



## The elemental composition of purple sea urchin (*Strongylocentrotus purpuratus*) calcite and potential effects of $p\text{CO}_2$ during early life stages

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**Abstract.** Ocean acidification will likely have negative impacts on invertebrates producing skeletons composed of calcium carbonate. Skeletal solubility is partly controlled by the incorporation of “foreign” ions (e.g. magnesium) into the crystal lattice of these skeletal structures, a process that is sensitive to a variety of biological and environmental factors. Here we explore effects of life stage, oceanographic region of origin, and changes in the partial pressure of carbon dioxide in seawater ( $p\text{CO}_2$ ) on trace elemental composition in the purple sea urchin (*Strongylocentrotus purpuratus*). We show that, similar to other urchin taxa, adult purple sea urchins have the ability to precipitate skeleton composed of a range of biominerals spanning low- to high-Mg calcites. Mg/Ca and Sr/Ca ratios were substantially lower in adult spines compared to adult tests. On the other hand, trace elemental composition was invariant among adults collected from four oceanographically distinct regions spanning a range of carbonate chemistry conditions (Oregon, Northern California, Central California, and Southern California). Skeletons of newly settled juvenile urchins that originated from adults from the four regions exhibited intermediate Mg/Ca and Sr/Ca between adult spine and test endmembers, indicating that skeleton precipitated during early life stages is more soluble than adult spines and less soluble than adult tests. Mean skeletal Mg/Ca or Sr/Ca of juvenile skeleton did not vary with

source region when larvae were reared under present-day, global-average seawater carbonate conditions ( $400\ \mu\text{atm}$ ;  $\text{pH}_T = 8.02 \pm 0.03$  1 SD;  $\Omega_{\text{calcite}} = 3.3 \pm 0.2$  1 SD). However, when reared under elevated  $p\text{CO}_2$  ( $900\ \mu\text{atm}$ ;  $\text{pH}_T = 7.73 \pm 0.03$ ;  $\Omega_{\text{calcite}} = 1.8 \pm 0.1$ ), skeletal Sr/Ca in juveniles exhibited increased variance across the four regions. Although larvae from the northern populations (Oregon, Northern California, Central California) did not exhibit differences in Mg or Sr incorporation under elevated  $p\text{CO}_2$  ( $\text{Sr/Ca} = 2.10 \pm 0.06\ \text{mmol mol}^{-1}$ ;  $\text{Mg/Ca} = 67.4 \pm 3.9\ \text{mmol mol}^{-1}$ ), juveniles of Southern California origin partitioned  $\sim 8\%$  more Sr into their skeletons when exposed to higher  $p\text{CO}_2$  ( $\text{Sr/Ca} = 2.26 \pm 0.08$  vs.  $2.09 \pm 0.005\ \text{mmol mol}^{-1}$  1 SD).

Together these results suggest that the diversity of carbonate mineralogies present across different skeletal structures and life stages in purple sea urchins does not translate into an equivalent geochemical plasticity of response associated with geographic variation or temporal shifts in seawater properties. Rather, composition of *S. purpuratus* skeleton precipitated during both early and adult life history stages appears relatively robust to spatial gradients and predicted future changes in carbonate chemistry. An exception to this trend may arise during early life stages, where certain populations of purple sea urchins may alter skeletal mineral precipitation rates and composition beyond a given  $p\text{CO}_2$

threshold. This potential for geochemical plasticity during early development in contrast to adult stage geochemical resilience adds to the growing body of evidence that ocean acidification can have differing effects across organismal life stages.

## 1 Introduction

Rising levels of atmospheric carbon dioxide (CO<sub>2</sub>) have resulted in increased dissolution of CO<sub>2</sub> in seawater and reduced pH of the upper ocean (Caldeira and Wickett, 2003; Byrne et al., 2010). This “acidification” of surface waters is associated with decreased carbonate ion concentration and reduced saturation states ( $\Omega$ ) of the calcium carbonate (CaCO<sub>3</sub>) minerals used by marine calcifiers to build shells and tests. A rapidly growing body of evidence has revealed a wide array of adult calcification responses to ocean acidification across invertebrate taxa (e.g. Orr et al., 2005; Fabry et al., 2008; Doney et al., 2009; Ries et al., 2009; Kroeker et al., 2010; Ries, 2011). Additional studies have focused on implications of ocean acidification for calcifying planktonic larvae, which are also sensitive to elevated CO<sub>2</sub> with important consequences for ensuing juvenile stages and population dynamics (Kurihara, 2008; Byrne, 2011; Gaylord et al., 2011; Hettinger et al., 2012). The character and magnitude of adult and larval responses, however, often diverge, which could be related to the fact that skeleton precipitated during early developmental stages is commonly composed of different calcium carbonate polymorphs (i.e. aragonite, calcite, high-magnesium calcite) than those used by adults, and often involves highly soluble amorphous precursor mineral phases (e.g. Weiss et al., 2002; Politi et al., 2006).

Biomineral solubility – a measure of how robust CaCO<sub>3</sub> structures may be to a depressed availability of carbonate ions – depends on skeletal mineral structure and elemental composition as well as seawater chemistry. In addition to crystal size, microstructure, and surface area, the substitution of “foreign ions”, such as magnesium (Mg) for calcium (Ca) into abiotic and biotic calcite crystal lattice, is known to substantially increase mineral solubility within a single polymorph (Chave et al., 1962; Walter and Morse, 1983; Morse et al., 2006, 2007). Some work has demonstrated, however, that the geochemical behavior of biologically precipitated minerals does not strictly follow predictions based on abiotic experimental results (Henrich and Wefer, 1986; Ries et al., 2009; Ries, 2011; Nash et al., 2012) and that synthetic minerals may not always be representative of biogenic skeleton (Bischoff et al., 1987). The degree to which ion substitution occurs is sensitive to environmental variables such as temperature (Chave, 1954), seawater composition (Ries, 2010), skeletal growth rate (Carpenter and Lohmann, 1992; De Choudens-Sánchez and Gonzáles, 2009), and seawater saturation state (Lee and Morse, 2010;

Ries, 2011). In some marine calcifiers, the sensitivity to temperature is reliable enough that Mg/Ca and Sr/Ca ratios preserved in the skeleton, shells, and tests of marine organisms can be utilized to reconstruct the thermal properties of past oceans (e.g. Beck et al., 1992; Rosenthal et al., 1997; Lea et al., 1999). In other groups, however, there is considerable variation in the elemental response to these parameters in both culture and inorganic calcite precipitation experiments (e.g. Mucci and Morse, 1983; Morse and Bender, 1990; Russell et al., 2004; Dissard et al., 2010). The effects of environmental variation on echinoderm skeletal Mg or Sr substitution rates can be large and variable across taxa, with recent work by Ries (2011) showing that coralline red algae exposed to elevated *p*CO<sub>2</sub> incorporate ~20% less Mg into their skeleton than under control conditions, whereas serpulid worms incorporate ~26% more Mg into their tubes. Portions of these differences may stem from variation in the sensitivity of species-specific mineralization pathways to *p*CO<sub>2</sub> (Ries, 2011).

