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Signals of resilience to ocean change: high thermal tolerance of early stage Antarctic sea urchins (*Sterechinus neumayeri*) reared under present-day and future pCO₂ and temperature

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Abstract We tested the hypothesis that development of the Antarctic urchin *Sterechinus neumayeri* under future ocean conditions of warming and acidification would incur physiological costs, reducing the tolerance of a secondary stressor. The aim of this study is twofold: (1) quantify current austral spring temperature and pH near sea urchin habitat at Cape Evans in McMurdo Sound, Antarctica and (2) spawn *S. neumayeri* in the laboratory and raise early developmental stages (EDSs) under ambient (-0.7 °C; 400 µatm pCO₂) and future ($+2.6$ °C; 650 and 1,000 µatm pCO₂) ocean conditions and expose four EDSs (blastula, gastrula, prism, and 4-arm echinopluteus) to a one hour acute heat stress and assess survivorship. Results of field data from 2011 to 2012 show extremely stable inter-annual pH conditions ranging from 7.99 to 8.08, suggesting that future ocean acidification will drastically alter the pH-seascape for *S. neumayeri*. In the laboratory, *S. neumayeri* EDSs appear to be tolerant of temperatures and pCO₂ levels above their current habitat conditions. EDSs survived acute heat exposures >20 °C above habitat temperatures of -1.9 °C. No pCO₂ effect was observed for EDSs reared at -0.7 °C. When reared at $+2.6$ °C, small but significant pCO₂ effects were observed at the blastula and prism stage, suggesting that multiple stressors are more detrimental than single stressors. While surprisingly tolerant overall, blastulae were the most sensitive stage to ocean warming and acidification. We conclude that *S. neumayeri* may be unexpectedly physiologically tolerant of future ocean conditions.

Introduction

Due to properties of persistent cold seawater, high-latitude marine ecosystems are ranked to be among the most sensitive regions to global change (Orr et al. 2005; Peck 2005; McClintock et al. 2008; Fabry et al. 2009; Turley et al. 2010). The Southern Ocean is expected to significantly change with respect to multiple factors within decades, due to anthropogenic release of carbon dioxide (CO₂) to the atmosphere (Orr et al. 2005; McNeil and Matear 2008). Specifically, unprecedented rates of ocean warming and acidification are predicted (IPCC 2007; Stammerjohn et al. 2008; Hönisch et al. 2012; Marcott et al. 2013). The fingerprints of this change are already being documented in Antarctic marine ecosystems (McClintock et al. 2008; Montes-Hugo et al. 2009; Bednaršek et al. 2012; Naveen et al. 2012; Steinberg et al. 2012). Due to the rate of environmental change and narrow adaptive capacity of Antarctic marine organisms, future shifts in the structure and function of Antarctic ecosystems are expected to be significant (Peck 2005; Fabry et al. 2009). A critical research priority is to assess the adaptive capacity of Antarctic organisms to future complex changes, which are often not considered together (Boyd 2011). In this light, the aim of this study was to assess the physiological cost of development of a circum-Antarctic marine invertebrate, the sea urchin *Sterechinus neumayeri*, under future multi-stressor scenarios.

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Assessing the adaptive capacity (sensu Dawson et al. 2011) of Antarctic organisms is a critical research priority as the rate of change of waters surrounding Antarctica is expected to be rapid. As atmospheric CO_2 dissolves into the surface layers of the ocean, due to differential partial pressures in the atmosphere and seawater, ocean pCO_2 increases and pH decreases. Atmospheric pCO_2 exceeded 400 ppm in May 2013 (http://www.esrl.noaa.gov/gmd/ccgg/trends/); an increase from 280 ppm pre-industrial time (Feely 2004). The Southern Ocean is the fastest acidifying ocean on the planet with an annual pCO_2 increase of 2.13 ± 0.64 µatm $year^{-1}$ since the 1980s (Takahashi et al. 2009). Atmospheric CO_2 is predicted to reach 1,000 ppm by 2100 resulting in a decrease of 0.4 ocean pH (IPCC 2007). However, biological ramifications in the Southern Ocean may become apparent earlier with seasonal aragonite undersaturation predicted to occur when atmospheric CO_2 levels reach 450 ppm, as early as 2030 (McNeil and Matear 2008). The Southern Ocean has warmed at twice the rate of global warming: 0.17 °C since the 1950s (Fyfe 2006; Gille 2002), and sea surface temperature is predicted to rise by an additional 2.6 °C in the next 90 years (IPCC 2007).

The 'double whammy' of ocean acidification and ocean warming will create unprecedented marine environments and is expected to severely impact functional traits and physiological processes of polar species (Fabry et al. 2009; Sewell and Hofmann 2011). In general, species and populations may exhibit three types of responses to environmental change: (1) migrate with the changing climate envelope (Parmesan and Yohe 2003), (2) genetically adapt (Hoffmann and Sgrò 2011), and (3) acclimatize using current physiological plasticity (Gienapp et al. 2008; Visser 2008; Hoffmann and Sgrò 2011).

Migration to colder water, the poleward shifts observed at lower latitudes, is not an option for polar organisms. Here, changes in bathymetric distribution are also not viable as there is no thermocline that might preserve colder water at depth. Genetic adaptation may also be limited. Due to cold adaptation and thermal stability of the Southern Ocean over evolutionary timescales, some Antarctic species have lost the genetic capacity to deal with environmental change (Pörtner et al. 2012; Somero 2012). For example, the Antarctic notothenioid fish *Trematomus bernacchii* has lost an inducible heat shock response as a result of evolutionary adaptation to cold temperatures, whereas temperate notothenioids have retained this trait (Place et al. 2004; Hofmann et al. 2000, 2005; Clark and Peck 2009). Such evolutionary adaptations may result in reduced capacities to withstand future multi-stressor environments (Enzor et al. 2013). Additionally, slow growth and long generation times of Antarctic invertebrate species reduce the potential for rapid genetic adaptation that may be required to flourish in a future ocean (Peck 2005; Pörtner

et al. 2007). Studies on marine invertebrates in general suggest that they are already operating at the limits of their thermal tolerance (Sunday et al. 2012), and so further acclimatization to warming may be limited, especially in polar species (Peck et al. 2009). Thus, success of Antarctic marine species in a future ocean likely depends on current physiological plasticity and acclimatization capacity of functional traits. The importance of present-day tolerances and functional traits has been emphasized in other ecosystems when predicting climate change responses (Buckley and Kingsolver 2012; Chown 2012).

