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Could the acid–base status of Antarctic sea urchins indicate a better-than-expected resilience to near-future ocean acidification?

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Abstract

Increasing atmospheric carbon dioxide concentration alters the chemistry of the oceans towards more acidic conditions. Polar oceans are particularly affected due to their low temperature, low carbonate content and mixing patterns, for instance upwellings. Calcifying organisms are expected to be highly impacted by the decrease in the oceans' pH and carbonate ions concentration. In particular, sea urchins, members of the phylum Echinodermata, are hypothesized to be at risk due to their high-magnesium calcite skeleton. However, tolerance to ocean acidification in metazoans is first linked to acid–base regulation capacities of the extracellular fluids. No information on this is available to date for Antarctic echinoderms and inference from temperate and tropical studies needs support. In this study, we investigated the acid–base status of 9 species of sea urchins (3 cidaroids, 2 regular euechinoids and 4 irregular echinoids). It appears that Antarctic regular euechinoids seem equipped with similar acid–base regulation systems as tropical and temperate regular euechinoids but could rely on more passive ion transfer systems, minimizing energy requirements. Cidaroids have an acid–base status similar to that of tropical cidaroids. Therefore Antarctic cidaroids will most probably not be affected by decreasing seawater pH, the pH drop linked to ocean acidification being negligible in comparison of the naturally low pH of the coelomic fluid. Irregular echinoids might not suffer from reduced seawater pH if acidosis of the coelomic fluid pH does not occur but more data on their acid–base regulation are needed. Combining these results with the resilience of Antarctic sea urchin larvae strongly suggests that these organisms might not be the expected victims of ocean acidification. However, data on the impact of other global stressors such as temperature and of the combination of the different stressors needs to be acquired to assess the sensitivity of these organisms to global change.

Keywords: acid–base regulation, Antarctica, echinoderms, ocean acidification, sea urchins, Southern Ocean

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Introduction

Since the beginning of the Industrial Revolution, the atmospheric carbon dioxide concentration (CO_2) has increased from 280 ppm to 398 ppm due to anthropogenic activities (April 2014; Ed Dlugokencky and Pieter Tans, NOAA/ESRL (www.esrl.noaa.gov/gmd/ccgg/trends/)). This increase leads to major chemical changes within the surface waters of the oceans as the CO_2 dissolves into seawater and its subsequent dissociation (Zeebe & Wolf-Gladrow, 2001). Consequences are a decreased seawater pH by 0.1 units since preindustrial times, and a decreased concentration in carbonate ions, leading to the shoaling of the saturation horizons of the different carbonate minerals. These processes are gathered under the term ocean acidification (OA) (Orr, 2011). However, these are global values and all oceans are not being affected the same way.

Polar oceans are absorbing a great part of the atmospheric CO_2 due to their low temperature (and thus higher solubility of CO_2) and mixing patterns such as upwellings and the deep water formation (Zeebe & Wolf-Gladrow, 2001; McNeil & Matear, 2008; Fabry *et al*., 2009; Takahashi *et al*., 2009; Orr, 2011). The Southern Ocean accounts for about 4% of the global uptake of CO_2 by the world oceans (Takahashi *et al*., 2009). This leads to an increased rate of acidification of the seawater but also a more rapid shoaling of the saturation horizons (Feely *et al*., 2004; Orr *et al*., 2005; McNeil & Matear, 2008; Fabry *et al*., 2009; Orr, 2011), putting the marine organisms of the Southern Ocean at a higher risk to find themselves in unfavorable waters, in particular regarding calcification processes. However, very

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few studies have been conducted to date on the possible impact of OA on Antarctic calcifying organisms (Cubillos *et al.*, 2007; McClintock *et al.*, 2009; Moy *et al.*, 2009; Cummings *et al.*, 2011; Seibel *et al.*, 2012; Walker *et al.*, 2013).

Because the saturation state of magnesium calcites cannot be currently calculated for the deep ocean, the aragonite saturation horizon (ASH) is used as a proxy (see Morse *et al.*, 2006). Presently, this ASH is situated on average at 730 m in the Southern Ocean south of 60°S. However, it is predicted that the ASH will shoal to the surface by 2030 during the winter in the latitudinal band range 65–70°S (McNeil & Matear, 2008). Moreover, as Mg-calcites are more soluble than aragonite, the saturation horizons for these minerals will shoal even sooner. Andersson *et al.* (2008) calculated that the Southern Ocean surface is already undersaturated for Mg-calcite of 12 mol% $MgCO_3$ or more and it is expected that the high-latitude surface waters will be undersaturated for Mg-calcite of 4–5 mol% $MgCO_3$ by the end of the century (Andersson *et al.*, 2008).

Echinoderms are very important components of the Antarctic macrobenthos in terms of carbon cycling and diversity, and are found throughout the Southern Ocean (Arntz *et al.*, 1994; Gutt & Starmans, 1998; Barnes & Brockington, 2003; David *et al.*, 2005a; Lockhart & Jones, 2008; Lebrato *et al.*, 2010; White *et al.*, 2012; Grange & Smith, 2013). They are considered highly sensitive to OA because their skeleton is composed of high-magnesium calcite (5.9 to 15.8 mol % $MgCO_3$), the most soluble form of crystallized calcium carbonate when magnesium content reaches 8 mol% (Weber, 1969; Morse *et al.*, 2006; McClintock *et al.*, 2011). This was also hypothesized by Sewell & Hofmann (2011) who predicted a potential susceptibility of the Antarctic sea urchins, on the basis of the nature of their skeleton and their present depth distribution, mainly above the calcite saturation horizon (CSH). However, ASH is a more relevant proxy for biogenic magnesium calcites than CSH (see Morse *et al.*, 2006). According to Sewell & Hofmann (2011), brooding irregular sea urchins would be among the most impacted Antarctic echinoderms because juveniles are exposed to the surrounding seawater when in the brooding chambers (bulges within the female skeleton in which embryos and juveniles are kept during development). In fact, the skeleton composition might not be the main concern for echinoderms facing ocean acidification. Indeed, the echinoderm skeleton is an endoskeleton and is, in most cases, not in direct contact with seawater. Furthermore, numerous species do efficiently calcify below the ASH, including the heavily calcified cidaroid urchins (David *et al.*, 2005b).

Tolerance to ocean acidification in metazoans is linked to the capacity to control their acid–base balance when facing disturbances (Seibel & Walsh, 2001; Melzner *et al.*, 2009; Pörtner *et al.*, 2011). Indeed, enzyme activities and protein structures are directly influenced by the pH of the ambient medium (either extra- or intracellular) (Clarke, 1998). Regulation of the intracellular pH is influenced by the extracellular pH (pH_e) since gradients and efficiency of ion exchanges will be modified by a change in pH_e (Pörtner *et al.*, 1998). Regulation of pH_e is in turn dependent on buffering capacities of the extracellular fluid, exchange with seawater (either passive or active) and possible changes in respiration intensity (Pörtner, 2008). The only studies that considered the impact of OA on Antarctic echinoderms focused on the effects on fertilization and larval development of the sea urchins *Sterechinus neumayeri* and *Arbacia dufresnii*, and the starfish *Odontaster validus* (Clark *et al.*, 2009; Ericson *et al.*, 2010, 2012; Catarino *et al.*, 2012a; Byrne *et al.*, 2013; Gonzalez-Bernat *et al.*, 2013; Ho *et al.*, 2013; Yu *et al.*, 2013; Kapsenberg & Hofmann, 2014). These studies revealed that the impact of reduced pH on development history was small except for a reduced growth rate and reduced survival of the sea star larvae (but see Sewell *et al.*, 2014). No study addressed the capacity of adult Antarctic echinoderms to control their acid–base balance probably due to the rather heavy logistics needed for such studies. Nevertheless, previous studies carried out on adult echinoderms from lower latitudes showed marked differences according to taxa, experimental design, and possibly environmental history (e.g., Dupont & Thorndyke, 2012; Spicer & Widdicombe, 2012; Stumpp *et al.*, 2012; Collard *et al.*, 2013, 2014; Kurihara *et al.*, 2013; Moulin *et al.*, 2014) precluding an inference from these data to Antarctic species.

Therefore, the aim of this study was to determine the acid–base balance in different taxonomical groups of Antarctic sea urchins, including brooding species, occurring naturally under different saturation conditions, to estimate their capacity of regulating their extracellular pH.

Materials and methods

Sampling

All samplings were done on freshly collected individuals during R/V Polarstern Cruise ANTXXIX/3 (summer, 01/2014-03/2014). Samples were collected with an Agassiz trawl in the Weddell Sea and the Bransfield Strait, between latitudes 60 and 65°S and longitudes 54 and 62°S (Table S1). Collection

of samples were carried out under research permit n°12/02 issued by the Belgian Federal Public Service 'Health, Food chain safety and Environment'.