CO<sub>2</sub> concentrations in the ocean also vary geographically, driven by differences in prevailing oceanographic conditions. This feature raises the additional untested possibility that individuals originating in different regions might exhibit local adaptation (e.g. Sanford and Kelly, 2011), and may therefore respond to environmental factors in unique ways due to underlying genetic variation. Because continued oceanic absorption of anthropogenic CO<sub>2</sub> will lower seawater calcite and aragonite saturation states ( $\Omega$ ), not only is it likely that the abundance of calcifiers precipitating less-stable minerals (e.g. high-Mg calcite) will decrease, but also life stages utilizing this more soluble mineral will be negatively impacted in future ocean ecosystems (Feely and Chen, 1982; Feely et al., 1988; Orr et al., 2005; Andersson et al., 2008; Doney et al., 2009). Therefore, understanding responses of skeletal Mg incorporation to current and predicted atmospheric CO<sub>2</sub>, including how trends vary both across and within species, and as a function of geography, will provide a key insight for predicting the future success of calcifying taxa.

Sea urchins are among the better studied of species thought to respond to ocean acidification, but while some studies suggest that adult and larval sea urchin development and calcification will be somewhat resilient to future changes in seawater carbonate chemistry (Martin et al., 2011; Ries, 2011; Pespenti et al., 2013), others have reported significant impacts on larval growth, development, and gene expression (Dupont and Thorndyke, 2009; Dupont et al., 2010; Martin et al., 2011; Stumpp et al., 2011a, b; Yu et al., 2011). The degree to which these disparate outcomes might be related to stage-, population-, or species-specific differences in skeletal mineralogy, geochemistry, or precipitation rate under elevated *p*CO<sub>2</sub>, as well as differences in experimental methods, remains unknown (Byrne, 2012).

The utilization of different calcium carbonate polymorphs by different life stages may have implications for how species respond to ocean acidification. Adult and larval purple sea

urchins have been shown to utilize an amorphous calcium carbonate (ACC) precursor phase prior to skeletal stabilization (Beniash et al., 1997; Politi et al., 2006). Although ACC is a transient phase, it is highly soluble, which could make the production of this disordered mineral phase more susceptible to lowered saturation state at the site of calcification than the stabilized skeleton (e.g. Ries, 2011; Weiner and Addadi, 2011). Given the unique biomineralization mechanisms that sea urchins employ, it is possible that calcification or mineralogical plasticity might buffer urchin larvae against ocean acidification, ultimately affecting foreign ion incorporation and, thus, mineral solubility.

Sea urchins have been shown to precipitate skeletal structures (ossicles) of highly variable geochemical composition (Weber, 1969). Adult purple sea urchin tests are more isotopically fractionated ( $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$ ) and nearly three times more enriched in Mg than spines (Weber and Raup, 1966). These geochemical offsets have led to the understanding that adult urchins utilize multiple calcification pathways that draw upon varying proportions of metabolic vs. inorganic carbon (Weber and Raup, 1966; Weber, 1969; Ebert, 2007; Ries, 2011).

In general, echinoderms produce high-Mg calcite intracellularly within vesicles formed by fused cellular membranes that regulate pH,  $p\text{CO}_2$ , and trace elemental composition of the calcification compartment (Weiner and Dove, 2003). Skeletal calcification in echinoderm plutei is also thought to be somewhat protected from external conditions due to its internal location within mesodermal tissue (Byrne, 2011). However, changes in seawater chemistry could indirectly influence echinoderm biomineralization by affecting the physiological cost of maintaining this intracellular chemistry (Knoll, 2003; Porter, 2007).

Recent studies suggest that growth and geochemical composition of adult sea urchins will be resistant to future acidification (*Eucidaris tribuloides* and *Arbacia punctulata*; Ries et al. 2009; Ries, 2011). However, no such studies have investigated differences in trace element incorporation between life stages, or among individuals of the same species that originate from distinct geographic areas. In addition, no research has examined impacts of ocean acidification on the incorporation of trace elements by delicate skeletal structures precipitated during early developmental life history stages. To investigate these unknowns, we examined (1) the geochemical composition of purple sea urchin (*Strongylocentrotus purpuratus*) skeleton precipitated during both adult and early life history stages; (2) potential differences in geochemical composition among individuals originating from regions spanning a broad latitudinal range encompassing a spectrum of oceanographic regimes; and (3) the impact of ocean acidification on “foreign ion” (Mg and Sr) incorporation into larval and juvenile *S. purpuratus* skeleton. Each of these study components provides important insights into the mineralogical plasticity of this ecologically vital species.

## 2 Materials and methods

### 2.1 Adult urchin skeleton

We analyzed the Mg and Sr content of adult purple sea urchin (*Strongylocentrotus purpuratus*) spines from a range of upwelling environments to determine whether skeletal composition varies across a natural gradient of carbonate chemistry. One to two spines were collected from five individuals from a site within each of four regions along the West Coast of the US: Oregon, Northern California, Central California, and Southern California; Table 1, Fig. 1). Two adult urchin tests were collected from Bodega Marine Reserve for comparison. Each spine and about half of each test was ground to a powder with an acid-cleaned ceramic mortar and pestle. Approximately 5 mg of powder from each sample was chemically cleaned following an oxidative method adapted from Pak et al. (2004) and Shen and Boyle (1988) to remove organics as well as any non-lattice bound material prior to Mg/Ca and Sr/Ca analysis via inductively coupled plasma-optical emission spectroscopy (ICP-OES; Sect. 2.3; Supplement).

### 2.2 Larval culturing

#### 2.2.1 Culturing design

Purple sea urchin (*Strongylocentrotus purpuratus*) larvae were cultured through their entire larval duration (~50 days) under modern global-mean atmospheric  $p\text{CO}_2$  levels (400  $\mu\text{atm}$ ) and a “fossil-fuel intensive” scenario predicted for 2100 (900  $\mu\text{atm}$ ) at Bodega Marine Laboratory (Solomon et al., 2007; Pespenti et al., 2013). In brief, 30 adult urchins collected from each of the four source populations were spawned in the laboratory, and the gametes from 10 females (~200 000 eggs female<sup>-1</sup>) and 10 males were pooled and used for fertilization for each population (Table 1; Fig. 1). Fertilized embryos were maintained at either control or elevated  $p\text{CO}_2$  for 24 h before hatched blastulae were transferred into culture jars ( $n = 3\text{--}4$  jars per population  $\times p\text{CO}_2$  level; 0.66 larvae mL<sup>-1</sup>) containing 3 L of filtered seawater (FSW) pumped into the laboratory from the adjacent outer coast and pre-equilibrated with NIST-certified, pre-mixed treatment gases (Airgas, Inc.). Jars fit into sealed boxes (3 per  $p\text{CO}_2$  level), which received  $\text{CO}_2$  air mixtures to minimize degassing of culture jars. The jars were held in seawater tables maintained at 14 °C ( $\pm 0.2$  °C) and were stirred using oscillating paddles. Every 2 days, 90 % of the culture water was removed via reverse filtration through a 60  $\mu\text{m}$  mesh and replaced with pre-equilibrated FSW. Larvae were fed an equal mixture of the unicellular algae *Rhodomonas* sp. and *Dunaliella* sp. immediately following each water exchange (2500 cells per mL of each species).

