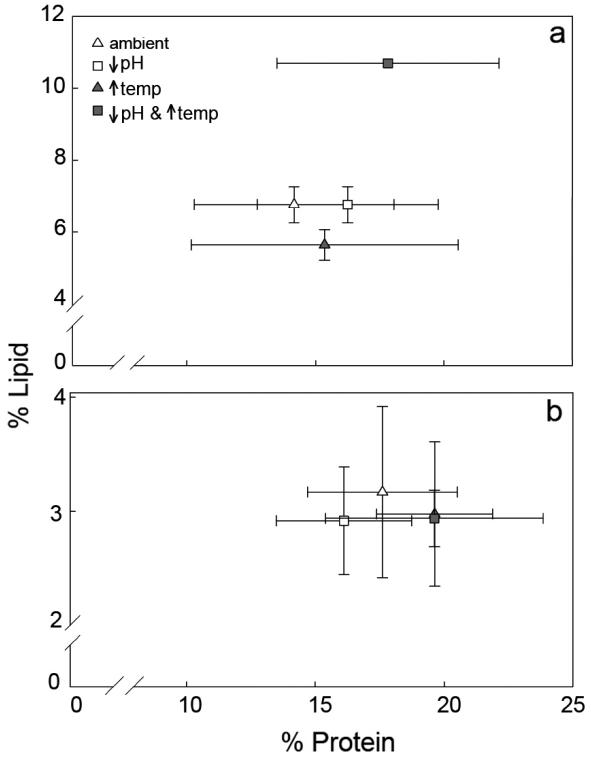

Fig. 5. Amphipod proximate body composition. Percent lipid as a function of the percent protein in whole body tissues of (a) *Gondogeneia antarctica* ($N_{protein}$ = 12, 7, 12, 7 and N_{lipid} = 4, 2, 4, 1 for ambient, reduced pH, elevated temperature, and reduced pH–elevated temperature treatments, respectively) and (b) *Paradexamine fissicauda* ($N_{protein}$ = 12, 6, 10, 6 and N_{lipid} = 12, 4, 11, 3 for ambient, reduced pH, elevated temperature, and reduced pH–elevated temperature treatments, respectively). Tissue composition was quantified following a 90 d exposure period to combinations of present-day (pH 8.0, 1.5°C) and near-future (pH 7.6, 3.5°C) experimental conditions. Symbols represent mean (±1 SE) percent lipid and protein

cation of lipid samples for *G. antarctica* in the reduced pH–elevated temperature treatment we were unable to include these data in the statistical analysis, making it difficult to draw conclusions from this analysis. In contrast to *G. antarctica*, there was a slight but significant increase in protein content of whole body tissues in *P. fissicauda* maintained at elevated temperature following the exposure period (Fig. 5a, Table 6). The whole body tissue lipid content of *P. fissicauda* exposed to reduced pH treatment tended to be lower than the whole body tissue lipid content of those maintained in the ambient treatment (Fig. 5b).

DISCUSSION

In the present study, we combined measurements of survival, growth, consumption rates, and proximate body composition to assess the potential impacts of reduced pH and elevated temperature on 2 common Antarctic benthic amphipod species. Both *Gondogeneia antarctica* and *Paradexamine fissicauda* exhibited decreased survival when exposed to reduced pH and combined reduced pH–elevated temperature. Our calculated hazard ratios, representative of the likelihood of mortality, indicated that CO_2-induced reductions in seawater pH posed a significant risk of mortality to both amphipod species. This is a noteworthy departure from the general resilience to reduced seawater pH predicted for the majority of crustaceans (Wittmann & Pörtner 2013). Despite maintaining lighter skeletons than other taxa studied, of those species exposed to 851 and 1370 µatm CO_2, approximately one-third were negatively affected (Wittmann & Pörtner 2013). Some of these negative effects may be attributed to disruption of normal ion regulation.