The sampled species included the cidaroids *Aporocidaris eltaniana*, *Ctenocidaris gigantea* and *Notocidaris gaussensis*, the regular euechinoids *Sterechinus antarcticus* and *Sterechinus neumayeri* and the irregular echinoids *Abatus cavernosus*, *Amphipneustes lorioli*, *Amphipneustes rostratus* and *Amphipneustes similis* (Table S2). The three cidaroids belong to the same clade, Ctenocidarinae, the two regular euechinoids belong to the derived monophyletic family Echinidae, and the four irregular echinoids are members of a monophyletic subclade of the family Schizasteridae. Therefore, the three sets are phylogenetically homogeneous. C. De Ridder and B. David identified species on board according to David *et al.* (2005b).

Measurements in seawater

At each sampling site, *in situ* parameters (temperature, salinity and oxygen saturation) were obtained with a CTD connected to a carousel of 24 water Niskin bottles (Table 1, Table S1). Seawater samples for pH, A_T and DIC (concentration and carbon isotopic signature) measurements were obtained from three different Niskin bottles at each site. These samples were the first to be collected to minimize gas exchange with the atmosphere.

pH and electromotive force (emf) were measured immediately after sampling with a Metrohm pH-meter (826 pH mobile, combined glass electrode Metrohm 6.0228.010) calibrated with CertiPUR® buffer solutions pH 4.00 and 7.00 (Merck, Darmstadt, Germany). All measurements were converted to total scale according to DelValls & Dickson (1998)'s method with TRIS/AMP buffers (kindly provided by the Biogeochemistry and Earth System Modeling Laboratory of the Université Libre de Bruxelles, Belgium). Salinity and temperature were also measured simultaneously to the pH measurement using a salinometer Multi 340i (WTW, Weilheim in Oberbayern, Germany).

The DIC samples were prepared following the method of Gillikin *et al.* (2007) adapted as in Collard *et al.* (2014). Briefly, the sample was transferred into a 3 ml gas tight Exetainer tube (Labco Ltd, Lampeter, UK). For analysis, a headspace of 0.75 ml of helium was created in the Exetainers using a gas tight syringe and 50 μl of 99% phosphoric acid were injected in the tubes, which were left overnight at constant temperature. Exactly 300 μl headspace were then sampled *per* gas tight syringe and injected through a GC port mounted in front of the copper reduction column of an Elemental Analyser (Flash 1112 series EA Thermo) coupled online via a Conflo III to an Isotope Ratio Mass Spectrometer (Delta V, Thermo at Vrije Universiteit Brussel) (Gillikin & Bouillon, 2007). The DIC calibration was performed using $NaHCO_3$ (Sigma Aldrich, St. Louis, MO, USA) solutions of known concentrations, and plotting DIC concentration vs. area of the total signal peak of CO_2 detected by the mass spectrometer(combined signals of masses 44, 45, 46; 'area all' in mV). Error on the measurements of standard certified seawater obtained from Andrew G. Dickson's Oceanic Carbon Dioxide Quality Control Laboratory (batch number 120) was of 4.2 ± 2.6% (mean ± SD; n = 8). For the carbon isotopic ratio, the samples were calibrated against the standard NBS-19 ($\delta^{13}C$ = +1.95 ‰) and data are reported as ‰VPDB using the conventional delta notation:

$$\delta^{13}C = (R_{sample}/R_{standard} - 1) * 1000$$

with

$$R = {}^{13}C/{}^{12}C$$

For A_T, 2 ml of water were transferred upon collection into a tube and $HgCl_2$ added (7% w/w). The tubes were stored at 4 °C and in the dark until further analysis. A_T of the seawater was determined by means of a potentiometric titration method (Gran, 1952) adapted to small volumes (Collard *et al.*, 2013) to have comparable results between seawater and coelomic fluid measurements (see below). Briefly, a potentiometric titration was realized on a 0.5 ml sample by adding first 5 μl of 0.1M HCl (Merck, Darmstadt, Germany) and then 1 μl at a time. After each addition, the sample was agitated and pH measured using a 3 mm diameter glass microelectrode (reference 6.0224.100; Metrohm, Darmstadt, Germany). The $A_{T\text{-}SW}$ was then calculated using Gran's function (Gran, 1952). Error on the measurements of standard certified material from Andrew G. Dickson's Oceanic Carbon Dioxide Quality Control Laboratory (batch number 120) was of 2.3 ± 1.2% (mean ± SD; n = 18).

Aragonite and calcite saturation state values (Ω) as well as pCO_2 and the concentrations of the carbonate system components were calculated from A_T, pH (total scale), salinity and temperature data (from the CTD measurements) using the software CO2SYS (Pierrot *et al.*, 2006) with the dissociation constants for carbonate from Mehrbach *et al.* (1973) refitted by Dickson & Millero (1987), and for K_{SO4} from Dickson (1990) (Table 1).

Physiological parameters of the sea urchins

Immediately after collection of the specimens, the coelomic fluid was collected with a syringe and a needle by puncturing either the peristomial membrane (regular euechinoids), the bottom of the brooding chambers where the skeleton is very thin (irregular echinoids) or through a small hole drilled in the test (cidaroids). The air-free syringes were then kept on ice until further measurement.

For all sea urchins, pH_T (pH_{CF}), total alkalinity ($A_{T\text{-}CF}$), and DIC (DIC_{CF}) were measured in the coelomic fluid (CF). pH_{CF} and $A_{T\text{-}CF}$ were measured as described in Collard *et al.* (2013). Briefly, 0.5 ml CF were taken from the sea urchin (see above) and transferred immediately into an Eppendorf tube. pH_{CF} was measured using the microelectrode described above within 1 h after the sea urchins were brought back on the deck. As pH_{CF} of sea urchins does not change upon a 24 h emersion (Spicer *et al.*, 1988), we are confident that these measurements reflect the *in situ* pH_{CF}. Once the initial pH was measured, A_T was measured as described previously for seawater (but samples were neither spiked with $HgCl_2$ nor stored). The obtained values for A_T were corrected by using standard certified seawater provided by Andrew G. Dickson's Oceanic Carbon Dioxide

Table 1 *In situ* measurements of seawater parameters at collection sites and carbonate system parameters calculated from CO2SYS using pH_T, A_T, *in situ* salinity and temperature (*in situ* measurements from Schröder *et al*., 2013)

	Measured parameters					Calculated *in situ* parameters (CO2SYS)					
Station	pH	T (°C)	S (PSU)	A_T	DIC	pCO_2	CO_2	HCO_3^-	CO_3^{2-}	Ω Ca	Ω Ar
116	7.86 ± 0.03	−1.542	34.474	2.41 ± 0.03	2.36 ± 0.03	632 ± 41	42 ± 3	2253 ± 27	64 ± 4	1.5 ± 0.1	1.0 ± 0.1
118	7.79 ± 0.01	−1.143	34.543	2.38 ± 0.02	2.35 ± 0.02	742 ± 18	49 ± 1	2243 ± 16	55 ± 1	1.3 ± 0.0	0.8 ± 0.0
160	7.75 ± 0.01	−1.743	34.431	2.26 ± 0.01	2.24 ± 0.02	765 ± 16	51 ± 1	2140 ± 15	47 ± 0	1.1 ± 0.0	0.7 ± 0.0
162	7.85 ± 0.00	−1.861	34.464	2.33 ± 0.03	2.28 ± 0.03	628 ± 14	42 ± 1	2181 ± 26	59 ± 0	1.4 ± 0.0	0.9 ± 0.0
163	7.83 ± 0.00	−1.765	34.513	2.26 ± 0.05	2.22 ± 0.05	629 ± 21	42 ± 1	2117 ± 51	56 ± 1	1.3 ± 0.0	0.8 ± 0.0
164	7.83 ± 0.01	−1.336	34.376	2.34 ± 0.02	2.29 ± 0.02	656 ± 13	43 ± 1	2190 ± 16	59 ± 0	1.4 ± 0.0	0.9 ± 0.0
185	7.55 ± 0.04	−1.595	34.470	2.38 ± 0.04	2.42 ± 0.04	1324 ± 129	89 ± 9	2303 ± 34	32 ± 3	0.8 ± 0.1	0.5 ± 0.0
193	7.73 ± 0.02	−0.995	34.534	2.02 ± 0.16	2.01 ± 0.17	738 ± 94	48 ± 6	1918 ± 158	41 ± 2	1.0 ± 0.0	0.5 ± 0.0
197	7.78 ± 0.02	−1.464	34.472	2.36 ± 0.02	2.33 ± 0.02	743 ± 32	49 ± 2	2229 ± 17	53 ± 2	1.3 ± 0.0	0.6 ± 0.0
199	7.80 ± 0.01	−1.326	34.477	2.38 ± 0.01	2.34 ± 0.02	728 ± 28	48 ± 2	2238 ± 15	55 ± 2	1.3 ± 0.0	0.8 ± 0.0
202	7.72 ± 0.01	−0.720	34.563	2.36 ± 0.06	2.34 ± 0.05	869 ± 12	56 ± 1	2237 ± 50	48 ± 3	1.2 ± 0.1	0.8 ± 0.0
218	7.80 ± 0.04	−0.799	34.558	2.14 ± 0.22	2.10 ± 0.22	647 ± 77	42 ± 5	2006 ± 206	52 ± 8	1.2 ± 0.2	0.7 ± 0.1
227	7.73 ± 0.04	−0.853	34.551	2.06 ± 0.05	2.04 ± 0.04	748 ± 47	49 ± 3	1953 ± 43	42 ± 4	1.0 ± 0.1	0.8 ± 0.1
234	7.70 ± 0.03	0.033	34.415	2.26 ± 0.07	2.24 ± 0.08	878 ± 80	55 ± 5	2142 ± 74	45 ± 1	1.1 ± 0.0	0.7 ± 0.0
246	7.71 ± 0.07	−0.15	34.445	2.35 ± 0.01	2.34 ± 0.03	919 ± 167	58 ± 11	2232 ± 26	47 ± 7	1.1 ± 0.2	0.7 ± 0.1
249	7.60 ± 0.05	0.091	34.530	2.13 ± 0.09	2.15 ± 0.10	1081 ± 161	68 ± 10	2046 ± 91	34 ± 3	0.8 ± 0.1	0.5 ± 0.0