**Table 1.** Sampling locations and coordinates for eight urchin populations. OR = Oregon, N. CA = Northern California, C. CA = Central California, S. CA = Southern California.

Location	Region	Lat	Long
<b>Adult Spines</b>			
Purisma Point, CA (PP)	S. CA	34.8	120.6
Terrace Point, CA (TP)	C. CA	37.0	122.1
Van Damme State Park, CA (VD)	N. CA	39.3	123.8
Fogarty Creek, OR (FC)	OR	44.8	124.1
<b>Cultured Metamorphs</b>			
Santa Barbara, CA (SB)	S. CA	34.3	119.6
Sand Hill Bluff, CA (SHB)	C. CA	37.0	122.2
Bodega Marine Reserve, CA (BMR)	N. CA	38.3	123.1
Strawberry Hill, OR (SH)	OR	44.3	124.1

### 2.2.2 Cultured metamorph sample preparation

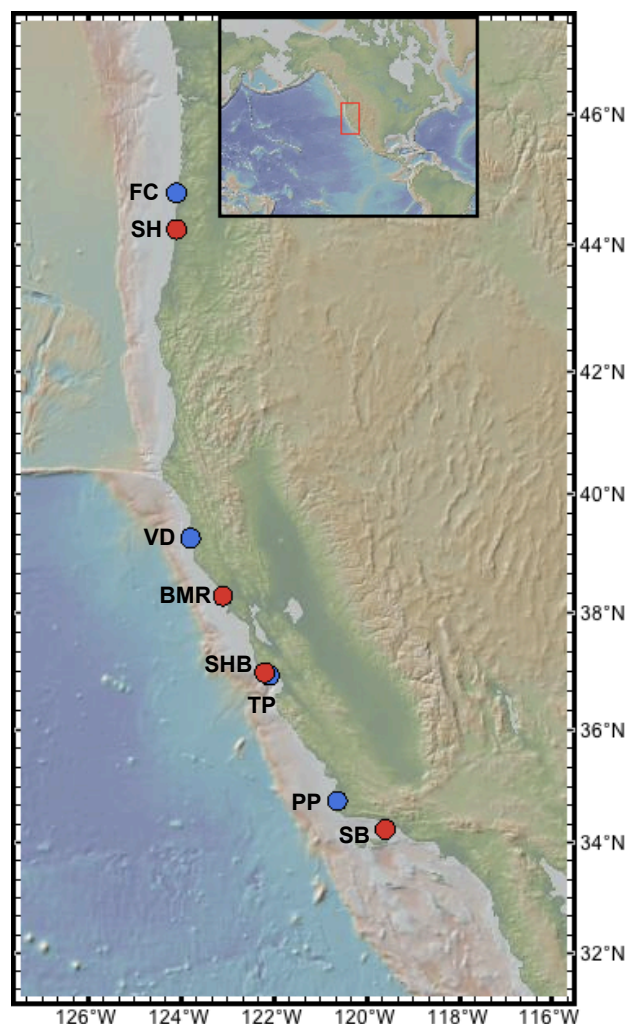
On day 48 and 49 (post-fertilization), we induced metamorphosis following established methods for sea urchin larvae (Cameron et al., 1989; Pearce and Scheibling, 1994). Larvae ( $n = 15\text{--}20$ ) were randomly sampled from each culture jar and placed in 70 mM KCl in FSW for 2 h, then transferred to FSW for 24 h in an incubator held at 14 °C. Twenty successfully metamorphosed larvae were pooled from each jar, rinsed with distilled water, dried at room temperature, and ashed in a muffle furnace to oxidize all organic tissue to CO<sub>2</sub> (500 °C; 4 h). For each sample, the ashed skeletons of 20 metamorphs were transferred to a single acid-cleaned micro-centrifuge tube prior to oxidative cleaning and Mg/Ca and Sr/Ca analysis via ICP-OES.

Five additional metamorphosed larvae from selected culture jars continued to grow on a mixed diet of several benthic diatom species in a common (non-CO<sub>2</sub> equilibrated) flow-through seawater table for an additional 5 months post-settlement before the tests were chemically cleaned and analyzed for Mg/Ca and Sr/Ca.

### 2.2.3 Seawater chemistry

#### Carbonate chemistry

Samples (250 mL) of culture jar water were collected during each water change. Seawater pH<sub>T</sub> and temperature were measured using a potentiometric pH/temperature meter (Accumet XL60). Raw pH readings (mV) were calibrated using two seawater buffers (2-amino-2-hydroxymethyl-1,3-propanediol (“TRIS”) and 2-aminopyridine/HCl (“AMP”) in synthetic seawater), converted to pH<sub>T</sub> (total scale) following Dickson et al. (2007), and checked against a TRIS buffer certified reference material (A. Dickson, Scripps Institute of Oceanography). Salinity of the source water was determined using a YSI Professional Plus multiparameter



**Fig. 1.** Map of study area noting collection sites of adult urchins (*Strongylocentrotus purpuratus*) for spine samples (blue circles) and individuals spawned for culturing experiment (red circles). Site abbreviations as in Table 1.

instrument. Total alkalinity was measured using automated Gran titration (Metrohm 809), and standardized using a certified reference material from A. Dickson. Other carbonate system parameters were estimated using CO<sub>2</sub>SYS (Lewis and Wallace, 1998) using  $K_1$  and  $K_2$  equilibrium constants from Mehrbach et al. (1973) refit by Dickson and Millero (1987), using  $K_{SO_4}$  from Dickson (1990), and employing pH<sub>T</sub> and alkalinity as the primary input variables (Table 2). Experimental pH<sub>T</sub>,  $pCO_2$ , and carbonate ion concentration data were analyzed using separate one-way ANOVAs. Salinity and temperature (mean  $\pm$  1 SD) were  $32.17 \pm 0.66$  and  $14.11 \pm 0.14$  °C, respectively. During all culturing, we verified that seawater chemistries of the rearing jars from the two  $pCO_2$  treatments were significantly different ( $n = 12\text{--}14$  culture jars per  $pCO_2$  level in each of the two trials) (ANOVA, “ $pCO_2$ ”,  $F_{1,161} = 4897$ ;  $p < 0.0001$ ; “pH<sub>T</sub>”,