Extracellular acid–base compensation has been demonstrated to be nearly complete in many crustacean species (Widdicombe & Spicer 2008). However, Antarctic crustaceans may have a lower ion regulation potential (Frederich et al. 2000). For instance, poor ion regulation of Mg^{2+} in polar decapods has been attributed as one reason that reptant crustaceans have been mostly absent in the Southern Ocean south of the Antarctic Convergence (Frederich et al. 2000). There has been speculation that there may be a synergistic interactive effect of Mg^{2+} and decreasing temperature on growth and reproduction of reptant decapods (Frederich et al. 2001), but this pattern may shift in the near future in rapidly warming seas around Antarctica (Aronson et al. 2015). Previous work with deep sea crustaceans has demonstrated that where species generally live a slow and hypometabolic life, they also have a reduced capacity for acid–base regulation (reviewed by Pörtner 2008). The ability to regulate extracellular balance is crucial to alleviating or mediating potential negative effects of exposure to reduced seawater pH due to the metabolic cost associated with maintaining cellular processes (Pörtner 2008). Of the 2 species discussed here, the species most adversely affected by reduced seawater pH and elevated temperature, *P. fissicauda*, typically exhibits a slower mode of life than *G. antarctica*, suggesting that acid–base regulation may in part explain some of the differences observed in the survival of these 2 species following a 3 mo exposure.

Reduced amphipod survival and growth following exposure to elevated temperature and decreased seawater pH has been documented previously (Poore et al. 2013). In contrast to the results of the present study, the temperate amphipod *Peramphithoe parmerong* exhibited decreased survival following exposure to combinations of elevated temperature and reduced pH, but in this case elevated temperature had a greater impact on survival than reduced pH (Poore et al. 2013). However, these results cannot be generalized to all temperate amphipods: the survival of the temperate neritic amphipod *Gammarus locusta* was not significantly impacted following exposure to a range of pH levels (8.1 to 7.6) for 2 and 4 wk (Hauton et al. 2009). However, the authors noted that mortality occurred most frequently among developing juveniles molting. In the present study we observed the sharpest declines in survival in both species after 20 to 30 d of exposure to experimental conditions, especially among individuals held in the reduced pH treatments. During the same time frame we also recorded the greatest incidence of molting. Accordingly, the co-occurrence of peaks in mortality and molt frequency suggests that the molts and the post-molt interval may be a particularly vulnerable time period for amphipods exposed to reduced seawater pH. Previous studies have found that reductions in seawater pH can interfere with post-molt calcification processes because of additional potential stress associated with additional acid–base balance demands (reviewed by Whiteley 2011).

Internal acid–base balance has been studied in a variety of crustaceans, including temperate and polar species occupying habitats with a range of pH levels associated with elevated pCO_2, such as kelp forests and tide pools (reviewed by Whiteley 2011, Dissanayake 2014). Shifts in environmental temperature and pCO_2 can influence 'animal performance', which has been defined as the sum of multiple relevant traits

contributing to ecological success (Melzner et al. 2009). For instance, following exposure to elevated seawater CO_2, the spider crab *Hyas araneus* experienced a narrowing of its thermal tolerance, characterized by changes in temperature-dependent hemolymph oxygen partial pressure as well as heart and ventilation rates (Walther et al. 2009). In the present study, we observed slight (but not significant) reductions in the percent survival of amphipods exposed to elevated temperature alone. In a previous study with temperate crabs, heat tolerance, carapace mineralization, and immune response were not affected by reduced seawater pH, but maintenance of these functions resulted in decreased metabolic rate and shifts in hemolymph chemistry (Small et al. 2010). It is possible that both amphipods investigated in the present study became less efficient at performing metabolic shifts under experimental conditions, resulting in the observed reduction in survival.

In the present study we observed that upon exposure to reduced pH the amphipod *G. antarctica* increased its consumption of macroalgal disks. This pattern occurred regardless of whether individuals were exposed to ambient or elevated temperatures. It is possible that *G. antarctica* experienced increased metabolic demands under conditions of reduced pH and consumed more food as a compensatory response. The increased consumption observed in *G. antarctica* at reduced seawater pH is similar to, but less dramatic, than the 3.5-fold increase in feeding rates observed in Antarctic krill *Euphausia superba* exposed to near-future reduced pH (Saba et al. 2012). Increased consumption rates may allow krill to behaviorally compensate for higher energy requirements induced by exposure to reduced seawater pH. By increasing consumption, krill can acquire sufficient energy to maintain growth and reproduction, provided that food availability remains of sufficient quality and quantity to satisfy energetic demands (Saba et al. 2012).