To investigate the breadth of current plasticity in a polar marine invertebrate with respect to seawater temperature and pH, we studied the early developmental stages (EDSs) of the Antarctic sea urchin *S. neumayeri*. *S. neumayeri* is an ideal study organism for ocean change experimental biology. With an extensive biogeographic range, it is the most abundant echinoid on the shallow Antarctic benthos and a critical member of the circum-Antarctic near-shore marine ecosystem (Bosch et al. 1987). Adults can easily be spawned in the laboratory, and larvae can readily be cultured (Bosch et al. 1987). In general, EDSs are predicted to be among the most sensitive stages to environmental change (Kurihara 2008; Dupont and Thorndyke 2009; Byrne 2011), especially with respect to synergistic stressors (Harvey et al. 2013). Due to a long pelagic larval duration of up to 115 days (Bosch et al. 1987), *S. neumayeri* spend a significant amount of time under direct influence of seawater conditions during its potentially most sensitive life history stage. Additionally, the slow development of *S. neumayeri* provides a unique opportunity to assess the physiological plasticity in response to multistressors at different EDSs. Previous studies of *S. neumayeri* EDSs have shown reduced growth (Clark et al. 2009; Byrne et al. 2013; Yu et al. 2013) and enhanced larval asymmetry (Byrne et al. 2013) under elevated pCO_2 conditions, suggesting that there is in fact a physiological cost of development under these conditions.

The goal of this study was to assess, in general, the sensitivity of *S. neumayeri* EDSs (from present-day spawning) to future abiotic conditions expected for the Southern Ocean. In order to assess its current physiological plasticity, we asked whether early development under elevated temperature and pCO_2 came at a cost of other physiological tolerances, in this case the tolerance of an acute heat stress. As an example, temperate sea urchin and abalone larvae reared under elevated pCO_2 showed reduced heat stress response following a one hour (1 h) acute heat exposure (O'Donnell et al. 2008; Zippay and Hofmann 2010a). This suggests that there are trade-offs associated with development at high pCO_2 where energy is diverted away from the cellular stress response, potentially leaving the organism ill-equipped to deal with secondary stressors.

Use of acute heat stress tests and laboratory-determined thermal tolerance limits can provide valuable insight into species' physiological trade-offs and energy allocations, current physiological plasticity and acclimation capacities (Tomanek 2010; Terblanche et al. 2011) and ultimately, resilience in the face of climate change (Chown and Gaston 2008; Barnes et al. 2010; Buckley and Kingsolver 2012; Chown 2012).

During austral spring when natural spawning occurs, we raised larvae in the laboratory at McMurdo Station, Antarctica, under six multi-stressor scenarios (two temperatures and three pCO_2 levels) and exposed four developmental stages to a 1 h heat stress test and assessed survivorship. We hypothesized that elevated pCO_2 and high temperature incur a physiological cost to *S. neumayeri* EDSs and predicted that embryos and larvae will have reduced survivorship following a 1 h temperature challenge compared to larvae reared under ambient conditions. In order to place the laboratory findings in an environmentally relevant context, we also measured field pH and temperature in the water column above a sea urchin population for the duration of the larval culturing. Here, we hope to address two questions: (1) what is the natural variability in the local seawater with respect to pH and (2) does development under future conditions of ocean acidification and warming alter the acute heat tolerance of *S. neumayeri* EDSs?

Materials and methods

Field pH measurements

In order to parameterize the CO_2 manipulation experiments of larval cultures, we measured pH near a *S. neumayeri* population using a SeaFET sensor with a Honeywell Durafet® pH electrode (Martz et al. 2010). SeaFET pH sensors were deployed above the benthos at 18 m depth on a 27 m benthic mooring at Cape Evans, Ross Island, Antarctica, at the sea urchin collection site (S 77°38.060′, E 166°24.918′) from late October through November, both years during 24 h daylight. In 2011, a temperature sensor was also deployed (Sea Bird Electronics SBE-37 SM, Matson 2012).

Raw voltage recorded by the SeaFET was converted to pH_{total} using one discrete seawater sample per sensor deployment. Single discrete calibration samples were collected via SCUBA following standard operating procedures (SOP) 1 and processed for spectrophotometric pH analysis (total hydrogen ion concentration pH scale) at 25 °C following SOP 6b (Dickson et al. 2007). Salinity was measured using a YSI 3100 Conductivity Instrument. Total alkalinity was determined according to SOP 3b, using an open-cell titrator (Mettler-Toledo T50) after measuring certified reference materials (CRMs) seawater standards from Dr. Andrew M. Dickson at Scripps Institution of Oceanography to within 10 μmol kg^{-1} accuracy (Dickson et al. 2007). In situ pH_{total} of the calibration sample was calculated in CO2Calc (Robbins et al. 2010) using in situ temperature recorded by the SeaFET at the time of sample collection and CO_2 constants from Mehrbach et al. (1973) refit by Dickson and Millero (1987).

Accuracy of SeaFET data depends on the quality of the calibration sample and is estimated to be ∼0.01 pH as compared to SOP 6b processing of CRMs (data not shown, Matson et al. 2011). pH was recorded every hour (2011) or 30 min (2012) and reported as hourly data after applying a 1 h low-pass filter.

Animal collection and larval cultures

In order to raise sea urchin EDSs under multi-stressor scenarios of temperatures and pCO_2 conditions, cultures were reared at −0.7 °C in 2011 and +2.6 °C in 2012 (hereinafter referred to as 'cold' and 'warm' cultures) at three pCO_2 levels (400, 650, and 1,000 μatm) as described in Yu et al. (2013). The warm culture temperature was chosen to be an extreme future scenario. Adult *S. neumayeri* were collected from the benthos by SCUBA at Cape Evans, McMurdo Sound, Antarctica (S 77°38.060′, E 166°24.918′), in October 2011 and 2012 during the spawning season (Pearse and Giese 1966; Brey et al. 1995; Stanwell-Smith and Peck 1998). Depth of the collection site was approximately 20 m. Adults were transported in coolers to McMurdo Station and maintained in ∼−1 °C flow-through seawater tables for approximately two weeks until spawning.

Adult *S. neumayeri* were induced to spawn by injection of ∼1 ml ice-cold 0.55 M KCl. Females were inverted over a beaker containing filtered seawater (FSW) on ice, to collect eggs; sperm was collected dry directly from the gonopores. Sperm and eggs were kept on wet ice until use. After successful test fertilization assays, eggs from 20 (15) females were pooled and fertilized using diluted sperm from one male to >90 % fertilization success for the cold (warm) culture. Embryos were split between culture buckets prefilled with control and two CO_2-acidified seawater treatments as described in Yu et al. (2013). Culture vessels were stocked at ∼160,000 embryos/12 l.