**Table 2.** Mean ( $\pm 1$  SD) culture conditions during the study.

Supplied or measured parameters		
Treatment gas $p\text{CO}_2$ ( $\mu\text{atm}$ )	$385 \pm 8$	$1000 \pm 19$
pH total	$8.02 \pm 0.03$ ( $n = 335$ )	$7.73 \pm 0.03$ ( $n = 332$ )
Total alkalinity ( $\mu\text{mol kg}^{-1}\text{SW}$ )	$2212 \pm 12$ ( $n = 81$ )	$2213 \pm 14$ ( $n = 82$ )
DIC ( $\mu\text{mol kg}^{-1}\text{SW}$ )	$2043 \pm 42$ ( $n = 67$ )	$2140 \pm 39$ ( $n = 66$ )
Mg/Ca ( $\text{mol mol}^{-1}$ )	$4.79 \pm 0.06$ ( $n = 23$ )	$4.80 \pm 0.05$ ( $n = 20$ )
Sr/Ca ( $\text{mMol mol}^{-1}$ )	$8.61 \pm 0.17$ ( $n = 23$ )	$8.62 \pm 0.17$ ( $n = 20$ )
Temperature ( $^{\circ}\text{C}$ )	$14.1 \pm 0.3$ ( $n = 335$ )	$14.1 \pm 0.3$ ( $n = 332$ )
Calculated parameters based on CO2SYS estimation program		
$p\text{CO}_2$ from $\text{pH}_{\text{tot}}/\text{TA}$ ( $\mu\text{atm}$ )	$410 \pm 27$	$871 \pm 53$
$p\text{CO}_2$ from $\text{TA}/\text{DIC}$ ( $\mu\text{atm}$ )	$490 \pm 138$	$1001 \pm 291$
$[\text{CO}_3^{2-}]$ ( $\mu\text{mol kg}^{-1}\text{SW}$ )	$137 \pm 7$	$76 \pm 4$
$\Omega_{\text{calcite}}$	$3.3 \pm 0.2$	$1.8 \pm 0.1$

$F_{1,665} = 17\,116$ ;  $p < 0.0001$ ; “[ $\text{CO}_3^{2-}$ ]”,  $F_{1,161} = 5372$ ;  $p < 0.0001$ ; “ $\Omega_{\text{calcite}}$ ”,  $F_{1,161} = 5372$ ;  $p < 0.0001$ ).

### Seawater Mg/Ca and Sr/Ca

The stability of culture water Mg/Ca and Sr/Ca was monitored in 8 out of the 28 total jars throughout the experiment (2 jars per  $p\text{CO}_2$  treatment and per site for the Northern CA and Southern CA populations: BMR and SB). Culture jar seawater samples (13 mL) were collected every 4 days, filtered through acid-cleaned  $0.45\ \mu\text{m}$  poly-sulfone syringe filters, acidified to  $\text{pH} < 2$  by addition of  $25\ \mu\text{L}$  of OPTIMA grade  $\text{HNO}_3$ , and diluted 10x prior to Mg/Ca and Sr/Ca analysis via ICP-OES (Field et al., 1999). We verified that culture water Mg/Ca and Sr/Ca ( $\text{Mg}/\text{Ca}_{\text{SW}}$  and  $\text{Sr}/\text{Ca}_{\text{SW}}$ ) in each of the 8 jars sampled was stable within  $< 2\%$  throughout the 50-day experiment, and there were no statistically significant differences in  $\text{Mg}/\text{Ca}_{\text{SW}}$  or  $\text{Sr}/\text{Ca}_{\text{SW}}$  among any of the 8 jars (Table 5; Mg/Ca; ANOVA,  $F_{7,35} = 0.2269$ ,  $p = 0.9762$ ; Sr/Ca; ANOVA,  $F_{7,35} = 0.8150$ ,  $p = 0.5811$ ). Therefore, any variability observed in the skeletal Mg/Ca and Sr/Ca data could be attributed to biological processes rather than differences in culture water composition.

### 2.3 Mg/Ca and Sr/Ca analyses

All trace element sample preparation and analyses followed standard laboratory protocols for Class 100 conditions. All plasticware was leached in 1 N HCl (reagent grade in 18 M $\Omega$ -cm Milli-Q water) at  $60\ ^{\circ}\text{C}$  for at least 4 h and rinsed thoroughly with Milli-Q water prior to use. All solutions were made with ultrapure reagents (OPTIMA grade, Seastar Chemicals Inc., BC, Canada) and Milli-Q water unless otherwise noted.

For Sr/Ca and Mg/Ca analysis, a JY-Ultima 2C ICP-OES (Horiba Scientific, NJ, USA) was equipped with a cyclonic quartz spray chamber, a glass peristaltic-pumped neb-

ulizer for 10 mL seawater samples (Meinhard, CO, USA), and a PFA MicroFlow 100 ( $100\ \mu\text{L min}^{-1}$ ) nebulizer for 250–500  $\mu\text{L}$  dissolved carbonate samples (Elemental Scientific Inc., NE, USA). The emission line ratios used for data interpretation were polychrometer lines Sr407/Ca317 and Mg285/Ca317 (monochrometer line 279 Mg was found to be optimal for seawater). Repeat measurements of seawater certified reference material was used to normalize data between analytical runs (CRM-SW; High Purity Standards, NC;  $n = 13$ ). Average reproducibility of duplicate seawater samples was 2% ( $n = 4$  duplicates) for Mg/Ca and Sr/Ca. Based on the method described by Schrag (1999), an in-house carbonate reference solution was analyzed between each carbonate sample to correct for instrumental drift within and between analytical runs. Within-run precision on replicate carbonate reference solution analyses (run as an unknown) was  $< 1\%$  RSD for both Mg/Ca and Sr/Ca ( $n = 3$ ). For the purpose of comparing our data with previous studies and given that any non-lattice bound Mg was removed via chemical cleaning prior to analysis, we assume that all of the Mg measured in our samples was present as  $\text{MgCO}_3$ . Most of the 20-metamorph samples contained enough carbonate for a single ICP-OES measurement. However, several of the pooled urchin metamorph samples fell below the analytical detection limits because of low sample weights, and were thus excluded from further analysis (13 samples excluded in total: 400  $\mu\text{atm}$   $p\text{CO}_2$  treatment: 3 Southern CA, 2 Oregon, 1 Monterey Bay; 900  $\mu\text{atm}$   $p\text{CO}_2$  treatment: 1 Southern CA, 1 Oregon, 1 Central CA, 4 Northern CA). Nevertheless, 2–4 pooled metamorph samples were analyzed per site/ $p\text{CO}_2$  treatment, thus ultimately representing mean Mg/Ca and Sr/Ca values based upon a total of 40–80 individual metamorph skeletons per site/ $p\text{CO}_2$  treatment.

### 3 Results and discussion

#### 3.1 *S. purpuratus* skeletal composition

Early studies revealed that adult sea urchins precipitate skeletal ossicles with a wide range of geochemical compositions (Weber and Raup, 1966; Weber, 1969). These findings led to the understanding that many sea urchin species exert a strong biological control on skeletal geochemistry rather than passively recording ambient conditions as other calcifiers appear to do (e.g. corals, foraminifera; Davies et al., 1972; Ebert, 2007). These biological “vital effects” make urchin skeleton a poor archive of the paleoceanographic environment. However, the strong vital effects also suggest a possible sensitivity of calcification to other processes that might be influenced by present-day gradients and future changes in ocean chemistry. Geochemical approaches can be utilized to explore the potential for such biological responses, including altered elemental incorporation and consequent shifts in solubility of purple sea urchin skeleton.