In a previous study conducted with the generalist *G. antarctica*, established amphipod consumption rates for a suite of weakly chemically deterrent macroalgae shifted from significant deterrence to no deterrence in response to acute exposure to elevated seawater temperature (3.5°C; Schram et al. 2015a). A change in feeding activity, such as this observed shift from discriminant to indiscriminant feeding following exposure to elevated temperature, has been suggested to be a potential behavioral sign of metabolic stress (Schram et al. 2015a). Alternately, following exposure to elevated CO_2, *E. superba* displayed elevated metabolic rates which may also be indicative of metabolic stress (Saba et al. 2012). In the present study, despite increased consumption rates upon exposure to reduced pH, *G. antarctica* displayed a declining trend in mean wet body mass. Poore et al. (2013) suggested that decreased amphipod survival and growth, as observed with the temperate amphipod *P. parmerong*, may induce potential direct and indirect effects of climate stressors on the strength of alga–herbivore interactions. Potentially reinforcing a shift towards smaller body sizes and their relation to trophic interactions, a recent meta-analysis concluded that despite elevated temperature increasing consumption rates in temperate and tropical species due to higher metabolic rates, this higher consumption rate did not directly translate to increased secondary production by invertebrates (Nagelkerken & Connell 2015).

Larger body size, like that associated with the polar gigantism of many Antarctic amphipod species (Chapelle & Peck 1999), may make it more difficult for some of the larger species because thermal extremes tend to influence large before small individuals, which tend to have wider thermal tolerance windows (Pörtner 2008). If elevated CO_2 exacerbates the effects of elevated temperature, the thermal windows for larger individuals could be further narrowed, leading to a shift towards smaller body sizes (Pörtner 2008) like those observed in the present study. However, it is important to note that the change in wet body mass should be interpreted with care due to relatively high degree of error associated with the group wet weight measurements resulting from the uneven amphipod group numbers at the end of the experimental period and high degree of variation within treatments reported here. Despite the net decrease in mean wet mass recorded in the present study, amphipods maintained consistent activity levels throughout the experimental period, suggesting that changes in mass were not solely the result of amphipods being unduly physiologically stressed or generally unhealthy (authors' pers. obs). However, to more fully understand and tease apart potential metabolic stress in Antarctic benthic amphipods, measures of respiration and excretion are needed.

Similar to *E. superba* (Saba et al. 2012), we did not observe a pH effect on whole body protein content in *G. antarctica* at reduced pH, but there appears to be some evidence of a trend of a pH–temperature interactive effect on lipid content. This potential synergistic interplay between temperature and pH may have contributed to the elevated percent lipid content of whole body tissues in individuals held in the reduced pH–elevated temperature treatment. Overall, our

findings suggest that *G. antarctica* is losing energy through a pathway not measured in the present study. However it is important to note that due to mortality and resultant small amount of dry tissue available, we had no replication for lipid content analysis for *G. antarctica* maintained in the reduced pH–elevated temperature treatment, making it impossible to compare statistically.

A significant pH–temperature interactive effect on consumption rates in *P. fissicauda* made it more difficult to tease temperature and pH effects apart. Individuals maintained in all treatments (except that with reduced pH–elevated temperature) experienced a decline in mean body mass. The surviving amphipods in the reduced pH–elevated temperature treatment exhibited higher mean body mass at the end of the experiment as well as the highest consumption rates. The high mortality rates in this treatment may have culled the individuals most sensitive to changes in pH and temperature, resulting in a cohort more fit to survive environmental conditions predicted for future oceans. The relatively high degree of variability in measured responses suggests that a subset of amphipods may be able to acclimate to predicted near-future conditions. If the surviving sub-populations maintain their fitness, this could alleviate some of the negative consequences of ocean acidification and warming at the ecosystem level (reviewed by Harvey et al. 2014). In an ecological context, it is important to note that both amphipods experienced relatively high mortality over a relatively brief 3 mo period of chronic exposure to reduced seawater pH (7.6). This pH level is predicted to occur in the Southern Ocean as early as the end of the century (IPCC 2014).