Experimental seawater acidification

For experimental sea urchin cultures, seawater was acidified with CO_2 gas. Control and CO_2-acidification of 0.32 μm-FSW followed methods modified from Fangue et al. (2010) and as described in Yu et al. (2013), with the exception that seawater tubing was not insulated. Briefly,

Table 1 Acute temperature exposure (1 h) of early developmental stages of *Sterechinus neumayeri* reared in two thermal environments: cold (−0.7 °C) and warm (+2.6 °C)

Culture	Stage	Temperature (°C) of 1 h exposure									Absolute change in vial temperature (°C)[a]
Cold	*Blastula*	−0.5	2.2	4.7	7.4	10.2	13.2	15.5	17.9	20.6	0.2 ± 0.1
	Gastrula	−0.4	2.3	5.0	7.5	10.2	13.0	15.3	17.8	20.3	0.2 ± 0.2
	Prism	−0.8	4.9	10.3	13.8	16.0	17.8	20.1	22.6	25.4	0.3 ± 0.3
	Pluteus	−0.6	5.3	10.9	14.4	16.4	18.4	20.6	22.8	25.2	0.1 ± 0.1
Warm	*Blastula*	2.6	7.1	10.0	13.0	14.8	17.7	19.6	22.7	25.5	0.4 ± 0.3
	Gastrula	2.8	7.3	10.2	13.1	15.0	17.9	20.2	22.5	24.7	0.2 ± 0.2
	Prism	2.8	7.3	10.2	13.2	15.2	18.1	20.0	22.0	24.2	0.5 ± 0.4
	Pluteus	3.0	7.3	10.2	13.2	14.9	17.8	19.9	22.0	24.4	0.2 ± 0.2

Maximum observed change in vial temperature during the 1 h incubation was 1.1 and 1.5 °C in cold and warm culture assays, respectively

[a] Values are given as mean ± SD, sample size is 54

pure CO_2 gas was mixed with dry air to desired levels and subsequently dissolved into FSW in three reservoir buckets by a venturi injector. We aimed for a control pCO_2 level of 400 μatm and treatment pCO_2 levels of 650 and 1,000 μatm in five replicate culture vessels for a total of 15 culture vessels. Reservoir and culture vessels were thermally controlled in flow-through seawater tables with ambient seawater (cold culture) and temperature-control immersion heaters (Process Technology) (warm culture).

Reservoir buckets were sampled daily for temperature, salinity, total alkalinity and pH according to methods listed under 'field pH measurements' for discrete seawater samples, with the exception that temperature was measured with a wire probe (Fluke 52 K/J Thermometer). Culture vessels were sampled daily for temperature and pH. In situ pH_{total} and carbonate parameters were calculated in CO2Calc (Robbins et al. 2010), using batch processing and CO_2 constants from Mehrbach et al. (1973) refit by Dickson and Millero (1987).

Survivorship assays

Acute thermal tolerance of *S. neumayeri* was tested at four stages of sea urchin early development—hatched blastula, mid-gastrula, prism, and 4-arm echinopluteus (hereinafter 'pluteus')—using a survivorship assay modified from Hammond and Hofmann (2010). A thermal gradient was established by cooling and warming two ends of an aluminum block containing 60 holes fabricated to fit 20 ml scintillation vials. Thermal tolerance was tested at nine temperatures, with two technical replicates per acute heat stress temperature and pCO_2 group for each stage (Table 1). Unlike ecologically relevant culture temperatures of −0.7 and +2.6 °C, acute heat stress temperatures (up to 25 °C) were chosen to study the physiology of EDSs and do not represent temperatures likely to be encountered

in the field. For optimum temperature range, two assays were required for each development stage. As a matter of protocol for all CO_2 treatments, the cooler assay was always run before the warmer assay. All assays were conducted in the Crary Laboratories' walk-in environmental rooms maintained at −1 and +4 °C for the cold and warm culture, respectively.

Prior to each assay, 20 ml scintillation vials were filled with 5 ml control seawater, capped, and allowed to equilibrate to temperatures within the aluminum block for two hours. Embryos and larvae were collected from all five replicate culture vessels, concentrated through 64-μm Nitex mesh and pooled. Concentrated larvae were kept on ice in environmental rooms until used for the survivorship assay at ~−1 and 0 °C for the cold and warm culture, respectively. Approximately 220–300 larvae were transferred to each temperature-equilibrated vial in no more than 250 μl. Location was randomized by CO_2 treatment within the aluminum block, and time of embryos/larvae addition was noted. Temperatures in the vials were measured immediately prior to embryos/larvae addition and upon removal of vials from the aluminum block after 1 h. The mean of these two temperatures is reported (Table 1). The brief temperature change induced by the addition of embryos/larvae was negligible. Embryos/larvae were allotted a 20 h recovery period at control temperatures (on ice in a −1 °C environmental room for the cold culture, and +2 °C water bath for the warm culture). For the first blastula stage assay of the cold culture, addition of larvae to the vials was staggered by 30 min for each temperature (cold to warm) and scored in order. For all remaining assays, larvae were added consecutively by temperature and scored randomly.

After a 20 h recovery period, larvae were concentrated by reverse filtration using a transfer pipette with 64-μm Nitex mesh. The first 100 embryos/larvae viewed under a

microscope on a Sedgewick Rafter counting cell slide were scored for survival in the environmental room. Abnormal embryos/larvae (due to abnormal development or evidence of cellular egression or regression) that still exhibited ciliary activity were considered dead. Tipping-point temperatures (TTs) were determined in order to compare thermal tolerance across the cold and warm cultures and are defined as the highest temperature at which both replicates of EDSs reared under control pCO_2 levels have greater than 80 percent survivorship.

Statistical analysis

Survivorship data of the 1 h temperature exposure were analyzed by developmental stage and separately for cold and warm larval cultures, using a second-order logistic model with a beta-binomial distribution (JMP 9) where pCO_2, temperature (T), and T^2 were considered main effects. There were no interactions between pCO_2 and temperature effects at any stage, and the interaction was removed from the final model.

Results

Field pH measurements

In general, pH was remarkably stable over the period of the SeaFET deployment in austral spring. Continuous pH data were recorded during two separate field seasons: 33 days in 2011 (October 29, 2011 to November 30, 2011, see Matson 2012) and 14 days in 2012 (October 31, 2012 to November 13, 2012, this study). pH was consistent over both field seasons (Fig. 1; Table 2). During these recording intervals, pH varied from 7.99–8.08 (2011) to 8.01–8.08 (2012) with median values of 8.01 during both seasons. Mean pH in 2011 and 2012 was 8.01 ± 0.02 and 8.02 ± 0.01, respectively. Temperature in 2011 ranged from −1.90 to −1.78 °C with a mean of -1.88 ± 0.03 °C (Matson 2012). Due to the SeaFET location under fast sea ice and stable SeaFET temperature data (not shown) and previously documented temperatures (Hunt et al. 2003), we assumed a constant temperature of −1.9 °C to calculate pCO_2 and carbonate ion saturation states (Ω) for the 2012 deployment (Table 2).