##### 3.1.1 Magnesium content

We found that the Mg contents of adult *S. purpuratus* spines and tests are within a factor of two of those found for previously studied sea urchin species (spines =  $2.8 \pm 0.2$  %  $\text{MgCO}_3$ ,  $n = 31$ ; tests =  $6.4 \pm 0.6$  %  $\text{MgCO}_3$ ,  $n = 16$ ; Table 3; Weber, 1969; Davies et al., 1972; Ries, 2011). Similar to other taxa,  $\sim 2.3 \times$  more Mg is incorporated into *S. purpuratus* adult tests than spines, suggesting that this sea urchin species also employs distinct calcification pathways for spine vs. test precipitation (Ebert, 2007). We do note, however, that *S. purpuratus* spines contain  $\sim 40$  % less  $\text{MgCO}_3$  than previously studied species, such that the range of *S. purpuratus* Mg values spans the transition between high- and low-Mg calcite (4 %  $\text{MgCO}_3$ ). According to typical definitions, the adult and 5.5-month post-settlement juvenile *S. purpuratus* tests are composed of high-Mg calcite (6–7 %  $\text{MgCO}_3$ ), while adult spines are composed of low-Mg calcite (Scoffin, 1987). Based on this pattern, one would therefore predict that tests would be more soluble than spines (Chave et al., 1962; Morse et al., 2006). However, additional solubility experiments on *S. purpuratus* calcite would be required to confirm this prediction. The composition of newly metamorphosed juvenile skeleton falls between these spine and test endmembers ( $5.3 \pm 0.3$  %  $\text{MgCO}_3$ ;  $n = 22$ ; Table 4). If the distinct calcification pathways that drive Mg/Ca offsets between adult spines and tests also influence skeletal Mg content in newly settled juveniles (e.g. Ebert, 2007), then these data indicate that larval/early juvenile *S. purpuratus* sea urchins also utilize a combination of biomineralization pathways, drawing upon both inorganic carbon directly from seawater and metabolic  $\text{CO}_2$  (Ebert, 2007). Carbon isotopic

data from sea urchin metamorph skeleton could validate this trace elemental evidence.

##### 3.1.2 Strontium content

We found that  $\sim 1.4 \times$  more Sr is incorporated into the tests of adult and 5.5-month post-settlement urchins ( $\text{Sr}/\text{Ca} = 2.72 \pm 0.1 \text{ mmol mol}^{-1}$ ,  $n = 16$ ;  $2.85 \pm 0.1 \text{ mmol mol}^{-1}$ ,  $n = 4$ , respectively) than into the low-Mg calcite of adult spines ( $\text{Sr}/\text{Ca} = 1.9 \pm 0.05 \text{ mmol mol}^{-1}$ ;  $n = 31$ ), with newly settled juveniles falling between the spine and test endmembers ( $\text{Sr}/\text{Ca} = 2.1 \pm 0.1 \text{ mmol mol}^{-1}$ ;  $n = 22$ ; Fig. 2; Table 4). Mucci and Morse (1983) demonstrated that the incorporation of Sr into inorganic calcites precipitated from seawater is dependent upon calcite  $\text{MgCO}_3$  content. The replacement of Ca by smaller Mg ions distorts the calcite crystal, allowing for more large Sr ions to be incorporated into the lattice (Mucci and Morse, 1983). Carpenter and Lohmann (1992) invoke this same mechanism to explain the positive linear relationship between Mg and Sr composition in both biotically and abiotically precipitated marine calcites. While the slopes of the abiotic and biotic marine calcite Sr vs. Mg regressions are similar, the y-intercepts of these two relationships are offset such that biotic calcites consistently contain more Sr than abiotic samples. Carpenter and Lohmann (1992) suggest that this offset is a direct result of differences in mineral precipitation rate, where Sr incorporation is kinetically controlled during the rapid biologically induced precipitation of biotic calcites, whereas Sr incorporation of abiotic calcites occurs at equilibrium. In order to interpret our sea urchin data in the context of these studies, we calculated Sr distribution coefficients ( $D_{\text{Sr}}$  values) for each of the sea urchin carbonate samples.

$$D_{\text{Sr}} = (\text{Sr}/\text{Ca}_{\text{calcite}})/(\text{Sr}/\text{Ca}_{\text{sw}}) \quad (1)$$

*S. purpuratus*  $D_{\text{Sr}}$  values calculated based on the mean Sr/Ca ratio measured in the experimental culture waters (derived from a local coastal seawater supply;  $8.61 \pm 0.17 \text{ mmol mol}^{-1}$ ; Table 5) were regressed against skeletal  $\text{MgCO}_3$  concentration (Fig. 2). In general, the positive relationship between *S. purpuratus*  $\text{MgCO}_3$  content and  $D_{\text{Sr}}$  agree with the trend observed for inorganic calcites by Mucci and Morse (1983; Fig. 2). Although skeletal Mg content is thought to be dependent upon biological controls and calcification mechanism (Ebert, 2007), Sr incorporation appears to follow the behavior of inorganic calcites precipitated in a non-biologically mediated seawater environment. This point suggests that once biological factors impart a given Mg composition to newly laid-down skeleton, strontium (and possibly other foreign ion) incorporation proceeds inorganically from the calcification fluid.

A similar relationship is observed when our data are plotted against over 30 species of other biotic marine calcifiers (Carpenter and Lohmann, 1992; Fig. 3). In general, except



**Table 3.** Magnesium and strontium content of adult *S. purpuratus* ossicles compared with other sea urchin taxa.

Species	Data source	Mg content (MgCO <sub>3</sub> % ± 1 SD)		Sr content (Sr / Ca mmol mol <sup>-1</sup> ± 1 SD)	
		spine	test	spine	test
<i>Strongylocentrotus franciscanus</i> (CA, USA)	Weber (1969)	5.8 ± 0.9	8.8 ± 0.7	–	–
<i>Strongylocentrotus drobachiensis</i> (ME, USA)	Weber (1969)	4.5	7.4	–	–
<i>Lytechinus variegatus</i> (FL, USA)	Carpenter and Lohmann (1992)	6.8	–	2.1	–
<i>Echinus</i> sp.	Carpenter and Lohmann (1992)	–	12.9 ± 0.6	–	2.5 ± 0.1
<i>Echinometra lacunter</i> (FL, USA)	Carpenter and Lohmann (1992)	–	–	–	2.2
Unidentified echinoid (FL, USA)	Carpenter and Lohmann (1992)	5.3 ± 0.7	12.2 ± 0.1	2.1 ± 0.1	2.5 ± 0.2
<i>Eucidaris tribuloides</i> (FL, USA)	Carpenter and Lohmann (1992)	5.8	–	2.2	–
<i>Eucidaris tribuloides</i> (FL, USA)	Ries (2011)	4.4 ± 0.1	9.4 ± 0.1	–	–
<i>Arbacia punctulata</i> (FL, USA)	Ries (2011)	4.9 ± 0.1	6.9 ± 0.2	–	–
<i>Arbacia punctulata</i> (NC, USA)	Davies et al. (1972)	5.3–6.7	–	2.0–2.5	–
<i>Strongylocentrotus purpuratus</i> (CA–OR, USA)	This study	2.8 ± 0.2	6.4 ± 0.6	1.9 ± 0.05	2.72 ± 0.09