Both amphipod species in the present study occupy similar macroalgal habitats (overlapping niches), however the smaller *G. antarctica* is commonly found actively swimming among the thalli of benthic macroalgae while the larger *P. fissicauda* is considerably less active (authors' pers. obs.). We observed a significant temperature effect on protein content of whole body tissues of *P. fissicauda* with the highest mean percent protein found in individuals held in the ambient temperature and pH treatments. Behavioral activity levels, and therefore metabolic and energetic demands, may differ between these 2 amphipod species and may explain some of the observed species-level differences. Overall, reductions in mesograzer abundances in coming decades may result in shifts in the structure of macroalgal–mesograzer assemblages along the WAP. A recent mesocosm study on the responses of Antarctic crustacean grazer assemblages demonstrated that grazer assemblages exhibit high resistance to initial exposure to decreased pH due to response diversity within functional groups (Schram et al. 2016a). One important outcome of functional shifts in mesograzer assemblages along the WAP could be unchecked fouling on the host macrophyte by diatoms and filamentous epiphytes, which are ultimately detrimental to many aspects of the hosts' fitness (Aumack et al. 2011b, also see Reynolds et al. 2014).

Changes in temperature tend to influence larger individuals more than smaller individuals due to their wider thermal tolerance windows (Pörtner 2008). Additionally, previous studies have demonstrated that exposure tolerance to seawater warming declines when combined with reduced pH in crustaceans as well as in several species of coral, echinoderms, and fish, suggesting that narrowed thermal tolerance may be a potential unifying principle across animal taxa (Wittmann & Pörtner 2013). In the present study, we observed a high incidence of mortality coincident with molting in both *G. antarctica* and *P. fissicauda* exposed to seawater acidification. Consistent with the predictions of the metabolic theory of ecology, the generally greater size and lower activity levels of *P. fissicauda* may have made this species more susceptible to reduced seawater pH and elevated temperature because of a potentially greater reduction in their thermal tolerance window compared to the smaller and more active *G. antarctica.* Decreased survival in common amphipods that occupy contrasting niches as grazers in macroalgal-grazer assemblages has the potential to disrupt the effectiveness of grazers in community responses to environmental change, directly and indirectly (Poore et al. 2013). Moreover, shifts in feeding preferences could alter the functional role of these common mobile mesograzers along the WAP.

Acknowledgements. The authors gratefully acknowledge the outstanding science and logistical support staff of Antarctic Support Contract for their invaluable support. Margaret Amsler and Kevin Scriber of the Department of Biology provided valuable field assistance. We appreciate the invaluable statistics consultation provided by Charles Katholi. Thanks are due to Kenan Matterson for his assistance with the protein analyses and Robert Thacker for use of his laboratory equipment. The present study was directly supported by NSF award ANT-1041022 (J.B.M., C.D.A., R.A.A.) from the Antarctic Organisms and Ecosystems program. J.B.M. also acknowledges partial support from NSF Award ANT-1141896, a collaborative grant award with R. B. Aronson (ANT-1141877). The UAB Department of Biology and an Endowed Professorship in Polar and Marine Biology provided additional support to J.B.M.

LITERATURE CITED

Amsler CD, Iken K, McClintock JB, Amsler MO and others (2005) Comprehensive evaluation of the palatability and chemical defenses of subtidal macroalgae from the Antarctic Peninsula. Mar Ecol Prog Ser 294:141–159

Amsler CD, McClintock JB, Baker BJ (2008) Macroalgal chemical defenses in polar marine communities. In: Amsler CD (ed) Algal chemical ecology. Springer, Berlin, p 91–103

Amsler CD, McClintock JB, Baker BJ (2014) Chemical mediation of mutualistic interactions between macroalgae and mesograzers structure unique coastal communities along the western Antarctic Peninsula. J Phycol 50:1–10