T: temperature; S: salinity; A_T: total alkalinity (mmol kg_{SW}^{-1}); DIC: dissolved inorganic carbon (mmol l^{-1}); pCO_2 in μatm; CO_2, HCO_3^-, CO_3^{2-} in μmol kg_{SW}^{-1}; Ω: saturation state, Ca: calcite, Ar: aragonite. Depth is the mean value during the Agassiz trawling.

Quality Control Laboratory (batch number 120). For DIC, we applied the method described in Collard *et al.* (2014). Four ml of CF were extracted and centrifuged at 400 *g* for 10 minutes to remove coelomocytes. Thereafter, the coelomic fluid was transferred into a 3 ml Exetainer tube and $HgCl_2$ was added (7% w/w) to eliminate any remaining biological activity. Tubes were then closed with a screw cap and septum and kept at 4 °C until further analysis (same as described above for seawater). Aragonite and calcite saturation state values (Ω) as well as pCO_2 and *in situ* concentrations of the carbonate system components were calculated from DIC, pH (total scale), salinity and temperature data (from the CTD measurements) using the software CO2SYS (Pierrot *et al.*, 2006) as described for seawater (Table 2). These calculations were performed only for sea urchins for which pH_{CF} and corresponding DIC_{CF} were available.

Brooding chambers

Individuals of the species *Amphipneustes rostratus* (n = 4) and *Amphipneustes similis* (n = 3) were used to determine the conditions within the brooding chambers of irregular sea urchins maintained in small aquaria on ice for the duration of the measurements (ca. 10 min). For each individual, pH was measured in each brooding chamber, in the surrounding seawater and in the coelomic fluid (microelectrode described above). Salinity and temperature were determined with a salinometer Multi 340i (WTW). Parameters of the carbonate system were determined as described above for seawater and coelomic fluid measurements (Table 3).

Statistical analysis

All ANOVA models were built according to the recommendations of Doncaster & Davey (2007). Coelomic fluid pH, ΔA_T, ΔDIC and $\delta^{13}C$ data were compared in between species (adding seawater as a 'species') using a one-way ANOVA, followed by a Tukey test for multiple comparisons. Groups which presented n = 1 for the analyzed parameter were left out of the statistical analysis. pH measured and pCO_2 calculated in the different compartments of brooding species were compared with a two-way ANOVA (species, fixed factor, and compartment, fixed factor). The differences between genders were analyzed using two-sample t-tests, in the three groups separately. All correlation analyses were carried out using simple correlation of Spearman with associated Bonferroni probabilities. All tests were realized using the software Systat 12 (Systat Software Inc., USA).

Results

Carbonate chemistry at the collection sites is reported in Table 1. Temperature ranged from −1.861 to 0.091 °C and salinity was very stable around 34.5. pH_T ranged from 7.60 to 7.86, with the exception of station 185 which had a particular carbonate chemistry and a pH_T around 7.55. Alkalinity (A_T) was typical of the

Table 2 Measured and calculated parameters of the echinoid coelomic fluid (mean ± SD) under *in situ* conditions (salinity and temperature from CTD measurements at collection site; Schröder *et al.*, 2013). Only sea urchins for which pH_{CF} and corresponding DIC_{CF} were available were used

		Measured			Calculated (CO2SYS)					
Species	N	DIC	pH_T	A_T	pCO_2	CO_2	HCO_3^-	CO_3^{2-}	Ω Ca	Ω Ar
Aporocidaris eltaniana	4	2.44 ± 0.19	7.20 ± 0.05	2.86 ± 0.61	2893 ± 339	187 ± 22	2238 ± 176	14 ± 2	0.3 ± 0.1	0.2 ± 0.0
Ctenocidaris gigantea	3	2.58 ± 0.19	7.02 ± 0.10	3.18 ± 0.63	4459 ± 1227	300 ± 84	2273 ± 135	9 ± 2	0.2 ± 0.1	0.1 ± 0.0
Abatus cavernosus	5	2.61 ± 0.08	7.05 ± 0.20	2.47 ± 0.10	4422 ± 1986	298 ± 134	2296 ± 142	11 ± 5	0.3 ± 0.1	0.2 ± 0.1
Amphipneustes lorioli	1	3.08	6.98	2.81	5745	375	2697	10	0.2	0.2
Amphipneustes similis	7	2.67 ± 1.10	7.08 ± 0.08	2.80 ± 0.09	4207 ± 1948	274 ± 128	2380 ± 976	11 ± 5	0.3 ± 0.1	0.2 ± 0.1
Sterechinus antarcticus	11	4.35 ± 0.95	7.19 ± 0.19	4.25 ± 0.84	5466 ± 2501	362 ± 167	3966 ± 916	27 ± 12	0.6 ± 0.3	0.4 ± 0.2
Sterechinus neumayeri	19	4.39 ± 0.47	7.26 ± 0.14	4.13 ± 0.49	4802 ± 1844	321 ± 121	4040 ± 419	19 ± 9	0.7 ± 0.2	0.4 ± 0.1

AT: total alkalinity (mmol $kgSW^{-1}$); DIC: dissolved inorganic carbon (mmol l^{-1}); pCO2 in µatm; CO2, HCO3, CO32 in µmol $kgSW^{-1}$; Ω: saturation state, Ca: calcite, Ar: aragonite.

Table 3 Measured and calculated parameters (mean ± sd) of the seawater in the brooding chambers *in situ* conditions (temperature from CTD measurements at collection site; Schröder *et al.*, 2013)

		Measured		Calculated (CO_2SYS)					
Species/Compartment	N	A_T	pH_T	DIC	pCO_2	CO_2	HCO_3^-	CO_3^{2-}	Ω Ca	Ω Ar
Amphipneustes rostratus										
CF	4	2.06 ± 0.05	6.96 ± 0.02	2.29 ± 0.01	5124 ± 207	242 ± 10	2036 ± 1	10 ± 0	0.2 ± 0.0	0.2 ± 0.0
BC	4	2.06 ± 0.05	7.55 ± 0.17	2.06 ± 0.05	1364 ± 638	64 ± 30	1957 ± 34	42 ± 13	1.0 ± 0.3	0.6 ± 0.2
Amphipneustes similis										
CF	3	2.06 ± 0.05	7.17 ± 0.08	2.18 ± 0.03	3112 ± 628	147 ± 30	2018 ± 8	17 ± 3	0.4 ± 0.1	0.3 ± 0.0
BC	3	2.06 ± 0.05	7.73 ± 0.04	2.01 ± 0.01	813 ± 77	38 ± 4	1914 ± 12	58 ± 5	1.4 ± 0.1	0.9 ± 0.1
SW	2	2.06	7.81	1.98	670	31	1883	71	1.7	1.1

AT: total alkalinity (mmol $kgSW^{-1}$); DIC: dissolved inorganic carbon (mmol l^{-1}); pCO2 in µatm; CO2, HCO3, CO32 in µmol $kgSW^{-1}$; Ω: saturation state, Ca: calcite, Ar: aragonite. CF: coelomic fluid; BC: brooding chambers; SW: seawater.

open ocean ranging from 2.02 to 2.41 mmol kg_{SW}^{-1}. While most stations had a saturation state for calcite (Ω_{Ca}) above 1 (from 1.0 to 1.5, except stations 185 and 249 where $\Omega_{Ca} = 0.8$), they were all undersaturated regarding aragonite ($\Omega_{Ar} = 0.5$ to 1.0).