**Table 4.** Mean (± 1 SD) trace elemental ratios of *S. purpuratus* life history stages.

	Mg / Ca (mmol mol <sup>-1</sup> )	D <sub>Mg</sub> (mmol mol <sup>-1</sup> calcite/ mmol mol <sup>-1</sup> SW)	Sr / Ca (mmol mol <sup>-1</sup> )	D <sub>Sr</sub> (mmol mol <sup>-1</sup> calcite/ mmol mol <sup>-1</sup> SW)
Adult Spines	33.8 ± 3.0	0.007 ± 0.0006	1.93 ± 0.05	0.22 ± 0.006
Adult Tests	82.5 ± 7.0	0.017 ± 0.001	2.72 ± 0.09	0.32 ± 0.011
5.5 Month-old Juvenile Tests	92.4 ± 2.5	0.019 ± 0.0005	2.85 ± 0.07	0.33 ± 0.008
Newly Metamorphosed Juveniles	67.0 ± 4.0	0.014 ± 0.0008	2.11 ± 0.08	0.25 ± 0.009

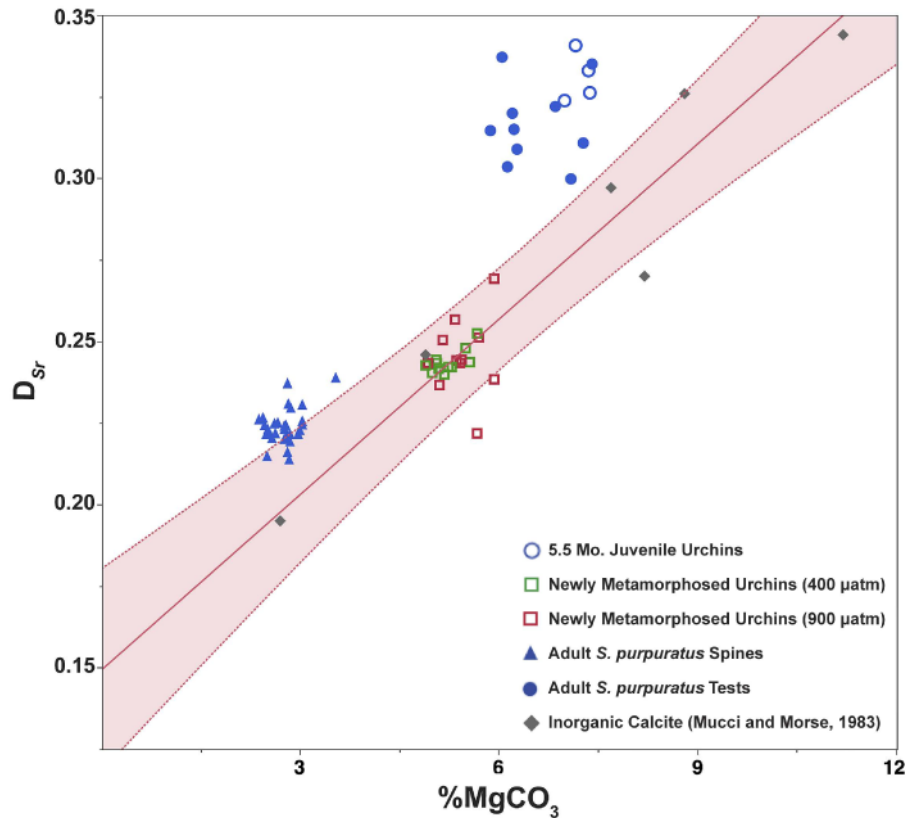
for the adult and 5.5-month old test samples, the *S. purpuratus* samples follow the positive relationship observed in the Carpenter and Lohmann (1992) data set, suggesting that the passive incorporation of Sr into *S. purpuratus* skeleton is also kinetically controlled (Fig. 3). The adult *S. purpuratus* spines and newly settled metamorphs plot close to the biotic calcite regression, whereas *S. purpuratus* adult and juvenile test  $D_{Sr}$  values are higher and more variable. This offset indicates that the precipitation of adult and juvenile *S. purpuratus* test calcite occurs further from equilibrium than spines and newly settled metamorphs, which could be a result of faster and more-variable mineral precipitation rates (Carpenter and Lohmann, 1992). This finding would be in agreement with early isotopic evidence of adult urchin ossicles (Weber and Raup, 1966; Weber, 1969; Ebert, 2007). This result furthermore suggests that the crystal precipitation rate at the site of calcification could be an inherently plastic trait during *S. purpuratus* test calcification.

### 3.2 Adult spine composition across a range of oceanographic regimes

The data from adult spines presented in Figs. 2 and 3 represent individuals from several distinct coastal upwelling regimes characterized by different levels of carbonate saturation within the California Current system (CCS; Feely et

al., 2008). The strength of coastal upwelling in the CCS is highly variable both spatially and temporally (Feely et al., 2008; Hauri et al., 2009). A recent monitoring effort has characterized the chemical variability of the intertidal CCS environment (Chavez et al., 2011; Hofmann et al., 2013). These data have shown that the frequency of low-pH events driven by upwelling range widely across sites. In one year, as much as one-fifth of Central Oregon pH values fell below 7.8 in contrast to < 2 % for Central and Southern California, with Northern California sites falling between these two extremes (Hofmann et al., 2013). Therefore, the more-northern sites across our latitudinal gradient experience cooler, more-acidic conditions, whereas the Southern CA site is the warmest and most buffered (Hofmann et al., 2013). Given that Sr, Mg, and Ca are conservative ions with concentrations generally scaling with salinity, Mg/Ca and Sr/Ca variations along this northeast Pacific latitudinal gradient are likely very minor (< ~ 0.5 %; Culkin and Cox, 1966; Brass and Turekian, 1974; de Villiers, 1999).

Adult spine Mg/Ca was not statistically different among populations spanning this natural environmental gradient, and Sr/Ca composition had minor differences among sites (Fig. 4; Mg/Ca ANOVA,  $F_{3,17} = 0.2441$ ,  $p = 0.8643$ ; Sr/Ca ANOVA,  $F_{3,17} = 4.7006$ ,  $p = 0.0144$ ). Therefore, the large-scale pH mosaic spanning the US West Coast does



**Fig. 2.** The relationship between skeletal magnesium content (expressed as %  $\text{MgCO}_3$ ; x-axis) and strontium partition coefficient ( $D_{\text{Sr}}$ ; y-axis) for *S. purpuratus* adult spines (blue filled triangles), adult tests (blue filled circles), 5.5-month-old cultured juvenile tests (open blue circles), and newly metamorphosed juveniles settled after 48–49 days in culture at 400 (green open squares) and 900 (red open squares)  $\mu\text{atm}$   $p\text{CO}_2$ . Linear regression and 95 % confidence intervals plotted in red shading derived from inorganic calcite precipitation experiments (grey filled diamonds; Mucci and Morse 1983).

not impact *S. purpuratus* spine Mg composition or Sr incorporation (a potential indicator of growth rate variability; Carpenter and Lohmann, 1992). This natural solubility gradient will continue to shift in the next several decades (Andersson et al., 2008; Gruber et al., 2012). As a consequence, although adult *S. purpuratus* skeleton precipitated in northern high upwelling and frequently low-pH regions are similar in elemental composition to skeleton precipitated under warmer, more-buffered conditions, the higher-latitude regions will likely become undersaturated with respect to high-Mg calcites ( $> 12\%$   $\text{MgCO}_3$ ) and aragonite before the lower-latitude sites (perhaps by the end of this century; Andersson et al., 2008).