Amsler MO, Amsler CD, von Salm JL, Aumack CF, McClintock JB, Young RM, Baker BJ (2013) Tolerance and sequestration of macroalgal chemical defenses by an Antarctic amphipod: a 'cheater' among mutualists. Mar Ecol Prog Ser 490:79–90

Aronson RB, Smith KE, Vos SC, McClintock JB and others (2015) No barrier to emergence of bathyal king crabs on the Antarctic shelf. Proc Natl Acad Sci USA 112: 12997–13002

Aumack CF, Amsler CD, McClintock JB, Baker BJ (2011a) Changes in amphipod densities among macroalgal habitats in day versus night collections along the western Antarctic Peninsula. Mar Biol 158:1879–1885

Aumack CF, Amsler CD, McClintock JB, Baker BJ (2011b) Impacts of mesograzers on epiphyte and endophyte growth associated with chemically defended macroalgae from the western Antarctic Peninsula: a mesocosm experiment. J Phycol 47:36–41

Bednaršek N, Tarling G, Bakker D, Fielding S and others (2012) Extensive dissolution of live pteropods in the Southern Ocean. Nat Geosci 5:881–885

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Brown JH, Gillooly JF, Allen AP, Savage VM, West GB (2004) Toward a metabolic theory of ecology. Ecology 85: 1771–1789

Caldeira K, Wickett ME (2003) Anthropogenic carbon and ocean pH. Nature 425:365

Calosi P, Turner L, Hawkins M, Bertolini C, Nightingale G, Truebano M, Spicer J (2013) Multiple physiological responses to multiple environmental challenges: an individual approach. Integr Comp Biol 53:660–670

Chapelle G, Peck LS (1999) Polar gigantism dictated by oxygen availability. Nature 399:114–115

Collard M, De Ridder C, David B, Dehairs F, Dubois P (2015) Could the acid–base status of Antarctic sea urchins indicate a better-than-expected resilience to near-future ocean acidification? Glob Change Biol 21:605–617

Constable AJ, Melbourne-Thomas J, Corney SP, Arrigo KR and others (2014) Climate change and Southern Ocean ecosystems I: how changes in physical habitats directly affect marine biota. Glob Change Biol 20:3004–3025

Crame A (2014) Evolutionary setting. In: De Broyer C, Koubbi P, Griffiths HJ, Raymond B and others (eds) Biogeographic atlas of the Southern Ocean. Scientific Committee on Antarctic Research, Cambridge, p 155–166

Cummings V, Hewitt J, Van Rooyen A, Currie K and others (2011) Ocean acidification at high latitudes: potential effects on functioning of the Antarctic bivalve *Laternula elliptica*. PLoS ONE 6:e16069

de la Haye KL, Spicer JI, Widdicombe S, Briffa M (2011) Reduced sea water pH disrupts resource assessment and decision making in the hermit crab *Pagurus bernhardus*. Anim Behav 82:495–501

Dickson AG (1990) Standard potential of the reaction: $AgCl(s) + \frac{1}{2}H_2(g) = Ag(s) + HCl(aq)$, and the standard acidity constant of the ion HSO_4^- in synthetic sea water from 273.15 to 318.15 K. J Chem Thermodyn 22:113–127

Dickson AG, Sabine CL, Christian JR (2007) (eds) Guide to best practices for ocean CO_2 measurements. PICES Spec Publ 3

Dissanayake A (2014) Ocean acidification and warming effects on Crustacea: possible future scenarios. In: Goffredo S, Dubinsky Z (eds) The Mediterranean Sea: its history and present challenges. Springer, Dordrecht, p 363–372

Dissanayake A, Clough R, Spicer J, Jones M (2010) Effects of hypercapnia on acid-base balance and osmo-/iono-regulation in prawns (Decapoda: Palaemonidae). Aquat Biol 11:27–36

Dodd LF, Grabowski JH, Piehler MF, Westfield I, Ries JB (2015) Ocean acidification impairs crab foraging behaviour. Proc R Soc B 282:20150333

Doyle SR, Momo FR, Brêthes JC, Ferreyra GA (2012) Metabolic rate and food availability of the Antarctic amphipod *Gondogeneia antarctica* (Chevreux 1906): seasonal variation in allometric scaling and temperature dependence. Polar Biol 35:413–424