Within our samples, regular euechinoids represented 48% of the samples analyzed, irregular echinoids 35% and cidaroids 17% (abundances calculated from the number of individuals used in our analysis).

Physiological parameters are presented in Fig. 1 and Table 2. The coelomic fluid pH (pH_{CF}; Fig. 1A) fluctuated in the different echinoid groups. Only the cidaroid *N. gaussensis* and the irregular *A. rostratus* had a significantly lower pH_{CF} than the two regular euechinoid species ($p_{ANOVA} < 10^{-3}$, $p_{Tukey} \leq 0.047$). The pH_{CF} of the other cidaroid and irregular echinoid species did not differ significantly from at least one of the regular euechinoids ($p_{Tukey} \geq 0.074$). None of the comparisons of pH_{CF} between the different cidaroid and irregular echinoid species were significant ($p_{Tukey} \geq 0.078$). The pH_{CF} of the two regular euechinoid species were not different from one another ($p_{Tukey} = 0.956$). pH_{CF} was not correlated with pH_{SW} in any of the three groups ($P \geq 0.483$).

Total alkalinity of the coelomic fluid (expressed as $\Delta A_T = A_{T\text{-}CF} - A_{T\text{-}SW}$; Fig. 1B) significantly differed according to species ($p_{ANOVA} < 10^{-3}$). The comparisons between the different cidaroid and irregular species were not significant ($p_{Tukey} \geq 0.721$), while they all had significantly lower ΔA_T than the regular euechinoids ($p_{Tukey} \leq 0.018$). ΔA_T of the two regular euechinoid species were not different from one another ($p_{Tukey} = 1.0$). $A_{T\text{-}CF}$ was not correlated with $A_{T\text{-}SW}$ in any of the groups ($P \geq 0.156$).

Dissolved inorganic carbon content (expressed as $\Delta DIC = DIC_{CF} - DIC_{SW}$; Fig. 1C) differed among the different groups ($p_{ANOVA} < 10^{-3}$). It was not significantly different when considering the comparisons between the cidaroid and irregular echinoid species ($p_{Tukey} \geq 0.985$), while they all had significantly lower ΔDIC than the regular euechinoids ($p_{Tukey} \leq 0.004$). ΔDIC of the two regular euechinoid species were not different from one another ($p_{Tukey} = 1.0$). The carbon isotopic signature ($\delta^{13}C$) of the DIC (Fig. 1D) of the coelomic fluid of the sea urchins was significantly more negative than that of the seawater ($p_{ANOVA} < 10^{-3}$; $p_{Tukey} < 10^{-3}$). The signals measured for the cidaroid *A. eltaniana* and the irregular echinoid *A. similis* did not differ ($p_{Tukey} = 0.986$) but they were both significantly more negative than that of the two regular euechinoids and the irregular *A. cavernosus* ($p_{Tukey} < 10^{-3}$). The $\delta^{13}C$ measured for the two regular euechinoid species and the irregular echinoid *A. cavernosus* were not different from each other ($p_{Tukey} \geq 0.072$).

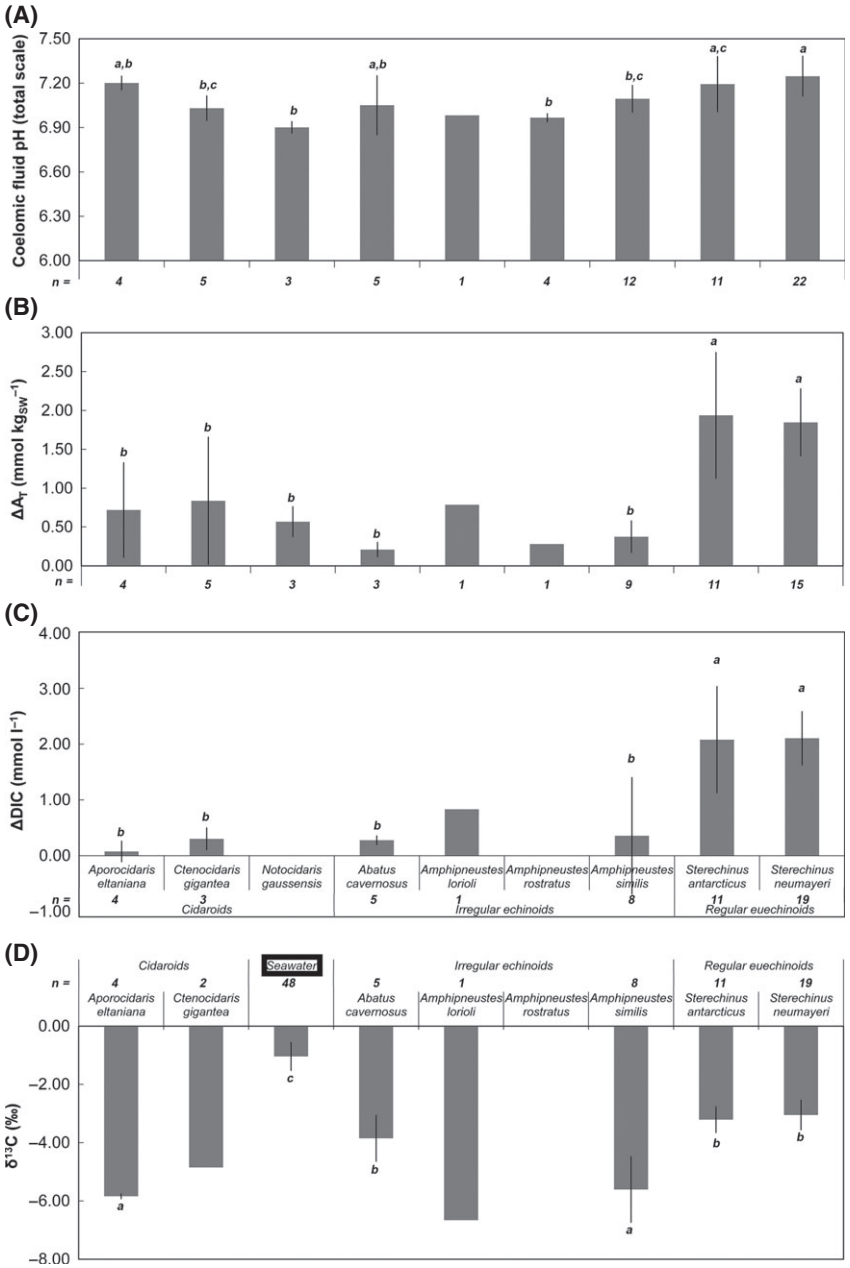

Fig. 1 Coelomic fluid acid–base parameters (mean ± SD) for the different sea urchin species studied. (A) pH_T, (B) buffer capacity (presented as $\Delta A_T = A_{T\text{-}CF} - A_{T\text{-}SW}$), (C) DIC content (presented as $\Delta DIC = DIC_{CF} - DIC_{SW}$), (D) carbon isotopic signature of DIC (sea urchin species and seawater). Means sharing the same letter are not significantly different ($P \geq 0.05$).

Size and gender had no impact on the measured pH_{CF}, ΔA_T and ΔDIC in all three groups of sea urchins ($P \geq 0.203$ for correlations between size and the physiological parameters and $P \geq 0.058$ for the differences between genders).

The pH measured in the different compartments of the brooding species (Fig. 2) showed significant differences. pH_{CF} of *A. rostratus* and *A. similis* were not significantly different (species*compartment $p_{ANOVA} = 0.303$, $p_{Tukey} = 0.168$). pH_{CF} was significantly lower than pH of the brooding chambers and seawater for both species ($p_{Tukey} < 10^{-3}$). pH of the brooding chambers were significantly different between the two species ($p_{Tukey} = 0.004$) but not from seawater ($p_{Tukey} \geq 0.059$). However, when pCO_2 was considered (Table 3), the pCO_{2CF} was significantly different between the two species (species*compartment $p_{ANOVA} < 10^{-3}$, $p_{Tukey} < 10^{-3}$), and in both cases was different from the seawater and the brooding chambers ($p_{Tukey} < 10^{-3}$). The pCO_2 in the brooding chambers of the two species

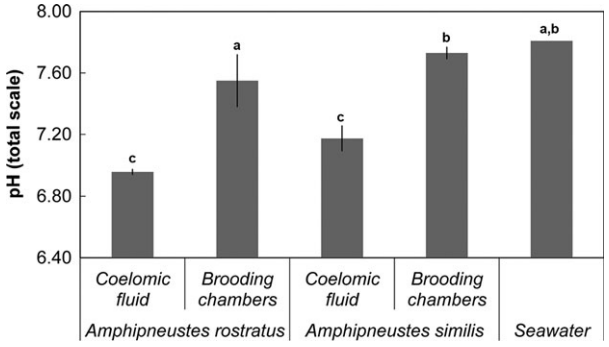

Fig. 2 pH (mean ± sd; $n = 4$ for *A. rostratus*, 3 for *A. similis*, 2 for seawater) measured in the different compartments (coelomic fluid, brooding chambers and surrounding seawater) of brooding species of irregular sea urchins. Means sharing the same letter are not significantly different ($P \geq 0.05$).

were not different from one another and from seawater ($p_{Tukey} \geq 0.328$).