Conditions of the laboratory sea urchin cultures

During the laboratory portion of the experiment, pH (as controlled by CO_2-mixing) and temperature in the sea urchin cultures were stable. The three CO_2 treatments resulted in similar pH conditions in both the cold (2011) and warm (2012) culture (Fig. 1). Average cold and warm culture temperatures were -0.7 ± 0.3 and $+2.6 \pm 0.1$ °C,
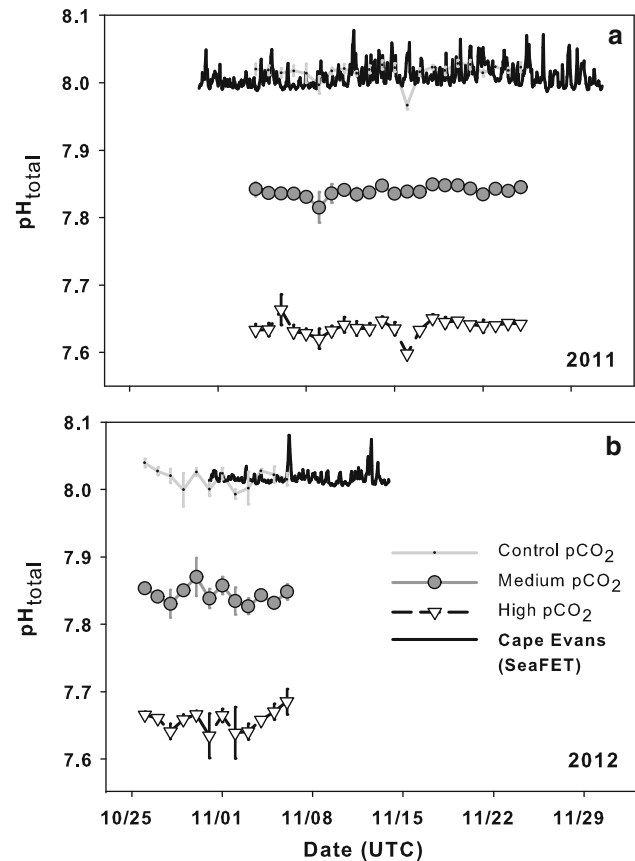

Fig. 1 Comparison of field pH_{total} and laboratory pH_{total} conditions of early developmental sea urchin cultures in 2011 (**a**) and 2012 (**b**). Field pH (*black line*) was measured by a SeaFET sensor at 18 m depth and above a *Sterechinus neumayeri* population at Cape Evans, McMurdo Sound, Antarctica. pH of CO_2-acidified experimental cultures (control pCO_2 = *light gray line* with *dot symbols*; medium pCO_2 = *dark gray circles*; high pCO_2 = *white triangles*) was measured via spectrophotometric analysis and reported as daily averages (*error bars* are SD). Culture temperature in 2011 was −0.7 and +2.6 °C in 2012. Culture pH in (**b**) is reported up to the prism stage (see text for details). Time is in Coordinated Universal Time

Table 2 Field conditions at Cape Evans, McMurdo Sound, Antarctica, in austral spring 2011 (29 October–30 November) and 2012 (31 October–13 November) using a SeaFET pH sensor

Field parameter	2011[a]	2012
T (°C)	-1.88 ± 0.03	–
pH_{total}	8.01 ± 0.02	8.02 ± 0.01
pCO_2 (µatm)	426 ± 16	419 ± 10
$\Omega_{aragonite}$	1.22 ± 0.04	1.25 ± 0.03
$\Omega_{calcite}$	1.95 ± 0.06	1.99 ± 0.04
Sample size	768	335

Saturation state (Ω) and pCO_2 for 2012 were calculated in CO2calc assuming −1.9 °C and 34.7 salinity and 2,346 µmol $kgSW^{-1}$ total alkalinity as measured in a discrete calibration sample

Values are given as mean ± SD

[a] Data are from Matson (2012)

Table 3 *Sterechinus neumayeri* culture conditions in 2011 and 2012

Parameter CO₂ treatment	Cold (2011)	Warm (2012)[a]
T (°C)		
Control	−0.7 ± 0.3 (98)	2.6 ± 0.1 (75)
Medium	−0.7 ± 0.3 (98)	2.6 ± 0.1 (70)
High	−0.7 ± 0.3 (88)	2.6 ± 0.1 (85)
pH$_{total}$		
Control	8.038 ± 0.015 (98)	8.009 ± 0.0268 (72)
Medium	7.858 ± 0.011 (98)	7.846 ± 0.020 (64)
High	7.652 ± 0.014 (88)	7.657 ± 0.021 (80)
pCO₂ (μatm)		
Control	402 ± 16 (98)	437 ± 31 (72)
Medium	626 ± 17 (98)	658 ± 33 (64)
High	1,032 ± 37 (88)	1,044 ± 55 (80)
Ω$_{aragonite}$		
Control	1.36 ± 0.04 (98)	1.46 ± 0.07 (72)
Medium	0.92 ± 0.02 (98)	1.04 ± 0.05 (64)
High	0.59 ± 0.02 (88)	0.69 ± 0.03 (80)
TA (μmol kgSW^{-1})		
Control	2,348 ± 9 (22)	2,348 ± 8 (17)
Medium	2,341 ± 9 (22)	2,349 ± 6 (17)
High	2,339 ± 10 (22)	2,347 ± 5 (17)
Salinity		
Control	34.2 ± 0.1 (22)	34.8 ± 0.1 (17)
Medium	34.2 ± 0.1 (22)	34.8 ± 0.1 (17)
High	34.2 ± 0.1 (22)	34.8 ± 0.1 (17)

Values are given as mean ± SD with sample size between parentheses

TA total alkalinity

[a] Excludes pH, pCO₂, Ω$_{arag}$, data on days of gas valve failures (see text for details)

respectively, with little variation between culture vessels. pH and pCO₂ levels of the cold culture remained stable throughout the experiment (Table 3). Control pH of the cold culture was 8.038 ± 0.015 (402 ± 16 μatm pCO₂) and slightly higher than observed field pH in 2011 and 2012, whereas control pH of the warm culture matched field pH at 8.009 ± 0.027 (437 ± 16 μatm pCO₂). Failure of mass flow control valves on day 12 of the warm culture resulted in a one day increase of pCO₂ to 2,651 ± 353 μatm in control culture vessels and decrease to 216 ± 18 μatm in the high CO₂ treatment. Subsequently on day 14, medium CO₂ treatment levels declined to 216 ± 18 μatm pCO₂ and failed to recover for the remainder of the experiment. The warm culture pluteus stage was the only larval stage affected by the valve

failures in this study. This stage was still included in the heat stress experiment.