Ducklow HW, Fraser WR, Meredith MP, Stammerjohn SE and others (2013) West Antarctic Peninsula: an ice-dependent coastal marine ecosystem in transition. Oceanography (Wash DC) 26:190–203

Egilsdottir H, Spicer JI, Rundle SD (2009) The effect of CO_2 acidified sea water and reduced salinity on aspects of the embryonic development of the amphipod *Echinogammarus marinus* (Leach). Mar Pollut Bull 58:1187–1191

Enzor LA, Zippay ML, Place SP (2013) High latitude fish in a high CO_2 world: synergistic effects of elevated temperature and carbon dioxide on the metabolic rates of Antarctic notothenioids. Comp Biochem Physiol A Mol Integr Physiol 164:154–161

Ericson JA, Lamare MD, Morley SA, Barker MF (2010) The response of two ecologically important Antarctic invertebrates (*Sterechinus neumayeri* and *Parborlasia corrugatus*) to reduced seawater pH: effects on fertilisation and embryonic development. Mar Biol 157:2689–2702

Fabry VJ, McClintock JB, Mathis JT, Grebmeier JM (2009) Ocean acidification at high latitudes: the bellwether. Oceanography (Wash DC) 22:160–171

Folch J, Lees M, Stanley GHS (1957) A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem 226:497–509

Frederich M, Sartoris FJ, Arntz WE, Portner H (2000) Haemolymph Mg^{2+} regulation in decapod crustaceans: physiological correlates and ecological consequences in polar areas. J Exp Biol 203:1383–1393

Frederich M, Sartoris F, Pörtner HO (2001) Distribution patterns of decapod crustaceans in polar areas: A result of magnesium regulation? Polar Biol 24:719–723

Gattuso JP, Magnan A, Billé R, Cheung W and others (2015) Contrasting futures for ocean and society from different anthropogenic CO_2 emissions scenarios. Science 349: aac4722

Gillooly JF, Brown JH, West GB, Savage VM, Charnov EL (2001) Effects of size and temperature on metabolic rate. Science 293:2248–2251

Gomes V, Passos MJACR, Rocha AJS, Santos TCA, Machado ASD, Ngan PV (2013) Metabolic rates of the Antarctic amphipod *Gondogeneia antarctica* at different temperatures and salinities. Braz J Oceanogr 61:243–249

Harvey BP, Al-Janabi B, Broszeit S, Cioffi R and others (2014) Evolution of marine organisms under climate change at different levels of biological organisation. Water 6:3545–3574

Hauton C, Tyrrell T, Williams J (2009) The subtle effects of sea water acidification on the amphipod *Gammarus locusta.* Biogeosciences 6:1479–1489

Huang YM, Amsler MO, McClintock JB, Amsler CD, Baker BJ (2007) Patterns of gammaridean amphipod abundance and species composition associated with dominant subtidal macroalgae from the western Antarctic Peninsula. Polar Biol 30:1417–1430

Hughes AR, Bando KJ, Rodriguez LF, Williams SL (2004) Relative effects of grazers and nutrients on seagrasses: a meta-analysis approach. Mar Ecol Prog Ser 282:87–99

IPCC (2014) Climate change 2013: impacts, adaptation, and vulnerability. Part A: global and sectoral aspects. In: Field CB, Barros VR, Dokken DJ, Mach KJ and others (eds) Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge

Johnson DE, Graybill FA (1972) An analysis of a two-way model with interaction and no replication. J Am Stat Assoc 67:862–868

Kaiser S, Brandão S, Brix S, Barnes DA and others (2013) Patterns, processes and vulnerability of Southern Ocean benthos: a decadal leap in knowledge and understanding. Mar Biol 160:2295–2317

Kapsenberg L, Hofmann GE (2014) Signals of resilience to ocean change: high thermal tolerance of early stage Antarctic sea urchins (*Sterechinus neumayeri*) reared under present-day and future pCO_2 and temperature. Polar Biol 37:967–980

Kawaguchi S, Kurihara H, King R, Hale L and others (2011) Will krill fare well under Southern Ocean acidification? Biol Lett 7:288–291