Discussion

The results of this study showed that Antarctic echinoids present the same patterns of acid–base status than temperate and tropical sea urchins (Stumpp *et al*., 2012; Calosi *et al*., 2013; Collard *et al*., 2013, 2014; Moulin *et al*., 2014; Taylor *et al*., 2014). Indeed, the regular euechinoid species (*Sterechinus antarcticus* and *S. neumayeri*) have an increased buffer capacity [evidenced as total alkalinity (A_T)] of the coelomic fluid compared to the cidaroids and irregular echinoids sampled and to seawater. This increased buffer capacity is also linked to an increased DIC and so increased bicarbonate concentration, and to a higher pH_{CF}. Indeed, $A_{T\text{-}CF}$, as well as the dissolved inorganic carbon (DIC) content, highly differs according to the considered group. The $\delta^{13}C$ of the DIC_{CF} indicates principally a metabolic origin of the CO_2 in cidaroids (very low signal; as was shown for *Eucidaris tribuloides*) whereas that of the regular euechinoids is less negative, probably due to a mixing of the signals of metabolic and seawater CO_2 (as was shown for *Paracentrotus lividus* and *Tripneustes ventricosus*; Collard *et al*., 2014). The situation of irregular echinoids is unclear as one species mirrors the observations in cidaroids and the other species is similar to regular euechinoids.

The Antarctic regular euechinoids present a pH_{CF} of about 7.25, a ΔA_T ($A_{T\text{-}CF} - A_{T\text{-}SW}$) of about 1.9 mmol kg_{SW}^{-1}, and a ΔDIC ($DIC_{CF} - DIC_{SW}$) of about 2.1 mmol l^{-1}. Although these values are similar to those reported for temperate and tropical species of regular euechinoids (Catarino *et al*., 2012b; Stumpp *et al*., 2012; Calosi *et al*., 2013; Collard *et al*., 2013, 2014; Moulin *et al*., 2014; Taylor *et al*., 2014), their response to OA may differ in some aspects. Because Antarctic invertebrates have evolved in a very cold and stable environment, many physiological characteristics may be altered. For instance, living in the cold implies changes in membrane viscosity (homeoviscous response) and in enzyme activities (Clarke, 1998). Indeed, at low temperatures, the available kinetic energy for enzyme activities is reduced, and this is further reinforced by a slowed metabolism (Pörtner *et al*., 1998). However, changes in temperature lead to changes in mechanisms used to regulate acid–base disturbances. Ion leakage (passive diffusion of ions through membranes, or through water channels) is usually unaltered by a decrease in temperature, whereas the active ion pumps may see their activity reduced. This is usually referred to as membrane decoupling (Hochachka, 1973; Hochachka & Somero, 2002). Many different strategies exist to compensate for this differential effect. Cold stenothermal organisms mainly rely on passive transport for pH_e adjustments and this allows them to compensate faster for acid–base base changes (Pörtner *et al*., 1998). This faster compensation is evidenced when comparing sea urchins from environments differing in ambient temperature (Table 4). For a similar seawater pH decrease, the time needed to achieve pH_{CF} compensation in *Strongylocentrotus droebachiensis* from the Arctic is about 5 days, whereas it is of 10 days for the same species from the Baltic Sea. The temperate sea urchin *Paracentrotus lividus* from Brittany fully compensates pH_{CF} after 10-15 days and finally, *Tripneustes ventricosus*, a tropical sea urchin, requires 18 days (Dupont & Thorndyke, 2012; Stumpp *et al*., 2012; Collard *et al*., 2014). It thus seems that acid–base disturbance is compensated faster in sea urchins living in cold environments. Interestingly, *P. lividus* and *S. droebachiensis* from the Baltic Sea experience similar ambient temperatures and show similar compensation rates, whereas *S. droebachiensis* specimens that live in two very different temperature regimes differ in the time needed to compensate for acidosis. This may mean that Antarctic regular euechinoids will be able to compensate for pH_e changes when pH_{SW} is reduced. Indeed, the pH_{CF} of *Sterechinus spp*. did not differ with pH_{SW} over the range 7.6–7.9, indicating that in field conditions, *Sterechinus spp*. are able to maintain their pH_e.

Furthermore, it was shown that the buffer capacity of the CF in regular euechinoids is highly dependent on nutritional state, with fed individuals showing a higher $A_{T\text{-}CF}$ and a better compensation of pH_{CF} when exposed to reduced pH_{SW} (Stumpp *et al*., 2012; Collard *et al*., 2013). This results in low or no impact of OA on physiological processes of fed animals, whereas fasted sea urchins showed negative impacts (Stumpp *et al*.,

Table 4 Time for compensation of the acidosis of the coelomic fluid pH due to a decreased seawater pH for different regular euechinoids

Species	Location Depth	Temperature range in the field	Temperature tested	Control pH	ΔpH tested	Days needed for compensation
Strongylocentrotus droebachiensis	Disko Bay, Greenland 1-2 m	−2 °C to 5 °C*,†	10 °C	8.2	−0.5	5 days‡
Strongylocentrotus droebachiensis	Baltic Sea (Kiel), Germany 17 m	3 °C to 18 °C§,**	10 °C	7.8	−0.3	10 days††
Paracentrotus lividus	Crozon (Brittany), France 0 m (intertidal)	10 °C to 17 °C‡‡,§§	10 °C	8.0	−0.6	10–15 days***
Tripneustes ventricosus	Discovery Bay, Jamaica 1-5 m	27 °C to 30 °C†††,‡‡‡	28 °C	8.0	−0.6	18 days***

*Madsen *et al.*, 2001; .
†Heide-Jørgensen *et al.*, 2007.
‡Dupont & Thorndyke, 2012.
§Thomsen *et al.*, 2010; .
**Seigel & Gerth, 2013.
††Stumpp *et al.*, 2012.
‡‡Goulletquer *et al.*, 2002.
§§Plouguerné *et al.*, 2006.
***Collard *et al.*, 2014.
†††Gayle & Woodley, 1998.
‡‡‡Quinn & Kojis, 2003.

2012; Taylor *et al.*, 2014). Food supply in Antarctic waters is highly variable in amount and time, and is expected to change with shortening periods of sea ice cover, as linked to global change (e.g., Smith *et al.*, 2006; Turner *et al.*, 2013). Usually, a high quantity of food is available in spring/summer following algal blooms and declines in fall/winter when ice covers the sea surface. This may preclude sea urchins from compensating acidosis during winter month, or reduce allocation of resources from their reserves for growth and/or reproduction to favor acid–base regulation. However, conditions may differ among different locations, due to differences in substrates. For instance, along the Western Antarctic Peninsula, Brockington *et al.* (2001) observed that *S. neumayeri* individuals were not feeding at all during winter months in Adelaide Island (west of the Antarctic Peninsula, Marguerite Bay, 67°S) as food availability is low, which also resulted in a decreased metabolism (Brockington & Peck, 2001). On the other hand, Wigham *et al.* (2008) and DeMaster *et al.* (in Smith *et al.*, 2006) showed that *S. antarcticus* never stopped feeding near Anvers Island (west of Antarctic Peninsula, Bellingshausen Sea, 65°S). The differences are probably linked to the different local conditions, particularly regarding substrates. Indeed, in the first case, *S. neumayeri* was found on rocky substrate whereas the other two locations are characterized by muddy sand bottom where organic matter may accumulate and serve as a continuous food source for detritivores (Norkko *et al.*, 2007; Wigham *et al.*, 2008).