Developmental progression of sea urchins in experimental cultures

Overall, development of *S. neumayeri* larvae occurred synchronously across all CO₂ treatments with development progressing faster in the culture maintained at +2.6 °C as compared to the culture maintained at −0.7 °C (Fig. 2). As measured by prevalence of >90 %, at −0.7 °C, embryos reached hatched blastula at 104 h, gastrula by day 11, prism by day 15, and pluteus by day 21. At +2.6 °C, *S. neuamayeri* reached these same stages at 84 h, and by day 6, 11, and 16, respectively. Sampling of developmental stages of the warm culture was timed to best match developmental stages of the cold culture in the previous year as determined by daily observation. Due to the rapid development of embryos in the warm culture, there may have been a mismatch at the gastrula stage where slightly developmentally younger stage gastrulae, with smaller archenterons, were sampled in the warm culture compared to the cold culture. Some mortality was observed across all treatments at the prism and pluteus stages during the experiment but was not quantified as the requirement for experiments, other than the one reported here, dictated the need for large numbers of larvae.

Survivorship assays

Survivorship assays were used to assess whether development at elevated temperature and pCO₂ would alter the tolerance of acute heat stress in EDSs of *S. neumayeri*. All EDSs survived temperatures that greatly exceeded their average habitat temperature of −1.9 °C. In general, survivorship curves look similar across all temperature and CO₂ treatments. Survivorship following acute temperature exposure was high up to a tipping-point temperature (TT) beyond which it declined rapidly (Fig. 3).

S. neumayeri EDSs reared at −0.7 °C were extremely robust to pCO₂, and no pCO₂ effect was observed in the survivorship assays (Fig. 3a–d; Table 4). In contrast, negative pCO₂ effects were observed for two warm culture EDSs, suggesting that multiple stressors are more detrimental than single stressors (Fig. 3e–h; Table 4). First, high pCO₂ (>1,000 μatm) reduced thermal tolerance of warm culture blastulae at temperatures beyond the TT of 15 °C (*p* = 0.0005, Fig. 3e). This trend was also observed for blastulae reared at −0.7 °C but was not significant (*p* = 0.21). Second, medium pCO₂ (650 μatm) reduced thermal tolerance of prism larvae reared at +2.6 °C (*p* = 0.0476, Fig. 3g).

Fig. 2 Sampling schedule for survivorship assays of *Sterechinus neumayeri* early developmental stages (blastula, gastrula, prism, and 4-arm pluteus) reared at −0.7 °C (*white dots*) and +2.6 °C (*black dots*). Sampling was conducted once >90 % of embryos or larvae reached the stage of interest. *Split dot* represents sampling of both cultures. Representative developmental stage photos are of *S. neumayeri* reared at +2.6 °C and assorted pCO$_2$ levels. *Scale bar* is 100 μm
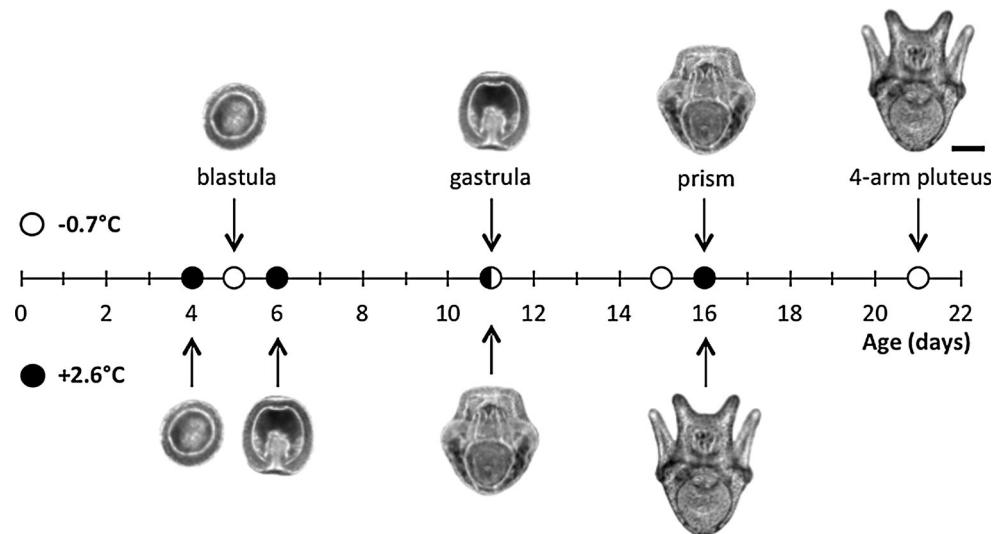
Finally, due to genotypic and potential sampling differences between the cultures, direct comparisons based on culture temperature should be interpreted with caution. However, based on TTs, there appears to be no strong effect of culture temperature on the thermal tolerance of *S. neumayeri* EDSs. In both the cold and warm culture, blastulae were less thermotolerant than post-blastula stages by ∼5 °C. Blastulae exhibited a TT of 15 °C. Post-blastula stages in both the cold and warm culture survived acute exposure up to 20 °C, with the exception of warm culture gastrulae which exhibited a TT of 18 °C (Fig. 4).

Discussion

The aim of this study was to assess the current physiological plasticity of the Antarctic sea urchin *S. neumayeri* in light of ocean warming and acidification and in the context of current ocean conditions. Here, we report two salient findings. First, as measured by autonomous pH sensors, we found that *S. neumayeri* EDSs currently experience extremely stable abiotic conditions in McMurdo Sound during the austral spring. Second, we found a remarkably high acute temperature tolerance of *S. neumayeri* EDSs reared at elevated pCO$_2$ and temperature. Although in general, Antarctic organisms are predicted to be sensitive to environmental change (Peck 2005; Somero 2012), we show that *S. neumayeri* EDSs may be more physiologically tolerant of future conditions than previously thought (Byrne et al. 2013).

Field pH measurements

Measurements of ocean temperature and pH at Cape Evans, McMurdo Sound, Antarctica, during two seasons (austral spring 2011, 2012) were extremely stable and match previously documented environmental stability in this region (Littlepage 1965; Matson et al. 2011). For example, in 2010, pH ranged from 8.002 to 8.050 from 26 October to 15 November (Matson et al. 2011). Collectively, median pH at Cape Evans over three consecutive austral spring seasons was 8.019 (2010, Matson et al. 2011), 8.005 (2011, Matson 2012), and 8.016 (2012, this study). Relative to other ecosystems, pH data from Cape Evans show extremely stable inter-annual pH conditions. By contrast, pH in temperate and tropical reefs can vary as much as 0.544 and 0.253 pH units over only 30 days (Hofmann et al. 2011).