Kawaguchi S, Ishida A, King R, Raymond B and others (2013) Risk maps for Antarctic krill under projected Southern Ocean acidification. Nat Clim Change 3:843–847

McDonald MR, McClintock JB, Amsler CD, Rittschof D, Angus RA, Orihuela B, Lutostanski K (2009) Effects of ocean acidification over the life history of the barnacle *Amphibalanus amphitrite*. Mar Ecol Prog Ser 385: 179–187

McNeil BI, Matear RJ (2008) Southern Ocean acidification: a tipping point at 450-ppm atmospheric CO_2. Proc Natl Acad Sci USA 105:18860–18864

Melzner F, Gutowska M, Langenbuch M, Dupont S and others (2009) Physiological basis for high CO_2 tolerance in marine ectothermic animals: Pre-adaptation through lifestyle and ontogeny? Biogeosciences 6:2313–2331

Nagelkerken I, Connell SD (2015) Global alteration of ocean ecosystem functioning due to increasing human CO_2 emissions. Proc Natl Acad Sci USA 112:13272–13277

Poore AGB, Graba-Landry A, Favret M, Brennand HS, Byrne M, Dworjanyn SA (2013) Direct and indirect effects of ocean acidification and warming on a marine plant–herbivore interaction. Oecologia 173:1113–1124

Pörtner HO (2008) Ecosystem effects of ocean acidification in times of ocean warming: a physiologist's view. Mar Ecol Prog Ser 373:203–217

Reynolds PL, Richardson JP, Duffy JE (2014) Field experimental evidence that grazers mediate transition between microalgal and seagrass dominance. Limnol Oceanogr 59:1053–1064

Ricevuto E, Lorenti M, Patti FP, Scipione MB, Gambi MC (2012) Temporal trends of benthic invertebrate settlement along a gradient of ocean acidification at natural CO_2 vents (Tyrrhenian sea). Biol Mar Mediterr 19:49–52

Robbins LL, Hansen ME, Kleypas JA, Meylan SC (2010) CO2calc: a user-friendly seawater carbon calculator for Windows, Max OS X, and iOS (iPhone). US Geological Survey Open-File Report 2010-1280. US Department of the Interior, Washington, DC

Roy RN, Roy LN, Vogel KM, Porter-Moore C and others (1993) The dissociation constants of carbonic acid in seawater at salinities 5 to 45 and temperatures 0 to 45°C. Mar Chem 44:249–267

Saba GK, Schofield O, Torres JJ, Ombres EH, Steinberg DK (2012) Increased feeding and nutrient excretion of adult Antarctic krill, *Euphausia superba*, exposed to enhanced carbon dioxide (CO_2). PLoS ONE 7:e52224

Schoenrock KM, Schram JB, Amsler CD, McClintock JB, Angus RA (2015) Climate change impacts on overstory *Desmarestia* spp. from the western Antarctic Peninsula. Mar Biol 162:377–389

Schoenrock KM, Schram JB, Amsler CD, McClintock JB, Angus RA (2016) Climate change confers a potential advantage to fleshy Antarctic crustose macroalgae over calcified species. J Exp Mar Biol Ecol 474:58–66

Schram JB, Schoenrock KM, McClintock JB, Amsler CD, Angus RA (2014) Multiple stressor effects of near-future elevated seawater temperature and decreased pH on righting and escape behaviors of two common Antarctic gastropods. J Exp Mar Biol Ecol 457:90–96

Schram JB, McClintock JB, Amsler CD, Baker BJ (2015a) Impacts of acute elevated seawater temperature on the feeding preferences of an Antarctic amphipod toward chemically deterrent macroalgae. Mar Biol 162: 425–433

Schram JB, Schoenrock KM, McClintock JB, Amsler CD, Angus RA (2015b) Multi-frequency observations of seawater carbonate chemistry on the central coast of the western Antarctic Peninsula. Polar Res 34:25582

Schram JB, Amsler MO, Amsler CD, Schoenrock KM, McClintock JB, Angus RA (2016a) Antarctic crustacean grazer assemblages exhibit resistance following exposure to decreased pH. Mar Biol 163:106