The coelomic fluid of cidaroids and irregular echinoids has a very low pH of about 7.05 (all species taken together), a ΔA_T ($A_{T\text{-}CF} - A_{T\text{-}SW}$) of about 0.5 mmol kg_{SW}^{-1}, and a ΔDIC ($DIC_{CF} - DIC_{SW}$) of about 0.3 mmol l^{-1}. This corresponds to values found previously for temperate and tropical cidaroid and irregular echinoid species (Spicer & Widdicombe, 2012; Collard *et al.*, 2013, 2014). The very low pH_{CF} found in most of these species will make changes in seawater pH/pCO_2 expected during the 21st century negligible and might allow for a very low acid–base balance maintenance with only limited energy needed for ion active transports (see Collard *et al.*, 2014). The only data available to date for a cidaroid species (tropical *Eucidaris tribuloides*) showed that the acid–base status of the sea urchin was not affected by progressive exposure to reduced seawater pH (−0.4 and −0.7 pH units from control pH 8.0; Collard *et al.*, 2014). On the contrary, data for one irregular echinoid (temperate *Brissopsis lyrifera*, from a depth of 70 m) showed an acidosis of the coelomic fluid pH when they were exposed without acclimation to reduced seawater pH for a maximum of 12 h (−0.7 pH units from control pH 7.7; Spicer & Widdicombe, 2012). This is the only available data to date, and it does not allow estimation of a possible compensation of pH_{CF} after longer periods of time. It is noteworthy that the pH_{CF} of irregular echinoids analyzed in this study did not correlate with pH_{SW} over the range 7.6–7.9.

The saturation state of the coelomic fluid for calcite was lower than 1 in regular euechinoids (0.6–0.7),

and in the cidaroid and irregular echinoid species (0.2–0.3). The aragonite saturation state was also lower than 1 for all species (0.1–0.4), although regular euechinoids showed a saturation state twice as high as the other species. Antarctic echinoids have a skeleton composed of high-magnesium calcite with percentages of $MgCO_3$ ranging from 5.9 to 15.8 mol% $MgCO_3$ (Weber, 1969; McClintock *et al.*, 2011). Thus, the solubility of their skeleton is similar or higher than that of aragonite (Morse *et al.*, 2006). Yet, the studied sea urchins showed an undersaturated state within their extracellular fluid under field conditions. Furthermore, 50% of the stations where echinoids were sampled showed undersaturated values for seawater aragonite saturation state (Table 1). So although the average depth of the ASH is 730 m today (McNeil & Matear, 2008), some shallower places are already undersaturated regarding aragonite during summer time (this condition might be only temporary; McNeil *et al.*, 2011). These values may explain decreased shell thickness in Antarctic euechinoids compared to values for temperate and tropical species (Watson *et al.*, 2012), although intertaxa comparisons are difficult to handle and many other factors, the principal one being wave exposure, are known to affect skeleton thickness, a highly plastic character (Lewis & Storey, 1984; Edwards & Ebert, 1991; Rogers-Bennett *et al.*, 1995). However, very low values of aragonite saturation state in the coelomic fluid are also observed for cidaroids both in tropical and polar environments (Collard *et al.*, 2014; this study). Despite this, most cidaroids have thick tests. On the contrary, most spatangoid irregular echinoids (*B. lyrifera*, *E. cordatum*, and species in this study) have thin tests whatever their origin. So it seems that test thickness is not linked to the acid–base status of the coelomic fluid nor of seawater, in the range of currently occurring values. Additionally, it was predicted that brooding irregular echinoids would be the most impacted group as the juveniles would be exposed to the surrounding seawater changes in chemistry (Sewell & Hofmann, 2011). Neither the pH nor the pCO_2 differed significantly between the surrounding seawater and that in the brooding chambers. This is probably explained by the epidermal cilia covering the female epidermis. Thus, respiration of the female and juveniles did not induce a hypercapnia within the brooding chambers. Consequently, brooded juveniles are submitted to similar seawater carbonate chemistry conditions than juveniles from sea urchins with an indirect development for which no brooding occurs.

In view of our results, Antarctic regular euechinoids seem equipped with the same acid–base regulation as tropical and temperate regular euechinoids. However, the costs of this regulation might be higher as the energy available to Antarctic ectotherms is well below that of temperate or tropical species due to differences in standard metabolic rates (Peck, 1998). However, this may be counterbalanced by relying principally on faster passive transport for pH_e regulation. The process of calcification itself will probably not be affected as these organisms already calcify in undersaturated external and internal environments. Antarctic cidaroids have a similar acid–base physiology to that of tropical cidaroids and will most probably not be affected by decreasing seawater pH, as seen for the only studied tropical cidaroid. Irregular echinoids might not suffer from reduced seawater pH as long as acidosis of the coelomic fluid pH does not occur when they are exposed to ocean acidification (response similar to that of cidaroids). However, more data on acid–base regulation in irregular echinoids facing experimental acidification are needed to assess the impact of OA on this group. Juveniles of brooding forms are unlikely to suffer more than juveniles of indirect developers from changes in seawater chemistry as the conditions in the brooding chambers did not differ from that of the surrounding seawater. Moreover, no major impact of ocean acidification was shown for larvae of Antarctic sea urchins (Clark *et al.*, 2009; Ericson *et al.*, 2010, 2012; Catarino *et al.*, 2012a; Byrne *et al.*, 2013; Ho *et al.*, 2013; Yu *et al.*, 2013; Kapsenberg & Hofmann, 2014). Furthermore, larvae of *S. neumayeri* were shown to better tolerate ocean acidification than temperate and tropical species (Clark *et al.*, 2009) and it is the only species for which no delayed development was reported (Kapsenberg & Hofmann, 2014). All these data strongly suggest that Antarctic sea urchins are not particularly at risk in front of ocean acidification if compared with temperate and tropical species.

However, this possible tolerance to ocean acidification does not predict the response of these organisms to the other factors linked to global change like warming or changes in the habitat due to ice melting. Indeed, previous studies have shown that temperature could be a greater stressor for Antarctic species (Peck, 2005; Peck *et al.*, 2013). Some organisms, including echinoderms, have been shown to lose some essential biological functions such as burying or righting behavior when exposed to increased temperature, possibly affecting other processes such as food collection (Peck, 2005). Acid–base regulation mechanisms can also be affected through the influence of temperature on enzymes and/or passive vs. nonpassive processes (Hochachka & Somero, 2002; Somero, 2004). Early-life stages were also shown to be more affected by temperature or the com-

bination of temperature and pH compared to pH as a single stressor (Ericson *et al*., 2012; Byrne *et al*., 2013; Kapsenberg & Hofmann, 2014). Moreover, climate change creates new challenges for Antarctic echinoids, such as the recent invasion by king crabs (Fabry *et al*., 2009). The lack of durophagous (crushing) predators in the Antarctic waters has resulted in marine invertebrates with weakly calcified skeletons. They could then become easy prey for those king crabs (Fabry *et al*., 2009).

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References

Andersson A, Mackenzie FT, Bates NR (2008) Life on the margin: implications of ocean acidification on Mg-calcite, high latitude and cold-water marine calcifiers. *Marine Ecology Progress Series*, **373**, 265–273.

Arntz WE, Brey T, Gallardo VA (1994) Antarctic zoobenthos. *Oceanography and Marine Biology: An Annual Review*, **32**, 241–304.

Barnes DK, Brockington S (2003) Zoobenthic biodiversity, biomass and abundance at Adelaide Island, Antarctica. *Marine Ecology Progress Series*, **249**, 145–155.

Brockington S, Peck LS (2001) Seasonality of respiration and ammonium excretion in the Antarctic echinoid *Sterechinus neumayeri*. *Marine Ecology Progress Series*, **219**, 159–168.

Brockington S, Clarke A, Chapman ALP (2001) Seasonality of feeding and nutritional status during the austral winder in the Antarctic sea urchin *Sterechinus neumayeri*. *Marine Biology*, **139**, 127–138.

Byrne M, Ho MA, Koleits L *et al*. (2013) Vulnerability of the calcifying larval stage of the Antarctic sea urchin *Sterechinus neumayeri* to near-future ocean acidification and warming. *Global Change Biology*, **19**, 2264–2275.

Calosi P, Rastrick SPS, Graziano M *et al*. (2013) Distribution of sea urchins living near shallow water CO_2 vents is dependent upon species acid-base and ion-regulatory abilities. *Marine Pollution Bulletin*, **73**, 470–484.

Catarino A, De Ridder C, Gonzalez M, Gallardo P, Dubois P (2012a) Sea urchin *Arbacia dufresnei* (Blainville 1825) larvae response to ocean acidification. *Polar Biology*, **35**, 455–461.

Catarino AI, Bauwens M, Dubois P (2012b) Acid-base balance and metabolic response of the sea urchin *Paracentrotus lividus* to different seawater pH and temperatures. *Environmental Science and Pollution Research*, **19**, 2344–2353.

Clark D, Lamare M, Barker M (2009) Response of sea urchin pluteus larvae (Echinodermata: Echinoidea) to reduced seawater pH: a comparison among a tropical, temperate, and a polar species. *Marine Biology*, **156**, 1125–1137.

Clarke A (1998) Temperature and energetics: an introduction to cold ocean physiology. In: *Society for Experimental Biology, Seminar Series: 66, Cold Ocean Physiology* (eds Pörtner HO, Playle RC), pp. 365–390. Cambridge University Press, New York, USA.