### 3.3 Impact of $p\text{CO}_2$ on skeletal composition during early life history stages

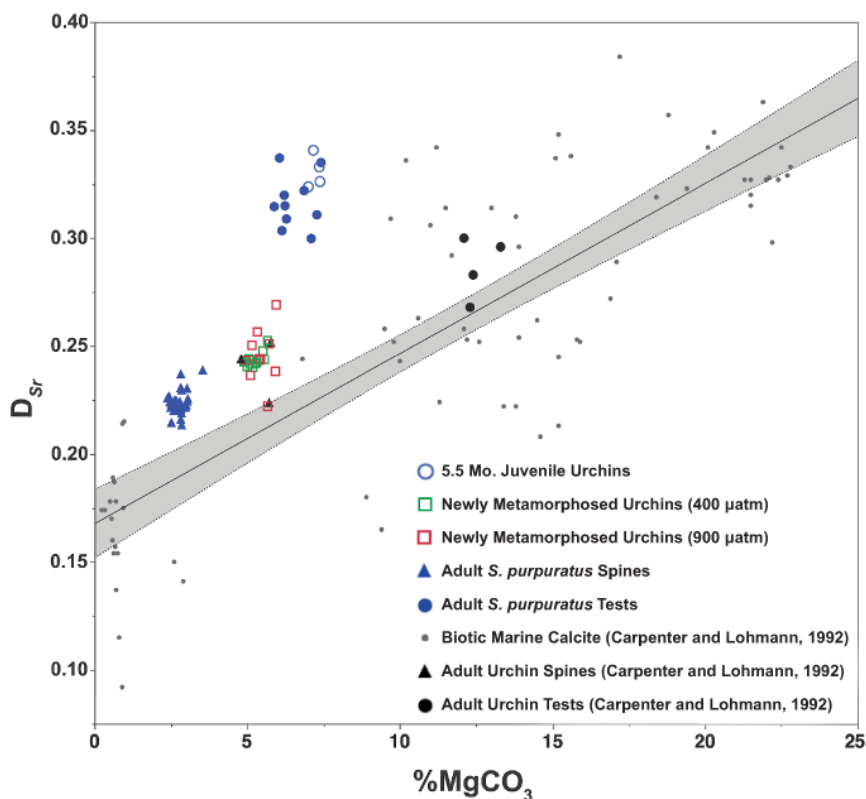
Although we found little evidence of effects of oceanographic regime on adult spine geochemistry, the larval utilization of skeleton with different trace element concentrations raises the question of whether earlier life stages might

be equivalently unperturbed by differences in seawater chemistry, in particular those associated with acidification.

Culture water Mg/Ca and Sr/Ca ( $\text{Mg}/\text{Ca}_{\text{SW}}$  and  $\text{Sr}/\text{Ca}_{\text{SW}}$ ) in each of the 8 larval culture jars was stable within  $< 2\%$  throughout the 50-day culturing experiment, and there was no statistically significant difference in  $\text{Mg}/\text{Ca}_{\text{SW}}$  or  $\text{Sr}/\text{Ca}_{\text{SW}}$  among any of the 8 jars (Sect. 2.2.3; Table 5). Therefore, any variability observed in the skeletal Mg/Ca and Sr/Ca data can be attributed to biological processes rather than differences in culture water composition.

We found that elevated  $p\text{CO}_2$  did not affect the incorporation of Mg or Sr in Oregon, Northern California, or Central California populations (Fig. 5; Tables 6 and 7), which is consistent with a lack of response of adult Atlantic urchins to acidification (Ries, 2011). There was no evidence for a difference in the Mg composition of newly settled metamorph skeleton among populations in response to elevated  $p\text{CO}_2$  (Table 6, Fig. 5). Elevated  $p\text{CO}_2$  did, however, impact the skeletal composition of the newly settled juveniles reared from Southern California, which incorporated 8 % more Sr into their skeleton under elevated  $p\text{CO}_2$





**Fig. 3.** The relationship between skeletal magnesium content (expressed as %  $\text{MgCO}_3$ ; x-axis) and strontium partition coefficient ( $D_{\text{Sr}}$ ; y-axis) for *S. purpuratus* adult spines (blue filled triangles), adult tests (blue filled circles), 5.5-month-old cultured juvenile tests (open blue circles), and newly metamorphosed juveniles settled after 48–49 days in culture at 400 (green open squares) and 900 (red open squares)  $\mu\text{atm}$   $p\text{CO}_2$ . Linear regression and 95 % confidence intervals plotted in grey shading derived from biotic marine calcite (grey filled circles) including adult urchin spines and tests (black filled triangles and circles, respectively; Carpenter and Lohmann, 1992).

**Table 5.** Mean ( $\pm 1$  SD) trace elemental ratios in culture jars.

$p\text{CO}_2$ treatment	Site	Jar #	Mg/Ca (mol mol <sup>-1</sup> )	Sr/Ca (mMol mol <sup>-1</sup> )	<i>n</i>
900	B	3	4.79 $\pm$ 0.06	8.64 $\pm$ 0.18	6
900	B	2	4.80 $\pm$ 0.03	8.62 $\pm$ 0.21	5
900	SB	3	4.81 $\pm$ 0.03	8.59 $\pm$ 0.15	5
900	SB	2	4.80 $\pm$ 0.08	8.62 $\pm$ 0.20	4
400	B	3	4.79 $\pm$ 0.11	8.59 $\pm$ 0.16	6
400	B	2	4.78 $\pm$ 0.03	8.57 $\pm$ 0.17	6
400	SB	3	4.77 $\pm$ 0.04	8.54 $\pm$ 0.12	6
400	SB	1	4.81 $\pm$ 0.06	8.76 $\pm$ 0.19	5
Mean (all samples)			4.79 $\pm$ 0.06	8.61 $\pm$ 0.17	43

( $F_{1,14} = 6.1670$ ;  $p = 0.0068$ ; Table 7). Given the small number of samples from each population and the unbalanced design of our ANOVA, we cautiously interpret these results as providing preliminary evidence that skeletal “foreign ion” incorporation in the population from Southern California responded differently to elevated  $p\text{CO}_2$ . This provides prelim-

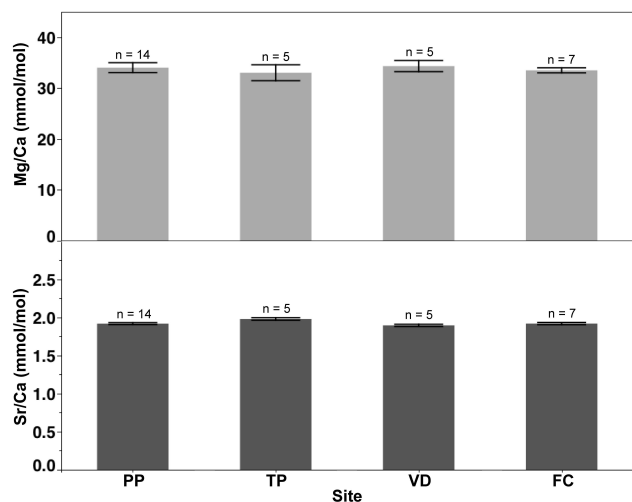
inary evidence for a possible physiological response linking the biomineralization pathways that dictate trace elemental composition of *S. purpuratus* skeleton and overall biological function in the Southern California population (Sect. 3.1).