The period of stable pH at Cape Evans coincides with the spawning season of *S. neumayeri* (Pearse and Giese 1966; Brey et al. 1995). Previous plankton tows throughout McMurdo Sound show the presence of blastulae and gastrulae from November to December (Bosch et al. 1987). Thus, depending on local retention time post-fertilization, it is likely that EDSs from the Cape Evans *S. neumayeri* spawning population consistently develop at seawater pH of 8.0–8.1 and temperature of approximately −1.9 °C. Future ocean acidification and carbonate ion undersaturation, such as the conditions used in the experimental portion of this study, will likely be unprecedented for *S. neumayeri* in the coming decades.

Survivorship assays of *S. neumayeri* EDSs

Here, we report the changes in survivorship following a 1 h acute heat stress of *S. neumayeri* EDSs reared at elevated temperature and pCO$_2$ that mimic predicted anthropogenic changes in the Southern Ocean. We hypothesized that development of *S. neumayeri* EDSs under seawater conditions outside their current ambient range would incur a

Fig. 3 Percent survivorship, following a 1 h acute heat stress and ~20 h recovery at culture temperatures, of four early developmental stages (**a**, **e** blastula; **b**, **f** gastrula; **c**, **g** prism; **d**, **h** 4-arm pluteus) of *Sterechinus neumayeri* reared at −0.7 °C (**a**–**d**) and +2.6 °C (**e**–**h**) under control pCO_2 (~400 µatm, *black squares* and *regression line*) and elevated pCO_2 (~650 µatm, *gray circles* and *regression line*; ~1,000 µatm, *white triangles* and perforated *regression line*) conditions. *Symbols* represent individual replicates ($n = 100$). *Asterisk* denotes significant pCO_2 effect
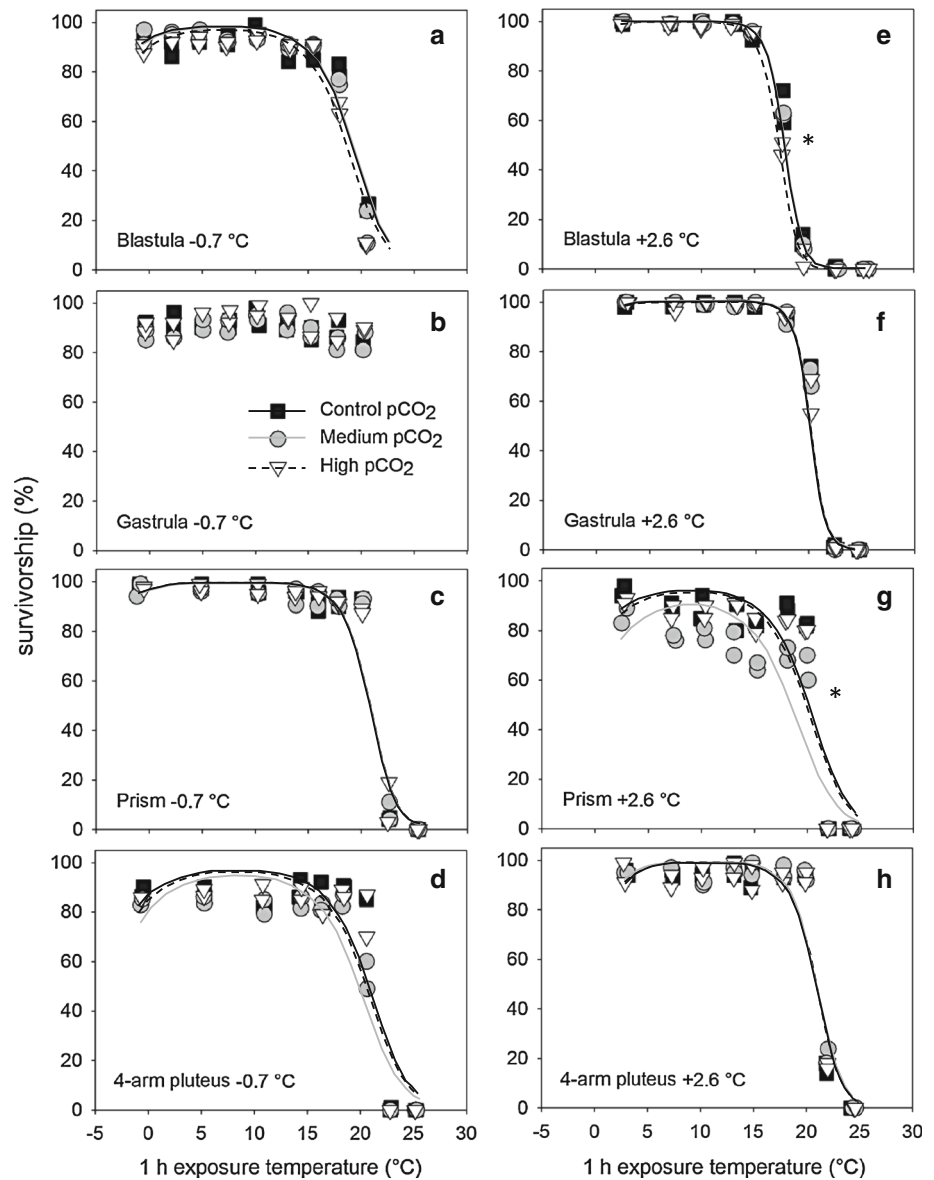
physiological cost, such that tolerance of a secondary stressor would be reduced. We measured this cost by assessing tolerance of acute heat stress (Terblanche et al. 2011). We found negative pCO_2 effects on acute thermal tolerance only when *S. neumayeri* were reared under elevated temperature of +2.6 °C (Fig. 4). While these results suggest a trade-off of development under a multi-stressor scenario and ability to cope with a secondary stress, the effect is relatively small from an ecological perspective. We therefore conclude that the effect of the multi-stressor scenario (ocean acidification and ocean warming together) on acute thermal tolerance is minor. *S. neumayeri* may be one of the more tolerant Antarctic marine invertebrates, exhibiting high physiological plasticity despite living under extremely stable conditions.