Schram JB, Schoenrock KM, McClintock JB, Amsler CD, Angus RA (2016b) Testing Antarctic resilience: the effects of elevated seawater temperature and decreased pH on two gastropod species. ICES J Mar Sci 73:739–752

Seibel BA, Maas AE, Dierssen HM (2012) Energetic plasticity underlies a variable response to ocean acidification in the pteropod, *Limacina helicina antarctica*. PLoS ONE 7:e30464

Sewell MA, Millar RB, Yu PC, Kapsenberg L, Hofmann GE (2014) Ocean acidification and fertilization in the Antarctic sea urchin *Sterechinus neumayeri*: the importance of polyspermy. Environ Sci Technol 48:713–722

Small D, Calosi P, White D, Spicer JI, Widdicombe S (2010) Impact of medium-term exposure to CO_2 enriched seawater on the physiological functions of the velvet swimming crab *Necora puber.* Aquat Biol 10:11–21

Spicer J, Raffo A, Widdicombe S (2007) Influence of CO_2-related seawater acidification on extracellular acid–base

balance in the velvet swimming crab *Necora puber*. Mar Biol 151:1117–1125

Stillman JH, Paganini AW (2015) Biochemical adaptation to ocean acidification. J Exp Biol 218:1946–1955

Suckling C, Clark M, Peck L, Cook E (2014) Experimental influence of pH on the early life-stages of sea urchins I: Different rates of introduction give rise to different responses. Invertebr Reprod Dev 58:148–159

Suckling CC, Clark MS, Richard J, Morley SA, Thorne MA, Harper EM, Peck LS (2015) Adult acclimation to combined temperature and pH stressors significantly enhances reproductive outcomes compared to short-term exposures. J Anim Ecol 84:773–784

Tortell PD, Asher EC, Ducklow HW, Goldman JA and others (2014) Metabolic balance of coastal Antarctic waters revealed by autonomous pCO_2 and ΔO_2/Ar measurements. Geophys Res Lett 41:6803–6810

Tukey JW (1949) One degree of freedom for non-additivity. Biometrics 5:232–242

Turner J, Maksym T, Phillips T, Marshall GJ, Meredith MP (2013) The impact of changes in sea ice advance on the large winter warming on the western Antarctic Peninsula. Int J Climatol 33:852–861

Turner J, Barrand NE, Bracegirdle TJ, Convey P and others (2014) Antarctic climate change and the environment: an update. Polar Rec (Gr Brit) 50:237–259

van Montfrans J, Wetzel RL, Orth RJ (1984) Epiphyte-grazer relationships in seagrass meadows: consequences for seagrass growth and production. Estuaries 7:289–309

Walther K, Sartoris FJ, Bock C, Pörtner HO (2009) Impact of anthropogenic ocean acidification on thermal tolerance of the spider crab *Hyas araneus*. Biogeosciences 6:2207–2215

Whiteley NM (2011) Physiological and ecological responses of crustaceans to ocean acidification. Mar Ecol Prog Ser 430:257–271

Widdicombe S, Spicer JI (2008) Predicting the impact of ocean acidification on benthic biodiversity: What can animal physiology tell us? J Exp Mar Biol Ecol 366:187–197

Wiencke C, Amsler CD (2012) Seaweeds and their communities in polar regions. In: Wiencke C, Bischof K (eds) Seaweed biology: novel insights into ecophysiology, ecology and utilization. Ecological studies, Vol 219. Springer-Verlag, Berlin, p 265–291

Wittmann AC, Pörtner HO (2013) Sensitivities of extant animal taxa to ocean acidification. Nat Clim Change 3: 995–1001

Zamzow JP, Amsler CD, McClintock JB, Baker BJ (2010) Habitat choice and predator avoidance by Antarctic amphipods: the roles of algal chemistry and morphology. Mar Ecol Prog Ser 400:155–163

Editorial responsibility: Edward Durbin, Narragansett, Rhode Island, USA

Submitted: November 23, 2015; Accepted: June 22, 2016
Proofs received from author(s): July 18, 2016