Although further study is required to elucidate these calcification mechanisms, the data presented here suggest that elevated  $p\text{CO}_2$  and reduced carbonate saturation state have the potential to affect mineral composition. The varied response among populations suggests that larval calcification and skeletal composition may depend upon a threshold response to elevated  $p\text{CO}_2$ . If so, then beyond 2100 the composition of skeleton calcified during purple sea urchin early life history stages in the more northern sites may also begin to shift as  $p\text{CO}_2$  levels exceed 900  $\mu\text{atm}$ .

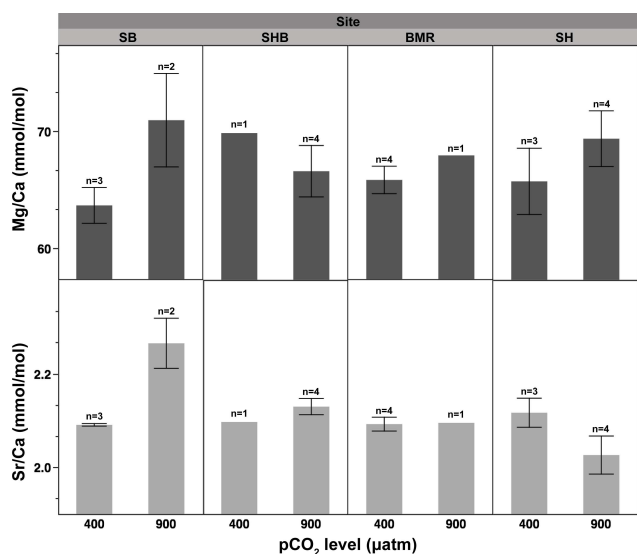
### 3.4 Possible controls on Mg and Sr incorporation in *S. purpuratus*

#### 3.4.1 Mg/Ca

The Mg content of *S. purpuratus* skeleton varies widely among skeletal ossicles and across life history stages. Such



**Fig. 4.** Mean Mg/Ca (upper) and Sr/Ca (lower) ratios for spines of adult *S. purpuratus* populations spanning the West Coast US shown in Fig. 1. Error bars represent  $\pm 1$  SE from mean.



**Fig. 5.** Mean Mg/Ca (upper) and Sr/Ca (lower) ratios of newly metamorphosed juvenile *S. purpuratus* urchins, settled after 48–49 days in culture at control (400 μatm) or elevated  $p\text{CO}_2$  levels (900 μatm). Cultures initiated from sites indicated in Fig. 1. Error bars represent  $\pm 1$  SE of 2–4 samples (consisting of  $\sim 20$  pooled metamorphs) from replicate culture jars from each population.

differences likely derive from strong biological controls on calcification and the use of multiple biomineralization pathways by *S. purpuratus* in different  $\text{CaCO}_3$  structures. Within a given skeletal structure, by contrast, and for the majority of the populations studied, we find that the Mg content of adults and newly metamorphosed *S. purpuratus* calcite is relatively insensitive to environmental conditions or projected changes in seawater carbonate chemistry by ocean acidifi-

**Table 6.** Two-way ANOVA: metamorph Mg/Ca.

Factor	df	Error df	F ratio	p
$p\text{CO}_2$	1	14	1.4232	0.2527
Source	3	14	0.0633	0.9784
$p\text{CO}_2 \times \text{Source}$	3	14	1.1043	0.3802

**Table 7.** Two-way ANOVA: metamorph Sr/Ca.

Factor	df	Error df	F ratio	p
$p\text{CO}_2$	1	14	1.3592	0.2631
Source	3	14	4.1020	0.0277
$p\text{CO}_2 \times \text{Source}$	3	14	6.1670	0.0068

cation. Further work on purple sea urchins, multiple stressors and threshold responses will be required to fully understand this response and distinctions from other species (Ries, 2011).

### 3.4.2 Sr/Ca

While the partitioning of Mg into sea urchin skeleton is thought to be biologically mediated, Sr incorporation appears to proceed inorganically across life history stages (Sect. 3.1; Lorens and Bender, 1980; Mucci and Morse, 1983; Morse and Bender, 1990). In addition to the broad relationship between calcification rate and Sr incorporation described by Carpenter and Lohmann (1992), several other studies also report a positive relationship between biotic and abiotic calcite Sr/Ca and calcification rate (driven by carbonate saturation state; e.g. Lorens and Bender, 1980; Russell et al., 2004). Most of the *S. purpuratus* populations cultured in our study revealed no significant change in Sr/Ca across a similar pH and carbonate ion concentration range. However, if a relationship between *S. purpuratus* mineral precipitation rate and skeletal Sr/Ca exists (Carpenter and Lohmann 1992), the Southern California population revealed a trend that may suggest faster precipitation rates of mineral crystals at the site of calcification during metamorphosis. We note that further study of the relationship between Sr incorporation and *S. purpuratus* calcite precipitation rates could explore this potential response of biologically mediated calcification to future acidification. The varied response across sites could indicate a possible biological threshold for *S. purpuratus* geochemical plasticity, which varies by regional upwelling, oceanography, and carbonate chemistry. If so, then Central and Northern California and Oregon larval purple sea urchin skeletal composition may eventually be affected at  $p\text{CO}_2$  levels beyond the range tested in this study.

#### 4 Conclusions

This study further illustrates the complexities involved in predicting marine calcifier outcomes due to anthropogenic ocean acidification. Although modeling results suggest that future changes in marine chemistry will favor calcite and low-Mg-calcite-precipitating organisms, many marine calcifiers, such as the purple sea urchin *S. purpuratus*, make use of a range of biominerals and biomineralization pathways and do not fall into a single mineralogical category (Ebert, 2007; Andersson et al., 2008). Taken together, the results presented here suggest that the geochemical composition of *S. purpuratus* skeleton precipitated during both early and adult life history stages is relatively insensitive to natural differences in chemical environment and future CO<sub>2</sub>-induced ocean acidification. However, elevated *p*CO<sub>2</sub> resulted in a change in the elemental composition of newly settled juvenile calcite from the warmest and most-buffered site (Southern California). While this study was designed to represent *p*CO<sub>2</sub> levels projected for 2100, a similar response may be evident in Central and Northern California and Oregon larval purple sea urchins when CO<sub>2</sub> concentrations exceed the levels studied here (beyond 2100, 900 μatm). These data provide evidence for potential geochemical plasticity during early life history stages that contrasts with the geochemical resilience observed in adult urchin calcite. Future research would quantify the degree to which the observed changes in Sr and Mg incorporation during early life history stages affect purple sea urchin skeletal stability and solubility under future ocean acidification. Coupled geochemical and biological experiments such as this one will continue to broaden our understanding of the biogeochemical mechanisms driving the wide array of observed biological responses to ocean acidification.

**Supplementary material related to this article is available online at:** <http://www.biogeosciences.net/10/3465/2013/bg-10-3465-2013-supplement.pdf>.

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