However, future multi-stressor scenarios may impact other physiological processes and long-term tolerances. For example, in recent research, *S. neumayeri* fertilization and early cleavage were negatively impacted by temperature (+1.5 and +3 °C) at a CO_2 concentration of 1,370 ppm (pH 7.5, Ericson et al. 2012). Inter-individual variation observed in *S. neumayeri* fertilization success under elevated pCO_2 may ameliorate such effects in the wild (Sewell et al. 2014). Furthermore, previous studies show compromised growth of *S. neumayeri* larvae reared at elevated pCO_2 (Byrne et al. 2013; Yu et al. 2013). However, warming may increase larval growth (Byrne 2011). Byrne et al. (2013) showed that the combined effects of warming and CO_2-acidification resulted in highly altered *S. neumayeri* larval body morphology and

Table 4 Second-order logistic model output

Culture	Source	df	L-R ChiSquare	Prob > ChiSquare
Cold	*Blastula*			
	pCO_2	2	3.1213056	0.21
	T	1	198.20761	<0.0001*
	$T \times T$	1	108.22541	<0.0001*
	Prism			
	pCO_2	2	0.0216827	0.9892
	T	1	331.1169	<0.0001*
	$T \times T$	1	54.107351	<0.0001*
	Pluteus			
	pCO_2	2	1.7438573	0.4181
	T	1	168.85749	<0.0001*
	$T \times T$	1	65.283207	<0.0001*
Warm	*Blastula*			
	pCO_2	2	15.29441	0.0005*
	T	1	3487.2801	<0.0001*
	$T \times T$	1	83.696576	<0.0001*
	Gastrula			
	pCO_2	2	0.1561852	0.9249
	T	1	657.08303	<0.0001*
	$T \times T$	1	38.055269	<0.0001*
	Prism			
	pCO_2	2	6.0902789	0.0476*
	T	1	155.09928	<0.0001*
	$T \times T$	1	38.090102	<0.0001*
	Pluteus			
	pCO_2	2	0.2318825	0.8905
	T	1	199.23477	<0.0001*
	$T \times T$	1	61.083177	<0.0001*

* Significant

asymmetry, suggesting that there is a significant physiological effect of multi-stressor scenarios on the development of this polar echinoderm. Byrne et al. (2013) also found that under a multi-stressor scenario of temperature (+1 °C) and pCO_2 (1,355 µatm, pH 7.6), development up to the gastrula stage was not affected but was negatively affected at the prism and pluteus stages with up to 83 percent of plutei showing abnormal development. Our findings show that these negative effects do not translate to secondary physiological trade-offs such that other functional traits, namely acute thermal tolerance, are compromised. Negative effects of warming and acidification on metamorphosis, juvenile growth and reproduction may swamp the small benefit of high physiological plasticity at the EDSs, although these have not yet been evaluated for *S. neumayeri*.

While normal development was not quantified in this study, culture temperature of +2.6 °C may have resulted in greater mortality of abnormal larvae such that the EDSs sampled in this study were selectively more thermotolerant than those observed by Byrne et al. (2013), which were reared at +1 °C. This type of outcome was observed in temperate purple sea urchin larvae, *Strongylocentrotus purpuratus*, where different genotypes were selected during culturing in various experimental future ocean acidification conditions (Pespeni et al. 2013). Ultimately, we assessed the embryos and larvae that survived culture conditions long enough to be sampled. For example, the pCO_2 effect observed at the prism stage is largely due to the presence of abnormal larvae in the medium CO_2 treatment (analysis not shown), and such larvae may not have survived to the pluteus stage. It should be noted that this treatment group was exposed to low pCO_2 due to valve
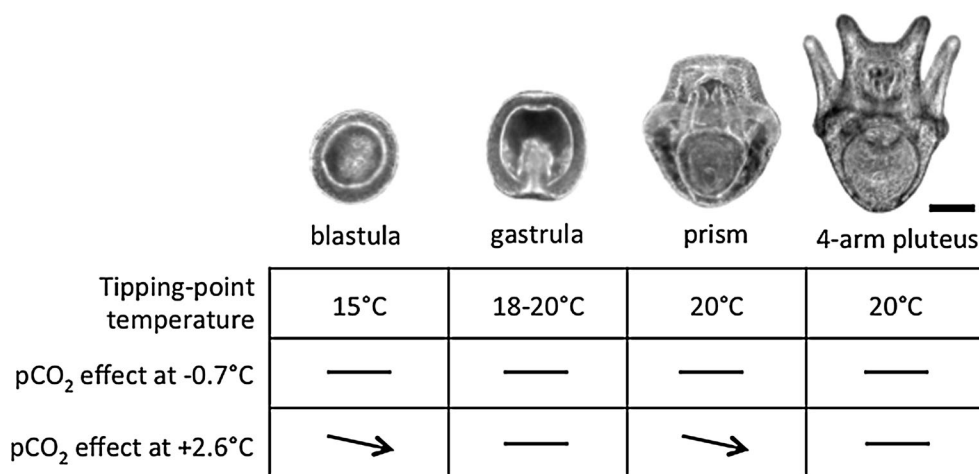

Fig. 4 Summary of salient finding for the physiological toll of exposure to future ocean scenarios during early development of *Sterechinus neumayeri*, as assessed by a 1 h heat stress survivorship assay at four developmental stages. *S. neumayeri* embryos and larvae exhibited high tipping-point temperatures independent of development at −0.7 °C and +2.6 °C. Elevated pCO_2 had an overall slight negative impact on blastulae and prisms reared at +2.6 °C. *Scale bar* is 100 µm

failures following sampling of the prism stage and higher survivorship of the pluteus stage could thus still be a factor of low pCO_2 exposure. Other studies on sea urchin species show impaired development at only a few degrees above ambient conditions (Sewell and Young 1999; Byrne et al. 2009; Sheppard Brennand et al. 2010; Delorme and Sewell 2013). It is likely that a culture temperature of +2.6 °C is near the upper temperature limit of *S. neumayeri* early development.

Within species, developmental stages respond differently to abiotic stressors. While all *S. neumayeri* EDSs survived temperatures that greatly exceed their average habitat temperature of −1.9 °C, blastulae appear to be the most sensitive stage to abiotic stressors. Blastulae reared at −0.7 °C exhibited a pattern of reduced acute thermal tolerance with increasing pCO_2. This pattern was statistically significant when blastulae were reared at +2.6 °C, suggesting that high pCO_2 affects the performance of blastulae. Additionally, in both the cold and warm culture, thermal tolerance increased as much as 5 °C from 15 to 20 °C from blastula stage to gastrula, prism and pluteus stages, respectively. Sensitivity of this EDS has been observed previously where developmental progression of *S. neumayeri* between −0.8 and 0 °C was only affected by high pCO_2 at the blastula stage (Yu et al. 2013).