Collard M, Laitat K, Moulin L, Catarino AI, Grosjean P, Dubois P (2013) Buffer capacity of the coelomic fluid in echinoderms. *Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology*, **166**, 199–206.

Collard M, Dery A, Dehairs F, Dubois P (2014) Euechinoids vs. Cidaroids: are pencil sea urchins better adapted to tolerate ocean acidification? *Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology*, **174**, 45–55.

Cubillos JC, Wright SW, Nash G *et al*. (2007) Calcification morphotypes of the coccolithophorid *Emiliana huxleyi* in the Southern Ocean: changes in 2001 to 2006 compared to historical data. *Marine Ecology Progress Series*, **348**, 47–54.

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

David B, Choné T, Festeau A, Mooi R, De Ridder C (2005a) Biodiversity of Antarctic echinoids: a comprehensive and interactive database. *Scientia Marina*, **69**, 201–203.

David B, Choné T, Mooi R, De Ridder C (2005b)*Antarctic Echinoidea*. *Synopses of the Antarctic Benthos* Vol. 10. Koeltz Scientific Books, Königstein.

DelValls TA, Dickson AG (1998) The pH of buffers based on 2-amino-2-hydroxymethyl-1,3-propanediol ('tris') in synthetic seawater. *Deep Sea Research I: Oceanographic Research Papers*, **45**, 1541–1554.

Dickson AG (1990) Standard potential of the reaction $AgCl_s + ½\ H_2 = Ag_s + HCl_{Aq}$ and the standard acidity constant of the ion HSO_4^- in synthetic seawater from 273.15-K to 318.15-K. *The Journal of Chemical Thermodynamics*, **22**, 113–127.

Dickson AG, Millero FJ (1987) A comparison of the equilibrium-constants for the dissociation of carbonic-acid in seawater media. *Deep Sea Research I: Oceanographic Research Papers*, **34**, 1733–1743.

Doncaster CP, Davey AJ (2007) *Analysis of Variance and Covariance*. Cambridge University Press, Cambridge, UK.

Dupont S, Thorndyke M (2012) Relationship between CO_2-driven changes in extracellular acid-base balance and cellular immune response in two polar echinoderm species. *Journal of Experimental Marine Biology and Ecology*, **424–425**, 32–37.

Edwards PB, Ebert TA (1991) Plastic response to limited food availability and spine damage in the sea urchin *Strongylocentrotus purpuratus*. *Journal of Experimental Marine Biology and Ecology*, **145**, 295–320.

Ericson JA, Lamare MD, Morley SA, Barker MF (2010) The response of tow ecologically important Antarctic invertebrates (*Sterechinus neumayeri* and *Parborlasia corrugatus*) to reduced seawater pH: effets on fertilization and embryonic development. *Marine Biology*, **22**, 160–171.

Ericson JA, Ho MA, Miskelly A, King CK, Virtue P, Tilbrook B, Byrne M (2012) Combined effects of two ocean change stressors, warming and acidification, on fertilization and early development of the Antarctic echinoid *Sterechinus neumayeri*. *Polar Biology*, **35**, 1027–1034.

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

Feely R, Sabine C, Lee K, Berelson W, Kleypas J, Fabry V, Millero F (2004) Impact of anthropogenic CO_2 on the $CaCO_3$ system in the oceans. *Science*, **305**, 362–366.

Gayle PMH, Woodley JD (1998) Discovery Bay, Jamaica. In: CARICOMP - Coral Reef, Segrass and Magrove Sites (ed. Kjerfve BJ). Costal region and small island papers, n°3.

Gillikin DP, Bouillon S (2007) Determination of $\delta^{18}O$ of water and $\delta^{13}C$ of dissolved inorganic carbon using a simple modification of an elemental analyzer-isotope ratio mass spectrometer (EA-IRMS): an evaluation. *Rapid Communications in Mass Spectrometry*, **21**, 1475–1478.

Gillikin DP, Lorrain A, Meng L, Dehairs F (2007) A large metabolic contribution to the $\delta^{13}C$ record in marine aragonitic bivalve shells. *Geochimica et Cosmochimica Acta*, **71**, 2936–2946.

Gonzalez-Bernat MJ, Lamare M, Barker M (2013) Effects of reduced seawater pH on fertilisation, embryogenesis and larval development in the Antarctic sea star *Odontaster validus*. *Polar Biology*, **36**, 235–247.

Goulletquer P, Bachelet G, Sauriau PG, Noel P (2002) Opean Atlantic Coast of Europe – a century of introduced species into french waters. In: *Invasive Aquatic Species of Europe: Distribution, Impacts and Management* (eds Leppäkoski E, Gollash S, Olenin S), pp. 276–290. Springer, The Netherlands.

Gran G (1952) Determination of the equivalence point in potentiometric titrations Part II. *Analyst*, **77**, 661–671.

Grange LJ, Smith CR (2013) Megafaunal communities in rapidly warming fjords along the West Antarctic Peninsula: hotspots of abundance and beta diversity. *PLoS ONE*, **8**, e77917.

Gutt J, Starmans A (1998) Structure and biodiversity of megabenthos in the Weddell and Lazarev Seas (Antarctica): ecological role of physical parameters and biological interactions. *Polar Biology*, **20**, 229–247.

Heide-Jørgensen MP, Laidre KL, Logsdon ML, Nielsen TG (2007) Springtime coupling between chlorophyll *a*, sea ice and sea surface temperature in Disko Bay, West Greenland. *Progress in Oceanography*, **73**, 79–95.

Ho MA, Price C, King CK, Virtue P, Byrne M (2013) Effects of ocean warming and acidification on fertilization in the Antarctic echinoid *Sterechinus neumayeri* across a range of sperm concentrations. *Marine Environmental Research*, **90**, 136–141.

Hochachka PW (1973) Basic strategies and mechanisms of enzyme adaptation to temperature. In: *Effects of Temperature on Ectothermic Organisms* (ed. Wieser W), pp. 69–81. Springer, Berlin, Germany.

Hochachka PW, Somero GN (2002) *Biochemical Adaptation: Mechanism and Process in Physiological Evolution*. Oxford University Press, New York, USA.

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 Biology*, **37**, 967–980.

Kurihara H, Yin R, Nishihara GN, Soyano K, Ishimatsu A (2013) Effect of ocean acidification on growth, gonad development and physiology of the sea urchin *Hemicentrotus pulcherrimus*. *Aquatic Biology*, **18**, 281–292.

Lebrato M, Iglesias-Rodriguez D, Feely RA *et al.* (2010) Global contribution of echinoderms to the marine carbon cycle: $CaCO_3$ budget and benthic compartments. *Ecological Monographs*, **80**, 441–467.

Lewis JB, Storey GS (1984) Differences in morphology and life history traits of the echinoid *Echinometra lucunter* from different habitats. *Marine Ecology Progress Series*, **15**, 207–211.

Lockhart SJ, Jones CD (2008) Biogeographic patterns of the benthic invertebrate megafauna on shelf areas within the Southern Ocean Atlantic sector. *CCAMLR Science*, **15**, 167–192.

Madsen SD, Nielsen TG, Hansen BW (2001) Annual population development and production by *Calanus finmarchicus*, *C. glacialis* and *C. hyperboreus* in Disko Bay, western Greenland. *Marine Biology*, **139**, 75–93.

McClintock JB, Angus RA, McDonald MR, Amsler CD, Catledge SA, Vohra YK (2009) Rapid dissolution of shells of weakly calcified Antarctic benthic macroorganisms indicates high vulnerability to ocean acidification. *Antarctic Science*, **21**, 449–456.

McClintock JB, Amsler MO, Angus RA *et al.* (2011) The Mg-calcite composition of Antarctic Echinoderms: important implications for predicting the impact of ocean acidification. *The Journal of Geology*, **119**, 457–466.

McNeil BI, Matear RJ (2008) Southern Ocean acidification: a tipping point at 450-ppm atmospheric CO_2. *Proceedings of the National Academy of Sciences of The United States of America*, **105**, 18860–18864.

McNeil BI, Sweeney C, Gibson JAE (2011) Natural seasonal variability of aragonite saturation state within two Antarctic coastal ocean sites. *Antarctic Science*, **23**, 411–412.

Mehrbach C, Culberso CH, Hawley JE, Pytkowic RM (1973) Measurement of apparent dissociation-constants of carbonic-acid in seawater at atmospheric-pression. *Limnology and Oceanography*, **18**, 897–907.

Melzner F, Gutowska MA, Langenbuch M *et al.* (2009) Physiological basis for high CO_2 tolerance in marine ectothermic animals: pre-adaptation through lifestyle and ontogeny? *Biogeosciences*, **6**, 2313–2331.