In general, blastulae are one of the more sensitive developmental stages in echinoderms, especially with respect to temperature (Roccheri et al. 1986; Sconzo et al. 1995; Giudice et al. 1999). Large increases in gene expression are associated with the transition from hatched blastula to gastrula and later stages (Giudice et al. 1968; Howard-Ashby et al. 2006). Such genes include, for example, genes coding for heat shock proteins, which can increase thermal tolerance of post-blastula stages (Roccheri et al. 1986; Sconzo et al. 1995; Giudice et al. 1999). It is interesting that increased thermal tolerance coincides with increased pCO_2 tolerance. Lower sensitivity of *S. neumayeri* post-blastula stages to pCO_2 and acute temperature stress may be related to alterations in the larval transcriptome. Such changes in gene expression may allow the organism to overcome the negative effects of pCO_2, demonstrating a link between gene expression and physiological plasticity that leads to tolerance of CO_2 acidification (Evans et al. 2013). Thus, *S. neumayeri* may already harbor some genetic plasticity that allow EDSs to tolerate future ocean change, at least to some degree.

Temperate sea urchin gastrulae of *S. purpuratus* exhibit large changes in gene expression relative to control conditions (pH 8.1/435 µatm pCO_2) when reared at naturally occuring levels of low pH (pH 7.77/813 µatm pCO_2) but not at pH below natural exposures (pH 7.59/1,255 µatm pCO_2). This suggests that gastrulae can compensate for pCO_2 stress within the limits of current pH variability

(Evans et al. 2013). Given that *S. neumayeri* EDSs experience a more stable pH environment than its temperate cousins, it is therefore unexpected that this species shows such high trait-based tolerance to elevated temperature and pCO_2. In association with this study, the transcriptomic response of these *S. neumayeri* EDSs is underway (Drs. G. Dilly and G.E. Hofmann unpublished results) and will be useful in elucidating the mechanisms that underlie the pCO_2 tolerance of this polar species.

Comparisons to other species

EDSs among marine invertebrates are inconsistently sensitive to multi-stressor scenarios (Pörtner and Farrell 2008; Byrne 2011). For example, our results contrast with the response of invertebrate larvae of both abalone *Haliotis rufescens* (Zippay and Hofmann 2010a) and red sea urchin *Strongylocentrotus franciscanus* (O'Donnell et al. 2008), which exhibit reduced thermal tolerance following development in CO_2-acidified seawater. Many studies report other deleterious impacts of CO_2 and temperature on EDSs stages of benthic marine invertebrates (Findlay et al. 2009, 2010; Parker et al. 2009, 2010; Sheppard Brennand et al. 2010; Anlauf et al. 2011; Byrne et al. 2011). For example, Anlauf et al. (2011) found that the combined effect of elevated pCO_2 and temperature had a greater negative effect on growth of the coral *Porites panamensis* primary polyps than pCO_2 alone. Findlay et al. (2010) also found that the post-larval growth rate of the barnacle *Semibalanus balanoides* was negatively impacted by reduced pH with a nonsignificant trend of further reduction in growth rate with increasing temperature of +4 °C. Parker et al. (2009) found reduced successful development of D-veliger larvae of oyster *Saccostrea glomerata* at high temperature and low pH treatments, compared to optimal temperature treatments. These studies highlight the importance of species-specific effects when attempting to predict biological changes to future multi-stressor scenarios, using measures of current physiological plasticity.

S. neumayeri temperature tolerance in context

Successful development of *S. neumayeri* nonfeeding EDSs at +2.6 °C and survival of acute exposure >20 °C above habitat temperatures is surprising for two reasons. First, Antarctic organisms are categorized as stenothermal due to evolutionary adaptation to the thermal stability of the Southern Ocean over evolutionary timescales (Peck 2005; Somero 2012). Second, gamete maturation in *S. neumayeri* occurs over the course of 12 to up to 24 months (Brockington et al. 2007) during which temperatures vary on an annual cycle of approximately −1.9 to −0.5 °C (Hunt et al. 2003).

Although longer temperature exposures will likely reduce upper temperature limits (Peck et al. 2009), the high acute temperature tolerance of *S. neumayeri* matches that of EDSs of eurythermal temperate species (Hammond and Hofmann 2010; Zippay and Hofmann 2010a, b). For example, gastrulae and 4-arm echinoplutei of temperate sea urchin *S. purpuratus* exhibit 50 percent survivorship following 1 h heat stress between 30 and 31 °C, a value up to 20 °C above average habitat temperatures (Hammond and Hofmann 2010). *S. neumayeri* are unlikely to ever experience temperatures up to 20 °C; however, assessing biological response to rapid warming can provide valuable insight into a species' overall physiological tolerance and impacts on ecology (Terblanche et al. 2011).

High thermal tolerance of *S. neumayeri* pelagic EDSs may be an ancestral trait, stemming prior to speciation (Díaz et al. 2011) that followed the opening of the Drake Passage and establishment of the Antarctic Circumpolar Current (Lee et al. 2004). Such trait conservation may contribute to *S. neumayeri*'s extensive biogeographic range including colonization of warmer Antarctic archipelagos (Barnes et al. 2010). Our findings support the hypothesis that may be one of the least stenothermal Antarctic marine ectotherms (Barnes et al. 2010).

While *S. neumayeri* may be highly tolerant of acute temperature stress, this tolerance does not appear to be physiologically adaptive. In other words, development at +3 °C above −0.7 °C did not shift TTs by the same magnitude. This has been shown for adult *S. neumayeri* as well where a 60-day exposure of adult sea urchins to +3 °C did not affect acute upper temperature limits, which were approximately 15 °C (Peck et al. 2010). The lethal temperatures reported here and in Peck et al. (2010) are much higher than current habitat temperatures and ecologically irrelevant for predictions of warming in the Southern Ocean. However, these data support the hypothesis that while *S. neumayeri* is less stenothermal than previously thought (Barnes et al. 2010), its physiological adaptive capacity is limited (Peck 2005; Somero 2012).

Conclusion

We show that present-day *S. neumayeri* embryos and larvae are resilient to relatively large short-term multi-stressor scenarios. Other studies show variable sensitivities of physiological measures in *S. neumayeri* EDSs (i.e., development, growth, and asymmetry), and it remains unclear how ocean acidification and warming will ultimately affect the life cycle of this species. These experimental results also point in the direction of a need to better understand adaptation and genetics in response to ocean acidification (Evans and Hofmann 2012; Kelly and Hofmann 2012). As the ocean

changes, new selection forces will act on the existing genetic structure. Individuals will be exposed to slowly changing environments which could enhance benefits of existing physiological plasticity, such as those described here, through maternal effects. It is significant that some studies show that traits of resilience are heritable (Sunday et al. 2011; Kelly et al. 2013) and local adaptation likely plays a strong role (Pespeni et al. 2013; Schaum et al. 2013). A high degree of present-day physiological plasticity and genetic variability may facilitate adaptation and long-term tolerance of future conditions. Given the predictions of rapid changes in oceans (IPCC 2007), identifying resilient and vulnerable species and variably tolerant populations is crucial in order to understand ecological impacts of ocean change.

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