Morse JW, Andersson AJ, Mackenzie FT (2006) Initial responses of carbonate-rich shelf sediments to rising atmospheric pCO_2 and 'ocean acidification': role of high Mg-calcites. *Geochimica et Cosmochimica Acta*, **70**, 5814–5830.

Moulin L, Grosjean P, Leblud J, Batigny A, Dubois P (2014) Impact of elevated pCO_2 on acid-base regulation of the sea urchin *Echinometra mathaei* and its relation to resistance to ocean acidification: a study in mesocosms. *Journal of Experimental Marine Biology and Ecology*, **457**, 97–104.

Moy AD, Howard WR, Bray SG, Trull TW (2009) Reduced calcification in modern Southern Ocean planktonic foraminifera. *Nature Geosciences*, **2**, 276–280.

Norkko A, Thrush SF, Cummings VJ, Gibbs MM, Andrew NL, Norkko J, Shwarz AM (2007) Trophic structure of coastal Antarctic food webs associated with changes in sea ice and food supply. *Ecology*, **88**, 2810–2820.

Orr JC (2011) Recent and future changes in ocean carbonate chemistry. In: *Ocean Acidification* (eds Gattuso JP, Hansson L), pp. 41–66. Oxford University Press, UK.

Orr JC, Fabry VJ, Aumont O *et al.* (2005) Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature*, **437**, 681–686.

Peck L (1998) Feeding, metabolism and metabolic scope in Antarctic marine ectotherms. In: *Society for Experimental Biology, Seminar Series: 66, Cold Ocean Physiology* (eds Pörtner HO, Playle RC), pp. 365–390. Cambridge University Press, New York, USA.

Peck L (2005) Prospects for surviving climate change in Antarctic aquatic species. *Frontiers in Zoology*, **2**, 9.

Peck L, Souster T, Clark MS (2013) Juveniles are more resistant to warming than adults in species of Antarctic marine invertebrates. *PLoS ONE*, **8**, e66033.

Pierrot D, Lewis E, Wallace DWR (2006) *MS Excel Program Developed for CO_2 System Calculations. ORNL/CDIAC-105a*. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, U.S. Department of Energy, Oak Ridge, Tennessee.

Plouguerné E, Le Lann K, Connan S, Jechoux G, Deslandes E, Stiger-Pouvreau V (2006) Spatial and seasonal variation in density, reproductive status, length and phenolic content of the invasive brown macroalga *Sargassum muticum* (Yendo) Fensholt along the coast of Western Brittany (France). *Aquatic Botany*, **85**, 337–344.

Pörtner HO, Hardewig I, Sartoris FJ, Van Dijk PLM (1998) Energetic aspects of cold adaptation: critical temperatures in metabolic, ionic and acid-base regulation? In: *Society for Experimental Biology, Seminar Series: 66, Cold Ocean Physiology* (eds Pörtner HO, Playle RC), pp. 88–120. Cambridge University Press, New York, USA.

Pörtner HO (2008) Ecosystem effects of ocean acidification in times of ocean warming: a physiologist's view. *Marine Ecology Progress Series*, **373**, 203–217.

Pörtner HO, Gutowska M, Ishimatsu A, Lucassen M, Melzner F, Seibel BA (2011) Effets of ocean acidification on nektonic organisms. In: *Ocean Acidification* (eds Gattuso JP, Hansson L), pp. 154–175. Oxford University Press, UK.

Quinn NJ, Kojis BL (2003) Variation in subsurface seawater temperature off Discovery Bay, Jamaica and the U.S Virgin Islands. *Revista de Biologia Tropical*, **51**, 181–187.

Rogers-Bennett L, Bennett WA, Fastenau HC, Dewees CM (1995) Spatial variation in red sea urchin reproduction and morphology: implications for harvest refugia. *Journal of Applied Ecology*, **5**, 1171–1180.

Schröder M, Wisotzki A, van Caspel M (2013) Physical oceanography measured on water bottle samples during POLARSTERN cruise ANT-XXIX/3. *Alfred Wegener Institute, Helmholtz Center for Polar and Marine Research, Bremerhaven*, doi:10.1594/PANGAEA.811818.

Seibel BA, Walsh PJ (2001) Potential impacts of CO_2 injection on deep-sea biota. *Science*, **294**, 319–320.

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.

Seigel H, Gerth M (2013) Sea surface temperature in the Baltic Sea in 2012. HELCOM Baltic Sea Environment Fact Sheets. Available at: http://www.helcom.fi/baltic-sea-trends/environment-fact-sheets/ (accessed 30 03 2014).

Sewell MA, Hofmann GE (2011) Antarctic echinoids and climate change: a major impact on the brooding forms. *Global Change Biology*, **17**, 734–744.

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. *Environmental Science and Technology*, **48**, 713–722.

Smith CR, Mincks S, DeMaster DJ (2006) A synthesis of bentho-pelagic coupling on the Antarctic shelf: food banks, ecosystem intertia and global climate change. *Deep-Sea Research II*, **53**, 875–894.

Somero G (2004) Adaptation of enzymes to temperature: searching for basic 'strategies'. *Comparative Biochemistry and Physiology B: Biochemistry & Molecular Biology*, **139**, 321–333.

Spicer JI, Widdicombe S (2012) Acute extracellular acid-base disturbance in the burrowing sea urchin *Brissopsis lyrifera* during exposure to a simulated CO_2 release. *Science of the Total Environment*, **427–428**, 203–207.

Spicer JI, Taylor AC, Hill AD (1988) Acid-base status in the sea urchins *Psammechinus miliaris* and *Echinus esculentus* (Echinodermata: Echinoidea) during emersion. *Marine Biology*, **99**, 527–534.

Stumpp M, Trübenbach K, Brennecke D, Hu MY, Melzner F (2012) Resource allocation and extracellular acid-base status in the sea urchin *Strongylocentrotus droebachiensis*. *Aquatic Toxicology*, **110–111**, 194–207.

Takahashi T, Sutherland SC, Wanninkhof R *et al.* (2009) Climatological mean and decadal change in surface ocean pCO_2 and net sea-air CO_2 flux over the global oceans. *Deep-Sea Research II*, **56**, 554–577.

Taylor JR, Lovera C, Whaling PJ, Buck KR, Pane EF, Barry JP (2014) Physiological effects of environmental acidification in the deep-sea urchin *Strongylocentrotus fragilis*. *Biogeosciences*, **11**, 1413–1423.

Thomsen J, Gutowska MA, Saphörster J *et al.* (2010) Calcifying invertebrates succeed in a naturally CO_2-rich coastal habitat but are threatened by high levels of future acidification. *Biogeosciences*, **7**, 3879–3891.

Turner J, Barrand NE, Bracegirdle TJ *et al.* (2013) Antarctic climate change and the environment: an update. *Polar Record*, **50**, 237–259.

Walker BJ, Miller MF, Bowser SS, Furbish DJ, Gualda GAR (2013) Dissolution of ophiuroid ossicles on the shallow Antarctic shelf: implications for the fossil record and ocean acidification. *Palaios*, **28**, 317–332.

Watson SA, Peck LS, Tyler PA, Southgate PC, Siangtan K, Day KW, Morley SA (2012) Marine invertebrate skeleton size varies with latitude, temperature and carbonate saturation: implications for global change and ocean acidification. *Global Change Biology*, **18**, 3026–3038.

Weber J (1969) The incorporation of magnesium into the skeletal calcites of echinoderms. *American Journal of Science*, **267**, 537–566.

White BA, McClintock J, Amsler CD *et al.* (2012) The abundance and distribution of echinoderms in nearshore hard-bottom habitats near Anvers Island, western Antarctic Peninsula. *Antarctic Science*, **24**, 554–560.

Wigham BD, Galley EA, Smith CR, Tyler PA (2008) Inter-annual variability and potential for selectivity in the diets of deep-water Antarctic echinoderms. *Deep-Sea Research II*, **55**, 2478–2490.

Yu PC, Sewell MA, Matson PG, Rivest EB, Kapsenberg L, Hofmann GE (2013) Growth attenuation with developmental schedule progression in embryos and early larvae of *Sterechinus neumayeri* raised under elevated CO_2. *PLoS ONE*, **8**, e52448.

Zeebe RE, Wolf-Gladrow D (2001) *CO2 in Seawater: Equilibrium, Kinetics, Isotopes*. Elsevier Science B.V, Amsterdam, The Netherlands. 346 pp.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Geographic coordinates and location of the different collection sites and *in situ* measurements of oxygen saturation at those sites. Depth is the mean value during the Agassiz trawling.

Table S2. Measured parameters of the coelomic fluid (CF) and seawater *in situ* (SW). A_T: total alkalinity (mmol kg_{SW}^{-1}); DIC: dissolved inorganic carbon (mmol l^{-1}); $\delta^{13}C$: carbon isotopic signature (‰). pH measurements are total scale except pH_{CF} of 26/01/2013.